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The Neuroendocrine Response to Stress in the Developing Rat Pup

Linda Joy Iny

A Thesis

in

The Department

of

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ABSTRACT

The Neuroendocrine Response to Stress in the Developing Rat Pup

Linda Joy Iny

This study was designed to examine the effects of stress on the activity of brain monoamines, as well as on pituitary and adrenal hormones, in rats of various ages. Significant increases in plasma β-endorphin-like immunoreactivity (β-EPLIR) were observed following exposure to ether-stress in pups 3, 7, 14, 21, and 35 days old. Moreover, Day 7 pups had higher basal levels of plasma β-EPLIR than adult animals. In contrast, no alteration in the plasma concentration of α-MSH was detected in Day 7 pups that were exposed to ether-stress, nor in plasma corticosterone levels following exposure to handling or ether vapours until Day 14. This latter finding is consistent with several previous reports on the absence of adrenocorticotropic and corticoid responses during stress in the neonate. The results suggest that the release of adrenocorticotropin, β-endorphin, and α-MSH in response to stress differ in the developing animal. Exposure to ether vapours was also found to increase the concentration of dopamine in the hypothalamus of animals 21 days and older, but not at the earlier periods.

The findings of the present study indicate that the concentrations of peptides and monoamines in the neonate, under both basal and stress conditions, are different from those observed in the adult, and that there is considerable variation in the maturation of these neuroendocrine responses to stress. These maturational variations may be understood in terms of the needs of the growing organism.

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The Neuroendocrine Response to Stress in the Developing Rat Pup

The response to stress constitutes a basic adaptive mechanism in mammals. In response to stress, the hypothalamic-pituitary-adrenal (H-P-A) axis is activated, leading to, among other things, increased vigilance, elevated glucose production, and increased basal and carbohydrate metabolic rates. Elevations in heart rate and blood pressure also occur, and blood flow is increased, especially to organs that are more physiologically active.

In the adult, such changes are central to the capacity to deal with both physical and psychological stressors, and are thus essential for the maintenance and survival of the organism. Because, however, these changes often involve catabolic and energy-requiring processes, they could interfere with the anabolic processes of growth and development. It has been of great interest, therefore, to find that the human and rodent neonate shows an attentuated H-P-A response compared to the older infant or adult (Milkovic and Milkovic, 1969; Schapiro, 1962).

Little is known, however, about the effect of stress on the central nervous system (CNS), per se, a fact that is somewhat surprising in view of the vast literature on the effect of early experiences, such as handling and maternal separation, on the behavioral functioning of the organism in later life. In adults, the catecholamine and indoleamine systems are known to be very responsive to stressors, and there has been considerable speculation that the long-term effects of early stress could be mediated by these systems. It seemed, therefore, that a close examination of the effects of stressors on these systems and a comparison of the effects of stress on the pituitary system could provide important

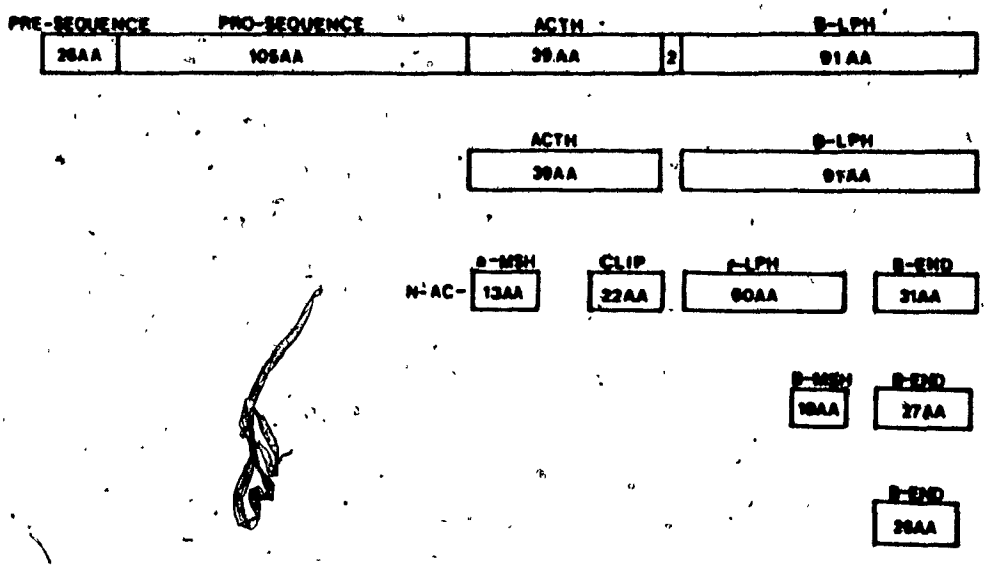
information about early vulnerability of the developing CNS.

The experiment to be reported in this thesis addresses these issues. In the following sections, the response of certain CNS, pituitary, and adrenal systems to stressors will be reviewed.

Anterior Pituitary

Corticotropin releasing factor (CRF), released by the medial basal hypothalamus, stimulates the processing of pro-opiomelanocortin (POMC) in corticotrophic cells in the anterior pituitary. Pro-opiomelanocortin is the parent molecule for the 39 amino acid peptide adrenocorticotropin (ACTH), the 91 amino acid peptide ß-lipotropin (ß-LPH), and ß-endorphin (ß-END) -(1-31), the 61-91 C-terminal sequence of ß-LPH (see Figure 1; Bloom, Battenberg, Rossier, Ling, Leppaluoto, Vargo, and Guillemin, 1977; Eipper and Mains, 1981; Guillemin, Vargo, Rossier, Minick, Ling, Rivier, Vale, and Bloom, 1977; Mains and Eipper, 1981; Pelletier, Leclerc, Labrie, Coté, Chretien, and Lis, 1977; Smythe and Zakarian, 1980; Voight, Weber, Fehm, and Martin, 1984; Weber, Martin, and Voight, 1979). In response to stress, secretion of CRF from the hypothalamus is increased, which in turn stimulates the processing of POMC, and the subsequent release of ACTH, ß-END and ß-LPH. This is reflected by an increase in the plasma concentration of POMC-derived peptides, and an initial decrease, followed by an increase, in the concentration of the peptides in the pituitary (Johnston, Spinedi, and Negro-Vilar, 1985; Kartesi, Palkovits, Kiss, Kanyicska, Fekete, and Stark, 1981; Lim and Funder, 1983; Mueller, 1981; Seggie and Brown, 1975).

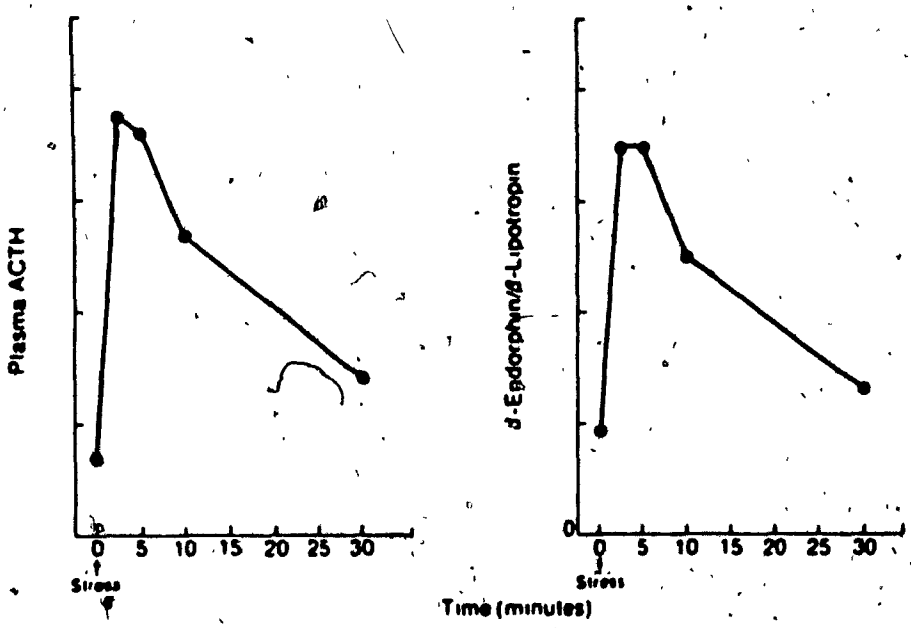
Figure 1. Post-translational processing of pro-opiomelanocortin.



de Souza and Van Loon (1985) examined the effect of a discrete, 2-minute restraint stress on the release of pituitary peptides in adult rats. They found parallel increases in plasma concentrations of ACTH and β-END/β-LPH 2.5 - 5 minutes after the onset of the stress. Plasma concentrations of these peptides returned to basal levels by about 30 minutes (see Figure 2). Guillemin et al. (1977) reported similar elevations in these peptides following stress induced by tibia-fibula breakage. Furthermore, de Souza and Van Loon (1985) found that adrenalectomized rats, lacking glucocorticoid negative feedback, had significantly higher basal concentrations of ACTH and β-END/β-LPH than intact rats. The adrenalectomized animals also secreted increased levels of the peptides in response to stress, suggesting that glucocorticoids exert a tonic, inhibitory effect on POMC-derived peptides.

In the anterior pituitary, POMC biosynthesis is regulated by glucocorticoids (Guillemin et al., 1977). Administration of glucocorticoids produces a dramatic reduction in the rate of POMC gene transcription that is apparent within 15 minutes of treatment (Eberwine and Roberts, 1984). Peptides derived from POMC in the anterior lobe have been reported to be under the influence of the same factors (Govoni, Pasinetti, Inzoli, Rozzini, and Trabucchi, 1984; Guillemin et al., 1977; Hollt, Przewlocki, and Herz, 1978). Thus, ACTH, β-END and β-LPH are stimulated by CRF and inhibited by glucocorticoids acting both directly on the pituitary and indirectly via inhibition of CRF production (Keller-Wood and Dallman, 1984; Plotsky, 1985; Rosa, Policastro, and Herbert, 1980; Vale, Rivier, Yang, Minick, and Guillemin, 1978; Vale, Speiss, Rivier, and Rivier, 1981; Vermes, Mulder, Smelik, and Tilders,

Figure 2. Comparison of the time course of plasma ACTH and β-END/β-LPH responses to a 2-minute restraint stress. (Based on the results of de Souza and Van Loon, 1985.)



1980).

The primary role of ACTH is to stimulate the adrenal cortex to secrete glucocorticoids, thereby serving to enhance the capacity of the organism to cope with stressors. The involvement of β-END in systems that regulate the body's response to stress is extensive; the autonomic nervous system has been shown to contain both endorphin-containing terminals (Schultzberg, Hokfelt, Lundberg, Terenius, Elfirm, and Elde, 1978) and opioid receptors (Young, Wamsley, Zarbin, and Kuhar, 1980), and central nuclei involved in the regulation of autonomic function, such as the nucleus of the solitary tract, are rich with opioids and their receptors (Akil, Watson, Young, Lewis, Khachaturian, and Walker, 1984). Endogenous opiates released during stress may attenuate stress-induced norepinephrine (NE) release (Tanaka, Kohno, Tsuda, Nakagawa, Ida, Iimori, Hoaki, and Nagasaki, 1983), thus providing a form of relief from stress for the animal.

In the mature rat, a fairly consistent set of physiological changes following exposure to noxious stimuli has been found, especially within the pituitary-adrenal system, whereas investigations conducted on young animals have suggested that the response to stress during the early stages of development may change over time.

