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NEUROCHEMICAL ADAPTATION FOLLOWING ACUTE AND CHRONIC STRESS:

IMPLICATIONS FOR PATHOLOGY

by

Jill Irwin

Dissertation presented 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
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December, 1984

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Abstract

A series of experiments was undertaken to assess the immediate and long term consequences of acute and chronic stressor application on central norepinephrine (NE) activity in CD-1 mice. Acute foot-shock stress increased the utilization of NE, and if the stressor was sufficiently severe, reductions of NE concentrations were evident. The magnitude of the amine reduction varied as a function of the stressor severity employed, the stressor controllability, and the brain region examined. In some instances, increased levels of serotonin (5-HT) were also noted following inescapable shock, but in general, the serotonergic system was less responsive to the aversive stimuli employed in the present series of experiments.

In contrast to the effects of an acute stressor, following chronic exposure to uncontrollable foot-shock (14-15 days), levels of NE reached or exceeded basal values. Determinations of NE utilization indicated that immediately following chronic stress, NE release was accelerated compared to that observed in the non-stress condition. Accordingly, it was postulated that the increased NE levels were due to increased synthesis, possibly reflecting an adaptive change which enables the organism to contend with sustained environmental demands.

Both acute and chronic stressors were found to have persistent effects on NE activity upon subsequent exposure to environmental insult. Specifically, presentation of a brief stressor 24 hr after acute uncontrollable shock provoked a marked decline of NE levels. Such an effect was noted even if a different form of aversive stimulation was administered during the reexposure session. It was thus suggested that the proactive effects of inescapable shock were due to

sensitization rather than conditioning processes. The sensitization of NE activity was also observed if mice were reexposed to a brief period of aversive stimulation as much as two weeks after the acute stress session. In mice that had previously been exposed to a chronic stress regimen, reexposure to a limited amount of footshock two weeks later resulted in increased NE levels. Since the elevated NE levels were accompanied by increased accumulation of the NE metabolite, MHPG, it was suggested that chronic stressor application resulted in a sensitization of NE synthesis. Accordingly, subsequent reexposure to the aversive stimuli resulted in accelerated NE synthesis.

Taken together, it appears that when confronted with a stressor, a sequence of adaptive changes occurs in order for the organism to contend with increased environmental demands. Acute stress results in accelerated NE release which is accompanied by an increased rate of synthesis. If the stressor is sufficiently severe, amine utilization exceeds synthesis, resulting in a net decline in levels of the transmitter. With repeated exposure to the stressor, synthesis is accelerated and eventually meets or exceeds the rate of amine utilization, resulting in NE concentrations which are higher than basal values. It is maintained that disturbances in this adaptive sequence may increase vulnerability to psychological and physical pathologies.

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To my mother and in memory of my father

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I. INTRODUCTION

There is considerable evidence, drawn largely from clinical studies, which suggests that stressful events play a significant role in promoting susceptibility to a variety of disease states. These include not only psychological disorders such as depression and schizophrenia, but diseases of the cardiovascular system, as well as immunologically mediated disorders and some forms of cancer. It has also become apparent, predominantly from basic animal research, that stressful events are associated with significant alterations of transmitters in the central nervous system, and these may come to influence pathological processes. In particular, it seems that variations in neurochemical activity may ultimately have either direct or indirect (e.g., via neuroendocrine alterations) effects on the immune system. Thus, it has been maintained that stressful events may contribute to the provocation or exacerbation of immunologically related disorders (see reviews in Fox, 1981; Irwin & Anisman, 1984; Monjan, 1981; Solomon, 1981; Stein, 1981). Furthermore, given that conditions such as depression are associated with central transmitter variations, it has been proposed that stressful events may also contribute to the provocation of affective disorders (e.g., Akiskal & McKinney, 1973; Anisman & Zacharko, 1982; Stone, 1983a,b). Accordingly, in evaluating the potential role of stressors in pathological states, it is important to examine the effects of psychological and physical insults on alterations of neurotransmitter activity.

The degree to which stressors will influence pathology, and produce changes in central neurotransmitter functioning, will depend upon a variety of factors. That is, neurochemical responses to

stressors will be influenced by individual differences including genetic factors, stress history, and the age of the organism. In addition, the nature of the stressor is an important determinant of the response. If, for example, the stressor is predictable, or if its offset is controllable, the neurochemical response may be limited. Similarly, stressor controllability may influence immunological processes (Laudenslager, Ryan, Drugan, Hyson & Maier, 1983) and susceptibility to pathology (e.g. neoplasia; Sklar & Anisman, 1979).

One particularly important determinant of the physiological consequences of aversive stimulation is the chronicity of the stressor. With respect to pathological processes for example, it has been demonstrated that although acute stressor application accelerated the growth of transplanted tumours, chronic exposure to a stressor of equivalent severity inhibited tumor development. Similarly, there is some evidence to suggest that acute stress results in suppression of the immune system, while chronic stress may provoke immunofacilitation (Monjan & Collector, 1977). It is particularly intriguing that parallel responses are noted in the central nervous system. That is, whereas acute stress may result in depletions of monoamines, and concomitant behavioral depression, with repeated application of stressors of the same magnitude, levels of the transmitter return to baseline. It might be argued that these neurochemical, immunological and pathological responses to chronic stress are all reflections of the adaptive capacity of the organism which enables it to respond to adverse environmental conditions. Before a meaningful analysis of the relationship between stressful experiences and diseases states can be undertaken, however, there must be an understanding of the basic

mechanisms subserving the response to stressors. Accordingly, it would be appropriate to assess the central neurochemical responses to stressful circumstances, and to examine those factors which may modify these responses.

The present paper will consider the effects of chronic stressor application on neurochemical processes. In the sections that follow, the responses of various transmitter systems to acute stressors will be described. This will be followed by a review of variables which limit these neurochemical responses, with particular attention devoted to stressor chronicity. Finally, a brief description of the behavioral consequences of stress exposure will be provided, with a view to exploring the potential contribution of neurochemical adaptation to various behaviors following chronic stress.

II. NEUROCHEMICAL RESPONSES TO STRESSORS

Exposure to environmental insults does not inevitably lead to either psychological or physiological disturbances. Among other things, those stimuli which are stressful to one individual may not be stressful to a second individual. Moreover, the neurochemical responses to a given stressor may differ considerably across subjects. It seems, as well, that a host of other experiential and organismic variables may come to determine the nature and magnitude of the neurochemical changes that occur following stressor application, as well as the pathological conditions that arise. There is reason to believe that organisms are capable of adapting to stressful events through a variety of physiological alterations. It is thought that when these adaptive processes are ineffective or do not function optimally, physical pathology ensues. According to such a formulation, analysis of the

effects of stressors should focus on the variables which increase vulnerability to such adaptive failures.

When an organism is confronted by environmental stressors, it will typically seek some way to diminish the potential impact of these insults. Presumably, it will adopt any one of several defensive strategies, which are likely to be determined by species and environmental considerations (see Bolles, 1970; Wahlsten, 1978). For instance, defensive styles of the rodent may consist of responses such as freezing, fleeing and fighting, and the specific behavior observed will depend upon the animal's previous experience in similar situations, as well as the environmental context in which the stressor appears. While the rat readily adopts a freezing posture in most threatening situations, the Mongolian gerbil is most likely to adopt a haphazard running pattern. Presumably, selection pressures (e.g., predators present in the respective environments of these rodent species) were influential in determining the particular response style highest in the defensive repertoire (see Anisman & Waller, 1973). Concomitant with such behavioral alterations, several physiological changes may occur in order to meet environmental demands. Some of these physiological alterations may blunt the impact of the physical insults, whereas others may be essential for the organism to emit appropriate defensive actions. Still others may be important in assuring that the animal is optimally prepared in the event that injury is sustained (e.g., alterations of energy metabolism, immunological responsivity).

Fundamental to the proposition that stressors contribute to pathology is the observation that upon exposure to physical insults a series of neurochemical alterations ensues. Briefly, the release of some neurotransmitters is increased, ostensibly as an adaptive response

in order to cope with the insult. Coupled with the increased release is an acceleration in the rate of synthesis in order to assure that adequate concentrations of the transmitter are available. Under some conditions, however, the release of transmitter may exceed synthesis, resulting in a net decline in the transmitter concentrations. It has been suggested that those variables which favor depletion of the transmitter will effectively increase vulnerability to pathology. Accordingly, it is necessary to determine the specific conditions and organismic factors which lend themselves to transmitter reductions.

II a. Norepinephrine

Norepinephrine (NE) is widely distributed throughout the brain and is thought to play a role in the mediation of various behaviors including consummatory responses and acquisition of appetitively and aversively motivated responses. Moreover, NE dysfunction has been associated with pathologies such as depression, and more recently, it has been postulated that alterations in NE may be associated with immunological disorders (see Irwin & Anisman, 1984).

Variations of NE concentrations seem to be consistent across various forms of stressors as well as a wide variety of species. In general, it appears that upon stressor inception the release and synthesis of NE are increased in many brain regions, and not unexpectedly, the extent of the turnover change varies as a function of stressor severity. Several lines of evidence have in fact pointed to the conclusion that stressors increase the turnover of NE. In particular, it has been demonstrated that physical insults increase the accumulation of the NE metabolite 3-Methoxy-4-hydroxy-phenylglycol (MHPG), and accentuate the NE depletion produced by inhibition of NE synthesis (Imori et al., 1982; Korf, Aghajanian & Roth, 1973a,b; Korf,

1976; Nakagawa, Tanaka, Kohno, Noda & Nagasaki, 1981; Tsuda et al, 1982). Moreover, it has been shown that stressors enhance the disappearance of exogenously administered ^3H -NE (Javoy, Thierry, Kety & Glowinski, 1968; Thierry, Blanc & Glowinski, 1971; Thierry, Javoy, Glowinski & Kety, 1968). Consistent with the suggestion that stressors enhance NE synthesis, it has been observed that the activity of the synthetic enzymes tyrosine hydroxylase and dopamine-beta-hydroxylase was accelerated after stressor application, and such treatments also increased the formation of ^3H -NE formed from exogenously administered precursors (Kobayashi, Palkovits, Kizer, Jacobowitz & Kopin, 1976; Kvetnansky et al., 1976; Thierry, 1973; Thierry, Fekete & Glowinski, 1968).

It is generally acknowledged that reductions of NE concentrations occur at a point when further increases in the rate of NE synthesis are not sufficient to accommodate the increased release of the amine. Additionally, it seems that stressor severity may influence the pool from which NE is released. For example, Thierry and her associates demonstrated that footshock of moderate severity increased the disappearance of ^3H -NE formed from tritiated precursors administered 2 to 20 minutes earlier, but did not affect ^3H -NE formed from tritiated precursors that had been administered 180 minutes earlier. In contrast, severe stressors resulted in increased disappearance of ^3H -NE formed from tritiated precursors irrespective of whether they were administered 20 or 180 minutes earlier. Thus, it was suggested that moderate stressors influence newly synthesized NE stored in the functional pools, while severe stressors also resulted in the release of previously synthesized transmitter stored in the reserve pools

(Thierry et al, 1971; Thierry, Javoy, Glowinski & Kety, 1968).

Stressors have been shown to induce changes of NE activity in a number of brain regions including limbic forebrain structures such as the hippocampus, amygdala, cerebral cortex as well as in the thalamus, hypothalamus and pons-medulla (Tsuda et al., 1982). It has been argued that the dorsal noradrenergic bundle is particularly sensitive to stressors since ablation of NE cell bodies in the locus coeruleus prevents the increased utilization of NE in ipsilateral terminal regions (Korf et al, 1973b). In addition there are considerable data indicating that terminal regions of the ventral noradrenergic pathway in regions such as the hypothalamus are equally or more sensitive to stress-induced alterations (Nakagawa et al., 1981; Tsuda et al., 1982). Recently it was suggested that physical stressors influence NE turnover in terminal regions of both the ventral and dorsal noradrenergic bundles, while psychological stressors preferentially influence NE activity in terminal regions of the ventral bundle (Iimori et al., 1982). However, it may simply be the case that hypothalamic neurons may be more sensitive to stressors than are limbic forebrain structures, and hence, milder forms of stress tend to have lesser effects in the latter brain regions. Indeed, using restraint stress it was demonstrated that increased turnover of NE was evident in several brain regions). The increased utilization of the transmitter was more readily detected in the hypothalamus than in regions such as hippocampus and cortex (Tanaka, Kohno, Nakagawa, Ida, Takeda & Nagasaki, 1982). Following a limited amount of stress, NE concentrations remain fairly stable, but as stressor duration or severity increases, transmitter depletions are apparent. Consistent with the observations of turnover,

it seems that depletion of the transmitter occurs more readily in the hypothalamus than in other structures.

II b. Dopamine

As in the case of the noradrenergic system, numerous functions have been attributed to dopaminergic activity, and among other things, it has been implicated in motor activity, arousal, attentional and motivational processes. Moreover, considerable evidence has suggested that dopaminergic dysfunction is significant in the etiology of schizophrenia. The data concerning the effects of stressors on dopamine (DA) variations are considerably less extensive than those concerning NE turnover and concentrations. While most of the early reports typically indicated that DA concentrations were either unaffected or increased following stressor application (see reviews in Anisman, 1978; Stone, 1975), more recent reports assessing DA neuronal activity have shown profound alterations which were restricted to a limited number of brain regions (see reviews in Anisman, 1984; Anisman & Zacharko, 1982; Dunn & Kramarcy, 1984).

When DA levels were assessed in whole hypothalamus, increased concentrations were typically observed (see discussion in Anisman, 1978; Weiss, Glazer, Pohorecky, Bailey & Schneider, 1979). However, evaluation of discrete nuclei within the hypothalamus revealed that DA reductions were evident in the arcuate nucleus (Kobayashi et al., 1976; Kvetnansky et al., 1976). Likewise, Monnet and Lichtensteiger (1981) reported increased DA fluorescence in this region following brief footshock. Inasmuch as the arcuate comprises only some 5% of the entire hypothalamus, it is not surprising that DA reductions were not observed when this entire region was assayed. However, it should be emphasized that the arcuate nucleus sends trajectories to the pituitary

via the median eminence, and thus this region may be fundamental in determining various neuroendocrine changes associated with stressors.

Several reports revealed brain regions other than the arcuate nucleus in which DA neurons are sensitive to stressors. For instance, Thierry, Tassin, Blanc and Glowinski (1976) reported that the depletion of DA following tyrosine hydroxylase inhibition was accelerated by stressors in the mesolimbic frontal cortex and the nucleus accumbens, while DA levels were apparently unaffected in the striatum or olfactory tubercle. Thus, it was suggested that stressors influence the mesolimbic DA system without affecting the nigrostriatal system. In support of these findings, studies of DA metabolism have indicated that concentrations of the DA catabolite dihydroxyphenylacetic acid (DOPAC) or the DOPAC/DA ratio were increased in the nucleus accumbens and/or frontal cortex following stress, while such effects were not evident in the striatum (Blanc, Herve, Simon, Lisoprawski, Glowinski & Tassin, 1980; Fekete, Szentendrei, Kanyicska, & Palkovits, 1981; Fadda, Argiolas, Mellis, Tissari, Onali & Gessa, 1978; Herman, Guillonneau, Dantzer, Scatton, Semerdjian-Rouquier & LeMoal, 1982; see also Tissari, Argiolas, Fadda, Serra & Gessa, 1979 concerning striatal synthesis after stress). Additionally, these experiments typically revealed that the frontal mesolimbic cortex was most sensitive to the effects of the stressor, the nucleus accumbens was somewhat less affected, while the striatum was not affected at all by the stressor. Indeed, exposure to footshock provoked more than a 100% increase of the DOPAC/DA ratio in the frontal cortex and more than a 50% increase in the cingulate cortex, without significantly affecting the nucleus accumbens or striatum (Lavielle et al., 1979). More recently it was also reported

that stressor application may reduce DA concentrations in the lateral septum, without influencing other portions of the septal region (Saavedra, 1982).

While the frontal cortex appears to be most sensitive to the effects of stressors on DA activity, under some conditions striatal activity may be altered. For example, although brief footshock exposure did not affect striatal DA (Dunn & Kramarcy, 1984), following 2 hr of cold restraint DA neuronal activity (as measured by the DOPAC/DA ratio) was increased in the striatum (Dunn & File, 1983). Similarly, Keller, Weiss, Schliefer, Miller and Stein (1981) had previously reported increased DA release in stressed animals, as determined by in vivo voltammetry, while Beley, Beley, Rochette and Bralet, (1976a) found increased DA synthesis in the striatum following stressor application. It is not clear why these few experiments revealed DA neuronal activation in the striatum whereas most investigators failed to detect dopaminergic alterations in this region. However, it might be noted that in the Dunn and File (1983) experiment the stressor applied was that of cold restraint, that of Keller et al (1981) involved cold water immersion, and Beley et al (1976a) employed exposure to a 4 C environment. Thus the possibility should be considered that striatal DA may be subject to alteration by only some forms of stressor (e.g., cold).

It has been suggested (see Dunn & File, 1983; Dunn & Kramarcy, 1984) that the heightened sensitivity of the frontal cortex to stressor application may be attributable to the fact that the DA terminals in this region lack autoreceptors (Bannon, Michaud & Roth, 1981). That is, in other brain regions the initial DA efflux prompted by stressor application would result in autoreceptor regulation of DA turnover,

whereas in frontal cortex the absence of these autoreceptors would prevent such regulation, resulting in an appreciable increase of turnover. However, it was recently reported that the increased DOPAC accumulation induced by footshock in prefrontal cortex and in piriform cortex was abolished by intracerebral infusion of a monoclonal antibody for substance P, despite the fact that only the former region lacks autoreceptors. Accordingly, it was suggested that the effects of the stressor on DA release was due to a substance P related mechanism rather than to the presence of autoreceptors (Bannon et al., 1983).

II c. Epinephrine

Considerably less attention has been devoted to stressor provoked changes of epinephrine (EPI) relative to that of norepinephrine and dopamine. Nevertheless, it does appear that EPI neurons in the brain are greatly affected by environmental insults. It was demonstrated, for instance, that footshock reduced EPI concentrations in hypothalamus, brainstem and locus coeruleus (Sauter, Baba, Stone & Goldstein, 1978). Likewise, hypothalamic and brainstem EPI was reported to decline following restraint (Saavedra, Kvetnansky, Kopin, 1979) and hypothalamic EPI was also reduced by swim or oscillation (Roth, Mefford & Barchas, 1982). The results of Sauter et al (1978) in fact, revealed that the EPI alterations were more sensitive to the stressor than were variations of NE activity. Sudo (1983) also reported more pronounced depletions of EPI than NE in hypothalamus, pons-medulla and midbrain following warm water swim; however, the duration of stressor exposure required to induce the EPI reductions was greater than that required to elicit the NE decline. In addition, it seems that the effects of stressors on EPI concentrations will vary as a function of

stressor severity and the brain region examined. Following 20 min of immobilization stress reductions of EPI were noted in the anterior hypothalamus and nucleus commissuralis, while increased concentrations were evident in the median eminence. With more protracted stress (240 min) reductions of the amine were noted in the anterior hypothalamus and in the periventricular and ventral medial nuclei. Such reductions were also noted in the nucleus tractus solitarius, nucleus commissuralis and the A1 and A2 regions which are the areas in which EPI fibres originate (Saavedra, 1980; Saavedra et al., 1979).

II d. Serotonin

Stressors have repeatedly been shown to increase the synthesis and release of serotonin (5-HT). Application of various forms of aversive stimulation were shown to increase the accumulation of the 5-HT metabolite, 5-hydroxyindoleacetic acid (5-HIAA) (Bliss, Thatcher & Ailion, 1972; Morgan, Rudeen & Pfiel, 1975; Sherman & Petty, 1980). Not unexpectedly, it seems that the accumulation of the metabolite increases with stressor duration in the hypothalamus, brainstem and cerebral cortex. In the striatum, increased 5-HIAA was evident only after very protracted stress (300 min of immobilization), while in the diencephalon, 5-HIAA concentrations were unaffected by stress (see Morgan et al., 1975).

Evidence of increased utilization of 5-HT in stressed animals was also gleaned from the finding that aversive stimulation accelerated the disappearance of 5-HT following administration of the synthesis inhibitor parachlorophenylalanine (see Thierry, 1973). In accordance with such findings, Thierry, Fekete and Glowinski (1968) and Thierry, Javoy, Glowinski and Kety (1968) reported that footshock resulted in increased formation of ^3H -5-HT formed from tritiated precursors.

Likewise, stressor administration resulted in a decline of exogenously administered ^3H -5-HT (see Kantak, Wayner & Stein, 1978 for similar effects with respect to the effects of food deprivation on 5-HT turnover). It appeared, however, that the 5-HT alterations were less readily attained than were NE variations. In particular, it was found that although 15 min of footshock was required to influence newly synthesized NE, 180 min of stress was necessary to alter the release of newly synthesized 5-HT (Thierry, Blanc & Glowinski, 1970, 1971; Thierry, Fekete & Glowinski, 1968; see review in Thierry, 1973).

The effects of stressors on 5-HT concentrations vary dramatically with the severity of the aversive stimulation, the time following stressor application, as well as the brain region examined. While brief periods of moderate stress seem to increase concentrations of 5-HT (see review in Welch & Welch, 1970), reductions of the amine may be achieved with more protracted stress. Telegdy and Vermes (1976) found that footshock applied over a 15-30 min period resulted in reduced hypothalamic 5-HT concentrations, had little effect on hippocampal or septal 5-HT, but engendered a slight decrease of the amine in mesencephalon and amygdala. If the stress period extended to 60 or 90 min, then the 5-HT alterations (either increased or decreased) were no longer evident. Alterations of 5-HT concentrations were likewise noted in a number of brain regions following various periods of exposure to restraint stress. Whereas brief periods of restraint (30 min) resulted in increased 5-HT levels in the ventromedial nucleus of the hypothalamus (Kiss, Culman, Mitro, Kvetnansky & Palkovits, 1980) protracted exposure (3 hr) resulted in a transient reduction of 5-HT

levels in the hypothalamus, amygdala and hippocampus (Palkovits, Brownstein, Kizer, Saavedra & Kopin, 1976).

II e. Acetylcholine

Several reports indicated that aversive stimuli would result in an initial decline of acetylcholine (ACh) concentrations, followed by an elevation which peaked about 40 min to 1 hr after stressor termination (e.g., Zajackowska, 1975; see also Anisman, 1978). When rats were tested 30 min after cold water swim (and subsequent exposure to a hot plate) ACh concentrations were found to increase in cerebellum, cerebral cortex and striatum. In hippocampus ACh concentrations increased, but not to a statistically significant extent, while in midbrain and brainstem amine concentrations were unaltered by the aversive treatment (Romano & Shih, 1983).

Measurement of the effects of stressors on the synthesis and utilization of ACh have not yielded consistent results. For instance, Gilad and McCarty (1981) reported that immobilization did not influence the activity of the synthetic enzyme, choline acetyltransferase, in the brainstem and locus coeruleus. Likewise, in hippocampus choline acetyltransferase activity was unaffected by a similar stress treatment, although ^3H -choline uptake was reduced (Gilad, Rabey & Shenkman, 1983). In contrast to the effects of a stressor on ACh synthesis, activity of the degradative enzyme, acetylcholinesterase, was reduced following acute immobilization plus cold (Gabriel & Soliman, 1983) suggesting decreased utilization of ACh. In accordance with these results, Costa, Tagliamonte, Brunello and Cheney (1980) found that immobilization did not affect the turnover of ACh in frontal

cortex and in hippocampus; however, it was noted that in hypothalamus prolonged immobilization (24 hr) reduced ACh turnover.

In contrast to the effects of restraint alone, the combination of immobilization plus cold exposure was found to decrease ACh turnover in cortex, hippocampus and hypothalamus, but such an effect was dependent on the duration of stressor application. That is, turnover of ACh in frontal cortex was reduced with 1 hr of cold exposure, but this effect was absent with 4 or 24 hr of treatment (Costa et al., 1980). In contrast, hypothalamic ACh turnover was reduced irrespective of whether rats were exposed to the cold environment for 1, 4 or 24 hr. Subsequent experiments (Brunello, Tagliamonte, Cheney & Costa, 1981) indicated that the nature (or severity) of the stressor influenced the alterations of ACh activity. While immobilization was without effect on ACh concentrations or turnover, cold exposure applied for equivalent time periods effectively reduced ACh turnover in the hypothalamus. Inasmuch as the ACh changes occurred in response to cold, but not to immobilization, it was suggested that the variations of this amine may be due to changes of body temperature as opposed to the effects of the stressor per se. Yet, it is possible that cold exposure was a more severe stressor for the animal, and pending parametric evaluation of the effects of different stressor severities on ACh turnover, a conclusion regarding this issue would be premature.

As indicated earlier, the effects of stressors on ACh turnover vary over time following stressor application. It was demonstrated that immediately following shock application (in a Sidman avoidance training paradigm) neither high affinity choline uptake nor ACh turnover in hippocampus were appreciably affected. However, within 1 hr of stressor termination both uptake and turnover of ACh were greatly increased

(Cooper, Schmidt & Barrett, 1983). In light of the finding that turnover of ACh was reduced immediately following cold exposure (see Costa et al., 1980; Brunello et al., 1981), the possibility needs to be examined that the diverse outcomes across studies are related to the time following stressor application at which ACh activity was determined.

The functional significance of the biphasic cholinergic change is not immediately evident. However, the variations of ACh turnover and concentrations in the hippocampus are paralleled by alterations in the motoric abilities that animals demonstrate in paradigms involving aversive motivation. In particular, soon after exposure to a stressor rodents display heightened arousal, after which depressed behavioral responding is evident. Inasmuch as cholinergic manipulations have been repeatedly demonstrated to differentially influence avoidance performance at various intervals following stressor application (see Anisman, 1975), it is possible that the initial decline of ACh turnover prepares the organism for active defensive response styles, whereas the subsequent increase of ACh turnover represents a phase of exhaustion or recuperation once the source of stress is absent.