Pro-opiomelanocortin has been reported to first appear in the anterior lobe of the pituitary on embryonic day 16 (Chatelain, Dupouy, and Dubois, 1979; Dupouy, 1980; Khachaturian, Alessi, and Lewis, 1983; Schwartzberg and Nakane, 1982). Rats are able to process POMC early in development (Sato and Mains, 1985; Seizinger, Hollt, and Herz, 1984), although during the neonatal period the pattern of POMC processing is

somewhat different from that in the adult. The total content of ß-END-like immunoreactivity (ß-EPLIR) in the anterior pituitary increases steadily with age. In contrast, concentrations of ß-EPLIR have been found to be higher in neonatal rats, decreasing to adult levels around the beginning of the second week of life (Seizinger et al., 1984). Presumably, this is due to a disproportionate increase in anterior pituitary cells that do not synthesize ß-END. The finding of elevated concentrations of ß-END in the neonate is supported by reports of high plasma concentrations of the peptide during the first few days following birth (Moss, Conner, Yee, Iorio, and Scarpelli, 1982; Panerai, Martini, Di Giulio, Fraioli, Vegni, Pardi, Marini, and Mantegazza, 1983). As in the adult animal, the anterior lobe of the neonate has been found to contain predominantly the biologically active, non-acetylated forms of ß-END (Sato and Mains, 1985; Seizinger et al., 1984), and contains adult-like ß-END/ß-LPH ratios (Seizinger et al., 1984). Interestingly, acetylated ß-END (Alessi, Khachaturian, Watson, and Akil, 1983) and a-melanocyte stimulating hormone (a-MSH; Khacharturian, Alessi, Munfakh, and Watson, 1983) have also been found in the anterior pituitary in concentrations greater than those found in adult animals, suggesting a greater overall level of POMC processing.

During the late fetal period and until shortly after birth, ACTH is functionally responsive to certain stresses and to CRF (Bartova, 1968; Cohen, 1976; Gray, 1971; Guillet and Michaelson, 1978; Schapiro, Geller, and Eiduson, 1962). Beginning at postnatal Day 1 to 2, however, the plasma ACTH response to stressors such as electric shock, ether vapours, and hypoxia is attenuated, and does not re-emerge until the second to

third week of life (Guillet and Michaelson, 1978; Hary, Dupouy, and Chatelain, 1981; Walker, Perrin, Vale, and Rivier, 1986; Walker, Sapolsky, Meaney, Vale, and Rivier, 1986). The effects of stress on the concentration of plasma β-END and β-LPH during this period have not been reported.

Neurointermediate Lobe of the Pituitary

In the neurointermediate lobe, the post-translational processing of POMC is different from that in the anterior pituitary; ACTH, β-LPH, and β-END-(1-31) serve predominantly as transient intermediates in the processing of other peptides (Crine, Gianoulakis, Seidah, Grossard, Pezzalla, Lis, and Chretien, 1978; Mains, Smith, and Ling, 1977). Enzymes present within the secretory granules of melanotropic cells initiate the cleavage of POMC to a-MSH, a 13 amino acid peptide, that corresponds to the N-terminal sequence of ACTH (see Figure 1). Following cleavage from the precursor protein, post-translational amidation of the C-terminus and acetylation of the N-terminus of the MSH molecule occurs, thus increasing both its melanotropic activity (Guttmann and Boissonnas, 1961; Rudman, Chawla, and Hollins, 1979) and the potency of the peptide in eliciting certain behavioral responses (O'Donohue, Handelmann, Chaconas, Miller, and Jacobowitz, 1981). Corticotropin-like intermediate lobe peptide (CLIP) is also cleaved from ACTH, and represents the 18-39 amino acid sequence of the molecule (Krieger, Liotta, Brownstein, and Zimmerman, 1980; Mains and Eipper, 1979). Post-translational processing of β-LPH results in β-melanocyte stimulating hormone (β-MSH), the 41-58 amino acid sequence of the molecule. Moreover, β-END-(1-31) undergoes N-acetylation (Ac-β-END), a process that completely eliminates its

capacity to bind with opiate receptors (Akil, Young, Watson, and Coy, 1981; Deakin, Dostrovsky, and Smyth, 1980), thus removing the analgesic property of the peptide (Smyth, Massey, Zakarian, and Finnie, 1979). Acetylated ß-END-(1-31) is further processed at its C-terminus to Ac-ß-END-(1-27) and -(1-26). Only a small proportion of ß-END in the neurointermediate pituitary is non-acetylated ß-END-(1-31) (Eipper and Mains, 1981). Thus, the neurointermediate lobe is characterized predominantly by a-MSH and ß-END derivatives (Jackson and Lowry, 1980; Liotta, Suda, and Krieger, 1978).

The secretory activity of neurointermediate lobe cells is regulated primarily by inhibiting dopamine-releasing neurons projecting from the arcuate nucleus to the median eminence of the hypothalamus (Bower, Hadley, and Hruby, 1974). Thus, administration of dopamine receptor antagonists to rats elevates POMC mRNA levels and induces the biosynthesis of POMC (Chen, Dionne, and Roberts, 1983), resulting in the increased conversion of the precursor to a-MSH and the acetylated forms of ß-END. Moreover, the rate of secretion of a-MSH and ß-END from the neurointermediate lobe are accelerated (Farah, Malcolm, and Mueller, 1982; Hollt and Bergmann, 1982), mimicking in this respect the response to stress. Akil, Shiomi, and Matthews (1985) stressed rats by exposing them to 30 minutes of intermittent footshock, and found an increase in the concentration of plasma Ac-ß-END-like material and a-MSH. Khorram, Bedran DeCastro, and McCann (1985) also found a significant increase in plasma levels of a-MSH five minutes following the onset of immobilization stress, as well as a significant elevation in the neurointermediate lobe content of the peptide. These results are in agreement with other

reports demonstrating an elevation in pituitary and plasma a-MSH following stress (Kastin, Schally, Viosca, and Miller, 1969; Usategui, Oliver, Vaudry, Lombardi, Rozenburg, and Mourre, 1976). Little is known about the physiological role of elevated plasma Ac-ß-END and a-MSH levels after stress. Plasma concentrations of a-MSH comparable to those reached after stress have been found, however, to stimulate aldosterone secretion (Vinson, Whitehouse, and Thody, 1980), suppress the release of leuteinizing hormone (Khorram et al., 1985) and prolactin (Wardlaw, Smeal, and Markowitz, 1986), and have also been found to increase the secretion of glucocorticoids (Llanos, Ramachandran, Creasey, Rudolph, and Seron-Ferré, 1979).

Pro-opiomelanocortin cells first appear in the intermediate lobe of the pituitary on embryonic day 17 to 18 (Chatelain et al., 1979; Dupouy, 1980; Khachaturian et al., 1983; Schwartzberg and Nakane, 1982). Processing of the precursor protein in this region closely resembles that of the adult, as a-MSH and ß-END account for most of the immunoreactivity (Sato and Mains, 1985), despite a dramatic increase in the content and concentration of the peptides between postnatal Day 1 and adulthood (Alessi et al., 1983; Seizinger et al., 1984). Moreover, as in the mature animal, a-MSH is largely di-acetylated (Leenders, Janssens, Theunissen, Jenks, and van Overbeeke, 1986; Sato and Mains, 1985). ß-Endorphin is both a-N-acetylated and C-terminally shortened, resulting in Ac-ß-END-(1-26), -(1-27), and -(1-31), although the extent of C-terminal shortening is not as great as that observed in the older animal (Leenders et al., 1986; Sato and Mains, 1985). No studies examining the effects of stress on Ac-ß-END or a-MSH during the early

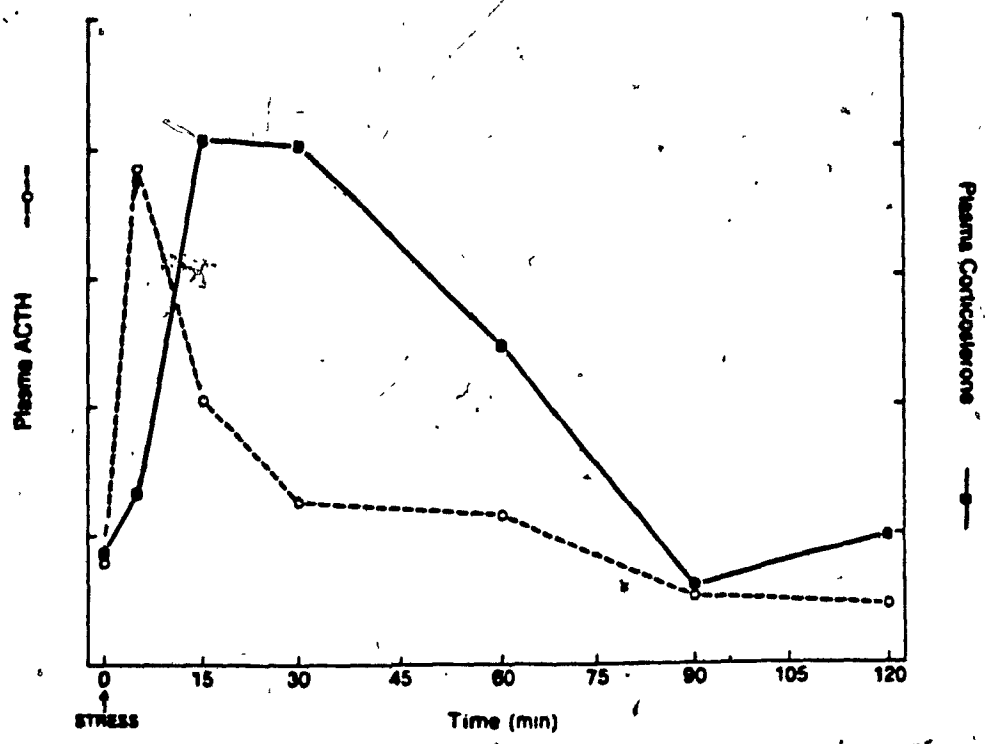
postnatal period have been reported to date.

Adrenal Corticoids

Adrenal corticoids are synthesized primarily from cholesterol taken up by the adrenal cortex from circulating plasma. Conversion of cholesterol to corticosterone, the principle glucocorticoid in the rat, involves a series of hydroxylations that result in the formation of the intermediate hormones pregnenolone, progesterone, and 11-deoxy-corticosterone. An additional hydroxylation, followed by activity of the enzymes 18-hydroxylase and 18-OH-dehydrogenase, results in the formation of aldosterone, the major mineralocorticoid. Synthesis of the corticoids is regulated by ACTH. Adrenocorticotropin influences the uptake of hepatic cholesterol from the plasma, and enhances intracellular cholesterol synthesis and hydrolysis of cholesterol esters (Dexter, Fishman, and Ney, 1970; Hadley, 1984). The pituitary peptide also regulates the transformation of cholesterol into pregnenolone (Hechter, 1958; Karaboyas and Koritz, 1965), the rate-limiting step in the synthesis of the corticoids.

The release of glucocorticoids in humans and animals in response to a variety of stressors is well known, dating back to the work of Selye (1950). de Souza and Van Loon (1982) have provided detailed information on the effect of acute stress on the time course of circulating corticosterone and ACTH levels in adult animals using a 2-minute restraint stress. They found an elevation in the concentration of plasma corticosterone that peaked at 15 to 30 minutes, shortly after the rise in plasma ACTH, and a decline to basal levels 60 to 90 minutes post-stress (see Figure 3).

Figure 3. Time course of plasma ACTH and corticosterone responses to a 2-minute restraint stress. (Based on the results of de Souza and Van Loon, 1982.)



The secretion of glucocorticoids by the adrenal cortex is a central feature of the stress response, and is essential for survival under sustained stress (Munck, Guyre, and Holbrook, 1984). Glucocorticoids serve to increase energy resources by increasing the rate of carbohydrate metabolism, enhancing sympathoadrenal activity, increasing the availability of catecholamines, increasing cardiovascular tone, and to inhibit the secretion of various hormones and neuropeptides.

During the late fetal period, basal plasma titers of corticosterone in the rat are within or slightly above adult range (Martin, Cake, Hartmann, and Cook, 1977; Meaney, Sapolsky, and McEwen, 1985), and pups are able to secrete the steroid in response to a variety of stressors as early as Day 17 of gestation (Milkovic and Milkovic, 1963, 1967; Ward and Weisz, 1984). Within the first two days of life, and especially in the first 6 to 8 hours after birth, corticosterone levels drop dramatically. During this period, there remains a small but statistically reliable increase in corticosterone in response to stress (e.g., Butte, Kakihana, Farnham, and Noble, 1973). From postnatal Day 2 until Day 14, however, pituitaryadrenal responsiveness decreases, resulting in a markedly attenuated response to stressors that, at other ages, reliably induce substantial hormone release (Walker, Perrin, Vale, and Rivier, 1986). Hyporesponsivity of the pituitary-adrenal system following stress during this critical period in the postnatal rat has been demonstrated consistently following exposure to a variety of stressors including handling, heat, histamine, electric shock, ether vapours, hypoxia, surgical invasion, and cold stressors (for a review, see Sapolsky and Meaney, 1986). In each case, animals between Day 3 and Day 14 have

failed to show an increase in corticosterone titers in response to stress (Butte et al., 1973; Coté and Yasmura, 1975; Gray, 1971; Guillet, Saffran, and Michaelson, 1980; Hiroshige and Sato, 1970; Schapiro et al., 1962; Walker, Perrin, and Rivier, 1985). Schapiro (1962) coined this the "stress nonresponsive period" (SNRP), which is characterized by the neonate's failure to mobilize a significant corticosterone secretion in response to stress. Recently, Sapolsky and Meaney (1986) have argued that this period is characterized by a relative, rather than absolute insensitivity to stress, and suggested the term "hyporesponsive" instead of "nonresponsive". A significant corticosterone response to stress is observed again by Day 15, coinciding with an increase in basal corticosterone titers that approximate adult levels (Henning, 1978; Meaney et al., 1985).

Monoamines

In recent years there have been many reports of alterations in cerebral catecholamine activity following stress in adult animals. The most consistent changes seem to occur in the noradrenergic systems. The locus coeruleus nucleus is the area with the richest concentration of NE-containing cell bodies (Fuxe, Hokfelt, and Ungerstedt, 1970), and is composed almost entirely of NE-producing neurons (Dahlstrom and Fuxe, 1964). Recent work indicates that neurons of the locus coeruleus, because of an extensive axonal collateralization, project to many areas of the CNS including the spinal cord, brain stem, hypothalamus, hippocampus, and cerebral cortex (Kuypers and Maisky, 1975; Nygren and Olson, 1977). Another group of NE-containing cell bodies exists within the lateral tegmental area and acts as a major source of hypothalamic

innervation, having the medial hypothalamus as its principal target (Olsen and Fuxe, 1972). Norepinephrine is also present, of course, in the periphery, within sympathetic neurons and the adrenal medulla.

Norepinephrine is synthesized from the amino acid tyrosine, which is present in the circulation. Following uptake into the cells, tyrosine undergoes a series of transformations: it is first converted by tyrosine hydroxylase, a catalytic enzyme considered to be a rate-limiting step in the biosynthesis of NE, to l-dihydroxyphenylalanine (l-DOPA), followed by conversion to dopamine (DA) and NE, with the synthesis of NE dependent upon the activity of the enzyme dopamine B-hydroxylase (DBH). Norepinephrine is synthesized in vesicles, where DBH is found, along with DA and adenosine triphosphate (ATP). Studies examining the noradrenergic system following acute exposure to stressful stimuli have found an increase in NE synthesis and release. The most consistent alterations seem to be a reduction in the content of NE in the hypothalamus and brainstem (Fuxe, Anderson, Eneroth, Siegel, and Agnati, 1983; Johnston et al., 1985; Reinstein, Lehnert, Scott, and Wurtman, 1984; Smythe, Bradshaw, and Vining, 1983), suggesting increased release of the catecholamine. Decreased concentrations of NE have also been reported in a wide variety of brain nuclei including the raphe, hippocampus, amygdala, locus coeruleus, thalamus, cerebral cortex, pons and medulla oblongata, and midbrain (e.g., Glavin, Tanaka, Tsuda, Kohno, Hoaki, and Nagasaki, 1983; Iimori, Tanaka, Kohno, Ida, Nakagawa, Hoaki, Tsuda, and Nagasaki, 1982; Reinstein et al., 1984). This stress-induced depletion has been found to be prevented by pretreatment with tyrosine (Lehnert, Reinstein, Strowbridge, and Wurtman, 1984). Furthermore, significant

increases have been reported in the synthesis of NE in the brain, in the activities of the enzymes and in the production of metabolites, indicating an increase in the turnover of the catecholamine (Glavin et al., 1983; Iimori et al., 1982; Sauter, Baba, Stone, and Goldstein, 1978; Stone, 1975).

Norepinephrine participates in the regulation of many neuroendocrine functions both under normal conditions and during stress, and therefore plays an important role in the homeostatic adaptation of the organism. Increases in NE metabolism in hypothalamic nuclei have been temporally correlated with acute changes in ACTH, B-END, prolactin, and growth hormone release induced by ether exposure (Johnston et al., 1985). Noradrenergic neurons innervating the hypothalamus appear to function as an inhibitory influence in regulating ACTH secretion (Ganong, 1984), and thus participate in feedback regulation of the H-P-A axis.

Stress-induced increases in the activity of dopaminergic neurons have also been found, although the response to stress does not appear to be as widespread or consistent as that in the noradrenergic system (Anisman, 1978; Stone, 1975). The most reliable change has been reported to occur in the mesolimbocortical DA neurons, whose cell bodies lie primarily in the ventral tegmental area, giving rise to axons that travel rostrally in the median forebrain bundle to terminate in forebrain areas such as the nucleus accumbens, the olfactory tubercle, the interstitial nucleus of the stria terminalis, the septum, the nucleus of the diagonal band of Broca, and the frontal cortex (Lindvall and Bjorklund, 1974). The nigrostiatal DA system has also been shown to respond to acute stress. This pathway contains cell bodies arising from the substantia

nigra and pars compacta, and reaches rostrally to innervate the caudate putamen and the dentral nucleus of the amygdala. Alterations in the tuberoinfundibular system were also found; this pathway originates from cells in the arcuate nucleus of the hypothalamus and projects to the median eminence and the pituitary gland. Laveille et al. (1979) investigated the effects of footshock stress and found that frontal cortex DA content fell within three minutes to a minimum level at ten minutes after the onset of the stress; moreover, an increase in DA utilization was found after 20 minutes, as indicated by elevated levels of the DA metabolite, dihydroxyphenylacetic acid (DOPAC). Furthermore, DA turnover increased rapidly and continued to rise throughout the 20 minute stress session, suggesting that the prolonged activation of the dopaminergic neurons not only enhanced the release of DA from terminals, but also stimulated DA synthesis. These results were confirmed by Reinhard, Bannon, and Roth (1982), who reported increases in DOPAC in the frontal cortex, while DA synthesis, as measured by DOPA accumulation following decarboxylase inhibition, was increased as well. Fadda, Argiolas, Tissari, Onali, and Gessa (1978) also found increases in DOPAC in the frontal cortex as well as in the nucleus accumbens after 20 minutes of electric footshock; however, no change in frontal cortex DA was detected, suggesting that synthesis of the catecholamine was keeping pace with release. Following combined cold and restraint stress, Dunn and File (1983) detected a decrease in the concentration of DA in both the striatum and frontal cortex, and an increase in the DOPAC/DA ratio in the striatum, nucleus accumbens, and frontal cortex. Johnston, Spinedi, and Negro-Vilar (1985) found that exposure to ether vapours significantly

enhanced DA metabolism in the rostral division of the arcuate nucleus, a finding that is consistent with earlier studies demonstrating decreased DA levels and increased DA synthesis in the whole arcuate nucleus following stress (Hedge, Van Ree, and Versteeg, 1976; Palkovits, Kobayashi, Kizer, Jacobowitz, and Kopin, 1975).

Stress has also been found to affect the serotonin (5-HT) system. Tryptophan is the primary precursor of 5-HT and is taken up into the cells where it is converted to 5-hydroxytryptophan (5-HTP) through the action of tryptophan hydroxylase. 5-Hydroxytryptophan then undergoes decarboxylation by 5-HTP decarboxylase, resulting in the formation of 5-HT. Cell bodies of 5-HT neurons are localized in nuclei of the raphe and reticular region of the brain stem (Dahlstrom and Fuxe, 1964), and their axons project widely throughout the brain. In the rat, the principal 5-HT neurons ascend in the ventral part of the median forebrain bundle in its medial and lateral parts (Dahlstrom and Fuxe, 1964; Fuxe and Johnsson, 1974), to innervate various limbic structures, the hypothalamus, the preoptic area, the cingulate cortex, the caudate nucleus, and the cerebellum. Similar ascending fibers have also been traced to the septal area, the hippocampus, the superior colliculi, and the neo- and mesocortex.

Stress does not appear to produce consistent changes in the actual content of brain 5-HT, but, in general, increases have been found in the concentration of the 5-HT metabolite, 5-hydroxyindoleacetic acid (5-HIAA), and brain 5-HT turnover. de Souza and Van Loon (1986) found that a 2-minute exposure to restraint stress significantly elevated concentrations of 5-HIAA in the hypothalamus, cerebral cortex, and brain

stem regions in the rat. Johnston, Demarest, and Moore (1984) immobilized rats for 30 minutes following a brief exposure to ether vapours and observed an increase in the rate of 5-HTP accumulation, suggesting increased synthesis of 5-HT, as well as an elevation in 5-HIAA in the suprachiasmatic and paraventricular nuclei. Azmitia and McEwen (1974) measured the activity of tryptophan hydroxylase following electric footshock, cold, and ether exposure, and found an increase in the concentration of the enzyme, thus providing further support for an increase in 5-HT synthesis. Stress-induced increases in 5-HT turnover have also been found to be accompanied by an increase in brain tryptophan (Knott, Joseph, and Curzon, 1973). Moreover, pretreatment of rats with valine, an amino acid that competes with tryptophan and thus, restricts its availability to the brain, prevented the increase in brain tryptophan that accompanied immobilization stress, and in turn, markedly attenuated the rise in 5-HIAA (Kennett and Joseph, 1981).

Thus, the effects of stress on catecholamine and indoleamine neuronal activity in the CNS of the mature animal have been studied extensively, and in general, the results suggest an increase in the synthesis and metabolism of the amines. Although some information is available on the ontogeny of amines in the CNS, information concerning the characterization of the effects of stress on brain monoamines during early development is scarce.