II f. Gamma-Aminobutyric Acid

There exists a great paucity of data concerning the effects of stressors on amino acid transmitters. However, several investigators had reported that stressors, such as restraint, were associated with increased concentrations of gamma amino butyric acid (GABA) (Bliss & Zwanziger, 1966). Likewise, aversive stimulation in the form of footshock was associated with heightened levels of GABA (McBride, Hingtgen & Aprison, 1976), although it appeared that several stress

sessions were necessary to provoke such a change (Chattopadhyay & Uniyal, 1975). There appears to be some question as to whether the GABA variations are due to the nociceptive attributes that may be associated with stressors. In particular, while 1 hr of restraint, a treatment which it will be recalled reliably influences catecholamine activity and concentrations, produced a decline of GABA, painful stimulation (10 sec on a 60°C hotplate) significantly increased GABA concentrations (Sherman & Gebhart, 1974). While these data are consistent with the suggestion that the GABA variations reflect a response to painful stimulation, it is not clear that the two treatments should be considered as being equally stressful. Besides, as indicated earlier, Bliss and Zwanziger (1966) reported that restraint increased GABA concentrations, a finding diametrically opposed to that reported by Sherman and Gebhart (1974). Unfortunately, the substantial procedural differences between these studies precludes specification of the salient factors responsible for these diverse outcomes.

More recent studies of the effects of stressors on GABAergic mechanisms have focussed on the influence of environmental factors on receptor density and binding. Following acute cold-swim exposure, increased GABA receptor binding was evident in forebrain, which was largely attributable to a moderate increase in the number of high affinity sites and a larger increase in the number of low affinity sites. The increased GABA binding is short-lived, being present immediately after a 3 min swim, but absent within 4 min of stressor termination (Skerritt, Trisdikoon & Johnston, 1981). In accordance with the effects of brief swim, it was reported that repeated handling of rats resulted in an increase of GABA receptor binding in cerebellum (Biggio, Corda, Demontis, Concas & Gessa, 1980), as well as in frontal

cortex and caudate (Biggio, Corda, Concas, Demontis, Rossetti & Gessa, 1981). Interestingly, the latter investigators observed that in handled animals the stress of footshock resulted in decreased GABA binding, but in otherwise naive animals footshock was without effect. These results were taken to suggest that the GABA binding values typically reported for control animals, and therefore considered to represent baseline levels, are in fact, reduced by the stress of handling prior to decapitation. By habituating animals to the handling procedure GABA levels were stable prior to decapitation, and hence the effects of physical stressors on GABA concentrations were more detectable.

Together, these data suggest that the GABA system is responsive to aversive stimulation; however, the direction of the effect and whether or not it is secondary to the activity of other transmitter systems remains to be determined. Nevertheless, stressor induced alterations of GABA are of interest given the putative role for this transmitter in cardiovascular function and in affective disorders.

III. VARIABLES MODIFYING STRESS-PROVOKED NEUROCHEMICAL CHANGE

It is clear from the preceding sections that stressors produce a variety of neurochemical alterations. In attempting to delineate the relationship between stressful events and the induction or exacerbation of pathological states it should be remembered that (a) stressors will not necessarily engender pathological states, (b) stressors may elicit different types of pathologies across species, strains within a species, as well as individuals within a strain, and (c) other things being equal, stressors may differentially influence neurochemical

activity as a function of diverse background (environmental) conditions, organismic variables and experiential factors. In effect, it is assumed that stressors will engender a great number of endogenous neurochemical variations, and it is thought that individuals or strains may differ with respect to that neurochemical which is most vulnerable to dysfunction. The specific neuronal mechanism(s) which are adversely affected by environmental insults will determine the nature of the pathology which ensues. Not unexpectedly, a number of variables will influence the reactivity of neurochemical systems, and it is imperative to consider the contribution of these factors in attempting to discern the stress-pathology profile. The ensuing sections will document the most widely studied factors known to influence stressor related neurochemical alterations.

III a. Behavioral Coping

In assessing the behavioral disturbances engendered by exposure to stressors in infrahuman subjects it has repeatedly been shown that the organism's ability to deal with the aversive stimuli through behavioral means was fundamental in determining whether subsequent behavioral disturbances would be manifested. For example, while exposure to escapable shock has typically been shown not to influence performance adversely in subsequent behavioral paradigms, exposure to an identical amount of uncontrollable shock (applied in a yoked paradigm) profoundly disrupted subsequent performance. Such deficits were demonstrated in numerous behavioral paradigms including shock and water escape performance (Maier & Testa, 1975; Irwin, Suissa & Anisman, 1980), antinociception in paw-lick and tail-flick tests (Jackson, Maier & Coon, 1979), alterations in social dominance hierarchies (Williams, 1982), variations of exploratory patterns (Bruto & Anisman, 1983),

induction of a response perseveration tendency (Anisman, Hamilton & Zacharko, 1984) and a marked reduction of responding for electrical stimulation from some brain regions (Zacharko, Bowers, Kokkinidis & Anisman, 1983).

In view of the potent influence of coping methods on behavioral disturbances, it was postulated that controllability over stressor termination might likewise be significant in the etiology of psychological disturbances such as depression (see Anisman & Zacharko, 1982), immunologically related disorders (Irwin & Anisman, 1984; see reviews in Fox, 1981; Monjan, 1981; Sklar & Anisman, 1980), as well as the classical psychosomatic illnesses such as gastric ulceration (Weiss, 1971a,b,c) and cardiovascular ailments (Galosy, Clarke, Vasko & Crawford, 1981). In an attempt to identify those factors subserving these stress-provoked behavioral and physiological disturbances, several investigators indicated that the organism's ability to deal with stressors through behavioral means might influence the neurochemical response to those stressors. For instance Weiss, Stone and Harrell (1970), and subsequently Anisman, Pizzino and Sklar, (1980) demonstrated that following exposure to inescapable footshock marked depletions in NE content were noted in various brain regions. In contrast, if animals were able to escape from the aversive stimuli, no alteration of NE content was apparent. In accordance with these findings, Swenson and Vogel (1983) reported that in hypothalamus escapable shock was without effect on NE concentrations, while inescapable shock produced a reduction of NE which was most dramatic 30 min following the termination of the stress session. These investigators observed that NE concentrations in the hippocampus were

decreased after a 60 min session of shock-escape training, whereas uncontrollable shock was without effect. It should be noted, however, that the effects of the stressor on hippocampal NE concentrations reported in this study are in marked contrast to those observed in other laboratories. That is, inescapable shock typically results in NE depletion while escapable shock has no effect on amine levels (see reviews in Anisman, Kokkinidis & Sklar, 1981; Stone, 1975; Weiss et al., 1979; Weiss et al., 1981).

The influence of controllable and uncontrollable stressors on hypothalamic NE concentrations appear to vary as a function of stressor duration (Tsuda, Tanaka, Nishikawa, Ida & Nagasaki, 1983). Following 3 hr of training in a fairly complex discriminated Sidman avoidance task, NE utilization was greater in animals exposed to escapable shock than in rats that received no shock treatment. Moreover, NE concentrations were reduced in escapably shocked animals relative to those rats that received inescapable shock or no shock. With longer stress sessions (6 hr) increased utilization of the amine was observed in both the escapable and inescapable shock groups, and reductions of NE levels were seen only in the escapable condition. Finally, once the response was well entrenched (21 hr of training) the utilization of NE in the escapable shock condition was reduced relative to that seen following lesser amounts of training, while in the inescapable shock condition, the increased NE utilization persisted and levels of the transmitter declined. These data were taken to suggest that the animal's ability to cope with a stressor will lead to reductions in the turnover of hypothalamic NE ordinarily produced by a stressor, but that such an effect will only be evident once animals have learned behavioral responses which terminate the aversive stimulation. In effect, the

differences between the inescapably and escapably shocked animals may not stem from the former group learning that shock offset is independent of responses, but rather, from the escapably shocked animals learning mastery over the experimental contingencies.

Further evidence suggesting that alterations of NE concentrations could be induced by the psychological rather than the physical attributes of the stressor comes from the finding that in mice trained to cope with a stressor behaviorally, subsequent exposure to inescapable shock did not provoke the depletion of NE ordinarily observed (Anisman, Irwin, Beauchamp & Zacharko, 1983; Anisman et al, 1980). Moreover, recent behavioral experiments indicated that the differential performance of escapably and inescapably shocked mice may be attributable to animals in the former condition acquiring competence in the demands of the task (Szostak & Anisman, 1984).

It has been suggested that the influence of coping factors on stressor induced alterations of NE are not restricted to the organism having the ability to determine shock offset. In particular, Stolk, Conner and Barchas (1974), demonstrated that the depletion of NE ordinarily produced by inescapable shock was prevented among rats that had been permitted to engage in fighting during the stress session. Moreover, the extent of the NE depletion was inversely related to the number of fighting bouts in which rats had engaged. In light of the fact that fighting was also shown to prevent both stressor induced gastric ulceration (Weiss, Pohorecky, Salman & Gruenthal, 1976) as well as the enhancement of tumor growth ordinarily elicited by stressors (Sklar & Anisman, 1980), the possibility exists that fighting acted as a method of coping with the physical insults. It might be noted

however, that although fighting appears to prevent the NE alterations produced by other forms of stress, fighting itself may dramatically influence amine levels and turnover (see Modigh, 1976; Welch & Welch, 1970). Indeed, even the psychological stress of observing conspecifics engaged in fighting led to NE depletion in mice (Welch & Welch, 1968).

In contrast to the relatively extensive literature concerning the influence of coping factors on NE alterations, few data are available concerning the contribution of this variable to changes in the activity of other neurotransmitters. It was reported, however, that in the lateral septum the reductions of 5-HT produced by inescapable shock were not evident in rats that had received escapable shock (Petty & Sherman, 1982). Moreover, Weiss et al (1981) reported that in the locus coeruleus, uncontrollable shock resulted in lower levels of 5-HT relative to non-stressed animals, although the uncontrollable shock did not significantly reduce 5-HT concentrations relative to rats that received escapable shock. Interestingly, Weiss et al (1981) reported that in the brainstem (excluding locus coeruleus), escapable stress resulted in increased 5-HT concentrations, while inescapable stress was without effect. Thus, it is conceivable that in the lateral septum and in the locus coeruleus the 5-HT depletion is a result of the inability to behaviorally deal with the stressor (which, incidentally, may also cause the stressor to be interpreted as being more aversive), while in the brainstem the increased 5-HT concentrations may be a result of the animal performing the coping response, rather than the stressfulness of the situation (see discussion in Weiss et al., 1981).

With respect to stressor invoked alterations of DA concentrations, it has yet to be adequately determined whether or not coping factors are a contributing factor. To be sure, there have been several reports

which examined the effects of controllable and uncontrollable stressors on DA alterations, but the neurochemical determinations have been conducted in either fairly large brain regions or in regions in which DA or DOPAC alterations are not typically observed. Typically, escapable and inescapable shock were not found to differentially influence DA concentrations in these brain regions (e.g., Anisman et al., 1980; Weiss et al., 1981). Interestingly, however, Swenson and Vogel (1983) observed that neither 10 nor 60 min sessions of escapable shock altered DA concentrations, while inescapable shock applied over a 10 min session led to a decline of DA. As the shock session continued (60 min) control levels of DA were attained, and within 30 min of the last shock trial concentrations actually exceeded those of rats exposed to controllable shock. It is tempting to speculate that the absence of DA determinations in discrete hypothalamic nuclei or mesocortical structures may have precluded observation of differential effects of controllable and uncontrollable shock in the Anisman et al. (1980) and Weiss et al. (1981) reports. However, it is equally likely that following a limited amount of shock a depletion of DA would have been evident. After all, it is conceivable that a compensatory increase of synthesis following a relatively large number of shock trials would have resulted in a return to basal levels of DA.

Paralleling the effects of stressors on DA activity in the frontal mesolimbic cortex, it was demonstrated that footshock may influence DA receptor binding in this region (Cherek, Lane, Freeman & Smith, 1980). Moreover, it appeared to be the case that stressor controllability was fundamental in determining this effect. While escapable shock administered over 4 consecutive daily 8 hr sessions was without effect,

an equivalent amount of inescapable shock increased low affinity receptor binding. While these data certainly do implicate controllability as a factor in determining the DA receptor alterations, the fact that several stress sessions of protracted duration were employed leads to the possibility that the chronicity of the stressor may have interacted with controllability in determining the observed outcome.

In light of the paucity of data concerning stressor effects on ACh concentrations and turnover, it is not surprising that still less information is available with respect to the role of coping in determining variations of this transmitter. There is indirect information, however, which suggests that this variable may be an important one in determining the ACh alterations. In particular, Aprison and Hingtgen (1970) observed that acquisition of the appropriate response in a Sidman avoidance task was associated with reductions of ACh concentrations. Furthermore, in an experiment in which rats were trained to escape shock, Karczmar, Scudder and Richardson (1973) reported that ACh concentrations were unaltered by the stress treatment. In a second study, however, in which rats were exposed to inescapable shock, increased ACh concentrations were noted. Unfortunately, since these studies were not conducted to assess the role of stressor controllability in determining the ACh changes (and hence a yoked paradigm was not employed), the effects of the controllability were confounded with the amount of shock rats received. Thus it cannot be concluded that the between studies differences were solely or primarily attributable to coping factors. More recently, however, evidence has become available suggesting that coping factors indeed influence cholinergic neuronal activity. Relative to rats that

received 4 sessions of escapable shock (8 hr duration), increased binding to cortical cholinergic receptors was evident in rats that received yoked inescapable shock (Cherek et al., 1980). Thus it appears that stressor controllability may come to modulate cholinergic neuronal functioning. However, as in the case of the DA changes described earlier, the possibility should not be dismissed that these results were due to the interaction between the chronicity and controllability of the stress regimen employed.

Taken together, it appears that the organism's ability to contend with stressors through behavioral means may be a particularly cogent variable in determining neurochemical responsivity. It appears that stressors elicit both behavioral and neurochemical changes to deal with the environmental insult. That is, in addition to attempts to eliminate or reduce the severity of the stressor, increases in the release of monoamines are also evident, coupled with a complementary increase in the rate of synthesis. Under conditions where behavioral methods of dealing with the stressor are not available, the burden of coping rests more fully on endogenous mechanisms. Thus, release of monoamines is increased, and if the stressor is sufficiently severe and/or protracted synthesis does not keep pace, consequently a net decline of the transmitter results. It has been the contention of some investigators that the reduction of amine levels represents a failure of the organism to adapt to the environmental demands, which may leave the organism more vulnerable to pathology (see Akiskal & McKinney, 1973; Anisman & Zacharko, 1982; Stone, 1975; Weiss et al., 1979; see also Irwin & Anisman, 1984; Anisman & Sklar, 1980; Stone 1983a).

III b. Prior Stress History

As indicated earlier, the alterations of catecholamine turnover and concentrations engendered by aversive stimulation are fairly transient. Stressors of moderate severity will induce neurochemical changes that persist for only a few hours. Despite the short-lived impact of stressors, it appears that traumatic experiences may have long-term repercussions. In particular, data are available which suggest that traumatic events may result in either the conditioning or sensitization of mechanisms subserving neurochemical activity, such that upon reexposure to the stressor or cues associated with the stressor a marked change of neurochemical activity ensues. It has been argued that some of the long-term behavioral disturbances attributable to uncontrollable stressors may, in fact, be due to conditioning or sensitization of central neurochemical states (see Antelman & Chiodo, 1984; Anisman, 1984; Anisman & Zacharko, 1982).

Several experiments conducted by Anisman and Sklar (1979, 1981) revealed that 24 hr following exposure to inescapable shock the turnover and concentrations of NE were comparable to those of naive animals. However, if mice were reexposed to an amount of shock which ordinarily was without effect, the NE reductions were reestablished. Similarly, Cassens, Roffman, Kuruc, Orsulak and Schildkraut (1980), demonstrated that presentation of cues that had been paired with footshock would elicit increased utilization of brain NE, as indexed by increased concentration of the NE metabolite MHPG. Thus, there is reason to suppose that conditioning factors may contribute to heightened neuronal activity. Together these data serve to illustrate that traumatic events may increase the reactivity of NE neurons to later stressors. While such an occurrence may have the beneficial

effect of preparing the organism to deal with later aversive events (i.e., owing to rapid induction of NE release), negative repercussions may be present in that animals may be more vulnerable to the amine depletion and hence the occurrence of pathology.

In the case of DA neuronal activity, as with that of NE, some data are available suggesting that release of the transmitter is sensitive to the animals previous stress experiences. For instance, it was reported that DOPAC concentrations in rats exposed to foot-shock over a 20 min period were appreciably increased in the antero-medial and sulcal frontal cortices, olfactory tubercle, nucleus accumbens and amygdaloid complex. Upon reexposure to the test environment in which shock had been delivered DOPAC concentrations increased in the anteromedial frontal cortex (Herman et al, 1982). Thus it appears that cues associated with a stressor may increase the release of DA, but it seems that DA neurons in some brain regions may be more sensitive to conditioning effects than in other brain regions. In addition to the mesocortical DA changes induced upon reexposure to a stressor, Monnet & Lichtensteiger (1981) found that in the substantia nigra a brief amount of foot-shock (0.5 mA for 10 sec) resulted in increased DA fluorescence. Upon reexposure to the environment associated with shock, the increased fluorescence appeared more rapidly in those animals that had previously been shocked (i.e., within 5 min) than in those that were otherwise naive (handling itself increased fluorescence, but not until 30 min after placement in the apparatus). It will be recalled that DA neuronal activity in substantia nigra is typically not altered by stressors; however, there is reason to believe that increased release of the amine may occur, but only after a brief amount of

stress. Thus, the data of Monnet and Lichtensteiger (1981) may be explained by conditioning of environmental cues associated with a relatively brief period of stress.

Additional data implicating sensitization of neurochemical processes by stressors stems from a number of pharmacological studies. Treatment with d-amphetamine in moderate doses has reliably been shown to induce a stereotyped pattern of responding, presumably due to the activation of DA neurons (see review in Antelman & Chiodo, 1984). In rats exposed to a stressor several days earlier, the stereotyped behavior ordinarily elicited by amphetamine was greatly enhanced (Antelman, Eichler, Black & Kocan, 1980). More recently, MacLennan and Maier (1983) observed a similar outcome following treatment with either amphetamine or cocaine. On the basis of such results, it was suggested that environmental stressors may result in the sensitization of DA neurons so that subsequent exposure to a DA stimulant would have an enhanced effect. Moreover, it was postulated that pathologies purportedly involving heightened DA neuronal activity (e.g., idiopathic and amphetamine engendered psychosis) may be influenced by the individual's previous stress history.

Only a single report is available concerning the potential of ACh neurons to be influenced by conditioning or sensitization processes. Using a conditioned emotional responding (CER) paradigm, Hingtgen, Smith, Shea, Aprison and Gaff (1976) demonstrated that a stimulus that had been paired with shock, which elicited response suppression, also engendered a rise of ACh concentrations. In this particular report catecholamine concentrations were evaluated as well, but alterations of these transmitters were not observed. It is possible that either the cholinergic system was more sensitive to the particular paradigm

employed, or that conditioning of catecholamine systems would more readily have been detected had turnover rather than levels been determined.

In summary, it appears that aversive events will not only have immediate effects on neurochemical systems, but may have lasting effects in that later presentation of aversive stimuli will result in a more rapid and pronounced response than in otherwise naive animals. It remains to be determined whether these effects are attributable to a general sensitization or a specific conditioning of mechanisms subserving the neurochemical change. Regardless of the specific nature of the process, these data serve as a reminder that the stress history of an organism should be considered in attempting to draw relationships between environmental events and the promotion or exacerbation of physical and psychological pathologies.

IIIc. Age

It is well documented that variations of neurotransmitter concentrations and turnover may occur as a function of the age of the organism (see review in McGeer & McGeer, 1980). Likewise, it appears that the effectiveness of stressors in modifying neurochemical activity varies with age. Older rats (7 months) tended to exhibit more pronounced and longer lasting depletions of hypothalamic and telencephalic NE concentrations than similarly treated 3 month old rats (Ritter & Pelzer, 1978). The younger animals were also inclined to show a greater release of NE following stressor application, possibly suggesting that the compensatory increase in synthesis was more effective in these animals than in the older rats (Ritter & Pelzer, 1980). Consistent with this supposition, Ida et al (1982a) found that

utilization of NE following restraint stress was greater in 2 month than in 15 month old rats, although comparable depletion was evident at both ages. In a second report Ida et al (1982b) also reported that in the basal ganglia, stress increased DA concentrations in younger animals but not older animals, leading these investigators to suggest that in the former group synthesis exceeded utilization following stress, presumably as an adaptive response to environmental demands. Indeed, it has been demonstrated that increased hypothalamic tyrosine hydroxylase activity was induced by cold stress in 4 month old rats but not 18 or 29 month old animals (Algeri, et al., 1982). Furthermore, these investigators reported that in limbic regions including the hippocampus, and in the substantia nigra, cold stress induced greater utilization of 5-HT in 29 month old rats than in similarly treated 18 month old animals, which in turn exhibited greater utilization of the amine than 4 month old rats.

Several experimenters examined the effects of stressors on catecholamine activity in juvenile animals. It seems that in rats as young as 10 days of age, the stress of repeated electrical stimulation of the lateral hypothalamus or delayed feeding increased synthesis of hypothalamic EPI, as determined by activity of the synthetic enzyme, phenylethanolamine-N-methyltransferase (Torda, 1976). Similarly, recurrent exposure to both stressors resulted in increased concentrations of hypothalamic NE (Torda, 1975). In animals exposed to the stress of separation from the mother (plus cool environment) at 5 days of age, long-term repercussions were detected with respect to NE, DA and 5-HT (Schellenberger, 1982). When these animals were tested in adulthood, concentrations of NE in the pons-medulla and midbrain were

reduced, DA concentrations in frontal cortex were increased, and 5-HT levels were reduced in cortex but increased in midbrain. Together these data suggest that early life trauma may have a considerable impact on developing neurotransmitter systems. As indicated by Sklar and Anisman (1980), however, in assessing the effects of early life trauma, it is essential to consider the specific time at which the stressor was applied, the form of the stressor, as well as its duration. In view of the diverse procedures that have been employed, it would be premature at this time to make specific conclusions concerning the potential neurochemical impact of stressors, or to determine the critical periods for such variations.

In addition to early life trauma, it appears that stressors applied to pregnant female rats will influence catecholamine concentrations in the offspring even as long as 90 days after birth. For instance, Moyer, Herrenkohl and Jacobowitz (1978) demonstrated that a compound stressor (heat, restraint plus bright light) applied from day 8 through day 21 of gestation led to NE reductions in the medial preoptic nucleus and median eminence of male offspring, and increased NE concentrations in entorhinal cortex of female offspring. Under these conditions DA concentrations were reduced in the periventricular nucleus of females and increased in the arcuate nucleus. The mechanisms responsible for these alterations, as well as their adaptive significance remains to be determined. Nevertheless, these data do indicate that prenatal stress may have long term consequences.

In accordance with the results of Moyer et al (1978), it was reported that stress (handling plus saline injection) applied throughout gestation greatly affected monoamine concentrations in the offspring (Peters, 1982). At 9 days of age NE concentrations in

hypothalamus were reduced, as was utilization of 5-HT. By 60 days of age, hypothalamic NE concentrations exceeded those of rats whose mother had not been stressed. Additionally, at 16 days of age 5-HT concentrations in the cortex and pons-medulla were increased in the offspring of the stressed female. It might be noted that not all reports of prenatal stress effects have revealed alterations of catecholamine activity or levels. Sobrian (1977), for example, found that footshock applied to rats from days 15 through 21 of gestation did not influence concentrations of NE, 5-HT or 5-HIAA in offspring. Inasmuch as this study employed a different form of stressor, applied at different times relative to the report of Moyer et al (1978) and Peters (1982), it is not possible to account for the divergent observations.

As discussed earlier, exposure to aversive events may have appreciable long-term repercussions on the reactivity of neurotransmitter systems to later stress experiences. It is tempting to speculate that trauma encountered prenatally or soon after birth may likewise result in the sensitization of neurochemical processes, thus leaving the organism more vulnerable to pathology during adulthood. Unfortunately, data are not yet available concerning such a possibility. Nevertheless, this hypothesis warrants consideration, particularly since human research has suggested that early life trauma (e.g., separation) may have profound effects on vulnerability to stressors encountered later in life (see Anisman, 1983).

III d. Genetic Factors

Behavioral responsivity to aversive stimuli appears to vary considerably across strains, as well as within members of a particular

strain, (see Wahlsten, 1978). Likewise, it has been demonstrated that considerable between-strain variation in neurotransmitter activity occurs in response to aversive stimulation. Thus, the proposition has been entertained that inherited characteristics may be significant in accounting for susceptibility to behavioral and physiological disturbances.

It is well established that neuroanatomical differences exist between strains of mice. These differences range from small variations in pathways to absence or agenesis of the corpus callosum (Lieblich, 1982; Wahlsten, 1982). Similarly, pronounced differences have been noted in the regional distribution, concentration and turnover of various neurotransmitters, including NE (Barchas et al., 1974; Ciaranello, Barchas, Kessler & Barchas, 1972; Eleftheriou, 1971, 1974; Kessler, Ciaranello, Shire & Barchas, 1972; Lemmer, Caspari-Irving, & Weimer, 1981), DA (Lemmer et al., 1981; Mandel, Ebel, Mack & Kempf, 1974), EPI (Perry, Stolk, Vantini, Guchhait & U'Prichard, 1983), ACh (Tunnicliff, Wimer & Wimer, 1973; Mandel, Ayad, Hermetet & Ebel, 1974), 5HT (Daszuta, Barrit & Faudon, 1982; Rosecrans & Schechter, 1972; Mitler et al., 1976), and GABA (Al-Ani, Tunnicliff, Rick & Kerkut, 1970; Rick et al., 1971).