Norepinephrine and DA have been reported to appear in the rat brain between Days 14 and 15 of gestation (Coyle and Henry, 1973), and show increases in concentration with maturity until adulthood. At birth, whole brain concentrations of both NE and DA are 15 to 30% of adult

levels, decreasing briefly in some areas during pubescence (Agrawal, Davis, and Himwich, 1966; Coyle and Henry, 1973; Karki, Kuntzman, and Brodie, 1960). This pattern of development has been found to correspond to age-related changes in the synthesizing enzymes tyrosine hydroxylase and dopamine beta-hydroxylase (Coyle and Axelrod, 1972), as well as to the proliferation of nerve terminals that take up and store the catecholamine (Coyle and Axelrod, 1971). The regional distribution of NE also appears to vary with age and parallels enzymatic activity. Initially, high levels of NE appear in areas that are near the cell bodies in the locus coeruleus, while later the concentration is more elevated in distant regions (Coyle and Henry, 1973).

Neuroblasts containing 5-HT have been reported to first appear in the rat CNS as early as the 12th day of gestation (Olson and Seiger, 1972). Tryptophan hydroxylase is first detected in the embryo on Day 16 (Wapnir, Hawkins, and Stevenson, 1971), and increases rapidly thereafter. At birth, 5-HT levels in the brain stem have been reported to be one-third to one-half of those found in the same region in adult rats (Agrawal et al., 1966; Karki et al., 1960), increasing progressively thereafter until postnatal weeks 5 to 7, when they reach concentrations slightly higher than those found in the adults (Agrawal, Glisson, and Himwich, 1968; Karki et al., 1960). Peripheral and central mechanisms have been found to favour tryptophan accumulation in the brain during early life. Brain tryptophan levels in the CNS are high during the early postnatal period, and may be 5 to 10 times higher than in adults for the first two days following birth (Bourgoin, Bauman, Benda, Glowinski, and Hamon, 1974). The metabolite of 5-HT, 5-HIAA, has also been found to be

relatively abundant in the brain of developing rats (Bourgoin et al., 1974). Levels of 5-HIAA in the brainstem were found to be as high at birth as in adults, while the concentration was reported to increase progressively both in the brain stem and the forebrain for the first three postnatal weeks, decreasing slowly and reaching adult values by the end of week five (Bourgoin et al., 1974).

The Present Experiment

A large number of studies have examined the effects of stress on brain monoamines and pituitary-adrenal hormones in the adult. Although some information on the ontogeny of these systems is available, little is known about how these systems respond to stress during early development. Since it is possible that the long-term effects of early stress may be mediated by hormones released from the CNS and the pituitary, it was considered worthwhile to further examine the nature of the stress response in these systems during the early postnatal period.

Method

The effects of stress throughout early development were examined in rats from the early postnatal, weaning, pubertal, and young mature periods. The predominant stressor used in this experiment was exposure to ether vapours, since it is clearly an aversive experience for the animals, and its common use lends itself readily to comparison with previous investigations. Handling-stress, which involves the brief removal of animals from their home cage, was also used, since the handling manipulation mimics the stressful conditions that the animals are naturally exposed to during the course of development, such as separation from their mother and removal from the nest. This stressor

was not used consistently, however, as it would have resulted in a greater number of samples than could be meaningfully analyzed, given the limitations of the sensitive biochemical procedures used in this study.

The effect of stress on brain activity was assessed by exposing rats to ether fumes and, following sacrifice, obtaining brains for the measurement of monoamine levels. Samples from the hippocampus, hypothalamus, and frontal cortex were selected, since these regions are heavily innervated by monoaminergic terminals, and have been reported to be responsive to stress in adult animals. Activity of the pituitary was assessed following exposure to ether-stress by measuring β-END/β-LPH levels in plasma, as well as following handling-stress.

In an attempt to distinguish between the contributions of the anterior and neurointermediate lobes of the pituitary during the early postnatal period, a measure of neurointermediate lobe activity was obtained. For this purpose, concentrations of a-MSH, a peptide secreted predominantly from the neurointermediate lobe of the pituitary, was measured in the plasma of Day 7 pups, an age considered to be characteristic of the so-called stress "hyporesponsive" period. Plasma levels of corticosterone were also measured in animals that were either exposed to ether vapours or handling, in order to establish the presence of a stress hyporesponsive period within the present investigation.

Subjects

Pregnant Long-Evans, New Colony hooded rats were obtained from Canadian Breeding Farms (St. Constant, Quebec) on the 17th or 18th day of gestation. They were individually housed in wire-mesh maternity cages (42 cm x 25 cm x 18 cm) partially filled with beta chips, allowed free

access to food (Agway Prolab animal diet) and water, and maintained on a reverse 12 hour light/dark cycle, with lights on at 2200 hours. The birth date of the pups was considered as Day 0. On Day 21 the mother was removed, and the pups were placed in smaller cages (18 cm x 18 cm x 25 cm) with between three and five littermates per cage. At 35 days of age, the animals were re-housed in groups of three according to sex. Animals were studied at 3, 7, 14, 21, and 35 days old, as well as on Day 63, an age that the rat is considered to be sexually mature. A total of 80 litters of rat pups were used in the study.

Experimental Design and Procedure

The animals were quasi-randomly selected from available litters to form the experimental groups. In order to obtain valid, basal plasma samples for pups in the control condition, home cages of the animals were undisturbed for a minimum of 90 minutes prior to sampling, and only two animals were removed from a litter at any one time. Rats in the control group were rapidly taken into an adjoining room and decapitated (<20 seconds). Trunk blood was collected in 1.5 ml microcentrifuge tubes that were pre-rinsed with heparin (400 units/ml; Organon Canada, Toronto, Ontario) to prevent clotting, and contained 5 μl sodium metabisulphite (333mg/2ml; Fisher Scientific, Montreal, Quebec) to protect against breakdown of the substrates (Renner and Luine, 1984). The samples were stored on ice until centrifugation. Following sacrifice, the brain was quickly removed and frozen on dry ice.

Animals in the handling-stress group were removed from their cages, again in pairs, and placed into a plastic container partly filled with beta chips in an adjoining room for a period of five minutes, following

which time they were decapitated, for the collection of blood and brains. Animals in the ether-stress group were placed in a covered, glass cylinder and exposed to ether fumes (Fisher Scientific) for two minutes, after which they were moved to a lined plastic container for three minutes until sacrifice (total: five minutes).

Following collection of the samples, the blood was spun at 3,000 r.p.m. in a pre-cooled (4°C) Savant high speed centrifuge for ten minutes. The plasma was then drawn off with a pipette, and the process was repeated until most of the plasma was aspirated. In order to obtain the appropriate amounts of plasma for the B-EPLIR and a-MSH radioimmunoassays, samples from Day 3 and Day 7 pups of the same sex and treatment group were pooled; no pooling was necessary at the later ages. Plasma and brain tissue were stored at -80°C until analyzed. Samples from animals of different litters were combined for the biochemical assays.

Measurement of plasma corticosterone immunoreactivity. Plasma corticosterone was measured using the radioimmunoassay of Krey, Butler, Hotchkiss, Piva, and Knobil (1975), with a corticosterone antiserum (B3-163) purchased from Endocrine Sciences, Tarzana, California. The antiserum was diluted to a final concentration of 1:64,000 in phosphate-buffered saline (PBS) (described below). The cross-reactivity relative to corticosterone was 4% for desoxycorticosterone and less than 1% for the other steroids tested, including aldosterone, cortisol, cortisone, desoxycortisol, dexamethasone, DHEA, estradiol, estrone, 17-hydroxy pregnenolone, 17a-hydroxy progesterone, 20a-hydroxy progesterone, 20ß-hydroxy progesterone, predisone, prednisolone,

pregnanediol, 5a-pregnanedione, 5ß-pregnanedione, pregnanetriol, pregnenolone, progesterone, testosterone, tetra hydro cortisol, and tetra hydro cortisone. Intra- and inter-assay coefficients of variability were 10 and 12% respectively. The absolute sensitivity of the assay was 10 pg.

To perform the assay, 10 μl of sample and 1 ml of ethanol (Alcools de Commerce, Montreal, Quebec) were pipetted into 12 x 75 mm borosilicate glass tubes (Fisher Scientific) and vortexed. The test tubes were vortexed again after 15 minutes, then centrifuged for 10 minutes at 3,000 r.p.m. in a Beckman J2-21 centrifuge at 4°C. A 100 μl aliquot of the supernatant was transferred into duplicate tubes. A standard curve was prepared in duplicate corresponding to 0, 10, 20, 50, 100, 200, 500 and 1,000 pg by pipetting aliquots of a standard solution of corticosterone (Sigma Chemical Company, St. Louis, Missouri), in absolute ethanol. A 100 μl aliquot of absolute ethanol was also pipetted into duplicate tubes to be used as a measure of the total count (TOT). The contents of all the tubes were dried down in a Labconco Freeze Dryer.

Phosphate-buffered saline was prepared, consisting of 143 g NaCl, 250 ml 0.5 M dibasic, 100 ml 0.5 monobasic (all products from Fisher Scientific), and 1.75 g merthiolate (Sigma Chemical Company), dissolved with glass distilled water to 17.5 liters, pH. 7.0. A gel was prepared by stirring 1 g commercial gelatin (Lenox) into one liter PBS over heat until dissolved, and was used to minimize nonspecific binding of reagents to glass and charcoal. The tracer mixture consisted of Corticosterone [1,2,6,7^{3}H(N)] (New England Nuclear, Lachine, Quebec) diluted to 12,000 to 14,000 c.p.m. using PBS gel. A 1/10 dilution was then mixed, the

proportions of which were altered to obtain a TOT count of 13,500 c.p.m. A 100 µl aliquot of the tracer was added to all tubes. The antiserum was added to standards and samples, with the exception of the TOT. The tubes were then covered with parafilm and placed in a cold room (4°C) for 12 to 24 hours. Nonspecific binding was assessed by incubating plasma extracts without the antiserum, and constituted 3 to 5% total binding.

Following incubation, 1 ml PBS gel was added to the TOT tubes, and 1 ml dextran-coated activated charcoal, made of 2.4 g Norite A (Aldrich Chemical Company, Milwaukee, Wisconsin) and 240 mg dextran (MW 71,200; Sigma Chemical Company) per liter PBS, was aliquoted into the other tubes to permit the separation of the antibody-bound from the free cortisol. After 15 minutes, all tubes were centrifuged at 4°C for 10 minutes at 3,000 r.p.m. The supernatant was then poured and tapped into plastic scintillation vials (Diamed Lab Supplies, Mississauga, Ontario). Four ml liquid scintillation cocktail (Liquiscent; National Diagnostics, Somerville, New Jersey) was then added, and the samples were counted on a Packard beta scintillation counter. Data reduction was done on an Apple IIE computer using a program for analysis of radioimmunoassay data based on a regression analysis.