Not only do basal levels and turnover vary across strains of mice and rats, but it appears that the neurochemical consequences of stressors will vary considerably as well. It has been shown, for instance, that the turnover of ACh and 5-HT following application of a stressor varies between strains of rats (Schmidt, Cooper & Barrett, 1980; Ray & Barrett, 1975), as does choline acetyltransferase activity and cholinergic muscarinic binding (Gilad et al., 1983). Indeed, stressors may have diametrically opposite effects on neurotransmitter

concentrations in different strains of mice. Thus, a stressor may increase hippocampal 5-HT concentrations in C57BL/6 mice, but decrease concentrations of this amine in DBA/2 mice. In frontal cortex 5-HT concentrations may be increased in the DBA/2 strain, but remain unaffected in C57BL/6. In the case of NE, amygdaloid concentrations may be increased in the C57BL/6 strain, but remain unaffected in DBA/2 (see Wimer, Norman & Eleftheriou, 1973; see also Church & Sprott, 1979). It appears as well that the effects of stressors on DA turnover vary with strain and brain region examined. While footshock was reliably found to increase the ratio of DOPAC to DA (indicating increased turnover), this increase was much greater in the Balb/c strain of mouse than in C57BL/6 (Herve et al., 1979). It was suggested that since the mesolimbic system may play an important role in emotional reactivity, the differential effects of stressors on mesolimbic DA neuronal functioning may contribute to the behavioral profiles characteristic of these strains.

The belief that stressful events may increase the incidence of pathology in "vulnerable" animals has prompted the search for correlates of neurotransmitter alterations in strains differentially sensitive to pathological states, as well as the effects of stressors on the induction of such pathologies. For instance, uncontrollable shock did not influence DA concentrations or turnover in either the relatively reactive Roman High avoidance (RHA) rat or in the Roman Low Avoidance (RLA) line. Footshock, however, produced a greater release of cortical NE in the RHA rats than in the RLA strain, while 5-HT in hypothalamus and pons-medulla was increased in the RLA strain, but reduced in the hypothalamus, hippocampus and cortex of the RHA strain (Driscoll, Dedek, Martin & Zivkovic, 1983). While it cannot be determined from the aforementioned data which, if any, of these

transmitter alterations are responsible for the behavioral differences seen in the two rat strains, it was maintained that 5-HT neuronal activity may play a significant modulatory role in the reaction to stressors (Driscoll et al., 1983).

Susceptibility to cardiac pathology may be influenced by genetic factors, and furthermore, cardiovascular responses to stressors vary across strains. For example, rats of the August strain generally exhibit more pronounced blood pressure changes than Wistar rats in the presence of emotionally arousing stimuli (Sudakov et al., 1980). The August rats demonstrated higher concentrations of DA in all brain regions, while the NE concentrations tended to be higher in the Wistar rat, particularly in hypothalamus. Following immobilization stress over a 30 hr period rats of both strains that were predisposed to cardiovascular alterations tended to exhibit a marked decline of hypothalamic NE concentrations. In the predisposed August rats, increased NE concentrations were also evident in the midbrain and to some extent in the medulla. In the predisposed Wistar rats DA concentrations increased in most brain regions, but most prominently in the midbrain, while in August rats increased concentrations of DA were observed in the hypothalamus and medulla, but not in the midbrain. In general, it appeared that a predisposition to cardiovascular alterations following stressor application were associated with increased DA activity in the midbrain as well as altered concentrations of hypothalamic NE (Sudakov et al., 1980).

In assessing the development of hypertension in relation to stressor-induced catecholamine activity, it was observed that in the spontaneously hypertensive rat stress was associated with increased

utilization of NE without a complementary increase in synthesis. In contrast, in normotensive rats NE synthesis was greatly increased while utilization was not (Zukowska-Grojec, 1980). These variations, it will be noted, first became evident during the early phase of hypertension (8 weeks of age), and progressed with age (24 weeks). Consequently, it was hypothesized that increased utilization without adequate synthesis, which results in reductions of amine concentrations, characterizes the hypertensive rat. This alteration of NE metabolism may be responsible for the pathogenesis of hypertension (Zukowska-Grojec, 1980).

Several attempts have been made to evaluate the effects of repeated stress exposure on neurotransmitter functioning in behaviorally reactive and nonreactive strains of rats. When tyrosine hydroxylase activity was measured 24 hr after a chronic stress regimen (shock applied over a 9 day period) increased enzymatic activity was evident in the hypothalamus and locus coeruleus of both the Maudsley reactive and Maudsley Nonreactive strains of rats. In hypothalamus the increased enzyme activity was greater in the reactive than nonreactive rats, while the opposite arrangement was noted in locus coeruleus (Blizard, Freedman & Liang, 1983). Unfortunately, because acutely stressed groups were not included in this study, it is not known to what extent the variations of catecholamine synthesis represents an adaptive response to the chronic stress regimen, nor is it clear whether the behavioral changes seen in these rats following various stress regimens correlates with neurochemical lability. Nonetheless, such data are suggestive of the central factors that might be associated with increased responsivity to environmental events.

Clearly, it is still premature to attempt to relate specific

neurochemical changes elicited by stressors to the provocation of pathological states in predisposed animals. The available data are correlational in nature, and at the very least, genetic analyses involving segregating generations will be required before particular substrates can be implicated in the etiology of particular pathologies. Nevertheless, the aforementioned data do serve to indicate that the effects of stressors will vary considerably across strains of animals as they do across individuals within a strain. On the one hand, it can be argued that too much variability exists concerning the effects of stressors, and hence it will be difficult to form relationships between neurotransmitter modification and pathological states. On the other hand, the availability of genetically inbred strains that differ in predisposition to pathology and vary with respect to various endogenous processes, may serve as a viable experimental tool to draw fracture lines between endogenous processes and the occurrence of psychological and physical illness.

IIIc. Psychosocial Factors

The effectiveness of stressors in determining pathological change may be dependent on the backdrop upon which the stressor was applied (see Irwin & Anisman, 1984). So too, it seems that the activity of neurotransmitter systems induced by stressors will vary with background environmental conditions.

Often overlooked variables, such as the conditions under which animals are housed, will influence neurochemical activity and will determine the nature of the changes provoked by stressors. In mice, isolated housing has been shown to reduce the turnover of brain NE (see Modigh, 1976; Welch & Welch, 1970), while inconsistent results have

been reported concerning DA turnover (see Modigh, 1976). Serotonin activity appears to be unaffected by such changes in housing conditions (Modigh, 1974; Nishikawa et al., 1974). In rats, turnover of NE was increased following isolated housing (Segal, Knapp, Kuczenski & Mandell, 1973; Stolk et al., 1974), and septal tryptophan hydroxylase activity was increased (Segal et al., 1973). However, analysis of discrete regions of rat brain indicated that steady state levels of NE were reduced in the hippocampus, amygdala, cingulate cortex, caudate, stria terminalis and paraventricular nucleus, and a decrease of DA turnover was evident in amygdaloid centralis (Thoa, Tizabi & Jacobowitz, 1977). In accordance with these results, Weinstock, Speiser and Askenazi (1978) reported that NE turnover was reduced in rats that had been isolated for 6-8 weeks, as was DA in some brain regions. Interestingly, when the isolated animals were placed in a novel environment (open field) the turnover of NE was markedly increased and animals displayed hyperemotionality. In effect, despite the reduction of basal rates of NE turnover, isolated animals appeared to be more reactive to environmental stimuli and this was accompanied by heightened catecholamine turnover. Along the same lines, Guisado, Fernandez-Tome, Garzon and Del Rio (1980) reported that isolated housing of rats for 12-15 months resulted in increased DA receptor binding. It was suggested that such a change might be responsible for the heightened reactivity induced by such a treatment.

The effects of isolation on brain monoamines also appear to vary with the age at which isolated housing was initiated. In rats that had been housed individually from weaning, increased NE concentrations were evident following either 6 or 9 weeks of isolation. In contrast, when

isolation was imposed during adulthood, NE concentrations were not altered (Nishikawa et al, 1976). While these data suggest an age dependent relationship between isolation and catecholamine variations, it has been postulated that changes in housing conditions (e.g. from grouped to isolated) may well influence both neurochemical and pathological processes (see Sklar & Anisman, 1980). Accordingly, the possibility exists that the results observed by Nishikawa et al (1976) may have been a reflection of the differential effects of changes in housing condition at different ages.

In addition to modifying NE turnover by itself, isolation has been shown to influence the effects of physical stressors on neurochemical activity. Anisman and Sklar (1981) reported that NE depletion was more readily induced among mice that had been housed individually than in grouped animals. Similarly, Blanc et al (1980) observed that 3 min of footshock applied to isolated mice increased DOPAC concentrations in frontal cortex to a greater extent than it did in grouped animals. Like Anisman & Sklar (1981), these investigators noted that the isolated animals also tended to accept the shock more passively, thus leading to greater performance disturbances in tasks where mice were required to maintain active responses in order to cope behaviorally with the stressor (see Anisman & Sklar, 1981).

Prolonged isolation has been observed to provoke fighting among some animals and this may be accompanied by alterations in neurochemical activity. For example, several reports have indicated that in animals that engaged in frequent fighting following isolation, NE concentrations in some brain regions were elevated relative to non-fighters or grouped animals (Modigh, 1974; Rolinski, 1975; Tizabi, Massari & Jacobowitz, 1980). Moreover, turnover of both DA and NE was

greater among aggressive animals than non-aggressive isolated controls (Modigh, 1974; Tizabi et al., 1980). The observation that fighting, like footshock or other physical stressors appears to increase monoamine turnover, might be taken to indicate that fighting itself is a stressor. However, as indicated earlier, under some conditions fighting tends to limit the pathological consequences otherwise induced by stressors, and it was suggested that aggressive behaviors may serve as a coping response. According to this formulation then, the increased monoamine turnover may serve as a preparatory mechanism to meet environmental demands. Yet, it should be considered that the effectiveness of fighting as an adaptive mechanism may depend upon whether the animal is dominant or subdominant in the encounter, as well as the animal's previous stress history (Tizabi et al., 1980).

III f. Chronic Exposure to Stressors

In the face of acute exposure to aversive events, a series of neurochemical changes occurs in order for the organism to meet the demands of the situation. That is, release of transmitter proceeds at an accelerated pace, and the synthetic rate of the amine is also increased. With prolonged exposure, synthesis may fail to keep pace with utilization, resulting in diminished amine concentrations. As indicated earlier, it is thought that the reduced transmitter levels leave the organism more vulnerable to pathology. One would think that with continued exposure to a stressor on repeated days, vulnerability to pathology might be substantially increased. However, it seems that such a stress regimen promotes adaptive changes which may serve to protect the organism. Indeed, it has been shown that some of the

behavioral disturbances associated with acute uncontrollable stressors are absent following a chronic stress regimen. Other pathological processes are apparently modified following repeated stressor exposure as well. For instance, unlike acute stressor exposure which promotes tumor growth, following a chronic regimen the rate of tumor development is inhibited (Sklar & Anisman, 1979). Similarly, Monjan and Collector (1977) observed that acute noise stress impaired the activity of the immune system, whereas immunofacilitation (i.e. increased T-lymphocyte activity) was observed following chronic stress. The possibility should be entertained that the alteration of central transmitter systems during repeated stressor exposure may mediate these pathological processes (see discussion in Irwin & Anisman, 1984).

Adaptive changes in neurochemical activity have been reported both within a protracted stress session and across repeated daily sessions. For example, Kvetnansky et al (1976) showed that the NE depletion evident after 20 minutes of immobilization stress was absent in rats following 180 minutes of such treatment. Likewise Weiss, Glazer, Pohorecky, Brick and Miller (1975), demonstrated that the depletion of NE that occurs after a single session of footshock was not evident in rats that received 14 such sessions. In accordance with these findings it was observed that hypothalamic and hippocampal concentrations of NE were increased by repeated oscillation stress (Roth et al., 1982) as well as by forced treadmill running (Brown et al., 1979).

It appears that the elevation of NE levels noted following chronic stressor application may be due to a compensatory increase of synthesis as indicated by increased tyrosine hydroxylase (TH) (Blizard et al,

1983; Stone, Freedman & Morgano, 1978; Stone and McCarty, 1983; Weiss et al., 1975) and dopamine-beta-hydroxylase activity (Kvetnansky, 1976). In particular, Weiss et al (1975) reported that whereas a single session of inescapable footshock was without effect on tyrosine hydroxylase activity, following 15 daily sessions, a significant elevation in TH activity was observed. Likewise, employing an in vitro measure of catecholamine synthesis, it was recently reported that hypothalamic NE synthesis was accelerated following a chronic regimen of inescapable footshock (Kramarcy, Delanoy & Dunn, 1984). Such an effect was absent, however, in the frontal cortex, hippocampus and brainstem. Parenthetically, in the Weiss et al (1975) study, if rats had been given a chronic footshock regimen and were exposed to cold swim stress immediately prior to decapitation, no such increase in TH activity was noted. Consequently, it may be the case that compensatory changes that occur following exposure to one form of stressor may be absent if a different stressor is subsequently introduced.

In addition to variations in enzyme activity, Weiss et al (1975) reported that uptake of labelled NE was reduced among rats that had received chronic exposure to a stressor. Accordingly, the adaptation consists not only of increased amine synthesis, but once released, the transmitter apparently remains in the synaptic cleft for a longer period of time, thereby increasing its functional efficacy.

Along with alterations in amine synthesis and uptake, it has been suggested that chronic stress alters the utilization rate of NE. In particular, Roth et al (1982) reported that following 7 days of oscillation stress, the utilization of NE was reduced relative to naive animals. This conclusion was based on the observation that in chronically stressed animals, a smaller reduction of NE was noted

following administration of a synthesis inhibitor (fusaric acid) than that seen in controls. However, since the baseline NE values for stressed and non-stressed animals were different (i.e. chronic stress exceeded controls) this may not have been the appropriate comparison. Indeed, if the stressed and non-stressed groups that were treated with the synthesis inhibitor are compared to their respective baseline values, it appears that chronic stressor exposure was without effect on NE utilization. It should also be noted that in the Roth et al (1982) experiment, utilization rates were assessed 24 hr following stressor termination, and it is conceivable that alterations in utilization would have been detected had release been assessed immediately following stressor termination.

In accordance with the suggestion that utilization is reduced following chronic stress, Stone and McCarty (1983) reported that immediately after the last session of a chronic stress regimen, concentrations of the NE metabolite MHPG were reduced relative to that observed in acutely stressed animals, suggesting that the transmitter was released at a slower rate. However, basal release rates in chronically stressed animals were elevated relative to non-stressed animals. Stone and McCarty (1983) suggested that the decreased utilization in response to stressor application serves to increase the animal's preparedness. That is, under conditions in which animals are exposed to sublethal levels of stress, utilization rates are decreased, presumably since further release is not necessary for survival. Consequently, in the event that more intense or protracted stressors are encountered, sufficient levels of transmitter would be available and would be released in order to contend with the aversive

stimulation.

Although it has been assumed that the increased synthesis and release of transmitters may be of adaptive significance and will increase the probability of the organism successfully coping with environmental demands, it should be understood that a persistent increase in the turnover of brain catecholamines could theoretically have negative repercussions (e.g., excessive DA activity has been implicated in schizophrenia, and excessive NE may come to influence affective states). It appears, however, that further adaptive neuronal alterations may occur under conditions of chronic stress. It has been demonstrated that following repeated exposure to an aversive stimulus the density of B-NE receptors declines (Stone & Platt, 1982; Nomura, Watanabe, Ueki & Nakazawa, 1981; U'Pritchard & Kvetnansky, 1980; Stone, 1979; Stone, 1983a) as does the formation of NE-stimulated cyclic AMP in brain slices may occur (Stone, 1983a; 1978, 1979, 1981). Such an effect has been noted after exposure to a variety of stressors and has been observed in several brain regions, but is particularly pronounced in the cortex. Indeed, following chronic exposure to inescapable footshock, subsensitivity of cortical B-NE receptors was noted, whereas other brain regions were largely unaffected (Nomura et al., 1981). More recently it was reported that chronic restraint stress resulted in a marked reduction in the cyclic-AMP response to catecholamines in cortical tissue (Stone, Platt, Trullas & Slucky, 1984). This response suppression was noted up to 24 hr following stressor termination, but the accompanying reduction in B-receptor density was noted only immediately after the end of the stress session. Thus it was suggested that there may have been a selective action on the non-Beta component of the noradrenergic response at relatively long

intervals after the termination of chronic stress.

In addition to altering Beta-receptors it has been demonstrated that chronic stressor application may provoke a reduction in the number and sensitivity of presynaptic alpha-2 receptors (Lynch et al., 1983). Upon stimulation by catecholamines, alpha-2 receptors have an inhibitory effect on transmitter release and consequently, a stress induced subsensitivity of these receptors would have the effect of increasing NE release. Thus, the down-regulation of alpha-2 receptors may be one of the mechanisms responsible for the induction of stress provoked increases of NE turnover, rather than a consequence of excessive activity of noradrenergic neurons during chronic stress.

Stone (1979) suggested that the down regulation of B-NE receptors may be fundamental in tempering the pathological consequences typically associated with stressors. For instance, it was reported that the reduction of food intake and the induction of gastric ulceration evident after relatively acute stress tended to disappear with repeated application of the stressor. Moreover, the timing of these events corresponded with the decreases in B-NE receptor density (Stone & Platt, 1982).

In attempting to relate the consequences of repeated stressor application to human affective disorders, Stone suggested that the induction of the NE receptor down regulation may be a critical feature in protecting against depression. In particular, accumulating evidence has favored the notion that the antidepressant qualities of pharmacological agents are due to their ability to reduce the sensitivity of the B-NE receptor and NE sensitive adenylate cyclase system (see Sulser, 1982). Essentially, Stone argued that the process

by which antidepressant agents produce such an effect may be similar to the endogenous adaptive changes engendered by chronic stressors. However, the processes are not identical since antidepressants predominantly desensitize the B-component of the cAMP response, whereas recent data suggest that the sensitivity of non-Beta component that is also reduced by chronic stress (Stone et al., 1984). Moreover, post-synaptic alpha-1 receptors may become supersensitive to catecholamine stimulation following some forms of antidepressant treatment (Vetulani, 1983). Such an observation is not necessarily at odds with the hypothesis offered by Stone (1983), since it was suggested that this up-regulation of alpha-1 receptors would be associated with an enhancement of noradrenergic activity in brain regions which are involved in the regulation of affective states (Vetulani, 1983). Moreover, sufficient data are not yet available to determine whether repeated stress influences alpha-1 receptors.

Regardless of the precise nature of the alterations of noradrenergic receptors, it appears that parallel processes may be operating during chronic stress and antidepressant treatment. This is not to say that a treatment for depression would be the application of a chronic stressor. Rather, it is thought that the individual who succumbs to depression does so because of a failure of such an adaptive mechanism, and antidepressant therapy is presumed to be effective because it compensates for, or enhances the course of endogenous adaptive processes.

The most recent account offered by Stone concerning the receptor down regulation and its functional significance has a new focus. According to this hypothesis, the down regulation does not reflect decreased activity within the system, so much as increased efficiency,

thereby reducing the necessity of excessive receptor stimulation (Stone, 1983b). When the organism first encounters a stressor, maximal transmitter output and receptor sensitivity may be necessary. The course of adaptation involves the adjustment of the activity or output of the neurochemical system to the level minimally required to sustain survival. Additionally, the efficiency of the transmitter system progressively improves, and ultimately a reduced receptor complement is necessary to deal with the environmental insults. While this hypothesis is an intriguing one, it unfortunately appears to be difficult to test (see commentaries in Stone, 1983b).

Only a few studies are available in which the effects of acute stressors on transmitters other than NE have been investigated; and even fewer have examined the consequences of chronic stressor application. It has been reported that following 40 immobilization sessions applied on successive days, DA concentrations were significantly increased in the dorsomedial and paraventricular nuclei of the hypothalamus (Kvetnansky et al., 1976; Kvetnansky, Palkovits, Mitro, Torda & Mikulaj, 1977), suggesting functional variations in these nuclei due to chronic stress treatment. As in the case of the NE alterations seen following a protracted session of immobilization stress, Telegdy, Fekete and Varszegi (1980) reported that both DA and NE concentrations were reduced following a single session of stress consisting of exposure to ether fumes. If the ether stress was repeated at 1 hr intervals, adaptation was seen with successive presentations of the aversive stimuli. However, when the stressor was applied at 30 min intervals, the depletion of the amines was accentuated. It is conceivable that the massed presentation of the aversive stimuli was

more stressful than the relatively spaced presentations, and was thus less amenable to adaptation.

With respect to turnover, Roth et al (1982) reported that the increased DOPAC concentrations evident after a single session of oscillation stress were not evident in rats that received 21 such sessions on successive days. Although these data suggest that as in the case of NE, utilization of DA declines as a consequence of chronic stress exposure, it should be noted that in these experiments animals were sacrificed immediately following acute stress, while metabolite levels of chronically stressed animals were assessed 24 hr following stressor termination. Given the transience of changes in DA turnover after acute stress (Kramarcy et al, 1984), it is possible that any changes in turnover resulting from chronic stress treatment had subsided within 24 hr.

In assessing the effects of a single protracted stress session, Beley et al (1976a), observed that synthesis of DA was increased in the striatum after 2.5 hr of cold stress (4 C), but returned to normal values following 24 hr of such treatment. This finding is somewhat surprising, since one might have expected a sustained increase of DA synthesis even after a protracted stress session. The fact that this did not occur may have been related to the stressor employed (note that here, as in several other reports, cold stress was found to produce outcomes that differed from that of other stressors), and DA was assessed in the substantia nigra where alterations of the amine following stressor application are limited.

In the case of 5-HT, it has been reported that immobilization increased the accumulation of the metabolite, 5-HIAA, with maximal concentrations being present following 120 min of the stress treatment.

Concentrations of the metabolite declined from peak levels within 300 min of immobilization stress, although the levels still exceeded those of non-stressed rats (Morgan et al, 1975). Elevations of 5-HIAA were also noted following protracted cold exposure; however in contrast to immobilization, there was no adaptation over the course of a 24 hr stress session (Algeri et al, 1982). Owing to the absence of studies which have directly compared the effects of acute versus chronic stressors on 5-HT activity, conclusions regarding the adaptive capacity of this neuronal system are pending.

It was indicated earlier that acute stress results in increased levels of ACh together with reduced turnover of the amine. It was recently demonstrated (Gabriel & Soliman, 1983) that the reduction of acetylcholinesterase seen after 1 hr of cold (4 C) plus restraint was still evident in rats that had been maintained in the cold environment for a 24 hr period after removal from restraint. These data provisionally suggest that adaptation of acetylcholinesterase activity does not occur during a protracted stress session. However, experiments conducted by Costa et al (1980) suggested that the intensity of the stressor may influence not only the duration of stimulation necessary to influence ACh turnover, but also the course of adaptation. Although up to 4 hr of immobilization stress did not affect ACh synthesis, 24 hr of such treatment decreased synthesis in hypothalamus, without altering turnover in either the frontal cortex or hippocampus. If the severity of the stressor was intensified (restraining wet animals in a temperature of 4 C), synthesis of ACh declined in each of the brain regions even after a short stress period. A brief period of cold exposure alone resulted in a sustained

reduction of ACh turnover in the hypothalamus, whereas in the frontal cortex such treatment produced a transient reduction in turnover, despite continued exposure to the cold environment. Thus it seems that within session changes of ACh turnover may occur, but such alterations are dependent on the severity of the stressor as well as the brain region examined.

Taken together, the available data suggest that the effects of acute stressors on neurochemical processes are reduced or eliminated among animals exposed to protracted or repeated stress sessions. As discussed earlier, some of these neurochemical alterations may serve to facilitate the animals' ability to meet environmental demands either by maintaining adequate stores of transmitter, or by modulating transmitter release to the minimum amount necessary to function effectively.

It may appear somewhat paradoxical that although re-exposure to an acute stressor enhances neurochemical depletions (see section IIIb), repeated exposure to a stressor results in neurochemical adaptation insofar as transmitter levels are concerned. In fact, such changes are not at all enigmatic. The second presentation of a stressor may result in a more rapid mobilization of transmitter from releasable pools in order to contend with environmental demands. Since the organism has only limited experiences with the stressor it would be biologically adaptive for maximal release of the transmitter to occur in order to deal with potentially lethal stimuli. Under conditions of repeated stressor application, utilization of the transmitter may also increase. As a consequence of previous encounters with the stressor, however, the transmitter release may not be so great as to render the animal incapable of dealing with further insults, yet sufficient to deal with

the current situation. Owing to the increased synthesis, adequate stores of the amine would be present to maintain sufficient transmitter for effective coping with further increases in stressor severity.

IV. BEHAVIORAL CONSEQUENCES OF STRESS

It is clear from the literature reviewed in the preceding sections, that exposure to stressors may have profound effects on the activity of several neurotransmitter systems. Stressors are also associated with significant changes of performance in a number of behavioral paradigms. Such changes, like neurochemical alterations, appear to be influenced by genetic and social factors, stress history and variables associated with aversive stimuli such as controllability and predictability. Of particular interest, is the observation that stressor chronicity may be an important determinant of the behavioral response to stress, given the apparent dichotomy in neurochemical responses to acute and chronic stressors. Accordingly, in the sections that follow, a brief review of some of the behavioral deficits associated with acute stress will be provided, as well as a description of those studies which examined the behavioral consequences of repeated aversive stimulation.