Measurement of plasma β-endorphin-like immunoreactivity. The β-EPLIR content in the serum was estimated by radioimmunoassay using an antiserum specific for the C-terminal of β-END at 1:30,000 final dilution. The antiserum gave almost 100% cross-reactivity with bovine β-LPH and a-N-acetylated β-END, and cross-reacted 70% with β-END-(1-27); it gave almost no cross-reactivity with ACTH, α-MSH, or the β-LPH fragments of -(61-65), -(62-67), and -(80-84). Thus, the antiserum

recognized POMC, β-LPH, β-END-(1-31) and β-END-(1-27) in both α-N-acetylated or nonacetylated forms, but did not recognize β-END-(1-16) and β-END-(1-17) (Gianoulakis, Drouin, Seidah, Kalant, and Chretien, 1981; Gianoulakis, Woo, Drouin, Seidah, Kalant, and Chretien, 1981). Due to the small quantity of serum available from each animal for estimation of β-EPLIR content in the serum, an aliquot of 100 μl of unextracted plasma was used. The minimum detectable quantity of β-EPLIR was 10 pg. Intra- and inter-assay variability was 8.9 and 10.1% respectively.

β-Endorphin was labeled with Iodine-125 (New England Nuclear) using a modification of a previously published procedure (Hunter and Greenwood, 1962). For iodination, 1 μg β-END in 10 μl of 0.001 N HCl was diluted with 25 μl of 0.05 M sodium phosphate (Fisher Scientific) buffer, pH. 7.6, and 10 μl of carrier-free $Na^{125}I$ (10 mCi/ml in 0.05 M phosphate buffer, pH. 7.6; New England Nuclear) was added. The reaction was initiated by adding 10 μl of freshly prepared chloramine T (Eastman Chemical Company, Rockville, Maryland) solution (5 mg/ml in 0.05 M phosphate buffer, pH. 7.6), and terminated after 5 to 10 seconds by the addition of 25 μl of freshly prepared sodium metabisulphite solution (5 mg/ml in 0.05 M phosphate buffer, pH. 7.6) and 1 ml of standard radioimmunoassay buffer (described below). The iodinated β-END was desalted by adsorption to Sep-Pak cartridges of ODS-silica (Waters Scientific, Lachine, Quebec), which were first conditioned by flushing with 5 ml acetonitrile (Fisher Scientific), followed by 10 ml of 0.1 N HCl. The iodination mixture (about 1.1 ml) was passed once through the cartridge and was then washed with 3.0 ml of 0.1 N HCl and 10 ml of 10% acetonitrile containing 0.1 N HCl. The bound peptide was eluted from the

resin with 1.5 ml of 60% acetonitrile/0.1 N HCl and the eluate stored at 4°C in an Eppendorf tube.

Standard radioimmunoassay buffer was composed of 0.05 M sodium phosphate buffer, 0.5% human serum albumin (Fisher Scientific), 0.2% (v/v) Triton X-100 (New England Nuclear), 0.2% (w/v) sodium azide and 1 mM ethylenediamine tetracetic acid (EDTA; Sigma Chemical Company), the final pH. of this solution being about 7.6. The radioimmunoassay was conducted in 12 x 75 mm polypropylene tubes (Sarstedt Company, Montreal, Canada) at 4°C, in a final volume of 300 μl containing 100 μl of tracer (10,000 c.p.m.), 100 μl of antiserum appropriately diluted with standard buffer, and 100 μl of peptide standard or serum. The assay included, in triplicate, a sample of total tracer; a blank containing tracer and normal rabbit serum diluted to the same extent as the antiserum; a standard curve containing 0 to 10,000 pg of β-END per tube; the samples (in duplicate or triplicate), and; neurointermediate lobe extract at two dilutions as inter-assay controls. All components were added at the start of the assay, mixed by vortexing, and incubated at 4°C for 48 hours. The incubation was terminated by the addition of 100 μl of 4% (v/v) normal rabbit serum and 100 μl goat anti-rabbit gama globulin (Bio-rad, Montreal, Quebec). Following an additional incubation for 16 to 24 hours at 4°C, the tubes were centrifuged at 3,000 r.p.m. in a Beckman J2-21 centrifuge at 4°C, the supernatants were aspirated, and radioactivity was measured in the pellets using an LKB 1282 Compugamma counter.

Measurement of plasma a-melanocyte stimulating hormone. The a-MSH content in plasma was measured by radioimmunoassay using a specific

antiserum (Bio-Mega, Montreal, Quebec). The cross-reactivity, relative to a-MSH, was less than 1% for H-ACTH-(1-39), ACTH-(1-16), ACTH-(1-24), ACTH-(4-10), ACTH-(1-10), B-END, B-MSH, B-LPH, and γ-LPH. a-Melanotrophe stimulating hormone (Institut Armand-Frappier, Laval, Quebec) was iodinated as described above for B-END, and diluted to approximately 20,000 c.p.m. in a buffer consisting of 0.06 M NaH_2PO_4 (Fisher Scientific), 0.1% Fraction V Powder 98-99% albumin (Sigma Chemical Company), 0.1% NaN_3 (Fisher Scientific), pH. 7.6, and 1% Trasylol (Miles Pharmaceuticals, Rexdale, Ontario), to a final pH. of 7.5.

The assay was done in a final volume of 300 μl containing 100 μl iodinated a-MSH, 100 μl of a-MSH standard in buffer (1 pg to 10,000 pg per tube) or sample, and 100 μl of antiserum diluted 2,000 times in buffer. After 24 hours incubation at 4°C, 300 μl of charcoal solution was added, consisting of 1% Norite A, 0.25% dextran T-70, and 1% human serum albumin purified. The samples were vortexed, and spun at 2,000 r.p.m. for 15 minutes in a Beckman centrifuge at 4°C. The supernatant was decanted into glass tubes and counted in an LKB 1282 Compugamma counter.

Measurement of Monoamines and Metabolites in Brain Tissue. Concentrations of NE, DA, DOPAC, 5-HT and 5-HIAA were quantified using reverse-phase High Performance Liquid Chromatography with Electrochemical Detection (HPLC-EC). Frozen brains were sliced in 300 μ sections at -10°C in a refrigerated cryostat (Ames), and the sections thaw-mounted on gel-coated (7.5 g gelatin to 750 ml glass distilled water, and 375 mg potassium sulphate; Fisher Scientific) glass slides. Samples from frontal cortex, hippocampal, and hypothalamic nuclei were dissected using

the microdissection technique of Palkovits (1980). Punches, with a diameter of either 1 mm or .5 mm, were expelled into an ice-cold medium of 4.3 ml/liter glacial acetic acid (pH. 5.0 with NaOH; Fisher Scientific), 0.15M sodium acetate (Fisher Scientific), and 0.1mM EDTA (Aldrich Chemical Company). The tissue punches in the diluent were disrupted by freeze-thawing. When samples were thawed, 5 μg ascorbic acid oxidase (Boehringer Mannheim, Dorval, Quebec) was added. The samples were then vortexed and centrifuged at 13,000 x r.p.m. for 5 minutes. The supernatant was transferred to vials for automated HPLC analysis.

Pellets were dissolved in 0.1 N NaOH (Fisher Scientific) for protein determination, using the method of Bradford (1976). A protein reagent consisting of 0.01% (w/v) Coomassie Brilliant Blue G (Sigma Chemical Company), 4.7% (w/v) ethanol, and 8.5% (w/v) phosphoric acid (Fisher) was prepared. Fraction V Powder 98-99% albumin in a concentration of 1 mg/ml 0.1 N NaOH was used as a protein solution. Samples were mixed by vortexing, and 15 μl was pipetted into 10 x 75 mm test tubes in triplicate. Following the addition of 1.5 ml of protein reagent, the tubes were vortexed. The absorbance was measured in a Perkin Aylmer Junior Model 35 spectrophotometer against a reagent blank prepared from 15 μl of 0.1 N NaOH and 1.5 ml of protein reagent. The protein concentration of the samples was predicted from a standard curve constructed using the absorbance values of known amounts of the protein (Fraction V) solution.

The HPLC-EC system consisted of a Waters Model 510 pump, a programmable sample processor (Waters 710-B WISP), and a 15 cm x 3.9 mm

Bondpack C18 column (Waters) with 4 μ packing. An amperometric electrochemical detector (Bioanalytical Systems, LC4B) with a glassy carbon electrode (Biochemical Systems, TL-5A) was used for electrochemical detection. The electrode potential was set at 0.76 V versus a Ag/AgCl reference electrode. The mobile phase was adapted from Renner and Luine (1984). Samples were eluted with a 0.15 M sodium acetate mobile phase containing 0.1 mM EDTA, 80 mg/liter octyl sodium sulfate (Eastman Kodak, Rochester, New York), and 3.3 % acetylnitrile (pH. 3.8 with glacial acetic acid; Aldrich Chemical Company). The mobile phase was pumped at 1.3 ml/min (1500 p.s.i.).

All samples contained 10 pg/μl DHBA as an internal standard. Sample monoamine and metabolite concentrations were estimated from the peak height ratios of known amounts of pure standards and samples to the internal standard (Sigma Chemical Company). The detection limit was defined by a minumum peak height of 3 x the width of the baseline. Data reduction was done on an Apple IIE computer using a program by J.B. Mitchell (unpublished).

Results

Plasma Corticosterone

Data from pups at 3, 7, and 14 days of age were analyzed using two-way analyses of variance (age x stress). The effects of handling- and ether-stress were examined in separate ANOVAs, since they represent qualitatively different stressors, and thus may not be meaningfully compared. Post-hoc analyses were conducted using Tukey tests. Data from male and female pups were pooled, since previous studies have not found any evidence for a sex difference in plasma levels of the steroid prior

to the onset of puberty (see Meaney et al., 1985).

Handling-stress. Examination of the results yielded significant main effects for both age, $F(2, 97) = 45.02$, $p<.001$, and stress, $F(1, 97) = 3.97$, $p<.05$, as well as a significant age x stress interaction, $F(2, 97) = 11.52$, $p<.001$. A summary of the ANOVA is presented in Table 1. Post-hoc analyses indicated that basal levels of plasma corticosterone in Day 3 pups were significantly lower than in pups 7 or 14 days of age ($p<.01$). Moreover, as illustrated in Figure 4, levels of corticosterone for pups in the control and handling-stress conditions were not found to differ significantly at either 3 or 7 days of age. A significant increase in the concentration of the steroid was detected, however, in plasma from handled pups 14 days of age ($p<.01$), as compared with basal levels, thus accounting for the age x stress interaction effect.

Ether-stress. This ANOVA yielded significant main effects for both age, $F(2, 99) = 45.14$, $p<.001$, and stress, $F(1, 99) = 8.5$, $p<.01$. A significant effect was also found for the age x stress interaction, $F(2, 99) = 14.86$, $p<.001$. A summary of the ANOVA is presented in Table 2. Tukey tests performed on basal values revealed that Day 3 pups had significantly lower concentrations of plasma corticosterone than pups 7 or 14 days old ($p<.01$). As illustrated in Figure 5, a significant increase in plasma concentrations of the steroid was observed only in Day 14 pups following exposure to ether-stress ($p<.01$), as compared to pups in the control group; no alterations were detected in corticosterone levels following ether-stress at either 3 or 7 days of age, accounting for the age x stress interaction effect.