IVa. Acute Stress

Paralleling the neurochemical changes observed following exposure to stressful stimuli, stress-induced changes in behavior have been observed following exposure to uncontrollable aversive stimuli. The most frequently studied behavior in this respect is the shock-escape deficit that is induced by inescapable shock. Typically, when animals that have previously been exposed to inescapable shock are tested in a

shuttle task in which escape is possible, they tend to accept the aversive stimulation passively. These deficits are fairly general, having been observed across a wide range of species and in several different types of escape paradigms. As in the case of neurochemical responses, these behavioral changes are dependent on whether the animal is able to control offset of the aversive stimuli during the initial stress session. Indeed, it has been consistently reported that animals that are given escapable footshock or are not exposed to shock, quickly acquire proficiency in subsequent shuttle escape testing. In contrast, inescapable shock treatment subsequently results in impaired performance (Anisman, deCatanzaio & Remington, 1978; Maier & Seligman, 1976; Seligman, Maier & Solomon, 1971; Weiss et al., 1975).

Several explanations have been offered to account for the profound behavioral impairments associated with uncontrollable aversive events. One of the earliest, and still a widely accepted proposition, is the "learned helplessness" hypothesis (see Maier & Seligman, 1976). Proponents of this model have argued that during exposure to inescapable shock animals learn that termination of the aversive stimulation is independent of their responses. In effect, they are helpless in determining outcomes. As a result of this cognitive change animals cease to make attempts to escape or to avoid the aversive stimuli. In addition, it is thought that uncontrollable stressors will engender associative disturbances such that animals will be deficient in acquiring response-outcome associations. Moreover, it is also thought that motivational processes are compromised, thereby further limiting performance (see Maier & Jackson, 1979; Maier & Seligman, 1976).

Although considerable support for the helplessness hypothesis has been forthcoming, several investigators have taken issue with this position. Among other things, it has been argued that alternative interpretations of the data are available, which account for the effects of several manipulations that are not amenable to a helplessness position. One of these hypotheses, the learned competing response view, asserts that during inescapable shock animals may learn unauthorized responses simply because shock offset is correlated with these responses (e.g., animals are relatively inactive at the time of shock offset and hence may adopt an inactive posture). These response tendencies are incompatible with those necessary for successful escape, thus leading to performance disturbances (see Anisman et al., 1978; Bracewell & Black, 1974; Glazer & Weiss, 1976a,b).

A second view, one not necessarily incompatible with the learned competing response hypothesis, is that the competing response tendencies provoked by inescapable shock are nonassociative in nature. In particular, it has been suggested that inescapable shock will result in deficits of response initiation to weak stimuli (e.g., a CS) and disturbances of response maintenance to strong stimuli (e.g., the shock itself). Consequently, disruption of escape performance will be most evident when the task is one that requires sustained active responding (Anisman et al., 1978; Weiss & Glazer, 1975; Weiss et al., 1976). For example, in a simple FR-1 shuttle task in which escape was possible immediately upon shock onset, latencies to escape in a group of mice that had been exposed to inescapable footshock were comparable to control values. If however, escape was prevented for several seconds, necessitating maintenance of motor activity, shuttle escape performance was significantly impaired (Anisman, Remington & Sklar,

1979). Likewise, if rats were required to emit a simple response such as a nose-poke, performance deficits were not evident in inescapably shocked mice, while pronounced disturbances were evident when rats were required to emit a vigorous running response. Moreover, if the motor demands in the latter task were increased by raising the height of the hurdle separating the two compartments of the shuttle box, the behavioral disturbances engendered by inescapable shock were magnified (Glazer & Weiss, 1976). Deficits in a swim escape task have also been noted following inescapable footshock, and consistent with the motor activation deficit hypothesis, performance was most profoundly impaired if escape was briefly prevented by means of a 3 to 5 sec gate delay. In addition, increasing energy expenditure by decreasing the water temperature, augmented the performance disruption (Irwin et al., 1980).

Although most attention has focussed on the escape deficits associated with inescapable shock, progressively more attention has been devoted to a variety of other behavioral disturbances that may be engendered by stressors. The use of these paradigms has not only been instrumental in delineating the range of behavioral impairments induced by stressors, but has also been fundamental in defining some of the mechanisms that may be essential for a more complete appreciation of the consequences of uncontrollable aversive events. In particular, it was demonstrated that inescapable shock resulted in biphasic variations of performance in a forced swim task. While motor depression was evident 24 hr after inescapable shock, a response invigoration was observed immediately after the stress session (Prince & Anisman, 1984). The invigoration was modifiable by benzodiazepine treatment (Prince, Collins & Anisman, 1984), while the depression was not affected by such

a treatment (Sherman, Sacquitne & Petty, 1982). Thus, it was suggested that uncontrollable stressors may provoke a transient anxiety reaction which may influence the expression of behavioral changes in other behavioral paradigms (Prince et al., 1984).

In addition to the motor changes seen in a forced swim task, there is evidence suggesting that inescapable shock will modify the defensive strategies that animals employ in aversively motivated tasks. Contrary to the assertion that uncontrollable stressors lead to disturbances in learning response-outcome associations (Jackson, Alexander & Maier, 1980), it has been proposed that uncontrollable stressors narrow the animals' defensive strategy. Consequently, they persist in emitting previously acquired response styles or those that are high in their innate repertoires, despite the fact that alternative response strategies would be more effective in terminating the aversive stimuli (Anisman, Hamilton & Zacharko, 1984). In particular, mice were trained in an aversively motivated position-discrimination task in a Y-maze, and were then exposed to uncontrollable footshock or they received no treatment. Upon subsequent testing in a reversal of the original task, acutely stressed mice exhibited impaired discrimination acquisition. It should be underscored, that once a particular response strategy was acquired, animals that were exposed to inescapable shock tended to persist in adopting this same response strategy. This defensive style was evident despite the fact that animals had received discrimination training previously, and hence had ostensibly learned that outcomes were not always independent of their responses. Since these effects were evident only when extrinsic cues did not signal the correct response, it was argued that uncontrollable stressors may limit the defensive strategies of animals regardless of their history with

respect to response-outcome contingencies.

Along the same line, it was reported that when placed in a water filled V-maze in which one arm was illuminated, mice tended to remain in the vicinity of light and avoid the dark, despite the fact that a ramp in the dark area provided an escape route. Pretreatment with inescapable shock intensified the tendency to approach the light and was also evident in animals that received only a few escapable shocks prior to swim testing. Interestingly, once animals learned the escape response proficiently, the propensity to approach the light declined. Thus, it was suggested that stressors in general elicit this defensive style, but once mastery over the task is learned, the predisposition to emit this highly prepared, stereotyped response pattern was eliminated (Szostak & Anisman, 1984).

The variation of response style exhibited by animals following inescapable shock is not limited to paradigms involving aversive motivation. Indeed, evidence has been forthcoming which indicates that stressors influence performance in exploratory tasks, as well as in tasks involving appetitive motivation. For instance Bruto and Anisman (1983) reported that inescapable shock altered the pattern of exploration mice displayed in an 8-arm radial maze. Naive mice ordinarily exhibit an exploratory pattern characterized by frequent entries into the least recently visited arm (spontaneous alternation). Following uncontrollable footshock, however, this tendency declined, while the frequency of perseverative responses increased. In effect, it appears that the range of responses to the novel environment was restricted by exposure to an uncontrollable stressor (Bruto & Anisman, 1983).

Inasmuch as exploration and habituation may be related to performance in those tasks involving appetitive motivation (see Carlton, 1969), it should come as no surprise that inescapable shock has been found to influence acquisition of a food reinforced bar press response (Rosellini, 1978). While Rosellini (1978) attributed these performance disturbances to associative and cognitive alterations, it is equally possible that these deficits were secondary to alterations of exploration or variations in motivation stemming from the stressor experience. Indeed, several recent reports have suggested that uncontrollable stressors influence motivational processes. In particular, it was demonstrated that although escapable shock did not influence responding for intracranial self stimulation, inescapable shock substantially reduced responding for stimulation from the nucleus accumbens and medial forebrain bundle, without affecting response rates in the substantia nigra (Zacharko et al., 1983). These data were taken to suggest that stressful events influence mechanisms subserving reward processes, but that these alterations are specific only to some brain regions, most notably those in which stressors alter DA neuronal functioning. Subsequent experiments confirmed that these deficits were neither due to motoric factors nor to the onset of an aversion to the brain stimulation.

In addition to the aforementioned performance alterations, stressors have also been shown to alter social hierarchies and the response to intruders (Williams, 1982), induce anorexia (Stone & Platt, 1982) and elicit an antinociceptive reaction (Maier & Jackson, 1979). A detailed discussion of these experiments is not germane to the present series of experiments; however, it is significant nonetheless to emphasize the broad range of behavioral disturbances associated with

acute stress.

To account for the numerous behavioral changes associated with uncontrollable stressors, several investigators have proposed that the neurochemical consequences of uncontrollable aversive events are primarily responsible for the behavioral deficits. Initially it was proposed that the NE depletion engendered by uncontrollable shock was principally responsible for the behavioral deficits. Indeed, in a series of experiments Weiss and his associates (Glazer, Weiss, Pohorecky & Miller, 1975; Weiss et al., 1979; 1981) demonstrated that drug treatments which reduced NE (as well as other monoamines) resulted in performance deficits in a shuttle test, and conversely, the effects of inescapable shock could be ameliorated by MAO inhibitors. Subsequently, it was demonstrated that the time course for the NE depletion in the locus coeruleus corresponded with the time course for the behavioral disturbances. Moreover, escape deficits were not evident under conditions where NE concentrations were comparable to those of naive animals (Weiss et al., 1981).

While not dismissing a role for NE, several experiments implicated other transmitters in the induction of the performance disturbances associated with inescapable shock. Among other things, drug treatments which reduced NE, blocked DA receptors, or increased ACh concentrations effectively mimicked the escape disturbances in inescapably shocked animals (Anisman, Irwin & Sklar, 1979). Conversely, catecholamine stimulants and anticholinergics were effective in alleviating the performance disturbances associated with inescapable shock (Anisman, Remington & Sklar, 1979). Along the same lines, experimental data reported by Sherman and Petty (1980; Petty & Sherman, 1982) revealed

that GABAergic and serotonergic manipulations successfully modified escape performance leading to the conclusion that these transmitters contributed to the escape interference.

The wide range of behavioral changes associated with inescapable shock, coupled with the fact that numerous pharmacological manipulations may influence performance, has led to the conclusion that multiple neurotransmitter alterations subserve the behavioral profile associated with stressful events. For instance, while NE alterations may subserve some aspects of escape performance (e.g., attention, maintenance of motor activity), alterations of DA may be responsible for motivational changes, variations of ACh may contribute to exploratory and habituation changes, and alterations of 5-HT and/or NE may influence the affective state of the organism. Clearly, it would be inappropriate to focus on only a single neurotransmitter in attempting to account for the diverse consequences of uncontrollable insults.

IVb. Chronic Stress

Although acute stressors have been shown to disrupt performance in a wide range of tasks, and to influence several components of performance, only a few studies have examined the behavioral consequences of chronic stressor application. Nonetheless, several of these experiments have revealed interesting parallels between the neurochemical and behavioral responses to repeated stressor application.

In contrast to the interference effect noted after a single session of inescapable shock, Weiss et al (1975) reported that rats that were tested in shuttle escape immediately after the last of 15 daily sessions of inescapable shock did not exhibit impairments in

avoidance-escape performance. Moreover, attenuation of the deficit was noted even if the chronic stressor differed from the aversive stimulus used during the test session. Such data are consistent with a neurochemical/ motor activation deficit hypothesis, since behavioral adaptation to the stressor was accompanied by recovery of the noradrenergic depletion provoked by acute stress. However, it was reported that repeated exposure to forced cold swim reversed the interference effect; yet this treatment did not reverse the depletion of NE noted following a single stress session. Weiss et al (1975) suggested that since chronic cold swim decreased NE uptake, this would have resulted in increased stimulation of noradrenergic receptors, resulting in the behavioral reversal.

More recently, Stone and his associates examined the consequences of chronic stress in the Porsolt forced swimming task. In this task, which was originally developed as a screening test for antidepressants (Porsolt, Anton, Blavet & Jalfre, 1978), animals are placed in a water-filled glass cylinder from which escape is not possible, and the amount of time spent floating (immobile) is determined. Whereas a single session of restraint stress was without effect on immobility, rats that had been exposed to 11 daily restraint sessions, exhibited reduced immobility (Platt & Stone, 1982). Moreover, this effect was mimicked by chronic treatment with antidepressants, suggesting that parallel processes may be operating between adaptation to stress and antidepressant therapy (Platt & Stone, 1982). In contrast to these data, Prince and Anisman (1984) noted that chronic exposure to inescapable footshock substantially increased the amount of immobility time exhibited by mice in the forced swim task. These divergent

findings may be due to differences in the type of stressor employed (i.e. restraint versus footshock) or to differences in the testing procedure (animals in the Platt & Stone study had been exposed to the swim apparatus prior to test day). Alternatively, these findings may represent genetic differences in the capacity of animals to adapt to adverse environmental conditions.

In addition to disrupting aversively motivated behaviors, it will be recalled that acute stressor application can influence responding for intracranial self-stimulation (Zacharko et al., 1983). It was recently reported that the depression of responding ordinarily observed immediately after a single session of inescapable shock was absent if mice had been exposed to repeated daily sessions of the stressor (Zacharko, Bowers, Priddy & Anisman, 1983). Thus, in contrast to the results of the forced swim task, it appears that mice did adapt to the demands of chronic stress regimen. It may therefore be the case, that adaptation to chronic stress will be evident with respect to only a limited number of behaviors. That is, whereas certain motoric behaviors may remain impaired, the disruption of motivational processes may be reversed following chronic stress exposure. However, such an hypothesis is only speculative, given the very limited data which address the behavioral consequences of chronic stressor application.

V. SUMMARY

In the face of acute stress a variety of neurotransmitter changes occur which ultimately may have detrimental effects on the capacity of organisms to respond appropriately to adverse environmental conditions. Moreover, it is thought that those conditions which favour the

depletion of neurotransmitters also favour several pathogenic processes. In order to examine such processes, it is important to consider those variables which maximize or limit the organism's physiological response to the increased environmental demands. These factors include the genetic and stress history of the organism, psychosocial factors and stressor variables such as controllability and predictability.

The available data suggest that although acute stress may have significant neurochemical consequences, organisms may exhibit a remarkable capacity to adapt to prolonged stressful conditions. Following protracted exposure to a stressor, catecholamine synthesis increases, ensuring that adequate amine stores are available. Limited data are also available which suggest that changes in uptake and release of the transmitter are modified as a consequence of chronic stress exposure. Additionally, several experiments have provisionally suggested that some form of behavioral adaptation may occur in concert with the compensatory changes in neurochemical activity that occur following repeated stressor application.

The present series of experiments was designed to assess (a) the variations of NE and 5-HT that occur following stress and to determine the course of adaptation of these amines to sustained stressor application, (b) some of the variables which influence the extent of the adaptation, and (c) the potential long-term consequences associated with acute and chronic stress.

EXPERIMENT 1

Time Course of Stress-provoked NE Alterations

The magnitude of the neurochemical alterations provoked by stressors is known to vary as a function of numerous factors, including the species or strain of animal employed (Herve et al, 1979; Wimer et al, 1973). Indeed, preliminary experiments conducted in this laboratory revealed that the behavioral and neurochemical consequences of stressors in the CD-1 strain of mouse, which is employed in the present series of experiments, differed quantitatively from those observed in earlier studies in which Swiss-Webster mice were used (eg. Anisman & Sklar, 1979, 1981). Accordingly, Experiment 1 was undertaken to assess the degree of NE reduction provoked by exposure to uncontrollable footshock, and to determine the time course of recovery of these alterations. In addition to assessing the course of NE alterations, variations of 5-HT concentrations were determined as well. It has been demonstrated that 5-HT levels may be influenced by exposure to a stressor and that the extent of the alterations may be dependent on the severity of the stressor employed, as well as the brain region examined (Telegdy & Vermes, 1976; Thierry, 1973).

Method

Subjects

A total of 54 experimentally naive, male CD-1 mice were obtained from Charles River (Canada), St. Constant, Quebec, at 50-55 days of age. Mice were housed in groups of 5, and were acclimatized to laboratory conditions for at least 7 to 10 days prior to being used in the experiment. Throughout the experimental period mice were housed in

standard opaque polypropylene cages, and were provided with ad lib access to water and lab chow.

Apparatus

Inescapable footshock was administered in four identical black Plexiglas chambers which measured 30 x 14 x 15 cm, and which were covered by red Plexiglas lids. The floor of each box was constructed of 0.32 cm stainless steel rods spaced 1.0 cm apart (center to center). The grids were connected in series with neon bulbs and shock (150 uA, 60 Hz, ac) was delivered through the grid floor from a 3,000 V source. The end walls of the boxes were made of stainless steel and were also wired in series with the grid floor.

Procedure

Mice were placed in the preshock boxes for a 1.1 hr period during which they were exposed to one of three treatments. Mice received either no shock, 60 shocks with a 60 sec interval between shocks, or 180 shocks spaced 16 sec apart. For both groups each shock was 6 secs in duration and an intensity of 150 uAmp was employed. The groups were further subdivided (n=6) and at intervals of either 1 min, 30 min or 24 hrs after the end of the treatment session, mice were decapitated and the brains were quickly removed and placed on a chilled glass plate for dissection. The hypothalamus was removed by first sectioning the underside of the brain so that the hypothalamus was separated from the temporal lobes at the entrance of the optic tracts. An anterior cut was made at the optic chiasm, and a posterior cut was made at the mamillary bodies. Using blunt dissection, the cortex was laterally displaced from the midline to the rhinal fissure and was cut. The fornices were then cut at the level of the septum. The exposed hippocampus was pulled posteriorly, ventrally transected at the

interrhinal cortices, and removed bilaterally as a single structure. Tissue samples were stored at -70°C until neurochemical analyses were conducted. Norepinephrine and serotonin concentrations were determined fluorometrically using a modified version of the hydroxyindole method (Lavery & Taylor, 1968; Maickel, Cox, Saillant & Miller, 1968).

Results and Discussion

Figure 1 shows the mean NE concentrations in the brain regions examined as a function of the time after the various shock treatments. Analysis of variance of the hypothalamic NE values revealed that NE concentrations varied with the Shock treatment mice received, $F(2,45)=5.52$, $p<.01$, and with the Time after such treatment, $F(2,45)=3.39$, $p<.05$. The interaction between these variables approached statistical significance, $F(4,45)=2.15$, $p=.09$. Newman-Keuls multiple comparisons of the simple effects revealed that both 60 and 180 shocks reduced NE concentrations at the 0 and .5 hr intervals, but at the 24 hr interval NE levels were comparable to those of non-shocked mice. As seen in Figure 1, the shock treatments produced a small reduction of NE in the hippocampus, but this effect was not statistically significant. Earlier experiments conducted in this laboratory using Swiss-Webster mice had also indicated that hippocampal NE reductions were less readily attained than the hypothalamic NE reductions (Anisman et al, 1980); however, the NE reduction in those experiments was statistically significant.

Analysis of the 5-HT content in the hippocampus indicated that there was a significant Shock Treatment x Time After shock interaction, $F(4,53)=2.83$, $p<.05$. Newman-Keuls multiple comparisons ($\alpha=.05$) of the means comprising this interaction revealed that 30 min after exposure

Figure 1: Mean ($\pm$ S.E.M.) concentrations of norepinephrine at various intervals following shock termination, as a function of shock treatment.

to 60 shocks, the levels of 5-HT were significantly higher than in nonshocked animals, but levels returned to normal within 24 hr. In the hypothalamus and cortex, the shock treatment was without effect.

EXPERIMENT 2

Cross-stressor Sensitization

Although the reduction of NE engendered by shock of moderate severity is relatively transient, it was reported that the amine reduction may be readily reestablished by reexposing animals to only a few shock presentations (Anisman & Sklar, 1979). It has been suggested that the mechanisms subserving NE activity may be subject to conditioning or sensitization processes, such that the amine turnover and concentrations are rapidly altered upon reexposure to a stressor. In fact, it was postulated that the sensitization of neurochemical processes may contribute to the long term behavioral disturbances provoked by inescapable shock. To date, the sensitization/conditioning of neurochemical changes has been shown only when the original stress session and the reexposure session involved the same stressor. It is important, however, to determine whether traumatic events will result in sensitization of neurochemical processes so that subsequent encounters with other forms of stress lead to enhanced neurochemical activity. Accordingly, Experiment 2 was undertaken to determine whether inescapable shock would increase vulnerability to NE alterations upon later encounters with shock, as well as with a stressor other than shock. Additionally, Experiment 2 was undertaken to determine whether a stress treatment would influence 5-HT concentrations upon subsequent exposure to aversive stimulation.

Method

Subjects and Apparatus

A total of 40 male CD-1 mice served as subjects. Inescapable shock was administered in preshock boxes as described in Experiment 1. For restraint stress mice were placed in a clear Plexiglas semicircular tube which measured 4.9 cm in length and 2.9 cm in height. Once the mouse was positioned in the restraint tube, a Plexiglas insert kept the mouse immobile. Cold stress consisted of placing the animal in a restraining tube which was then placed in a cold chest at 4°C.

Procedure

Mice were placed in the preshock boxes for 1.1 hr and exposed either to 60 inescapable shocks (150 uA, 6 sec duration, presented at 60 sec intervals) or they received no shock treatment. Following the end of the preshock session mice were individually housed. Twenty four hr later, equal numbers of mice ($n=5$) were exposed to either a single 30 sec footshock, 30 minutes in the restraining tube, 5 min of cold exposure, or they were briefly handled, but not otherwise stressed. Immediately following the treatment session, animals were quickly sacrificed by decapitation, their brains were removed and dissected, and transmitter content was analyzed fluorometrically as previously described.

Results and Discussion

The concentrations of NE as a function of the initial shock treatment and the reexposure stress condition are shown in Figure 2. Analysis of variance of the percent hypothalamic NE content revealed that the initial treatment that mice received influenced the levels of the amine, $F(1,69)=4.83$, $p<.05$, and that the Reexposure Stress Treatment approached statistical significance, $F(3,69)=2.57$, $p=.06$.

Although the Initial shock x Reexposure treatment did not reach statistical significance, Newman-Keuls multiple comparisons ($\alpha=.05$) were conducted on the simple main effects since a priori predictions had been made concerning the interaction (Winer, 1971). These comparisons revealed that although inescapable shock administered on day 1 reduced NE levels, the extent of the amine reduction in mice that were not reexposed to a stressor was moderate and did not reach statistical significance. In contrast, a significant NE reduction was evident among mice that were reexposed to a single shock ($\alpha=.05$). Likewise, in the absence of prior shock the restraint treatment produced a reduction of NE, which was further accentuated in animals that had been exposed to inescapable shock 24 hr earlier. In contrast, cold exposure alone produced a small decline of NE which was not influenced by the initial shock treatment mice had received. Unlike the hypothalamic alterations, the stress treatments were not found to influence NE concentrations systematically in the hippocampus. Likewise, serotonin values did not vary significantly as a function of the initial stressor or reexposure treatment in any of the brain regions examined.

Taken together, it appears that exposure to a stressor may influence NE concentrations upon reexposure to a stressor, even if the latter insult was distinctly different from the one to which animals had initially been exposed. In an analysis of the effects of stressors on NE concentrations, Weiss et al (1981) observed that inescapable shock had effects on NE concentrations which persisted for as long as 48 hr. The proposition was entertained that these effects, like those reported by Anisman and Sklar (1979), were related to conditioning of neurochemical changes (i.e. handling, apparatus cues, transport of

Figure 2: Mean (+ S.E.M.) concentrations of norepinephrine as a function of pretreatment and test day re-exposure treatment.

animals, etc. may have acted as a CS). Indeed, these investigators indicated that body temperature was altered by cues associated with the stressor. Weiss et al. (1981) indicated, however, that at the 72 to 96 hr interval following the stressor, the NE depletion was absent despite the fact that the cues associated with the stressor were presented to the animal. Accordingly, it was proposed that at this time a compensatory increase of NE synthesis was induced. It remains to be determined whether this assumption is correct; however, the possibility should be considered that the effects of stress reexposure (or cues associated with the stressor) will vary as a function of the interval between the initial stress experience and subsequent reexposure.

In contrast to the NE alterations, variations of 5-HT concentrations were not observed when animals were reexposed to either a similar or dissimilar stress treatment. Unfortunately, because the time course for the 5-HT variations was not assessed in Experiment 2, the possibility exists that alterations in 5-HT levels occurred at longer intervals after the reexposure treatment. Nevertheless, the finding that 5-HT variations were less readily observed than the NE alterations is consistent with earlier reports indicating that more severe stress was required to elicit variations in 5-HT activity (see Thierry, 1973).

EXPERIMENT 3

Transmitter Variations Following Chronic Stressor Exposure

The results of the preceding experiments confirmed the earlier finding that concentrations of NE are reduced following uncontrollable footshock. In contrast to the effects of an acute stressor, it has been

reported that following repeated stressor application over successive days the NE reduction is absent (Roth et al, 1982; Weiss et al, 1976). Inasmuch as alterations of NE following acute stress may vary as a function of factors such as severity of the stressor and the brain region examined (Nakagawa et al, 1981; Tanaka et al, 1982), it was of interest to determine whether an increase of NE levels following repeated stress would likewise depend on these variables. It is conceivable that as in the case of tolerance to pharmacological compounds, adaptation to a stressor would be more pronounced among animals repeatedly exposed to a relatively severe stressor and subsequently challenged with a mild stressor, than among animals exposed to the mild stressor throughout. Alternatively, it is possible that owing to the limited NE alterations associated with moderate stressor application, adaptation would progress more rapidly than after severe stressor application. Experiment 3 assessed the NE alterations that occur following various numbers of stress sessions of differing severity. In addition, variations in 5-HT levels were determined since it was of interest to assess whether alterations of this amine would be subject to adaptation, and also whether such an effect was dependent on stressor severity.

Method

Subjects and Apparatus

Eighty naive male CD-1 mice were employed as experimental subjects, as described earlier. Throughout the experimental period mice were individually housed and were provided with ad lib access to food and water. Inescapable footshock was administered in 4 identical preshock boxes described in Experiment 1.