Plasma β-Endorphin-like Immunoreactivity

Table 1

Plasma corticosterone levels following handling-stress

Summary table of the ANOVA

Source	df	Mean Square	F	p
Age	2	560.25	45.02	.001
Stress	1	49.44	3.97	.05
Age x stress	2	143.47	11.52	.001
Error	73	12.46		

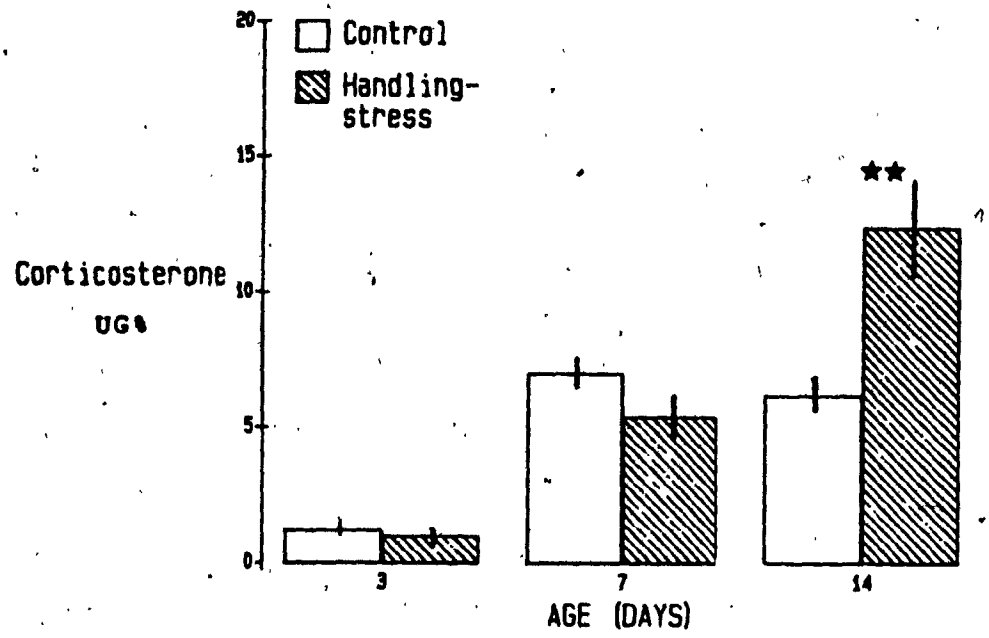


Figure 4. Plasma corticosterone concentrations in control (C) and handled (H) pups 3, 7 and 14 days old. Statistically reliable difference at ** $p<.01$. Sample sizes are as follows: Day 3, C=12, H=12; Day 7, C=20, H=22; Day 14, C=17, H=17.

Table 2

Plasma corticosterone levels following ether-stress

Summary table of the ANOVA

Source	df	Mean Square	F	p
Age	2	696.51	45.14	.001
Stress	1	131.21	8.50	.01
Age x stress	2	229.24	14.86	.001
Error	99	15.43		

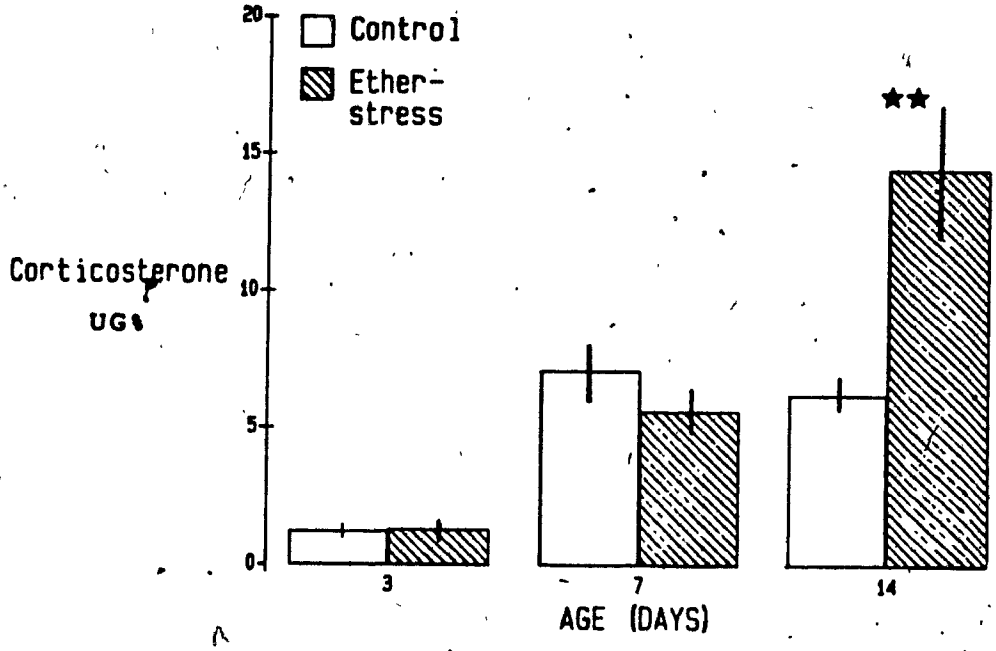


Figure 5. Plasma concentrations of corticosterone in control (C) and ether-stressed (E) pups 3, 7 and 14 days of age. Statistically reliable difference at ** $p<.01$. Sample sizes are as follows: Day 3, C=12, E=15; Day 7, C=20, E=20; Day 14, C=17, E=21.

Data from male and female pups of the same age and treatment group were pooled, since the preliminary ANOVAs revealed no sex differences in the concentration of plasma B-EPLIR.

Handling stress. Examination of the two-way ANOVA (age x stress) revealed a significant main effect for stress, $F(1, 70) = 19.90$, $p<.001$. No effect was observed due to the age of the animals, $F(3, 70) = 1.25$, n.s., or the age x stress interaction, $F<1$. A summary of the ANOVA is presented in Table 3. As illustrated in Figure 6, levels of B-EPLIR were increased following handling-stress, relative to controls, in pups 21 days of age ($p<.05$).

Ether-stress. The ANOVA (Table 4) comparing the concentration of B-EPLIR in control and ether-stressed animals yielded significant main effects for stress, $F(1, 134) = 33.29$, $p<.001$, and age, $F(5, 134) = 4.37$, $p<.001$. The age x stress interaction term was not significant, $F<1$. As can be seen in Figure 7, post-hoc comparisons revealed a significant increase in the plasma concentration of B-EPLIR following exposure to ether fumes at all ages, except for the adults $p<.05$. Table 5 illustrates the percentage of increase in plasma B-EPLIR in stressed pups as compared with controls throughout the study.

Basal. Given the absence of an age x stress interaction, the age differences in B-EPLIR could only be investigated by comparing means derived from both control and stressed animals. Since this analysis was considered meaningless, a separate one-way analysis on the basal B-EPLIR values was performed. Examination of the results comparing plasma concentrations of B-EPLIR in rats of different ages was significant, $F(5, 59) = 3.54$, $p<.01$ (see Table 6). As may be seen in Figure 7, further

Table 3

Plasma β-endorphin-like immunorectivity following handling-stress

Summary table of the ANOVA

Source	df	Mean Square	F	p
Age	3	15,565.86	1.25	.30
Stress	1	247,157.21	19.90	.001
Age x stress	3	8,374.41	0.67	
Error	70	12,421.56		

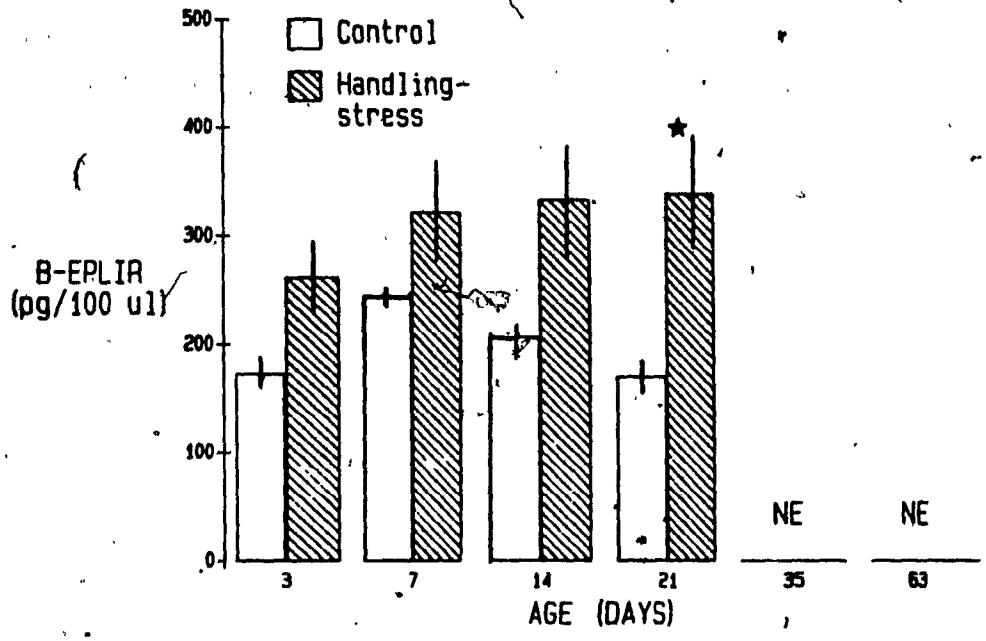


Figure 6. Plasma concentration of B-EPLIR in control (C) and handling-stressed (H) pups 3, 7, 14, and 21 days of age. Statistically reliable difference at * $p<.05$. Sample sizes are as follows: Day 3, C=10, H=11; Day 7, C=13, H=11; Day 14, C=7, H=9; Day 21, C=8, H=9. NE = not examined.

Table 4

Plasma B-endorphin-like immunoreactivity following ether-stress

Summary table of the ANOVA

Source	df	Mean Square	F	p
Age	5	89,084.05	4.37	.001
Stress	1	678,169.73	33.29	.001
Age x stress	5	11,810.78	0.58	
Error	134	20,371.58		

Figure 7. Plasma concentrations of β-EPLIR in control (C) and ether-stressed (E) animals. Statistically reliable differences at * $p<.05$, ** $p<.01$. Sample sizes are as follows: Day 3, C=10, E=11; Day 7, C=13, E=13; Day 14, C=7, E=11; Day 21, C=8, E=14; Day 35, C=9, E=17; Day 63, C=18, E=15.