Procedure

Mice were individually placed in the shock boxes for a 1.1 hr period on each of 15 successive days. Independent groups of mice ($n=8/\text{group}$) received shock exposure on either 0, 1, 3 or 15 days. For groups that received fewer than 15 shock sessions, the shock was applied on the last day, and on the preceding days mice were exposed to the apparatus but were not shocked. For half the mice shock consisted of 60 trials of 6 sec duration (150 μA) at intervals of 60 sec, whereas for the remaining mice, 180 shocks of 6 sec duration were delivered at 16 sec intervals. Two additional groups were exposed to 3 and 15 days of shock respectively. These groups received 180 shocks on all but the last day when 60 shocks were administered. Thus, these mice were exposed to the chronic shock regimen, but on the last day were challenged with reduced severity of the stressor. Immediately after the last stress session mice were decapitated, brains removed and dissected and frozen in liquid nitrogen. Concentrations of NE and 5-HT were subsequently determined fluorometrically.

Results and Discussion

Analysis of variance of the percent change of hypothalamic NE content indicated that concentrations of the amine did not vary as a function of the number of shocks administered per session, $F(1,41)=.88, p>.1$. However, NE levels were found to vary with the number of days of treatment that mice received, $F(2,41)=5.55, p<.05$. Newman-Keuls multiple comparisons ($\alpha=.05$) indicated that NE levels of mice that received 15 sessions of footshock significantly exceeded those of animals that received a single footshock session (see Figure 3). Although it appears that NE levels of acutely stressed mice were slightly reduced relative to baseline values, Dunnett's tests revealed

Figure 3: Mean ($\pm$ S.E.M.) concentrations of norepinephrine as a function of number of days of shock treatment and stressor severity. Values are reported as a percentage of the no shock treatment group.

that reductions of NE concentrations in mice that received one or three days of footshock did not reach statistical significance.

As depicted in Figure 3, increased NE concentrations were apparent in the hippocampus and cortex after chronic shock. Dunnett's tests confirmed that hippocampal NE levels were significantly higher after 15 sessions of 60 shocks relative to that seen in the no shock condition ($\alpha=.05$). The increased hippocampal NE levels in mice that received 14 sessions of 180 shocks followed by a single session of 60 shocks approached, but did not reach statistical significance. Moreover, Dunnett's tests confirmed that relative to non-shocked controls, in animals that received 15 sessions of 60 shocks, or 14 sessions of 180 followed by one session of 60 shocks, NE concentrations in the cortex were significantly elevated. The increase in cortical NE concentrations seen in animals that were given 15 sessions of 180 shocks also approached statistical significance.

In the hypothalamus and cortex, serotonin concentrations did not systematically vary with shock treatment; however, analysis of variance of hippocampal 5-HT levels revealed a significant Shock Treatment x Days interaction, $F(2,43)=7.43$, $p<.05$. Multiple comparisons of the simple main effects indicated that animals exposed to 3 days of 180 shocks per session exhibited elevated 5-HT levels compared to non-shocked animals and these levels returned to baseline with 15 days of such treatment. If mice were given fewer shocks per session (60), the increase of 5-HT levels in the hippocampus did not appear following 1 or 3 days of shock exposure, but after 15 stress sessions concentrations of this amine did significantly exceed control values.

Consistent with earlier reports, the results of the present study

demonstrated that with repeated exposure to uncontrollable footshock, an increase of NE levels occurs. It is of interest that increased NE concentrations were noted in all three brain regions examined, and they were observed even if the shock treatment did not provoke a significant decline in NE levels when administered acutely. The elevation in NE concentrations following chronic shock did not appear to vary as a function of the stressor severity employed either on the test day or on the preceding 14 days. Thus, unlike pharmacological tolerance, the extent of the adaptation was not dependent on the amount of shock administered during the chronic phase of the experiment. Moreover, if animals were given a less severe stressor on the test day than had earlier been administered (i.e. the 180-60 group), the magnitude of the NE increase in all three brain regions was comparable to that observed in the group that received the more severe stressor throughout. Indeed, even in the hippocampus, in which chronic administration of 60 shocks provoked a larger NE increase than did chronic exposure to 180 shocks, a reduction in stressor severity on the test day did not result in a further increase of NE levels. It would be expected that if tolerance had developed, the NE levels of the 180-60 group would have been comparable to those mice that were exposed to a chronic regimen of 60 shocks throughout the experiment.

As in the case of acute stressor application, it appears that the serotonergic system may be less responsive to chronic aversive stimulation. With more intense stressor application, increases of 5-HT levels were evident in the hippocampus, and the extent of the increase varied as a function of the stressor severity.

EXPERIMENT 4

Time-dependent Variations in Amine Levels After Chronic Shock

The preceding study demonstrated that the depletion of NE produced by acute stress was absent following a chronic stress regimen. In fact, under some conditions concentrations of NE of chronically stressed animals actually exceeded control levels, a finding consistent with that reported by other investigators (Roth et al, 1982; Weiss et al, 1975). It was postulated that the increased amine concentrations may be attributable to reduced utilization of the transmitter coupled with increased synthesis (Roth et al, 1982). It should be noted that amine concentrations and turnover in the experiments of Roth et al, (1982) were assessed 24 hr following the last stress session. Thus, these experiments provide an index of the relatively long-term consequences of the repeated stress sessions, but they do not address the nature of the amine alterations that occur during and/or immediately following exposure to a chronic stress regimen. Experiment 4 was undertaken to assess the variations of catecholamine concentrations that occur both immediately and 24 hr after repeated application of a stressor.

Method

Subjects and Apparatus

Thirty two naive male CD-1 mice were employed as described in Experiment 1 and these animals were group housed throughout the study. Inescapable shock was administered in the preshock boxes described earlier.

Procedure

Mice were randomly assigned to four treatment conditions (n=8/group). One group of mice received 180 shocks (6 sec duration, 150-

uA at 16 sec intervals) on each of 14 successive days. Twenty-four hours following the last stress session mice were decapitated, and brains were removed and dissected as in Experiment 1. A second group received the same shock treatment; however, prior to decapitation on the fifteenth day mice received a session of 60 shocks (6 sec duration, 150 uA at 60 sec intervals). The third group was handled on each of 14 days, and on the fifteenth day they were given a single session of 60 shocks (6 sec duration, 150 uA at 60 sec intervals) and were decapitated immediately afterward. The fourth group served as a handling control and animals were not exposed to shock. The stress parameters selected were based on those used in the preceding experiment, which resulted in adaptation of NE concentrations without an appreciable increase of NE relative to nonstressed animals. As in the other experiments, tissue was stored at -70°C until subsequent fluorometric determination of NE and 5-HT levels.

Results and Discussion

As seen in Figure 4, acute exposure to 60 shocks produced a small, but significant reduction of hypothalamic NE levels. In mice that received the chronic stress regimen and were again shocked immediately prior to decapitation, the NE levels exceeded those of mice that received only the acute stress session, but did not differ significantly from non-stressed mice. In contrast, in animals that were decapitated 24 hr after the chronic stress session, NE levels were increased relative to that of non-stressed mice. Analysis of variance of the percentage change of hypothalamic NE concentrations confirmed that NE levels were increased among mice that received the chronic shock treatment, $F(1,23)=8.08, p<.05$, and reduced in mice that received shock immediately prior to decapitation, $F(1,23)=5.28, p<.05$.

Figure 4: Mean ($\pm$ S.E.M.) concentrations of norepinephrine, immediately and 24 hr following stressor termination, as a function of acute and chronic shock treatment.

Consistent with the effects observed in the preceding studies, acute shock exposure did not reduce NE in the hippocampus. However, in animals that received the chronic stress treatment, NE levels were appreciably increased, $F(1,23)=5.72$, $p<.05$. Although the Chronic Shock x Acute Shock treatment interaction was not statistically significant, it is clear from Figure 4 (and confirmed by Newman-Keuls multiple comparisons, $\alpha=.05$) that the increased NE levels were evident only in those animals decapitated 24 hr after the last stress session. In cortex, the chronic shock treatment significantly elevated NE concentrations, $F(1,23)=5.76$, $p<.05$. In addition, the interaction between the Chronic and Acute Shock Treatments approached statistical significance, $F(1,23)=3.59$, $p=.072$. Pairwise comparisons revealed that acute shock treatment reduced NE levels somewhat ($p<.1$), whereas in chronically shocked mice, NE concentrations exceeded those of naive animals.

Analysis of variance revealed that hypothalamic 5-HT concentrations varied as a function of the Chronic x Acute shock interaction, $F(1,23)=5.10$, $p<.05$. Exposure to a single session of shock significantly increased 5-HT concentrations. The extent of the increase was largely reduced in mice exposed to chronic shock and decapitated 24 hr later, and was entirely absent in those animals that received chronic stress and were decapitated immediately after the last stress session. The same pattern of results was evident in the cortex and the hippocampus; however, only in the cortex did the Chronic shock x Acute shock interaction approach statistical significance, $F(1,23)=3.94$, $p<.06$.

Taken together, it appears that acute stress provoked a

significant reduction of NE in the hypothalamus, which was eliminated in animals that had received the chronic shock treatment. Moreover, in hypothalamus and hippocampus the concentrations of NE seen 24 hr after exposure to the chronic stress regimen actually exceeded those of non-stressed mice. In contrast to the NE reductions, concentrations of 5-HT were increased following a single session of shock. With repeated exposure to the stressor, the magnitude of the 5-HT increase was minimized or entirely eliminated.

EXPERIMENT 5

Stressor Effects on NE Utilization

Following repeated exposure to a stressor the activity of tyrosine hydroxylase and dopamine-beta-hydroxylase has been shown to increase (Roth et al, 1982; Weiss et al, 1976). It has been hypothesized that the compensatory increase of NE synthesis is responsible for the heightened NE concentrations noted after chronic stressor application. In addition, however, it was reported that 24 hr following repeated stressor application the rate of NE utilization is appreciably reduced (Roth et al, 1982). It is conceivable that the increased synthesis evident during repeated stressor application represents an adaptive mechanism which ensures that adequate amine supplies will be available to meet the immediate demands placed upon the organism. A further adaptive change may occur as well, so that upon termination of the daily stress session, utilization rates decline while heightened synthesis persists. Consequently, high concentrations of the amine are assured, thus leaving the organism better prepared to deal with

impending physical or psychological insults. Experiment 5 assessed the variations of NE utilization evident immediately and 24 hr following stressor termination. The proposition was entertained that upon shock inception amine utilization would increase regardless of the organism's stress history. However, among chronically stressed animals the rate of amine utilization may decline rapidly upon stressor termination.

Method

Subjects and Apparatus

A total of 120 naive male CD-1 mice served as subjects. The subject particulars and apparatus specifications were the same as those of Experiment 1.

Procedure

Mice were placed in the shock chambers for a 1.1 hr period on each of 14 consecutive days. During this time, half the mice received exposure to 180 shocks (6 sec, 150 μ A) as described in the preceding experiment, while the remaining mice were placed in the boxes but shock was not administered. On the fifteenth day, mice of each group were subdivided such that half the mice of each condition received intraperitoneal injection of the NE synthesis inhibitor alpha-methyl-para-tyrosine (α -MpT; 250 mg/kg), while the remaining mice received saline injection. These groups were further subdivided such that 2 hr after drug administration animals were exposed to either 60 shocks (150 μ A; 6 sec duration at 60 sec intervals) or no shock. Immediately after the shock treatment mice were decapitated and brains were removed. Tissue was dissected, rapidly frozen in liquid nitrogen and stored at -70° C for fluorometric determination of amine levels.

Results and Discussion

Analysis of variance of the percent change of hypothalamic NE concentrations revealed that NE levels were increased in animals that received the Chronic shock treatment, $F(1,108)=14.41$, $p<.01$ (see Figure 5). In contrast, a reduction of NE was induced by Acute Shock, $F(1,108)=15.10$, $p<.01$, and by treatment with α -MpT, $F(1,108)=223.97$, $p<.01$. Thus it appears that exposure to a single session of shock reduced NE levels, and that the extent of the reduction was further enhanced by treatment with α -MpT. Exposure to chronic shock resulted in an increase of NE concentrations. The latter effect, it should be noted, was evident regardless of whether mice received shock treatment on the fifteenth day.

The interaction between Chronic Shock x Acute Shock x Drug Treatment did not reach statistical significance, $F(1,108)=2.63$, $p=.10$. Nevertheless, Newman-Keuls comparisons of the simple main effects ($\alpha=.05$) were conducted since a priori predictions had been made concerning this interaction. These comparisons revealed that mice that received chronic shock and were treated with α -MpT 24 hr later (without exposure to shock at that time) exhibited higher NE levels than chronically shocked that received an additional session of shock following the drug treatment. Moreover, in animals that received a single shock session following treatment with α -MpT, the concentrations of NE were reduced relative to those that received α -MpT without the shock treatment ($p<.10$). These data indicate that utilization of NE was increased immediately after exposure to both acute and chronic shock. However, 24 hr after the last stress session the rate of NE release was attenuated in chronically stressed animals. In accordance with the findings of Roth et al (1982) it appeared that in α -MpT treated mice,

Figure 5: Mean ($\pm$ S.E.M.) concentrations of norepinephrine as a function of test day drug treatment and chronic shock pretreatment conditions. Values are reported as a percentage of the no shock, saline treated group mean.

escapable and yoked groups, but shock was withheld.

Immediately following the end of the treatment session, mice were decapitated and the brains were quickly removed, dissected and stored at -70°C for subsequent amine determination as described in Experiment 1.

Results and Discussion

Analyses of variance of the percent NE levels revealed significant Shock Treatment effects in the hypothalamus $F(2,26)=3.35$, $p<.05$, and in the hippocampus, $F(2,25)=3.49$, $p=.05$. As seen in Figure 7, in both of these brain regions, NE levels were significantly reduced by the inescapable shock treatment, but concentrations of NE in escapably shocked mice did not differ from control values ($\alpha=.05$). Consequently, it appears that the element of controllability over shock offset, rather than the shock per se was the important determinant of the NE neuronal response to footshock stress. Unlike the alterations noted in the hypothalamus and hippocampus, cortical NE levels were apparently unaffected by the administration of footshock treatment. Consistent with the results of the preceding studies, exposure to inescapable footshock did not alter 5-HT concentrations in any of the brain regions examined.

Together with the results of the preceding experiment, these data indicate that the neurochemical consequences of brief inescapable footshock (2 sec) are comparable to those observed when footshocks of longer duration are employed. In subsequent experiments these brief footshock parameters were therefore used in order to examine the processes involved in neurochemical adaptation to chronic footshock stress.

2

higher NE levels were evident 24 hr after chronic shock than in nonshocked mice. As in the study conducted by Roth et al., however, basal NE levels in the chronic condition exceeded that of nonstressed mice, precluding definitive conclusions concerning utilization rates.

Analysis of variance of the hippocampal and cortical changes of NE revealed that the drug treatment significantly reduced amine concentrations, $F_{s(1,111)}=110.48, 90.14, p,s<.05$, respectively. Although the Shock x Drug Treatment interactions were not significant, it was the case that NE concentrations were somewhat lower among mice that received the drug followed by shock, than in those that received α -MPT treatment but were not shocked. In neither brain region was there any indication that the chronic shock treatment altered the rate of NE utilization.

Consistent with the results of the preceding study, repeated footshock treatment resulted in an increase of NE concentrations in the hypothalamus. This increase was apparently due to an acceleration in the rate of amine synthesis, since the utilization rate following chronic shock was comparable to that observed in acutely stressed animals.

EXPERIMENT 6

NE Alterations After Varying Amounts of Brief Shock

In contrast to the effects of acute inescapable footshock, the data presented so far indicate that levels of NE reach or exceed baseline values following repeated stressor application. Such an outcome may be a result of adaptive processes engendered by repeated stressor application. Alternatively, this finding may be attributable,

in part, to the acquisition of competing motor responses. That is, in the preceding experiments, subjects were exposed to footshocks of 6 sec duration throughout the chronic stress regimen. The suggestion has been made that shock presentations of long duration favour the development of particular response styles owing to the adventitious reinforcement of behaviors, which although ineffective in eliminating a stressor, may be correlated with its offset (Anisman et al, 1978; Glazer & Weiss, 1976). It is conceivable that the adoption of such response styles influenced noradrenergic activity, either because these responses reduced the perceived aversiveness of the stressor or because they served as a method of "coping" with the aversive stimulus (see Anisman et al, 1978). In order to reduce the possibility that such motor tendencies would develop, subsequent studies employed shocks of brief duration, a treatment which does not lend itself to the development of learned response tendencies (see Anisman et al., 1978; Glazer & Weiss, 1976a,b).

Before assessing neurochemical responses evident after chronic exposure to brief duration footshock, it was necessary to establish that acute exposure to such treatment produces neurochemical effects similar to those seen following acute exposure to 6 sec inescapable footshock. Experiment 6 assessed the neurochemical consequences of various numbers of 2 sec footshocks.

Method

Subjects and Apparatus

A total of 30 naive male CD-1 mice served as subjects. The subject and apparatus specifications were identical to those described in Experiment 1.

Figure 6: Mean ($\pm$ S.E.M.) concentrations of norepinephrine as a function of the number of 2 sec footshocks administered. Values are presented as a percentage of the non-shocked group mean.

Procedure

Mice were randomly assigned to one of five treatment groups and were given either 0, 60, 180, 360 or 540 inescapable footshocks of 2 sec duration over a 1.1 hr period. Immediately following the end of the shock session animals were decapitated, the brains were removed and dissected as described in the procedure section of Experiment 1. Brain tissue was stored at -70°C until it was fluorometrically analyzed for norepinephrine and serotonin content.

Results and Discussion

The mean levels of NE in the hypothalamus, hippocampus and cortex are displayed in Figure 6 as a function of the shock treatment. Analysis of variance of the hypothalamic NE content revealed a significant Shock Treatment effect, $F(4,25)=6.04$, $p<.05$. Newman-Keuls multiple comparisons ($\alpha=.05$) indicated that groups that received either 360 or 540 footshocks exhibited significantly lower NE levels than non-shocked mice ($\alpha=.05$). However, the administration of 2 sec footshocks was without effect on hippocampal or cortical NE levels, $F's(4,25)=.55$ and 1.00 respectively, $p>.05$. Likewise, 5-HT values did not vary as a function of shock treatment in any of the brain regions examined. It appears that the magnitude of the effect produced by a single session of brief footshock on NE levels in the hypothalamus was comparable to that seen in earlier studies which employed 6 sec shocks, and it was therefore appropriate to use these parameters in subsequent investigations of adaptation to chronic footshock.

EXPERIMENT 7

Stressor Controllability

The element of controllability over stressor offset has repeatedly been shown to be an important determinant of both the behavioral and

neurochemical responses to aversive stimuli (Maier & Seligman, 1976; Weiss et al., 1970, 1981). Indeed following uncontrollable footshock, NE concentrations were reduced in several brain regions, whereas escapable footshock was without effect on levels of this transmitter (Anisman et al., 1980). In the latter study the pattern and duration of inescapable shock differed from those used in the present series of experiments. Consequently, an auxiliary study was conducted in order to confirm that within the current experimental parameters, controllability over the stressor influences neurochemical alterations.

Method

Subjects

Thirty naive male CD-1 mice were employed as subjects according to the specifications previously outlined.

Apparatus

Footshock was administered in three identical black Plexiglas shuttle boxes with interior dimensions of 26.4 x 9.0 x 15.5 cm. The floor of each box consisted of .32 cm stainless steel rods spaced 1.0 cm apart (center to center), and connected in series with neon bulbs, through which shock (150 μ A, 60 Hz, AC) was delivered from a 3,000 V source. Stainless steel plates lined the end walls of the boxes and these were connected in series with the grid floor. The two compartments of each shuttle box were separated by a stainless steel wall which held a solenoid-controlled gate that opened horizontally. In the open-gate position, a 1.0 cm high stainless steel hurdle separated the two sides of the shuttle box, and both the gate and the hurdle were connected in series with the grid floor.

At a distance of 1.5 cm from either side of the hurdle, there were

two infrared photodetector units, situated 1.0 cm and 4.0 cm above the grid floor. If the beams on both sides of the hurdle were crossed simultaneously, as in the case of a mouse being halfway across the hurdle, the cells would not trigger. The photocells would trigger only when the beam in the safe compartment was crossed, causing the gate to close and the shock to terminate. Additional photocells were located 2.5 cm from the end walls and 1.0 cm above the hurdle. Thus, if a mouse jumped over the cells at the gate, the latter photocells would be triggered. The shuttle boxes were housed in sound-attenuated chambers. A microcomputer system controlled the sequencing of trials for each shuttle box and recorded the escape latencies.

Procedure

Mice were randomly assigned to one of three treatment groups and received either escapable shock, yoked inescapable shock or no shock treatment. Mice in the escape group were individually placed in the shuttle boxes and were given escape training with a shock intensity of 150 μ A. On each trial shock was delivered through the grid floor and 1.5 sec later, the gate opened, permitting access to the adjacent non-shock compartment. If a subject did not cross the hurdle and enter the non-shock side of the shuttle box within 24 sec, the gate closed and the shock was terminated. Mice were given 360 trials with an intertrial interval of 9 sec. Animals in the yoked group were individually placed in the shuttle boxes but were not permitted to escape from the shock. Since the inescapable shock boxes were connected in series with those of the escapable shock group, yoked animals received the same pattern and amount of shock as that administered to their partners in the escapable condition. Mice in the no shock condition were individually placed in the shuttle boxes for an equivalent amount of time as the

escapable and yoked groups, but shock was withheld.

Immediately following the end of the treatment session, mice were decapitated and the brains were quickly removed, dissected and stored at -70°C for subsequent amine determination as described in Experiment 1.

Results and Discussion

Analyses of variance of the percent NE levels revealed significant Shock Treatment effects in the hypothalamus $F(2,26)=3.35$, $p<.05$, and in the hippocampus, $F(2,25)=3.49$, $p=.05$. As seen in Figure 7, in both of these brain regions, NE levels were significantly reduced by the inescapable shock treatment, but concentrations of NE in escapably shocked mice did not differ from control values ($\alpha=.05$). Consequently, it appears that the element of controllability over shock offset, rather than the shock per se was the important determinant of the NE neuronal response to footshock stress. Unlike the alterations noted in the hypothalamus and hippocampus, cortical NE levels were apparently unaffected by the administration of footshock treatment. Consistent with the results of the preceding studies, exposure to inescapable footshock did not alter 5-HT concentrations in any of the brain regions examined.

Together with the results of the preceding experiment, these data indicate that the neurochemical consequences of brief inescapable footshock (2 sec) are comparable to those observed when footshocks of longer duration are employed. In subsequent experiments these brief footshock parameters were therefore used in order to examine the processes involved in neurochemical adaptation to chronic footshock stress.

Figure 7: Mean ($\pm$ S.E.M.) concentrations of norepinephrine as a function of acute shock treatment (NS = no shock, ES = escapable shock, YIS = yoked, inescapable footshock).

EXPERIMENT 8

Transmitter Alterations After Brief Chronic Shock

Inescapable footshock of brief duration (2 sec) provoked depletions of NE in several brain regions, in a manner which paralleled the findings of earlier experiments employing 6 sec footshocks. Experiment 8, therefore, assessed whether a compensatory neurochemical response could be invoked with repeated application of very brief duration shocks, a procedure which was found to prevent or limit the acquisition of competing motor responses. Furthermore, it will be recalled that a variety of environmental and experiential factors influence the behavioral and neurochemical consequences of acute stressor application. The social housing conditions in which animals are maintained have been shown to profoundly influence the concentration and turnover of transmitters, and may also influence the organism's response to other stressors. For example, it was reported that social isolation accentuated the behavioral disruption provoked by a single session of uncontrollable shock, and also exacerbated the depletion of brain NE (Anisman & Sklar, 1981). Accordingly, in Experiment 8 it was of interest to assess whether social conditions would influence the response to acute stressors and whether this factor would alter the course of the adaptive neurochemical response to chronic uncontrollable footshock.

Method

Subjects and Apparatus

A total of 50 male CD-1 mice served as subjects, and the subject and apparatus specifications were the same as those described in Experiment 1.

Procedure

Mice were assigned to either isolated or grouped housing conditions (5 per cage) and further divided into one of five treatment conditions. Animals received a daily 1.1 hr session of footshock (360 shocks, 2 sec in duration, presented at 9 sec intervals) for either 1, 3 or 15 days. In the two remaining control conditions, mice were placed in the boxes for a 1.1 hr period without footshock or they received no handling whatsoever throughout the experiment. Immediately following the last treatment session (Day 15) mice were decapitated and their brains were removed, dissected and frozen for subsequent fluorometric determination of amines.

Results and Discussion

Analysis of variance of the hypothalamic NE levels revealed a significant Shock Treatment effect, $F(4,108)=5.67$, $p<.05$. Newman-Keuls multiple comparisons indicated that NE levels for animals exposed to 15 days of shock treatment were significantly higher than those in any other treatment condition ($\alpha=.05$). Although the Shock Treatment $\times$ Housing Condition interaction did not reach statistical significance, $F(4,108)=1.97$, $p=.104$, inspection of Figure 8 clearly shows that the NE profile varied with both the shock and housing condition. Indeed, Newman Keuls multiple comparisons ($\alpha=.05$) indicated that if animals had been isolated, and were not handled or exposed to the footshock chambers, hypothalamic NE levels were significantly lower than in group-housed, non-handled animals ($\alpha=.05$). However, if isolated animals were habituated to the handling procedures, NE levels were comparable to those of group-housed non-shocked animals. Not unexpectedly, in group housed animals where stimulation was presumably

Figure 8: Mean ($\pm$ S.E.M.) concentrations of norepinephrine as a function of the housing condition and the number of days of shock treatment. Values are expressed as a percentage of the group-housed non-handled control group.

maintained at a higher level (i.e. social interactions), the handling manipulation was without effect. However, in the group-housed animals, exposure to a single session of inescapable footshock reduced hypothalamic NE by approximately 17% ($\alpha < .1$); whereas in isolated animals acute shock was without effect. Analyses of variance of the NE content in the other brain regions examined failed to reveal significant treatment effects. Likewise, housing conditions and chronic shock treatment were without effect upon 5-HT levels in any of the brain regions examined.