Figure 7

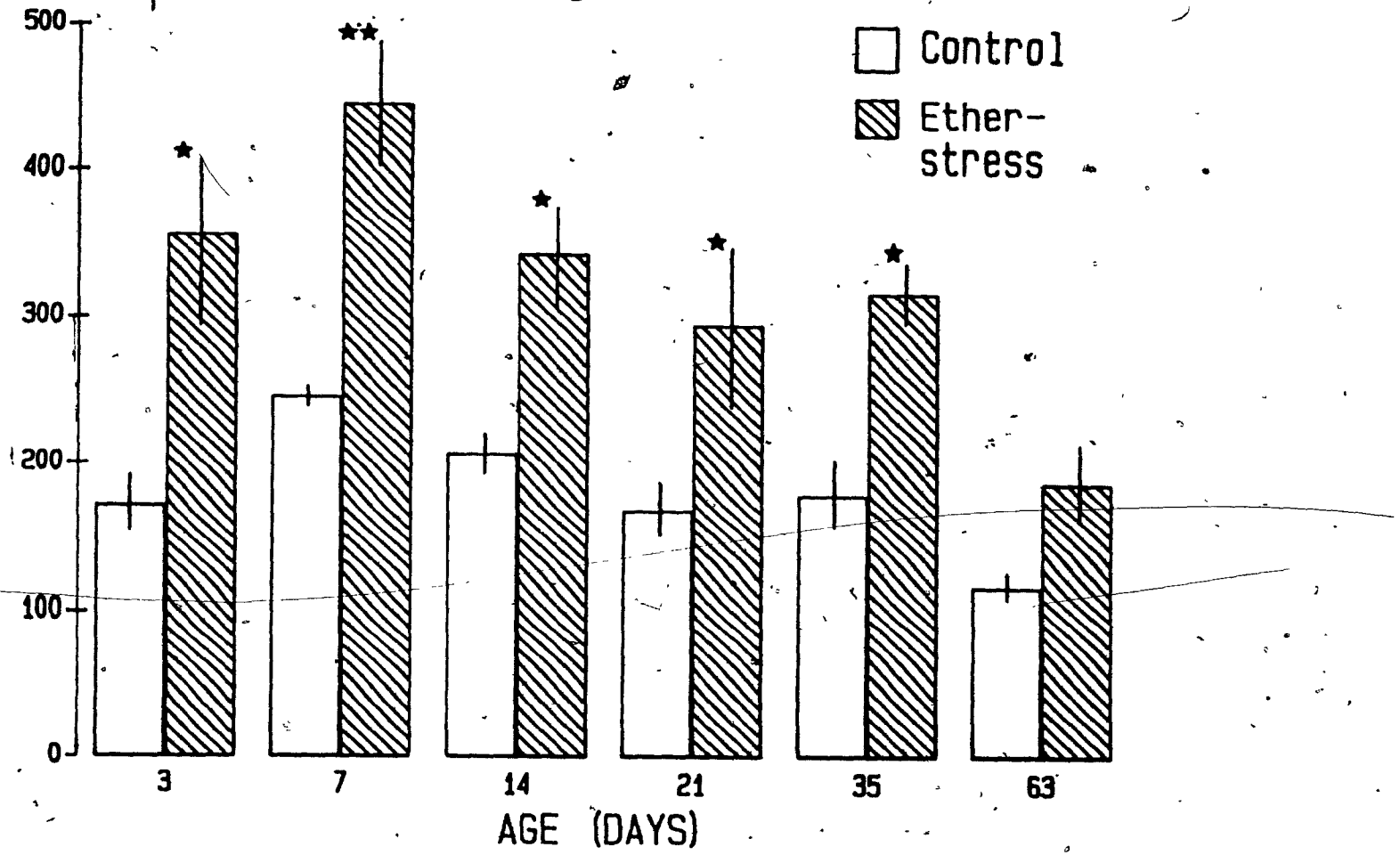

Control
Ether-
stress
B-EPLIR
(pg/100 ul)
500
400
300
200
100
0
3
7
14
21
35
63
AGE (DAYS)

Table 5

Age	% Increase following handling	% Increase following ether
3	151.3	181.1
7	130.0	210.5
14	161.2	165.4
21	173.7	172.9
35	NE	175.3
63	NE	160.1

NE = not examined

Percentage increase (controls = 100%) in plasma β-EPLIR levels with handling- or ether-stress in rats. Values are based on those presented in Figures 6 and 7.

Table 6

Basal levels of plasma B-endorphin-like immunoreactivity

Summary table of the ANOVA

Source	df	Mean Square	F	p
Age	5	17,964.01	3.54	.007
Error	59	5,081.34		

investigation of the difference using a Tukey test indicated that Day 7 pups had higher basal levels of plasma B-EPLIR than adult animals ($p<.01$).

a-Melanotrophe Stimulating Hormone

Analysis of the data using a two-way ANOVA (stress x sex) revealed no significant difference in a-MSH concentrations between male and female pups 7 days of age, $F(1, 26) = 1.89$, n.s., nor was there any effect of ether-stress on levels of the peptide, $F<1$ (see Figure 8).

Regional Brain Monoamines and Metabolites

Concentrations of NE, DA, 5-HT, 5-HIAA, and the 5-HIAA/5-HT ratio were analyzed separately for males and females, as per each HPLC run, using two-way analyses of variance (stress x age). Concentrations of DOPAC were not subjected to statistical analysis, as levels were generally below the detectable limit. Post-hoc analyses were conducted using Tukey tests.

Hippocampus: Norepinephrine. In males, statistical analysis yielded a significant main effect of age in NE levels, $F(5, 55) = 30.57$, $p<.001$ (see Table 7). As may be seen in Figure 9a, the difference can be attributed to 3 day-old pups having lower levels of NE than animals at each of the other ages examined. Exposure to ether-stress produced no significant effect, $F<1$, and there was no age x stress interaction, $F(5, 55) = 1.23$, n.s.

Levels of NE in the hippocampus of Day 3 and Day 7 female pups were below the detectable limit, and therefore, were not included in the analysis. As shown in Table 8, the ANOVA conducted on values for Day 14, 21, 35 and adult animals yielded no significant main effects of age, $F(3,$

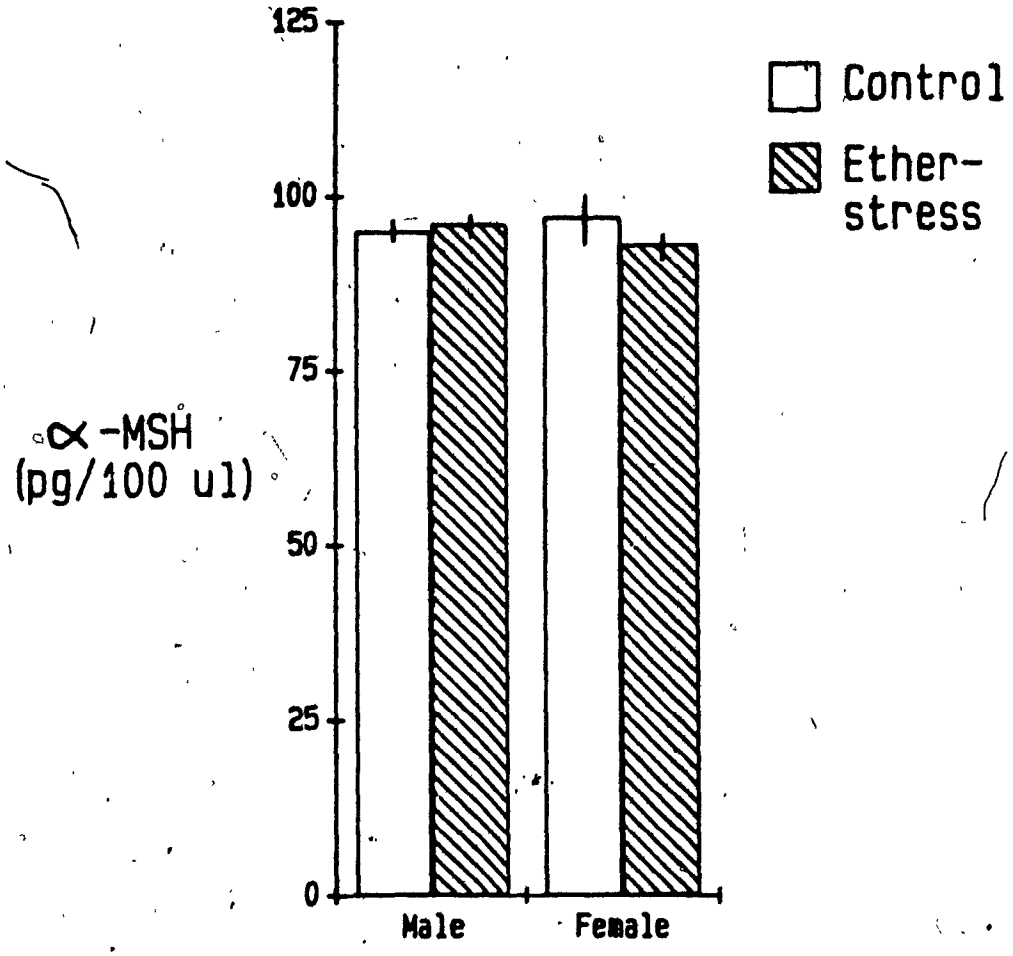


Figure 8. Plasma concentrations of a-MSH in control (C) and ether-stressed (E) day 7, male and female pups. Sample sizes are as follows: Males, C=8, E=8; Females, C=7, E=7.

Table 7

Concentrations of norepinephrine in the hippocampus of male rats

Summary table of the ANOVA

Source	df	Mean Square	F	p
Age	5	19.61	30.57	.001
Stress	1	0.60	0.10	
Age x stress	5	0.79	1.23	.31
Error	55	0.64		

Figure 9. Concentrations of NE in the hippocampus of control (C) and ether-stressed (E) (9a) male, and (9b) female rats of various ages. Sample sizes are as follows: Males- Day 3, C=4, E=6; Day 7, C=4, E=6; Day 14, C=6, E=6; Day 21, C=6, E=6; Day 35, C=6, E=5; Day 63, C=6, E=6; Females- Day 3, C=3, E=3; Day 7, C=5, E=5; Day 14, C=6, E=4; Day 21, C=6, E=5; Day 35, C=5, E=4; Day 63, C=5, E=6.