Several investigators have reported that although isolation does not alter levels of catecholamines, the turnover of NE in mice may be reduced by such a treatment (Welch & Welch, 1970). Moreover, isolation may interact with other stressors to provoke greater transmitter alterations (Anisman & Sklar, 1981; Blanc et al, 1980). The data of Experiment 8 raised the possibility that isolation may have rendered animals more susceptible to the effects of a mild stressor such as the handling that preceded decapitation. In fact, as already indicated, the isolated non-handled animals exhibited NE levels comparable to those of group-housed animals that received a single inescapable footshock session. Habituation to the handling procedure resulted in an increase of NE levels, such that the amine concentrations were not different from group-housed controls. Such a finding is not unique to the present investigation, and has been observed with respect to GABA activity (Biggio et al., 1981). Likewise, Riley, Fitzmaurice and Spackman (1981) reported that the effects of stressors on plasma corticoid concentrations varied with the background "noise" conditions upon which the stressor was applied. Indeed, it was maintained that the ordinary smells and noises

associated with a laboratory setting influence corticoid concentrations, thereby making it difficult to assess the genuine effects attributable to experimental stressors. The present results concerning central NE activity are consistent with conclusions provided by Biggio et al. (1981) and by Riley et al. (1981), in that analysis of stressor effects must consider that absolute basal values may vary as a function of the control procedures adopted.

Although in the present experiment acute shock did not provoke a depletion of NE in the isolated animals, it should be noted that chronic shock did result in a significant increase of hypothalamic NE. Thus, it appears that although housing conditions may influence the extent to which an acute stressor reduces NE levels, neurochemical adaptation following chronic stress exposure occurs regardless of whether animals are isolated or group-housed. Furthermore, such an effect was evident using very brief footshock which has been shown to limit the development of competing motor tendencies. Accordingly, the increased hypothalamic NE levels noted following chronic exposure to these shock parameters likely reflected a genuine neurochemical adaptation to the stressor, rather than an effect secondary to the acquisition of competing motor responses.

EXPERIMENT 9

Time Course of NE Alterations Following Chronic Stress

Acute stressor application has been shown to result in regional variations in the extent of the initial amine depletion, as well as the time course of the amine alterations. It may also be the case that the neurochemical effects of chronic stressors vary as a function of the time following stressor termination. In order to assess the duration of the transmitter alterations, amine determinations in

Experiment 9 were made in different brain regions at several intervals up to one week following the end of a chronic stress regimen.

Method

Subjects and Apparatus

A total of 45 male CD-1 mice were used according to subject and apparatus specifications previously described.

Procedure

In Experiment 9, animals were assigned to one of three treatment conditions and were exposed to either 15 daily sessions of inescapable shock as described in the preceding experiment (chronic), 14 days of no shock treatment followed by a single session of inescapable shock on day 15 (acute shock), or they were placed in the boxes each day without receiving shock (no shock). Equal numbers of animals from each of these groups ($n=5$) were sacrificed either immediately, 24 hr or 168 hr following the final treatment session. Brains were quickly removed, dissected and frozen in liquid nitrogen. Tissue was stored at -70°C for subsequent fluorometric determination of amine content.

Results and Discussion

Regional variations in NE content are shown in Figure 9 as a function of the time after footshock exposure. Analysis of variance revealed that hypothalamic NE levels were significantly reduced in animals that received a single session of inescapable footshock compared to mice that were chronically stressed, $F(1,51)=4.63$, $p<.05$. In addition, there was a significant main effect of the Time After treatment, $F(2,51)=3.46$, $p<.05$. Newman-Keuls multiple comparisons ($\alpha=.05$) indicated NE that levels were higher one week following stressor termination, than immediately following shock offset. As shown in

Figure 9: Mean ($\pm$ S.E.M.) concentrations of norepinephrine at various intervals following acute or chronic footshock exposure. Concentrations are reported as a percentage of the non-shocked group mean.

Figure 9, however, this effect apparently is due in large part to the marked reduction of NE seen immediately following acute footshock treatment. Because predictions had been made concerning the immediate effects of acute shock, Newman-Keuls multiple comparisons were conducted at each level of the shock treatment and time after shock application. The tests confirmed that immediately following shock offset, acutely stressed animals exhibited NE levels that were significantly lower than the chronically shocked group and that levels returned to baseline within 24 hr of acute stress termination.

Footshock treatment did not alter NE levels in either the cortex or hippocampus at any of the intervals observed $F_s(2,52)=1.46$ and $.34$, respectively, $p>.10$. Consistent with earlier experiments, 5-HT levels in the brain regions examined were unaltered by this treatment.

In accordance with the data from other experiments in this series, acute shock provoked a reduction of NE in the hypothalamus which was evident immediately following stressor termination and levels of this amine returned to baseline within 24 hr. Moreover, animals that were exposed to repeated application of footshock exhibited NE levels which did not differ from control values. Unlike the results of Experiment 4, however, there was little difference between the NE levels of chronically stressed animals which were sacrificed immediately after shock offset and those that were sacrificed 24 hr later. It is possible that the failure to find such temporal variations following chronic shock may be attributable to differences in shock parameters employed in the two experiments. That is, acute application of the footshock parameters employed in Experiments 3 and 4 produced substantially smaller depletions than that observed in the present experiment. It may

be the case that when the severity of a stress regimen is increased the degree of the neurochemical rebound following chronic stressor application is limited.

Although variations in hypothalamic NE were minimal 24 hr following chronic shock, time-dependent variations in amine utilization would be expected. Indeed, it was demonstrated in Experiment 5 that immediately following chronic shock offset NE release was accelerated, and 24 hr later this rate was attenuated. In order to confirm this finding, a supplementary experiment was conducted to assess variations of the NE metabolite MHPG using the shock parameters employed in Experiment 9. Equal numbers of mice ($n=14$) were exposed to a chronic shock regimen (14 daily sessions of 360 inescapable footshocks of 2 sec duration) or they were placed in the shock chambers but shock was withheld (no shock condition). Half the animals in each group were sacrificed immediately after the last footshock session, while the remaining mice were sacrificed 24 hr later.

Analysis of variance indicated that hypothalamic NE levels of chronically shocked mice were not significantly different from control levels at either of the intervals observed. As can be seen in Figure 10, however, MHPG levels varied as a function of the Shock Treatment x Time After Treatment interaction, $F(1,22)=11.47$, $p<.05$. Newman-Keuls multiple comparisons of the simple main effects ($\alpha=.05$) revealed that immediately after shock offset MHPG levels of chronically shocked mice were significantly higher than controls ($\alpha=.05$). Twenty four hrs following the last treatment session the MHPG levels of chronically shocked mice were substantially reduced, and were not significantly different from those of non-shocked mice. Although it appears that control MHPG levels increased 24 hr after placement in the no shock

Figure 10: Mean ($\pm$ S.E.M.) concentrations of NE and MHPG, immediately and 24 hr following chronic stressor application. Values are presented as a percentage of the no shock control group.

chambers, Newman-Keuls multiple comparisons indicated that this difference was not statistically significant ($\alpha > .05$)

In the hippocampus, as in the hypothalamus, NE levels were not significantly altered by chronic shock treatment. As can be seen in Figure 10, MHPG levels did appear to vary as a function of shock treatment. Although the Shock Treatment x Interval interaction did not reach statistical significance, Newman-Keuls multiple comparisons ($\alpha = .05$) were conducted since a priori predictions regarding this interaction had been made. These comparisons revealed that immediately following the chronic shock treatment MHPG levels were increased relative to control values, whereas 24 hrs later MHPG accumulation was not significantly different from control values.

Together these data confirm that immediately following chronic shock utilization of NE is accelerated relative to non-stressed animals, but that this pace was attenuated over time following stressor offset. Although the MHPG levels did not significantly differ from control values 24 hr after the chronic shock treatment, the data shown in Figure 10 suggest that recovery was not complete within 24 hr, and that the effects of chronic stress on NE release may be evident for relatively long intervals following stressor termination.

EXPERIMENTS 10A & 10B

Re-exposure to Stress at Long Intervals after Acute and Chronic Shock

It is clear from the results of the present investigation, as well as those of other investigators (Anisman and Sklar, 1980), that while alterations in NE levels produced by acute stressors are

relatively transient, subsequent exposure to even a small amount of stress may provoke a rapid depletion of NE. Indeed the sensitization effect may be observed even when the re-exposure stress is qualitatively different from that used in the initial stress session. Thus it appears that exposure to inescapable stress may have lasting implications for future stressful experiences. Inasmuch as repeated stressor application results in neurochemical adaptation, it would be of interest to determine whether a chronic stress regimen would also have persistent effects on NE neuronal functioning when animals are subsequently reexposed to the stressor. Experiment 10 assessed whether the compensatory neurochemical alterations noted following chronic stress exposure could be provoked by reexposure to a limited amount of stress, and whether such an effect would be present even when a relatively long period intervened between the end of the chronic stress regimen and the reintroduction of the stressor. A preliminary experiment (10a) was conducted in order to determine the parameters necessary to provoke a reexposure effect 24 hr following the initial stress session.

Method

Subjects and Apparatus

Forty-five and 30 subjects were employed in experiments 10a and 10b respectively. All subject and apparatus specifications conform to those described in Experiment 1.

Procedure

In both experiments equal numbers of mice ($n=15$ in Experiment 10a and $n=10$ in Experiment 10b) were assigned to one of three treatment conditions. Animals in the chronic shock condition were given 15 daily 1.1 hr sessions of inescapable footshock as described previously (360

shocks, 2 sec duration presented at 9 sec intervals). Animals in the acute shock group were exposed to the apparatus but shock was withheld during the first 14 days of the experiment, and then a single 1.1 hr footshock session was administered on day 15 (360 shocks, 2 sec duration, 9 sec interval). Those animals in the no shock condition were exposed to the apparatus each day for 15 days but were not given footshock.

In Experiment 10a, mice were returned to their home cages following the end of the last treatment session on day 14. Twenty four hours later the 3 treatment groups were further subdivided and equal numbers of mice ($n=5$) were exposed to either no shock, 15 shocks or 60 shocks (150 μ A, 2 sec duration, presented at 9 sec intervals). Immediately after the treatment session animals were removed from the apparatus and decapitated. The brains were removed, dissected and stored at -70°C for subsequent neurochemical analysis.

Animals in Experiment 10b were returned to their home cages for 2 weeks following the end of the last chronic shock session, and during this time they were handled only to change the cage bedding on day 7. On the 14th post-stress day, half of the animals from each the 3 treatment conditions were given 60 inescapable footshocks as described in Experiment 10a, and the other half were exposed to the apparatus for an equivalent amount of time but received no footshock. As in Experiment 10a animals were immediately decapitated and their brains removed and dissected. In both experiments, brain tissue was analysed using high performance liquid chromatography with electrochemical detection using a modification of the method of Mefford (1981; see Appendix 1).

Results and Discussion Experiment 10A

Figures 11 and 12 depict the NE and MHPG concentrations in the hypothalamus and hippocampus as a function of the preshock and reexposure treatments administered. Analysis of variance of NE levels in the hypothalamus (% no shock control) revealed a significant Shock Pretreatment x Reexposure Treatment interaction, $F(4,34)=9.33$, $p<.05$. Newman-Keuls multiple comparisons of the simple main effects comprising this interaction indicated that the administration of either 15 or 60 inescapable shocks to mice that had not previously been shocked resulted in a significant NE depletion ($\alpha=.05$). In contrast, if mice had received chronic shock treatment on the days preceding the administration of a brief period of footshock, NE levels were not significantly different from control values. Relative to controls, NE levels were significantly reduced by exposure to 60 footshocks on the test day in those animals that had previously been exposed to a single footshock session.

An analysis of variance of MHPG levels revealed that regardless of the nature of the pretreatment animals had received, accumulation of the metabolite was significantly increased following exposure to 60 shocks, $F(1,34)=30.39$, $p<.05$. It appears that with as little as 10 minutes of intermittent inescapable footshock, the rate of NE release was accelerated, and in some instances this was accompanied by a decline in the concentration of NE. The failure to observe a sensitization effect in acutely stressed animals was somewhat surprising, as this effect has been noted on several occasions in this laboratory. However, in the present investigation the shock parameters employed during the reexposure session produced a depletion in the

Figure 11: Mean ($\pm$ S.E.M.) concentrations of NE and MHPG in the hypothalamus, as a function of the shock pretreatment and re-exposure treatment condition. Concentrations are reported as a percentage of the group that received the no-shock treatment on both days.

Figure 12: Mean (+ S.E.M.) NE and MHPG levels in the hippocampus as a function of the pretreatment and re-exposure conditions.

order of 20%, and consequently a further decline in NE levels may have been precluded due to a floor effect. Moreover, it is conceivable that alterations in turnover produced by the acute stress pretreatment carried over to the reexposure session, again precluding the observation of a sensitization effect.

As seen in Figure 12, hippocampal NE levels were not significantly altered by the shock pretreatment or the reexposure treatments. However, analysis of variance of the MHPG levels yielded a significant main effect of Shock pretreatment, $F(2,34)=3.56$, $p<.05$, and Newman-Keuls multiple comparisons ($\alpha=.05$) revealed that MHPG levels were elevated in animals that received chronic footshock treatment. It is of interest that this elevation was noted even in those animals that were exposed to the apparatus without receiving footshock on the test day. These data suggest that accelerated turnover may be provoked in the hippocampus by exposure to cues previously paired with shock, a finding consistent with that reported by Cassens et al (1980). Cortical concentrations of NE and MHPG were apparently unaltered by either the pretreatment or reexposure treatments administered.

In addition to measuring NE and MHPG levels, in the present experiment HPLC determination was also made of tissue concentrations of epinephrine, dopamine and its metabolites HVA and DOPAC, and serotonin and its metabolite, 5-HIAA. Separate analyses of variance were calculated for each compound in each of the 3 brain regions examined. These analyses failed to reveal any systematic variations as a function of either the pretest or reexposure treatments administered.

Results and Discussion - Experiment 10B

In Experiment 10B, which assessed the long-term consequences of exposure to either acute or chronic stress, hypothalamic NE levels

varied as a function of the Shock Pretreatment x Reexposure Treatment interaction, $F(2,24)=9.24$, $p<.05$ (See Figure 13). Newman-Keuls multiple comparisons of the simple main effects indicated that in animals that did not receive preshock treatment, the application of 60 reexposure shocks did not significantly reduce NE levels. If, however, mice had received a single session of inescapable shock two weeks earlier, reexposure to footshock provoked a significant reduction of hypothalamic NE ($\alpha=.05$). In contrast, chronically shocked mice exhibited NE levels significantly above baseline following reexposure to 60 shocks ($\alpha=.05$). Analysis of variance of the Hypothalamic MHPG concentrations indicated that the Shock Pretreatment x Reexposure Treatment interaction approached, but did not reach statistical significance, $F(2,23)=2.98$, $p=.07$. Because predictions had been made concerning this interaction, Newman-Keuls multiple comparisons as well as Dunnett's tests were conducted. Both tests indicated that chronically shocked animals that were reexposed to the shock treatment exhibited MHPG levels that were significantly higher than control values. Among acutely stressed animals reexposure to the apparatus in which shock had previously been administered resulted in a significant increase in MHPG.

Analysis of variance of NE concentrations in the hippocampus revealed a significant Shock Pretreatment effect, $F(2,22)=4.01$, $p<.05$ (see Figure 14). Multiple comparisons indicated that regardless of the reexposure treatment, NE levels of mice that received the acute shock pretreatment did not differ from controls, whereas the chronically stressed groups exhibited a significant elevation of NE levels. Moreover, placement in the preshock boxes with or without footshock

Figure 13: Mean ($\pm$ S.E.M.) NE and MHPG levels in the hypothalamus as a function of the shock pretreatment and re-exposure treatment conditions. Values are expressed as a percentage of the no-shock group mean.

Figure 14: Mean ($\pm$ S.E.M.) NE and MHPG concentrations in the hippocampus as a function of the shock pretreatment and re-exposure conditions. Concentrations are presented as a percentage of the no-shock group mean.

administration resulted in increased MHPG levels in both chronically and acutely stressed mice, although this effect was not statistically significant. The finding that NE levels were elevated following placement in the footshock chambers suggests that simply exposing animals to cues associated with footshock was sufficient to induce a sensitization of hippocampal NE synthesis in chronically stressed animals. However, in order to confirm that cues alone could elicit such an effect, it would be necessary to include the appropriate control group (i.e. a group which is not handled or re-exposed to the test apparatus on test day).

As in Experiment 10a, analyses of variance failed to reveal any significant variations in hypothalamic, hippocampal or cortical concentrations of dopamine, epinephrine or serotonin. Furthermore, the amine metabolites HVA, DOPAC and 5-HIAA were unaltered by the various stress treatments. It will be recalled, however, that other investigators have reported that under some conditions footshock stress will provoke increases in DA turnover in discrete nuclei of the dopaminergic system (eg. Kobayashi et al, 1976; Monnet & Lichtensteiger, 1980).

Together, the results of Experiments 10A and 10B indicate that the noradrenergic system is subject to sensitization, and the nature of this effect is dependent upon the type of stressor to which animals had previously been exposed, as well as the length of the interval between the pretreatment and the reexposure stress. In particular, it appears that at relatively long intervals, the reintroduction of a few minutes of intermittent footshock will provoke a marked NE reduction in animals previously exposed to an acute stressor. In contrast, if mice had been exposed to a chronic stress regimen to which animals would

ordinarily adapt, then the subsequent reintroduction of a brief stressor provoked an increase in NE levels and NE release. Thus it appears that adaptation to chronic stress may have a lasting influence on the response to later encounters with a stressor.

General Discussion

Consistent with several earlier reports (Anisman et al, 1980a; Thierry, et al; 1971; Weiss et al, 1981), application of physical and psychological stressors provoked increases in NE utilization in several brain regions. Moreover, depending on the stressor severity and the brain region examined, the increased utilization was accompanied by decreased levels of the transmitter. Such alterations were noted irrespective of whether long or short duration footshocks were employed as the aversive stimuli, although it was found that the magnitude of the NE variations was directly related to stressor severity. In any event, levels of the transmitter returned to basal concentrations within 24 hr. of stressor termination. In some instances increased levels of 5-HT were also noted following inescapable shock, but in general it seems that the serotonergic system was less responsive to the aversive stimuli used in these experiments. Such data are consistent with earlier reports indicating that more severe stressor parameters are required in order to elicit detectable changes in 5-HT levels (Thierry, Fekete & Glowinski, 1968; Thierry, 1973).

Although a great many variables may influence the neurochemical response to stressors, the degree to which an organism is able to exert control over aversive stimuli, is thought to be fundamental in determining whether NE reductions would be observed (Anisman et al, 1980; Weiss et al, 1970, 1979, 1981). In agreement with these observations, the results of the present investigation revealed that NE depletions were noted only in those animals that were not given the opportunity to control footshock offset. If animals were able to escape

from the footshock, NE levels were comparable to those of non-stressed mice. Evidently, when behavioral means of coping with aversive stimuli are available to the organism, the extent of the neurochemical disruption is limited. This is not to suggest that neurochemical processes are unaltered following escapable footshock; to the contrary it seems that although the physiological demands placed upon the organism may be increased (requiring accelerated NE release), the system is better able to meet these demands through increased synthesis, thereby precluding amine depletion.

The NE depletion observed after acute shock is not evident after repeated exposure to an uncontrollable stressor. Whereas 1 or 3 days of inescapable shock tended to produce a small NE reduction, concentrations of the amine reached or exceeded control levels following 15 days of inescapable shock. Determinations of NE turnover following chronic shock indicated that relative to non-stressed animals, release of the transmitter was accelerated during the shock session. Accordingly, the increased NE concentrations are attributable to enhanced NE synthesis, which likely reflects an adaptive change in order for the organism to meet the increased environmental demands.

The extent of the NE elevations associated with chronic stress appear to vary with time following stressor termination. In particular, immediately after the last day of a chronic stress regimen NE concentrations were comparable to those of non-stressed mice; however, when determinations were made 24 hr later, NE levels exceeded baseline. Although such a biphasic effect in NE concentrations was not always observed following chronic shock, estimates of turnover (using NE synthesis inhibition or measurement of metabolites) confirmed that NE utilization was accelerated immediately following chronic shock offset,

but was attenuated 24 hr after the last stress session. Moreover, the extent of the increased release was comparable to that seen in acutely stressed animals, again suggesting that a compensatory increase in amine synthesis must have occurred in order for NE levels to meet or exceed basal values. This conclusion is commensurate with the data of Thierry, Javoy, Glowinski and Kety (1968) which indicated that 24 hr following 3 days of footshock stress, endogenous NE concentrations were increased relative to those of non-stressed animals. Such an outcome was attributed to increased synthesis of the transmitter persisting over the 24 hr period. Interestingly, however, these investigators observed that although utilization rates (as gauged by the disappearance rate of exogenous $^3\text{H-NE}$) were increased immediately after acute stress, they were reduced 24 hr after repeated stress. In effect, the increased endogenous NE concentrations evident at some time following a chronic stress regimen may be a result of the combined effects of sustained compensatory increases in the rate of NE synthesis coupled with a decrease in release.

It will be recognized that the biphasic variations of NE activity represent an exquisitely adaptive response to environmental insults. Upon exposure to a stressor the increased amine utilization permits the organism to cope with the immediate environmental demands placed upon it. The persistent increase of synthesis and reduced release that occur after stressor termination may represent an ideal method of accumulating and conserving amine stores in order for the organism to contend with impending stressors.

In considering the effects of acute and chronic stressors on noradrenergic functioning it is important not only to assess the

immediate neurochemical variations, but also the potential for altered responsivity to subsequent environmental challenges. It has been suggested that acute uncontrollable footshock results in sensitization or conditioning of neurochemical activity, such that reexposure to even a limited amount of stress re-induces the amine depletion (Anisman & Sklar, 1979). In those experiments, however, the re-exposure session occurred 24 hr after the initial stress experience and consequently it was not clear (a) whether the re-induction of the depletion was partially attributable to some form of carry-over effects of the initial stress session, and (b) whether the sensitization/conditioning effects of the initial session were, in fact, relatively permanent.

The results of the present investigation confirmed that in previously shocked mice, re-exposure to a limited amount of shock provoked a more pronounced reduction of NE levels. Moreover, such an effect was evident even if immobilization was used as the aversive stimulus during the second stress session. Given that this effect was observed when the re-exposure stressor was qualitatively different from the initial stressor, it is likely that the proactive effects of the the inescapable shock were due to sensitization rather than conditioning processes. Of course, it is conceivable that other factors associated with both stress sessions (eg. handling, or the internal state accompanying the stressful situation) acted as conditioned stimuli to elicit the amine alterations. Indeed, Cassens et al (1980) demonstrated that cues associated with a stressor effectively increased the accumulation of the NE metabolite MHPG, and the results of the present investigation are certainly consistent with this finding. In addition, the present investigation revealed that the sensitization effect occurred even if a two week period intervened between the

Figure 15: Schematic representation of the noradrenergic response sequence following stress exposure.

initial footshock and the subsequent re-exposure session. Clearly, uncontrollable aversive events increase vulnerability to NE alterations upon later stressful encounters even when relatively long intervals separate the two events.

In assessing the potential long term repercussions of stressors, it is important to consider the nature of the initial stressful experience. While re-exposure to an aversive stimulus provoked a more pronounced NE depletion in animals previously exposed to acute uncontrollable shock, this was not the case in animals that had earlier received repeated aversive stimulation. In fact, two weeks following a chronic stress regimen, re-exposure to a small amount of inescapable shock resulted in elevations hypothalamic and hippocampal NE concentrations. The increased transmitter levels were accompanied by heightened MHPG accumulation as well. These data suggest that repeated stress exposure altered the capacity of the animal to respond to aversive stimuli. Evidently, chronic stressor application results in the sensitization of neurochemical mechanisms such that the presumably adaptive processes associated with chronic stress are reintroduced upon subsequent encounters with similar environmental insults.

Taken together, the results of the present investigation suggest that a series of adaptive changes occur following application of stressors. The sequence of neurochemical responses that occur under stressful conditions are shown in Figure 15. The proposition is provisionally offered that dysfunction at any stage in this series may render the organism more vulnerable to psychological pathologies, as well as physical disorders which may directly or indirectly be influenced by NE activity (see for example Irwin & Anisman, 1984; Sklar

& Anisman, 1980). When confronted by a stressor, an animal makes vigorous efforts to limit the aversive stimulation. The specific response profile exhibited by the animal is of course, dependent on species factors, experiential variables and the environmental context in which the stressor is applied. These behavioral responses, as indicated earlier, are accompanied by increased neurochemical activity which probably represents one component of the physiological response sequence that enables the organism to meet the environmental challenge. Among other things, NE is released and synthesized at an accelerated pace, and if the behavioral coping attempts lead to elimination of the aversive stimuli, utilization of the stressor is not excessive. However, if behavioral attempts to cope with the stressor are unavailable (or unsuccessful) then the release of NE proceeds at an accelerated rate and synthesis of the transmitter may not keep pace. As a result, a net decline of NE may follow.

It has been postulated (Irwin & Anisman, 1984; Sklar & Anisman, 1981; Weiss et al, 1979) that those factors which increase the propensity for NE reductions likewise increase vulnerability to behavioral and physical pathology. For instance, the backdrop upon which stressors are applied may have a profound effect on neurochemical lability. In mice housed in isolation, acute stressors are more likely to induce alterations in transmitter levels and predictably, both behavioral disturbances (Sklar & Anisman, 1981), and physical pathology are more apt to occur (Dechambre & Gosse 1973; Plaut & Friedman, 1981; Sklar, Bruto & Anisman, 1981). Similarly, behavioral disturbances induced by stressors are more pronounced in older animals in which NE synthesis does not readily keep pace with utilization, relative to that seen in young adult animals (Ritter & Pelzer, 1978; 1980).

Additionally, it is known that alterations of NE levels are more readily provoked in some strains of mice and rats than others (Blizard et al, 1983; Sudakov et al, 1980; Zukowska-Grojec, 1980), and the possibility exists that these neurochemical changes correspond to vulnerability to stress-related disturbances (Anisman, Grimmer, Irwin, Remington & Sklar, 1979).

Under conditions of chronic stress a further sequence of adaptive changes may occur. Specifically, with repeated application of a stressor the organism responds with increased release of the amine. Gradually, however, a compensatory increase of tyrosine hydroxylase and dopamine-beta-hydroxylase activity occurs (i.e. synthesis rate increases) (Kvetnansky et al, 1976; Weiss et al, 1976). As seen in Figure 15, the net result of the increased synthesis is that the organism exhibits amine levels at or above basal values. Accordingly, the organism should be better prepared to deal with immediate environmental demands. As indicated earlier, immediately following stressor termination, the increased synthesis apparently persists for some time, while the rate of release declines. Consequently, a surplus of amine stores is established, thereby rendering the organism better prepared to deal with impending environmental insults.

The view adopted here (see also Irwin & Anisman, 1984) is that the aforementioned sequence of events in response to chronic stressors represents an adaptive change to ensure the survival of the organism. If a breakdown occurs in this adaptive sequence, then vulnerability to a variety of pathologies would be expected to increase. It should be underscored however, that this does not necessarily imply that pathology will invariably occur. Rather, whether an illness develops

will depend on a host of factors, including the presence of a pathogen, genetic predisposition to a particular disease state, nutritional status, and so forth. For example, if an individual is subjected to severe stress and adaptation does not occur, vulnerability to an infectious disease state may be increased provided that a pathogen is present. Likewise, it is not being suggested that stressors would be capable of causing affective disorders or physical illnesses, but rather that they may contribute to the creation of an internal environment which favours the development of the disease.

The notion that chronic stressors result in adaptive changes should not be misconstrued to imply that an appropriate treatment for stress-related disorders is one of continued stressor application. On the contrary, the contention is offered that the aforementioned adaptive sequence represents the course of events that ordinarily occurs. When stress-related pathology arises, it does so because of a failure of adaptive systems, and therefore further insults could only serve to aggravate the pathological state (see discussion in Anisman & Zacharko, in press). It is proposed that when a failure of adaptive processes does occur, pharmacological treatment may be necessary to either augment or mimic the endogenous adaptive processes. For instance, Stone (1979) suggested that chronic stress and antidepressant drugs influence similar neurochemical processes, leading to the belief that the antidepressant treatment is effective because it stimulates the induction of adaptive changes which would ordinarily occur in the individual who is not adversely affected by stressors.

No doubt the organism has at its disposal several adaptive networks of which the noradrenergic response described is but one. The possibility should be entertained that (a) the particular pathology

engendered by chronic stressors may be related to a specific neurochemical system which is adversely influenced by environmental or psychological insults, and (b) the more extensive the adaptive networks the better equipped the animal is in its ability to deal with stressors. In effect, in the canalized organism (either due to genetic factors or because of concurrent demands placed upon it) stressors may increase pathological outcomes simply because the organism has fewer alternative mechanisms available for dealing with the demands placed upon it.

Unfortunately, few data are available concerning the conditions under which chronic stressors lend themselves to pathological outcomes. It has been demonstrated that behavioral and somatic disturbances associated with acute uncontrollable stress are not evident in chronically stressed animals. For instance, Weiss et al (1975) reported that the shuttle escape disturbances associated with acute shock were not observed after chronic shock, and Platt and Stone (1982) observed similar outcomes in evaluating performance in a forced swim paradigm. Along the same lines, it has been shown that the immunosuppressive effects of acute stress and the tumour enhancement associated with such treatment are not evident in chronically stressed animals. In fact, under such conditions immunofacilitation has been observed and the rate of tumour development was reduced (Monjan & Collector, 1977; Sklar & Anisman, 1979). However, in many of these paradigms a very large degree of variability in chronically stressed animals has been observed. Whereas some animals undergo adaptation to stressor effects, in a subset of animals the detrimental effects of stressors become exaggerated. Indeed, as yet unpublished data collected in this

laboratory assessing immunocompetence in acutely and chronically stressed animals indicated that the activity of Natural Killer (NK) cells (i.e. a component of the immune system thought to offer protection against tumour development), was reduced in acutely stressed mice relative to non-stressed controls ($0.19 \pm .024$ vs $0.30 \pm .053$ lytic units/ 10^6 cells). In chronically stressed animals a bimodal distribution was evident. Whereas a subgroup of chronically shocked mice exhibited a marked enhancement of NK cell activity ($0.72 \pm .13$ lytic units/ 10^6 cells), another subgroup of chronically shocked animals displayed a marked suppression in NK lytic ability ($0.14 \pm .02$). In this instance, the characteristic that distinguished between the animals that did or did not fare well in response to chronic stressor exposure was related to social dominance hierarchy. That is, those animals that were subjected to repeated attacks by a dominant male exhibited low levels of NK cell activity, whereas mice that were not attacked exhibited high levels of NK cell lytic activity. Clearly, a more complete appreciation of the adaptive pattern associated with repeated stressor requires a more discrete analysis of the organismic, experiential and environmental backdrop upon which the stressor is superimposed.

On the basis of the adaptive changes outlined thus far, it might be supposed that when compensatory increases in transmitter synthesis and levels are effected, behavioral pathology will be less likely to occur. It should be underscored, however, that adequate levels of transmitter by no means provide such assurances. After all, in the chronically stressed animal turnover is greatly accelerated, and if sustained, could potentially be detrimental to the organism. Stone (1979, 1983a) has argued that following chronic stressor application

the sensitivity of B-receptors may be reduced and this is accompanied by down-regulation of the NE sensitive cyclic AMP response. More recently, Stone et al (1984) also suggested that modifications in non-Beta receptor systems may also occur (see also Vetulani, 1982 regarding up-regulation of alpha-1 NE receptor systems). The down-regulation of these systems would thus counteract the adverse effects of excessive receptor stimulation. While the results of the present investigation do not address this possibility, the model adopted here is not inconsistent with the proposition offered by Stone, and changes in receptor sensitivity may reflect an additional phase of the adaptive sequence.

As discussed earlier, acute and chronic stressor will not only have immediate effects on neurochemical lability, but may also influence the NE alterations evident upon reexposure to a stressor. Of particular interest in the present investigation was the finding that the effects of the reexposure treatment were dependent on the stress experience that animals had previously received. Whereas reexposure to aversive stimuli in acutely stressed animals produced a marked NE reduction, in chronically stressed animals the very same treatment resulted in elevated NE levels coupled with increased utilization. Thus it is likely that chronic stressor exposure resulted in sensitization or conditioning of the mechanisms subserving NE synthesis, such that upon stressor reexposure a marked and rapid increase in synthetic processes was activated. Such a change would have obvious adaptive significance in that it would allow for immediate mobilization of amine stores with a decreased risk of a reduction of transmitter concentrations.

Inasmuch as stressful events may have proactive neurochemical effects, analysis of the stress-pathology relationship should not only consider the immediate impact of stressors, but also the long-term neurochemical or hormonal consequences of reexposure to stressors or to cues associated with a stressor. Indeed, the argument has been advanced that stress reexposure experiences may not only influence neurochemical functioning, but might potentially affect immunological processes, thereby altering vulnerability to physical as well as psychological disorders (Irwin & Anisman, 1984). Additionally, several investigators have reported that uncontrollable aversive events may influence responses to subsequent pharmacological treatments. For instance, uncontrollable stressors were shown to enhance the stereotypy elicited by both cocaine and amphetamine (Antelman et al, 1980; McLennan & Maier, 1983), as well as the stimulus perseveration ordinarily elicited by amphetamine (Anisman, Hahn, Hoffman & Zacharko, in press 1985). These investigators suggested that since the response to amphetamine in animals may represent an animal analogue of idiopathic and drug-induced psychosis, the possibility should be entertained that stressful events may proactively influence the development of schizophrenia.

It might be added that human clinical data are available indicating that life stress events may lead to depressive episodes. Interestingly, the incidence of depression following a stress experience was reported to be particularly high in women who, during their early years, had lost their mothers (see Brown, 1979). While numerous factors could have contributed to such an outcome, the possibility exists that the early trauma resulted in a neurochemical sensitization effect such that more profound amine alterations were

engendered upon exposure to stressors in adulthood, thus contributing to the onset of the depressive symptomatology (see Anisman, 1984).

An additional issue warrants consideration at this point. In modelling the effects of stressors on pathology in humans, the view was adopted that the evaluation of chronic stressor effects might represent a more appropriate tactic than that of evaluating acute stressor effects. This assumption was based largely on the fact that the stressors encountered by humans are typically chronic in nature, particularly when ruminations over the stressor are considered. In her analysis of the stress-pathology relationship, Burchfield (1979), in fact, differentiated between several forms of stress. Whereas some chronic stressors are continuous in nature (e.g. physical disability), other stressors occur on an intermittent basis. It might be added as well that intermittent stressors may occur on a relatively predictable basis (e.g. same time each day) or on an unpredictable basis. Therefore, it may be important to construct animal models involving various forms and patterns of chronically applied stressors.

In fact, in proposing a sensitization model of affective disorders, Post, Rubinow and Ballenger (1984) have argued that temporal aspects of stress exposure may be critical in determining whether pathology develops. They suggested that stress-induced sensitization is particularly apt to occur when stressors are presented on an intermittent basis. It might be supposed that intermittent stressful events may limit the development of anticipatory behaviors which ordinarily help the organism to contend with the aversive situation. Indeed, Burchfield (1979) reported that when chronic intermittent stress was applied on a predictable schedule, the corticoid response typically elicited by a stressor ultimately developed in anticipation

of stressor application. Post et al (1984) have likewise suggested that after a sufficient number of stressful experiences, subsequent reexposure to stressors or to symbolic stimuli associated with the stressful event, may result in a compensatory neurochemical alteration following the initial amine response. As a result, the depressive response style may revert to one of mania. In effect, under some conditions (e.g. after intermittent stress) one form of pathology may ultimately give rise to a different illness rather than to neurochemical and behavioral adaptation as described previously. It is conceivable as well that in the absence of predictable patterns of stressor presentation, anticipatory responses might not occur, and also neurochemical adaptation may not develop. In fact, such an unpredictable, intermittent pattern of stressor presentations could theoretically result in sensitization effects with respect to neurochemical depletion. Accordingly, under such conditions vulnerability to illness may be maximized.

It will be recognized that although the preceding discussion has focussed primarily on noradrenergic consequences of stressors, the contribution of other transmitter systems to stress-related pathology should not be dismissed. As indicated earlier, stressors influence dopamine, acetylcholine, endorphins, and GABA, as well as several endocrine systems. No doubt, variations of these endogenous substances may contribute to pathological states associated with stressors, and a comprehensive analysis of the stress-pathology topography must consider both the individual and interactive effects of these systems.

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Catecholamines and Stress: Recent Advances. New York: Elsevier-
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APPENDIX A

Assay Procedures

I. Fluorometric Catecholamine Assay

(Ref.: Lavery, R. & Taylor, K.M. The fluorometric assay of catecholamines and related compounds: Improvements and extensions to the hydroxyindole technique. (1968) Analytical Biochemistry, 22, 269-279.)

Brain tissue samples were weighed, sonified in acidified butanol, and centrifuged at 3000 r.p.m. for 10 minutes. The supernatant was transferred to another test tube containing heptane and HCl, and the solution was mixed and centrifuged. Two aliquots were transferred to test tubes containing a buffer (pH=7.0) for use in the NE analysis. A third aliquot was transferred to a tube containing o-phthalaldehyde (OPT)/methanol and concentrated HCl, for use in the 5-HT analysis.

Standards were prepared by dilution of stock solutions of NE and 5-HT to .05 g/.10 ml 1N HCl. The standard solution and .1N HCL were added to tubes containing the buffer. For the 5-HT analysis, an aliquot of tissue extract was added to the tube containing OPT and this was heated at 65 C for 15 min. The samples were then ready for reading in the fluorometer.

For the NE analysis buffer was added to each test tube, and for the tissue samples, the following solutions were added:

iodine solution (oxidation reaction)
EDTA (conversion reaction)
glacial acetic acid (acidification reaction)

The same solutions were added in reverse order to the second set of tubes for the preparation of tissue blanks. All the samples were left to stand at room temperature for at least 20 minutes. The NE samples were read at 380/480 nm (excitation/emission wavelengths), and the 5-HT samples were read at 360/470, using an Aminco-Bowman spectrophotofluorometer.

II. HPLC Assay for Catecholamines & Metabolites

(Ref.: Mefford, I.N., (1981) Application of high performance liquid chromatography with electrochemical detection to neurochemical analysis: Measurement of catecholamines, serotonin and metabolites in rat brain. Journal of Neuroscience Methods, 3, 2077-224).

Frozen tissue sections were weighed and then sonified in 0.2M perchloric acid. The homogenate was then centrifuged and the supernatant was filtered through a .2 um regenerated cellulose filter. An aliquot of this filtrate was injected onto a C₁₈ reversed-phase column in a high-performance liquid chromatography system equipped with an electrochemical detector. The mobile phase used with this aliquot (0.1M citrate/acetate with 20% methanol) allowed for the separation of 5-HT, 5-HIAA, MHPG, HVA, and DOPAC. In order to separate NE, EPI, and DA, a second aliquot of the filtrate was added to 10 mg of acid-washed alumina and Tris buffer (pH=8.6). The catecholamines were then desorbed from the alumina with 0.2M perchloric acid, and an aliquot of this extract was injected into the HPLC system, along with a mobile phase composed of 0.1M acetate, 0.02M citrate and 15% methanol. Sodium octyl sulphate (50 mg/l) was added as an ion-pairing agent and EDTA (100 mg/l) was added to act as an anti-oxidant.

APPENDIX B

Table 1a

Analysis of Variance of NE Content in Hypothalamus

(Experiment 1)

<u>Source</u>	<u>df</u>	<u>SS</u>	<u>MS</u>	<u>F</u>	<u>SIG</u>
Shock Treatment (S)	2	.18707	.09353	5.52	p=.007
Time after treatment. (T)	2	.11475	.05737	3.39	p=.043
S X T	4	.14598	.03649	2.15	p=.090
within cells	45	.76266	.01694		

Table 1b

Analysis of Variance of NE Content in Hippocampus

(Experiment 1)

<u>Source</u>	<u>df</u>	<u>SS</u>	<u>MS</u>	<u>F</u>	<u>SIG</u>
Shock Treatment (S)	2	.00597	.00298	2.24	p=.118
Time after treatment (T)	2	.00782	.00391	2.93	p=.063
S X T	4	.00403	.00101	.76	p=.559
within cells	45	.06002	.00133		

Table 1c

Analysis of Variance of NE Content in Cortex

(Experiment 1)

<u>Source</u>	<u>df</u>	<u>SS</u>	<u>MS</u>	<u>F</u>	<u>SIG</u>
Shock Treatment (S)	2	.00246	.00123	1.07	p=.352
Time after treatment (T)	2	.00290	.00125	1.25	p=.295
S X T	4	.00695	.00173	1.5	p=.217
within cells	45	.05215	.00115		

Table 2a

Analysis of Variance of 5-HT Content in Hypothalamus

(Experiment 1)

<u>Source</u>	<u>df</u>	<u>SS</u>	<u>MS</u>	<u>F</u>	<u>SIG</u>
Shock Treatment (S)	2	.1196	.0598	.50	p=.607
Time after treatment (T)	2	.48325	.2416	2.04	p=.143
S X T	4	.16883	.0422	.36	p=.839
within cells	44	5.2195	.1186		

Table 2b
 Analysis of Variance of 5-HT Content in Hippocampus
 (Experiment 1)

<u>Source</u>	<u>df</u>	<u>SS</u>	<u>MS</u>	<u>F</u>	<u>SIG</u>
Shock Treatment (S)	2	.00884	.00442	.60	p=.553
Time after treatment (T)	2	.03377	.01688	2.29	p=.113
S X T	4	.0836	.0209	2.83	p=.035
within cells	45	.3319	.0073		

Table 2c

Analysis of Variance of 5-HT Content in Cortex

(Experiment 1)

<u>Source</u>	<u>df</u>	<u>SS</u>	<u>MS</u>	<u>F</u>	<u>SIG</u>
Shock Treatment (S)	2	.00168	.00084	.19	p=.828
Time after treatment (T)	2	.01294	.00647	1.46	p=.243
S X T	4	.00327	.00817	.18	p=.945
within cells	45	.19927	.00442		

Table 3a

Analysis of Variance of NE Content in Hypothalamus
(Experiment 2)

<u>Source</u>	<u>df</u>	<u>SS</u>	<u>MS</u>	<u>F</u>	<u>SIG</u>
Shock Treatment (S)	1	665.267	665.267	4.83	p=.03
Reexposure Treatment (R)	3	1063.86	354.619	2.57	p=.06
S X R	3	276.139	92.046	.67	p=.50
within cells	69	9503.475	137.731		

Table 3b

Analysis of Variance of NE Content in Hippocampus

(Experiment 2)

<u>Source</u>	<u>df</u>	<u>SS</u>	<u>MS</u>	<u>F</u>	<u>SIG</u>
Shock Treatment (S)	1	161.727	161.727	.72	p=.39
Reexposure Treatment (R)	3	278.863	92.9545	.42	p=.74
S X R	3	1451.94	483.982	2.16	p=.100
within cells	71	15892.08	223.832		

Table 3c
 Analysis of Variance of NE Content in Cortex
 (Experiment 2)

<u>Source</u>	<u>df</u>	<u>SS</u>	<u>MS</u>	<u>F</u>	<u>SIG</u>
Shock Treatment (S)	1	252.944	252.944	.93	p=.33
Reexposure Treatment (R)	3	457.544	152.515	.56	p=.64
S X R	3	1853.72	617.907	2.27	p=.08
within cells	69	19570.78	271.816		

Table 4a

Analysis of Variance of 5-HT Content in Hypothalamus
(Experiment 2)

<u>Source</u>	<u>df</u>	<u>SS</u>	<u>MS</u>	<u>F</u>	<u>SIG</u>
Shock Treatment (S)	1	92.079	92.079	.57	p=.45
Reexposure Treatment (R)	3	365.407	128.151	.75	p=.52
S X R	3	384.453	137.73	.79	p=.50
within cells	69	11228.39	162.73		

Table 4b

Analysis of Variance of 5-HT Content in Hippocampus

(Experiment 2)

<u>Source</u>	<u>df</u>	<u>SS</u>	<u>MS</u>	<u>F</u>	<u>SIG</u>
Shock Treatment (S)	1	36.486	36.486	.72	p=.69
Reexposure Treatment (R)	3	555.054	185.018	.76	p=.51
S X R	3	1561.95	520.649	2.15	p=.10
within cells	71	17196.35	242.202		

Table 4c

Analysis of Variance of 5-HT Content in Cortex
(Experiment 2)

<u>Source</u>	<u>df</u>	<u>SS</u>	<u>MS</u>	<u>F</u>	<u>SIG</u>
Shock Treatment (S)	1	94.021	94.021	.70	p=.40
Reexposure Treatment (R)	3	308.882	102.961	.76	p=.51
S X R	3	257.866	85.955	.64	p=.59
within cells	72	9739.18	135.26		

Table 5a
 Analysis of Variance of NE Content in Hypothalamus
 (Experiment 3)

<u>Source</u>	<u>df</u>	<u>SS</u>	<u>MS</u>	<u>F</u>	<u>SIG</u>
Shock Treatment (S)	1	275.612	275.612	.88	p=.35
Days of Treatment (D)	2	3465.13	1732.56	5.55	p=.007
S X D	2	87.09	43.54	.14	p=.87
within cells	41	12803.17	312.272		

Table 5b

Analysis of Variance of NE Content in Hippocampus

(Experiment 3)

<u>Source</u>	<u>df</u>	<u>SS</u>	<u>MS</u>	<u>F</u>	<u>SIG</u>
Shock Treatment (S)	1	.20525	.20525	.00	p=.98
Days of Treatment (D)	2	1888.02	944.01	1.83	p=.17
S X D	2	1073.56	536.77	1.04	p=.36
within cells	43	22138.4	514.84		

Table 5c

Analysis of Variance of NE Content in Cortex

(Experiment 3)

<u>Source</u>	<u>df</u>	<u>SS</u>	<u>MS</u>	<u>F</u> [*]	<u>SIG</u>
Shock Treatment (S)	1	.14701	.14701	.00	p=.98
Days of Treatment (D)	2	1310.58	655.29	1.67	p=.93
S X D	2	56.20	28.10	.07	p=.93
within cells	43	16877.7	392.50		

Table 6a

Analysis of Variance of 5-HT Content in Hypothalamus

(Experiment 3)

<u>Source</u>	<u>df</u>	<u>SS</u>	<u>MS</u>	<u>F</u>	<u>SIG</u>
Shock Treatment (S)	1	62.37	62.37	.54	p=.46
Days of Treatment (D)	2	437.29	218.64	1.88	p=.16
S X D	2	401.63	200.81	1.72	p=.19
within cells	41	4777.0	116.51		

Table 6b
 Analysis of Variance of 5-HT Content in Hippocampus
 (Experiment 3)

Source	df	SS	MS	F	SIG
Shock Treatment (S)	1	89.87	89.87	.58	p=.45
Days of Treatment (D)	2	84.85	42.42	.27	p=.76
T X D	2	2307.71	1153.86	7.73	p=.001
within cells	43	6675.69	155.24		

Table 7a

Analysis of Variance of NE Content in Hypothalamus
(Experiment 4)

<u>Source</u>	<u>df</u>	<u>SS</u>	<u>MS</u>	<u>F</u>	<u>SIG</u>
Acute Shock (A)	1	764.13	764.13	5.28	p=.025
Chronic Shock (C)	1	1169.3	1169.3	8.08	p=.006
A X C	1	49.71	49.71	.34	p=.560
within cells	58	8390.12	144.65		

Table 7b

Analysis of Variance of NE Content in Hippocampus

(Experiment 4)

<u>Source</u>	<u>df</u>	<u>SS</u>	<u>MS</u>	<u>F</u>	<u>SIG</u>
Acute Shock (A)	1	13.91	13.91	.05	p=.821
Chronic Shock (C)	1	1541.98	1541.98	5.72	p=.020
A X C	1	275.96	275.96	1.02	p=.316
within cells	58	15648.0	269.79		

Table 7c

Analysis of Variance of NE Content in Cortex

(Experiment 4)

<u>Source</u>	<u>df</u>	<u>SS</u>	<u>MS</u>	<u>F</u>	<u>SIG</u>
Acute Shock (A)	1	672.87	672.87	1.79	p=.186
Chronic Shock (C)	1	2167.82	2167.82	5.76	p=.186
A X C	1	231.84	231.84	.62	p=.435
within cells	57	21447.9	376.27		

Table 8a

Analysis of Variance of 5-HT Content in Hypothalamus

(Experiment 4)

<u>Source</u>	<u>df</u>	<u>SS</u>	<u>MS</u>	<u>F</u>	<u>SIG</u>
Acute Shock (A)	1	669.80	669.80	8.11	p=.006
Chronic Shock (C)	1	54.12	54.12	.66	p=.421
A X C	1	421.46	421.46	5.10	p=.027
within cells	58	4789.4	82.57		

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Table 8b

Analysis of Variance of 5-HT Content in Hippocampus
(Experiment 4)

<u>Source</u>	<u>df</u>	<u>SS</u>	<u>MS</u>	<u>F</u>	<u>SIG</u>
Acute Shock (A)	1	416.82	416.82	1.91	p=.171
Chronic Shock (C)	1	20.60	20.60	.09	p=.759
A X C	1	185.75	185.75	.85	p=.359
within cells	57	12407.4	217.6		

Table 8c

Analysis of Variance of 5-HT Content in Cortex
(Experiment 4)

<u>Source</u>	<u>df</u>	<u>SS</u>	<u>MS</u>	<u>F</u>	<u>SIG</u>
Acute Shock (A)	1	58.42	58.42	.59	p=.443
Chronic Shock (C)	1	15.39	15.39	.16	p=.693
A X B	1	387.27	387.27	3.94	p=.051
within cells	57	5597.0	98.19		

Table 9a

Analysis of Variance of Norepinephrine Content in Hypothalamus
(Experiment 5)

<u>Source</u>	<u>df</u>	<u>SS</u>	<u>MS</u>	<u>F</u>	<u>SIG.</u>
Chronic Treatment (C)	1	3730.19	3730.19	14.41	p=.0002
Drug Treatment (D)	1	57973.5	57973.5	223.9	p<.001
Acute Shock (A)	1	3908.14	3908.14	15.10	p<.001
C x D	1	854.75	854.75	3.30	p=.072
C x A	1	290.68	290.68	1.12	p=.291
D x A	1	612.40	612.40	2.37	p=.126
C x D x A	1	681.97	681.97	2.63	p=.107
within cells	108	27954.98	258.84		

Table 9b

Analysis of Variance of Norepinephrine Content in Hippocampus

(Experiment 5)

<u>Source</u>	<u>df</u>	<u>SS</u>	<u>MS</u>	<u>F</u>	<u>SIG</u>
Chronic Treatment (C)	1	274.71	274.71	.41	p=.524
Drug Treatment (D)	1	74265.5	74265.5	110.48	p<.001
Acute Shock (A)	1	1221.44	1221.44	1.82	p=.180
C x D	1	626.10	626.10	.93	p=.336
C x A	1	136.23	136.23	.20	p=.653
D x A	1	769.56	769.56	1.14	p=.287
C x D x A	1	9.22	9.22	.01	p=.96
within cells	111	74612.4	672.18		

Table 9c

Analysis of Variance of Norepinephrine Content in Cortex

(Experiment 5)

<u>Source</u>	<u>df</u>	<u>SS</u>	<u>MS</u>	<u>F</u>	<u>SIG</u>
Chronic Treatment (C)	1	4.59	4.59	.01	p=.934
Drug Treatment (D)	1	60212	60212	90.14	p<.001
Acute Shock (A)	1	256.03	256.03	.38	p=.537
C x D	1	274.96	274.96	.41	p=.522
C x A	1	100.39	100.39	.15	p=.699
D x A	1	71.95	71.95	.11	p=.743
C x D x A	1	945.43	945.43	1.42	p=.236
within cells	109	72814.78	72814.78		

Table 10a

Analysis of Variance of Norepinephrine Content in Hypothalamus

(Experiment 6)

<u>Source</u>	<u>df</u>	<u>SS</u>	<u>MS</u>	<u>F</u>	<u>SIG</u>
Shock Treatment	4	2498.24	624.55	6.04	p=.0015
within cells	25	2586.13	103.44		

Table 10b

Analysis of Variance of Norepinephrine Content in Hippocampus
(Experiment 6)

<u>Source</u>	<u>df</u>	<u>SS</u>	<u>MS</u>	<u>F</u>	<u>SIG.</u>
Shock Treatment	4	659.927	164.98	.55	p=.698
within cells	25	4811.415	192.456		

Table 10c

Analysis of Variance of Norepinephrine Content in Cortex
(Experiment 6)

<u>Source</u>	<u>df</u>	<u>SS</u>	<u>MS</u>	<u>F</u>	<u>SIG</u>
Shock Treatment ,	4	792.394	198.098	1.00	p=.4286
within cells	24	4771.091	198.795		

Table 11a

Analysis of Variance of Serotonin Content in Hypothalamus

(Experiment 6)

<u>Source</u>	<u>df</u>	<u>SS</u>	<u>MS</u>	<u>F</u>	<u>SIG</u>
Shock Treatment	4	1025.97	256.492	.64	p=.641
within cells	24	9660.01	402.50		

Table 11b

Analysis of Variance of Serotonin Content in Hippocampus

(Experiment 6)

<u>Source</u>	<u>df</u>	<u>SS</u>	<u>MS</u>	<u>F</u>	<u>SIG</u>
Shock Treatment	4	1906.32	476.58	2.58	p=.070
within cells	25	4811.41	192.46		

Table 11c

Analysis of Variance of Serotonin Content in Cortex

(Experiment 6)

<u>Source</u>	<u>df</u>	<u>SS</u>	<u>MS</u>	<u>F</u>	<u>SIG</u>
Shock Treatment	4	558.954	139.739	.51	p=.725
within cells	25	6518.067	271.586		

Table 12a

Analysis of Variance of NE Content in Hypothalamus

(Experiment 7)

<u>Source</u>	<u>df</u>	<u>SS</u>	<u>MS</u>	<u>F</u>	<u>SIG</u>
Shock Treatment	2	2236.33	1118.17	3.35	p=.05
within cells	26	8666.67	333.33		

Table 12b

Analysis of Variance of NE Content in Hippocampus

(Experiment.7)

<u>Source</u>	<u>df</u>	<u>SS</u>	<u>MS</u>	<u>F</u>	<u>SIG</u>
Shock Treatment	2	2555.96	1277.98	3.49	p=.046
within cells	25	9149.35	365.97		

Table 12c

Analysis of Variance of NE Content in Cortex

(Experiment 7)

<u>Source</u>	<u>df</u>	<u>SS</u>	<u>MS</u>	<u>F</u>	<u>SIG</u>
Shock Treatment	2	958.00	479.00	.21	p=.810
within cells	25	56344.38	2253.77		

Table 13a

Analysis of Variance of 5-HT Content in Hypothalamus
(Experiment 7)

<u>Source</u>	<u>df</u>	<u>SS</u>	<u>MS</u>	<u>F</u>	<u>SIG</u>
Shock Treatment	2	1484.22	742.10	2.32	p=.1178
within cells	26	8299.85	319.22		

Table 13c

Analysis of Variance of 5-HT Content in Cortex

(Experiment 7)

<u>Source</u>	<u>df</u>	<u>SS</u>	<u>MS</u>	<u>F</u>	<u>SIG</u>
Shock Treatment	2	155.916	77.958	.68	p=.517
within cells	26	2999.515	115.365		

Table 14a
 Analysis of Variance of NE Content in Hypothalamus
 (Experiment 8)

<u>Source</u>	<u>df</u>	<u>SS</u>	<u>MS</u>	<u>F</u>	<u>SIG</u>
Housing Condition (H)	1	387.02	387.02	1.18	p=.280
Shock Treatment (T)	4	7464.01	1866.00	5.67	p<.001
H X S	4	2589.59	647.39	1.97	p=.104
within cells	108	35554.96	329.21		

Table 14b

Analysis of Variance of NE Content in Hippocampus

(Experiment 8)

<u>Source</u>	<u>df</u>	<u>SS</u>	<u>MS</u>	<u>F</u>	<u>SIG</u>
Housing Condition (H)	1	242.85	242.85	.58	p=.448
Shock Treatment (T)	4	1737.54	434.38	1.04	p=.394
¹² H X S	4	1341.02	335.25	.80	p=.528
within cells	103	43186.8	419.28		

Table 14c
 Analysis of Variance of NE Content in Cortex
 (Experiment 8)

<u>Source</u>	<u>df</u>	<u>SS</u>	<u>MS</u>	<u>F</u>	<u>SIG</u>
Housing Condition (H)	1	757.38	757.38	2.75	p=.099
Shock Treatment (T)	4	2021.89	505.47	1.84	p=.126
H X S	4	229.91	57.47	.21	p=.933
within cells	110	30255.65	275.05		

Table 15a

Analysis of Variance of 5-HT Content in Hypothalamus

(Experiment 8)

<u>Source</u>	<u>df</u>	<u>SS</u>	<u>MS</u>	<u>F</u>	<u>SIG</u>
Housing Condition (H)	1	1283.01	1283.01	2.38	p=.125
Shock Treatment (T)	4	1514.33	378.58	.70	p=.591
H X S	4	22.84	5.71	.01	p=.999
within cells	108	58200.4	538.89		

Table 15b

Analysis of Variance of 5-HT Content in Hippocampus
(Experiment 8)

<u>Source</u>	<u>df</u>	<u>SS</u>	<u>MS</u>	<u>F</u>	<u>SIG</u>
Housing Condition (H)	1	.064166	.064166	.00	p=.988
Shock Treatment (T)	4	1892.4	473.1	1.66	p=.165
H X S	4	328.0	82.01	.29	p=.885
within cells	103	29392.8	285.36		

Table 15c
 Analysis of Variance of 5-HT Content in Cortex
 (Experiment 8)

<u>Source</u>	<u>df</u>	<u>SS</u>	<u>MS</u>	<u>F</u>	<u>SIG</u>
Housing Condition (H)	1	816.71	816.71	3.22	p=.075
Shock Treatment (T)	4	107.41	107.41	.11	p=.980
H X S	4	1442.08	360.51	1.42	p=.231
within cells	110	27893.64	253.57		

Table 16a

Analysis of Variance of NE Content in Hypothalamus

(Experiment 9)

<u>Source</u>	<u>df</u>	<u>SS</u>	<u>MS</u>	<u>F</u>	<u>SIG</u>
Shock Treatment (S)	1	2261.24	2261.24	4.63	p=.036
Time After Treatment (T)	2	3385.25	1692.63	3.46	p=.038
S X T	2	1787.42	893.7	1.83	p=.171
within cells	51	24926.2	482.75		

Table 16b

Analysis of Variance of NE Content in Hippocampus

(Experiment 9)

<u>Source</u>	<u>df</u>	<u>SS</u>	<u>MS</u>	<u>F</u>	<u>SIG</u>
Shock Treatment (S)	1	1227.29	1227.29	2.73	p=.1045
Time After Treatment (T)	2	575.99	287.99	.64	p=.531
S X T	2	301.97	150.98	.34	p=.716
within cells	52	23374.38	449.50		

Table 16c

Analysis of Variance of NE Content in Cortex

(Experiment 9)

<u>Source</u>	<u>df</u>	<u>SS</u>	<u>MS</u>	<u>F</u>	<u>SIG</u>
Shock Treatment (S)	1	634.10	634.10	2.23	p=.141
Time After Treatment (T)	2	365.21	182.60		p=.530
S X T	2	586.29	293.14		p=.364
within cells	51	14520.18	284.70		

Table 17a

Analysis of Variance of 5-HT Content in Hypothalamus

(Experiment 9)

<u>Source</u>	<u>df</u>	<u>SS</u>	<u>MS</u>	<u>F</u>	<u>SIG</u>
Shock Treatment (S)	1	280.43	280.43	1.04	p=.310
Time After Treatment (T)	2	413.71	206.85	.77	p=.466
S X T	2	226.54	113.27	.42	p=.657
within cells	68	18260.2	268.53		

Table 17b

Analysis of Variance of 5-HT Content in Hippocampus

(Experiment 9)

<u>Source</u>	<u>df</u>	<u>SS</u>	<u>MS</u>	<u>F</u>	<u>SIG</u>
Shock Treatment (S)	1	262.14	262.14	.52	p=.472
Time After Treatment (T)	2	1867.40	933.70	1.86	p=.165
S X T	2	567.59	283.79	.57	p=.570
within cells	51	25538.78	500.76		

Table 17c
 Analysis of Variance of 5-HT Content in Cortex
 (Experiment 9)

<u>Source</u>	<u>df</u>	<u>SS</u>	<u>MS</u>	<u>F</u>	<u>SIG</u>
Shock Treatment (S)	1	261.59	261.59	.96	p=.33
Time After Treatment (T)	2	357.12	178.56	.66	p=.52
S X T	2	369.67	184.83	.68	p=.51
within cells	52	14132.43	271.77		

Table 18a

Analysis of Variance of NE & MHPG Content in Hypothalamus

(Experiment 9a)

<u>Source</u>	<u>Variate</u>	<u>Statistic</u>	<u>F</u>	<u>DF</u>	<u>P</u>
Shock Treatment (S)	NE	SS=149.76 MS=149.76	.83	1	p=.376
	MHPG	SS=13206 MS=13206	12.98	1	p=.001
Time After Treatment (T)	NE	SS= 59.22 MS= 59.22	.33	1	p=.574
	MHPG	SS=504.65 MS=504.65	.50	1	p=.488
S X T	NE	SS=218.27 MS=218.27	1.21	1	p=.288
	MHPG	SS=11672.2 MS=11672.2	11.47	1	p=.002
within cells	NE	SS=2890.91 MS= 180.68		16	
	MHPG	SS=22380 MS= 1017		16	

Table 18b

Analysis of Variance of NE & MHPG Content in Hippocampus

(Experiment 9a)

<u>Source</u>	<u>Variate</u>	<u>Statistic</u>	<u>F</u>	<u>DF</u>	<u>P</u>
Shock Treatment (S)	NE	SS=960.04 MS=960.04	2.41	1	p=.134
	MHPG	SS=21699.5 MS=21699.5	3.38	1	p=.078
Time After Treatment (T)	NE	SS=126.861 MS=126.861	.32	1	p=.578
	MHPG	SS=912.49 MS=912.49	.14	1	p=.709
S X T	NE	SS=935.73 MS=935.73	2.34	1	p=.139
	MHPG	SS=9040.0 MS=9040.0	1.41		p=.247
within cells	NE	SS=9179.26 MS= 399.09		23	
	MHPG	SS=147659.0 MS= 6419.9		23	

Table 19a

Analysis of Variance of NE & MHPG Content in Hypothalamus

(Experiment 10A)

<u>Source</u>	<u>Variate</u>	<u>Statistic</u>	<u>F</u>	<u>DF</u>	<u>P</u>
Shock Pretreatment (S)	NE	SS= 4577.38	9.33	2	p<.001
		MS= 2288.69			
	MHPG	SS= 223.40	1.00	2	p<.001
		MS= 111.70			
Reexposure Treatment (R)	NE	SS=1119.63	2.28	2	p=.1176
		MS= 559.81			
	MHPG	SS=6764.21	30.39	2	p<.001
		MS=3382.11			
S X R	NE	SS=2609.65	2.66	4	p=.0494
		MS= 652.412			
	MHPG	SS= 826.42	1.86	4	p=.1409
		MS= 206.60			
within cells	NE	SS=8342.96		34	
		MS= 245.38			
	MHPG	SS=3784.19		34	
		MS= 111.29			

Table 19b

Analysis of Variance of NE & MHPG Content in Hippocampus

(Experiment 10A)

<u>Source</u>	<u>Variate</u>	<u>Statistic</u>	<u>F</u>	<u>DF</u>	<u>P</u>
Shock Pretreatment (S)	NE	SS=1222.05 MS= 611.02	1.68	2	p=.201
	MHPG	SS=1370.24 MS= 685.11	3.56	2	p=.038
Reexposure Treatment (R)	NE	SS= 437.71 MS=218.85	.60	2	p=.553
	MHPG	SS= 35.89 MS= 17.94	.09	2	p=.911
S X R	NE	SS=1527.94 MS= 381.98	1.05	4	p=.395
	MHPG	SS=1592.23 MS= 398.05	2.07	4	p=.105
within cells	NE	SS=12351.52 MS= 363.28		34	
	MHPG	SS=6931.38 MS= 192.53		34	

Table 19c

Analysis of Variance of NE & MHPG Content in Cortex

(Experiment 10A)

<u>Source</u>	<u>Variate</u>	<u>Statistic</u>	<u>F</u>	<u>DF</u>	<u>P</u>
Shock Pretreatment (S)	NE	SS= 925.88			
		MS= 426.94	2.86	2	p=.070
	MHPG	SS=1539.38			
		MS= 769.68	.76	2	p=.473
Reexposure Treatment (R)	NE	SS= 101.98			
		MS= 50.99	.31	2	p=.731
	MHPG	SS= 152.09			
		MS= 76.04	.08	2	p=.927
S X R	NE	SS= 608.74			
		MS= 152.18	.94	4	p=.452
	MHPG	SS=2143.53			
		MS= 535.88	.53	4	p=.712
within cells	NE	SS=5668.03			
		MS= 161.94		35	
	MHPG	SS=35217.73			
		MS=1006.221		35	

Table 19d

Analysis of Variance of 5-HT & 5-HIAA Content in Hypothalamus

(Experiment 10A)

<u>Source</u>	<u>Variate</u>	<u>Statistic</u>	<u>F</u>	<u>DF</u>	<u>P</u>
Shock Pretreatment (S)	5-HT	SS= 453.04 MS= 226.521	1.02	2	p=.371
	5-HIAA	SS=5002.89 MS=2501.44	1.24	2	p=.304
Reexposure Treatment (R)	5-HT	SS= 638.45 MS= 319.25	1.44	2	p=.251
	5-HIAA	SS=3772.11 MS=1886.06	.93	2	p=.404
S X R	5-HT	SS= 533.48 MS= 133.37	.60	4	p=.665
	5-HIAA	SS=20609.9 MS=5152.47	2.55	4	p=.059
Within cells	5-HT	SS=7554.15 MS= 222.18		34	
	5-HIAA	SS=60664.3 MS= 2022.1		30	

Table 19e

Analysis of Variance of 5-HT & 5-HIAA Content in Hippocampus

(Experiment 10A)

<u>Source</u>	<u>Variate</u>	<u>Statistic</u>	<u>F</u>	<u>DF</u>	<u>P</u>
Shock Pretreatment (S)	5-HT	SS=1722.92 MS= 861.46	2.14	2	p=.132
	5-HIAA	SS=2207.12 MS=1103.56			
Reexposure Treatment (R)	5-HT	SS= 329.93 MS= 164.96	.41	2	p=.667
	5-HIAA	SS=23420.8 MS=11710.4			
S X R	5-HT	SS=3097.26 MS= 774.31	1.92	4	p=.128
	5-HIAA	SS=15152.2 MS= 3788.05			
within cells	5-HT	SS=14523.9 MS= 403.4		36	
	5-HIAA	SS=174195.9 MS= 4977.0			

Table 19f

Analysis of Variance of 5-HT & 5-HIAA Content in Cortex

(Experiment 10A)

<u>Source</u>	<u>Variate</u>	<u>Statistic</u>	<u>F</u>	<u>DF</u>	<u>P</u>
Shock Pretreatment (S)	5-HT	SS=3819.59 MS=1909.79	.73	2	p=.488
	5-HIAA	SS=22846.3 MS=11423.2	1.17	2	p=.322
Reexposure Treatment (R)	5-HT	SS= 469.38 MS= 234.69	.09	2	p=.914
	5-HIAA	SS=14430.3 MS= 7215.1	.74	2	p=.484
S X R	5-HT	SS=6501.49 MS=1625.37	.62	4	p=.649
	5-HIAA	SS=57346.7 MS=14336.7	1.47	4	p=.233
within cells	5-HT	SS=91289.7 MS= 2608.2		35	
	5-HIAA	SS=321528.9 MS= 9743.3		33	

Table 19g

Analysis of Variance of DA, HVA & DOPAC Content in Hypothalamus

(Experiment 10A)

<u>Source</u>	<u>Variate</u>	<u>Statistic</u>	<u>F</u>	<u>DF</u>	<u>P</u>
Shock Pretreatment (S)	DA	SS= 387.97 MS= 193.98	.01	2	p=.989
	HVA	SS=1569.12 MS= 784.56	1.53	2	p=.232
	DOPAC	SS=2816.55 MS=1408.28	.14	2	p=.867
Reexposure Treatment (R)	DA	SS=12067.8 MS= 6033.9	.32	2	p=.728
	HVA	SS= 769.70 MS= 384.85	.75	2	p=.481
	DOPAC	SS=13936.2 MS= 6968.1	.71	2	p=.499
S X R	DA	SS=59902.5 MS= 14975.6	.80	4	p=.537
	HVA	SS=4553.89 MS=1138.47	2.21	4	p=.089
	DOPAC	SS=28026.1 MS= 7006.51	.71	4	p=.588
within cells	DA	SS=621598 MS= 18836		33	
	HVA	SS=16454.4 MS= 514.20		32	
	DOPAC	SS=333976.7 MS= 9822.8		34	

Table 19h

Analysis of Variance of DA, HVA & DOPAC Content in Hippocampus

(Experiment 10A)

<u>Source</u>	<u>Variate</u>	<u>Statistic</u>	<u>F</u>	<u>DF</u>	<u>P</u>
Shock Pretreatment (S)	DA	SS=11852.2 MS= 5926.0	.85	2	p=.435
	HVA	SS= 773.89 MS= 386.54	.54	2	p=.585
	DOPAC	SS=3060.91 MS=1530.45	2.92	2	p=.066
Reexposure Treatment (R)	DA	SS= 6546.0 MS= 3273.0	.47	2	p=.628
	HVA	SS=1424.75 MS= 712.37	1.00	2	p=.377
	DOPAC	SS= 1723.1 MS= 861.5	1.64	2	p=.207
S X R	DA	SS=115272. MS= 28818.	4.14	4	p=.007
	HVA	SS=1922.65 MS= 480.66	.67	4	p=.613
	DOPAC	SS= 542.9 MS= 135.7	.26	4	p=.902
within cells	DA	SS=250712 MS= 6964.2		36	
	HVA	SS=25643.0 MS= 712.3		36	
	DOPAC	SS=18879.9 MS= 524.4		36	

Table 191

Analysis of Variance of DA, HVA & DOPAC Content in Cortex

(Experiment 10A)

<u>Source</u>	<u>Variate</u>	<u>Statistic</u>	<u>F</u>	<u>DF</u>	<u>P</u>
Shock Pretreatment (S)	HVA	SS= 430.83 MS= 215.41	.20	2	p=.820
	DOPAC	SS=1374.42 MS= 687.20	.93	2	p=.405
Reexposure Treatment (R)	HVA	SS=1070.64 MS= 535.32	.49	2	p=.614
	DOPAC	SS= 1011.1 MS= 505.55	.68	2	p=.512
S X R	HVA	SS=1587.53 MS= 396.88	.37	4	p=.831
	DOPAC	SS=3080.67 MS= 770.16	1.04	4	p=.4018
within cells	HVA	SS=37980.1 MS= 1085.1		35	
	DOPAC	SS=3080.67 MS= 770.16		35	

Table 20a

Analysis of Variance of NE & MHPG Content in Hypothalamus

(Experiment 10B)

<u>Source</u>	<u>Variate</u>	<u>Statistic</u>	<u>F</u>	<u>DF</u>	<u>P</u>
Shock Pretreatment (S)	NE	SS=1373.74 MS= 686.86	6.22	2	p=.006
	MHPG	SS= 944.36 MS= 472.18	.75	2	p=.483
Reexposure Treatment (R)	NE	SS= 173.31 MS= 173.31	1.57	1	p=.225
	MHPG	SS= 911.25 MS= 911.25	1.45	1	p=.241
S X R	NE	SS=2041.8 MS=1020.9	9.24	2	p=.001
	MHPG	SS=3747.8 MS=1873.9	2.98	2	p=.070
within cells	NE	SS=2651.8 MS= 110.5		24	
	MHPG	SS=14482.9 MS= 629.6		23	

Table 20b.

Analysis of Variance of NE & MHPG Content in Hippocampus

(Experiment 10B)

<u>Source</u>	<u>Variate</u>	<u>Statistic</u>	<u>F</u>	<u>DF</u>	<u>P</u>
Shock Pretreatment (S)	NE	SS=3064.79 MS=1532.4	4.01	2	p=.032
	MHPG	SS=6978.9 MS=3489.4	1.49	2	p=.250
Reexposure Treatment (R)	NE	SS=4.978 MS=4.978	.01	1	p=.910
	MHPG	SS= 937.16 MS= 937.16	.40	1	p=.534
S X R	NE	SS= 174.23 MS= 87.11	.23	2	p=.798
	MHPG	SS=1276.12 MS= 638.05	.27	2	p=.764
within cells	NE	SS=8410.9 MS= 382.31		22	
	MHPG	SS=44419.5 MS= 2337.8		19	

Table 20c

Analysis of Variance of 5-HT Content in Hypothalamus

(Experiment 10B)

<u>Source</u>	<u>Variate</u>	<u>Statistic</u>	<u>F</u>	<u>DF</u>	<u>P</u>
Shock Pretreatment (S)	5-HT	SS=51576.3 MS=25788.2	.72	2	p=.4978
Reexposure Treatment (R)	5-HT	SS=29268.8 MS=29268.8	.82	1	p=.375
S X R	5-HT	SS=153759 MS= 76879.7	2.14	2	p=.139
within cells	5-HT	SS=861629.5 MS= 35901.2		24	

Table 20d

Analysis of Variance of 5-HT & 5-HIAA Content in Hippocampus

(Experiment 10B)

<u>Source</u>	<u>Variate</u>	<u>Statistic</u>	<u>F</u>	<u>DF</u>	<u>P</u>
Shock					
Pretreatment (S)	5-HT	SS=9290.2 MS=4645.12	1.79	2	p=.198
	5-HIAA	SS=3369.4 MS=1684.7	2.20	2	p=.138
Reexposure					
Treatment (R)	5-HT	SS= 49.24 MS= 49.24	.02	1	p=.892
	5-HIAA	SS= 183.87 MS= 183.87	.24	1	p=.629
S X R					
	5-HT	SS= 483.82 MS= 241.91	.09	2	p=.911
	5-HIAA	SS= 152.89 MS= 76.44	.10	2	p=.905
within cells					
	5-HT	SS=41415.6 MS= 2588.5		16	
	5-HIAA	SS=14555.9 MS= 766.1		19	

Table 20e
 Analysis of Variance of DA & DA Metabolites in Hypothalamus
 (Experiment 10B)

<u>Source</u>	<u>Variate</u>	<u>Statistic</u>	<u>F</u>	<u>DF</u>	<u>P</u>
Shock Pretreatment (S)	DA	SS=5429.69 MS=2714.85	.88	2	p=.426
	DOPAC	SS=7979.06 MS=3989.53	1.28	2	p=.298
Reexposure Treatment (R)	DA	SS=2001.68 MS=2001.68	.65	1	p=.427
	DOPAC	SS=3826.75 MS=3826.75	1.22	1	p=.280
R X S	DA	SS=1346.71 MS= 673.35	.22	2	p=.805
	DOPAC	SS=5397.36 MS=2698.68	.86	2	p=.435
within cells	DA	SS=73861.70 MS= 3077.57		24	
	DOPAC	SS=71948.79 MS= 3128.20		23	

Table 20f

Analysis of Variance of DA & DA Metabolites in Hippocampus

(Experiment 10B)

<u>Source</u>	<u>Variate</u>	<u>Statistic</u>	<u>F</u>	<u>DF</u>	<u>P</u>
Shock Pretreatment (S)	DA	SS=4464.85 MS=2232.43	.27	2	p=.766
	HVA	SS=1175.51 MS= 587.75	.16	2	p=.849
	DOPAC	SS= 458.16 MS= 229.08	.11	2	p=.892
Reexposure Treatment (R)	DA	SS=1053.25 MS=1053.25	.13	1	p=.725
	HVA	SS=6760.39 MS=6760.39	1.89	1	p=.186
	DOPAC	SS=2697.6 MS=2697.6	1.35	1	p=.258
R X S	DA	SS=36908.5 MS=18454.3	2.22	2	p=.135
	HVA	SS=9239.65 MS=4619.82	1.29	2	p=.299
	DOPAC	SS=7400.97 MS=3700.48	1.85	2	p=.182
within cells	DA	SS=157596.4 MS= 8294.5		19	
	HVA	SS=64484.25 MS= 3582.45		18	
	DOPAC	SS=42049.4 MS= 2002.3		21	

END

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