Figure 9a

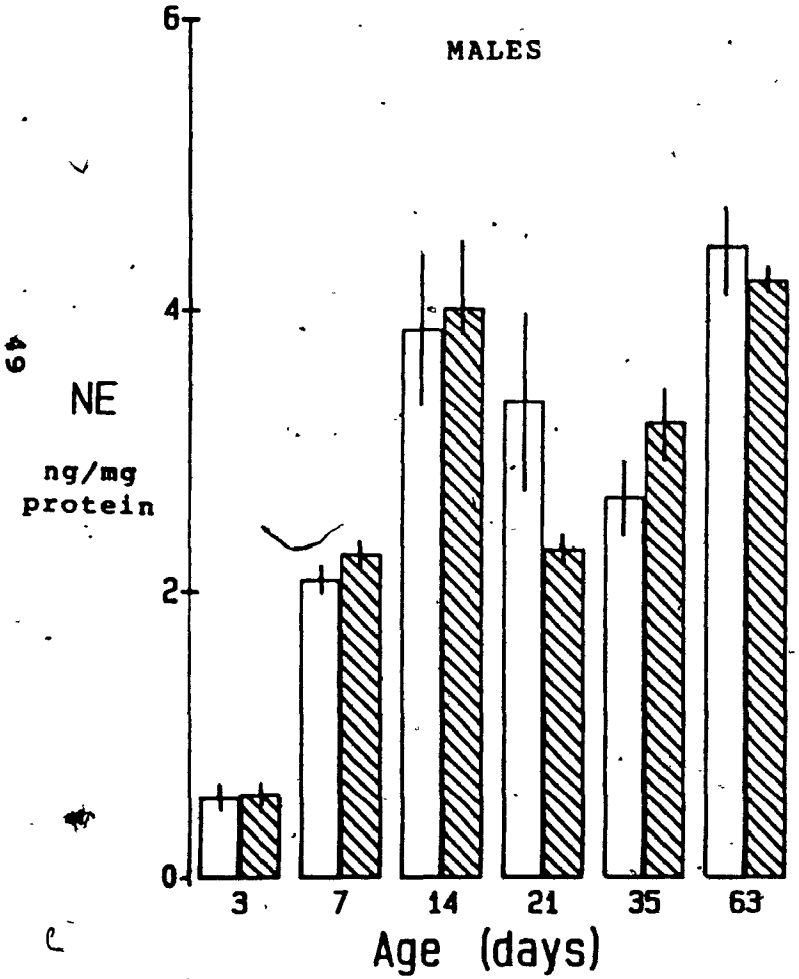


Figure 9b

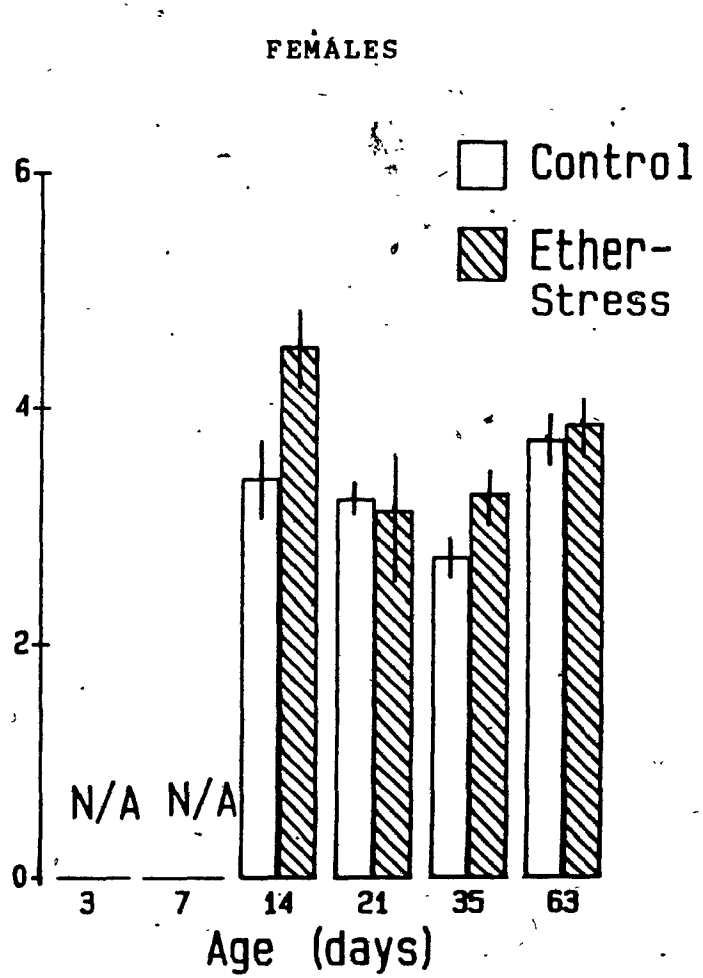


Table 8

Concentrations of norepinephrine in the hippocampus of female rats

Summary table of the ANOVA

Source	df	Mean Square	F	p
Age	3	1.84	2.40	.10
Stress	1	1.53	2.00	.17
Age x stress	3	0.61	0.79	
Error	28	0.77		

28) = 2.40, n.s., or stress, $F(1, 28) = 2.0$, n.s., or a significant age x stress interaction, $F<1$ (see Figure 9b).

Hippocampus: Dopamine. Concentrations of DA were below the detectable limit in both male and female rats.

Hippocampus: Serotonin. In male pups, a significant age-related difference in the concentration of 5-HT was detected, $F(5, 56) = 16.38$, $p<.001$. As illustrated in Figure 10a, the difference appears to be due to elevated levels of 5-HT for Day 14 pups compared with rats of other ages. The main effect of stress and the age x stress interaction terms were not significant, $F<1$. A summary of the ANOVA is presented in Table 9.

A very different pattern of 5-HT levels was found in the hippocampus of female rats. The analysis of variance detected significant main effects for both age, $F(5, 46) = 37.12$, $p<.001$, and stress, $F(1, 46) = 7.05$, $p<.01$, as well as a significant age x stress interaction, $F(5, 46) = 4.26$, $p<.003$ (see Table 10). Post-hoc comparisons revealed a decrease in 5-HT levels following exposure to ether fumes in pups aged 3 ($p<.05$), and 7 days of age ($p<.01$), but not in the older animals. As illustrated in Figure 10b, the age difference can be attributed to greater concentrations of 5-HT in pups 3 and 7 days of age than in the older animals ($p<.01$). Although direct statistical comparisons were not made since samples from male and female animals were assayed separately, concentrations of 5-HT in female pups 3 and 7 days of age were approximately ten-fold greater than in male pups at the same age, despite comparable levels of 5-HT at the older ages examined.

Hippocampus: 5-Hydroxyindoleacetic acid. Examination of the results

Figure 10. Concentrations of 5-HT in the hippocampus of control (C) and ether-stressed (E) (10a) male, and (10b) female rats of various ages. Statistically reliable differences at * $p<.05$, ** $p<.01$. Sample sizes are as follows: Males- Day 3, C=4, E=5; Day 7, C=5, E=6; Day 14, C=6, E=6; Day 21, C=6, E=6; Day 35, C=6, E=6; Day 63, C=6, E=6; Females- Day 3, C=2, E=2; Day 7, C=4, E=4; Day 14, C=5, E=6; Day 21, C=6, E=6; Day 35, C=6, E=6; Day 63, C=5, E=6.

Figure 10a

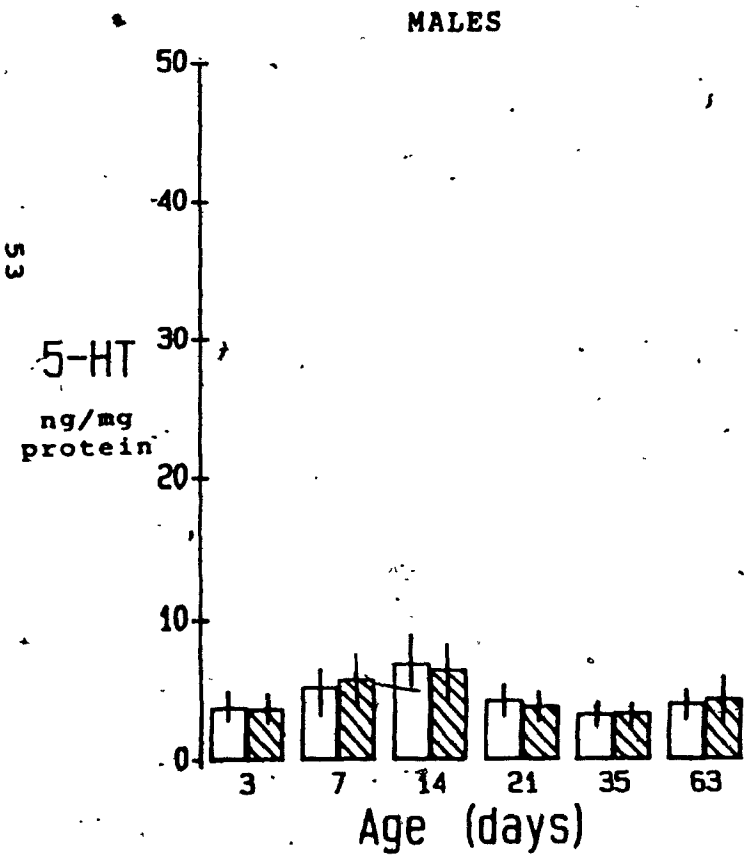


Figure 10b

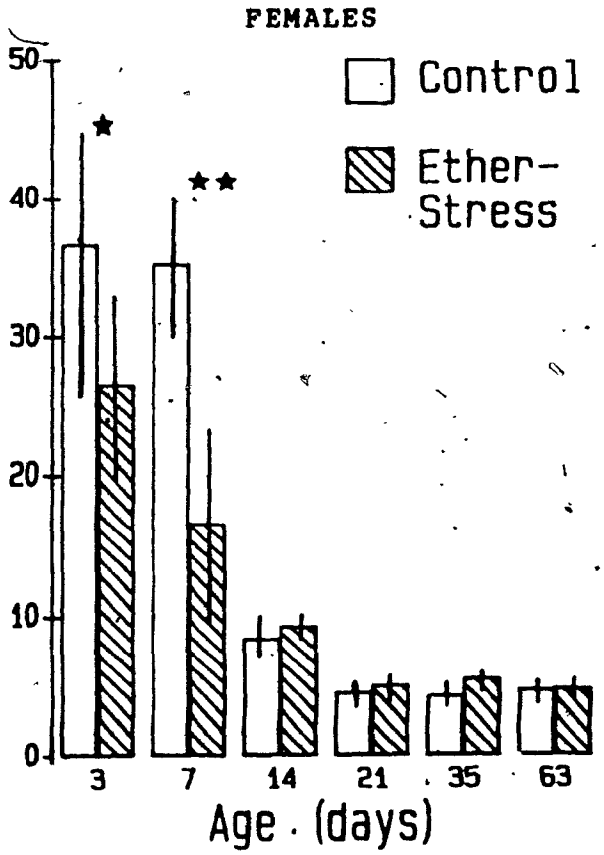


Table 9

Concentrations of serotonin in the hippocampus of male rats

Summary table of the ANOVA

Source	df	Mean Square	F	p
Age	5	19.37	16.38	.001
Stress	1	0.01	0.01	
Age x stress	5	0.49	0.42	
Error	56	1.18		

Table 10

Concentrations of serotonin in the hippocampus of female rats

Summary table of the ANOVA

Source	df	Mean Square	F	p
Age	5	1,246.23	37.12	.001
Stress	1	236.61	7.05	.01
Age x stress	5	143.13	4.26	.01
Error	46	33.57		

revealed a significant difference in levels of 5-HIAA in male pups according to age, $F(5, 56) = 18.75$, $p<.001$. As may be seen in Figure 11a, 5-HT metabolism appeared higher in Day 14 males than at most other ages. There were no significant changes in levels of this 5-HT metabolite following stress, however, $F<1$, and the age x stress interaction term was not significant, $F<1$ (see Table 11).

In females, the ANOVA yielded a significant effect of age, $F(5, 49) = 2.70$, $p<.05$ (see Figure 11b). As shown in Table 12, the main effect of stress $F<1$, and the age x stress interaction, $F(5, 49) = 1.94$, were not significant.

Hippocampus: 5-HIAA/5-HT. A summary of the ANOVA is presented in Table 13. Analysis of the 5-HIAA/5-HT ratio in males revealed a significant main effect for age, $F(5, 56) = 9.09$, $p<.001$, while the effect of stress and the age x stress interaction were not significant, $F<1$ (see Figure 12a).

A significant main effect of age was also found for females, $F(5, 45) = 26.70$, $p<.001$ (see Table 14), although the pattern was different from that observed in the males. As may be seen in Figure 12b, the 5-HIAA/5-HT ratio increased with age, suggesting that turnover of 5-HT is lower in females during the first week of life than in older animals.

Hypothalamus: Norepinephrine. Monoamine levels for male rats aged 35 and 63 days old are not available for this region, as a result of technical difficulties. Therefore, the statistical analysis presented here was conducted on values obtained for male pups 3, 7, 14, and 21 days old. An examination of the NE levels in the hypothalamus revealed a significant main effect of age for both males, $F(3, 39) = 16.31$, $p<.001$,

Figure 11. Concentrations of 5-HIAA in the hippocampus of control (C) and ether-stressed (E) (11a) male, and (11b) female rats of various ages. Sample sizes are as follows: Males- Day 3, C=4, E=6; Day 7, C=5, E=6; Day 14, C=5, E=6; Day 21, C=6, E=6; Day 35, C=6, E=6; Day 63, C=6, E=6; Females- Day 3, C=2, E=3; Day 7, C=5, E=4; Day 14, C=6, E=6; Day 21, C=6, E=6; Day 35, C=6, E=6; Day 63, C=5, E=6.

Figure 11a

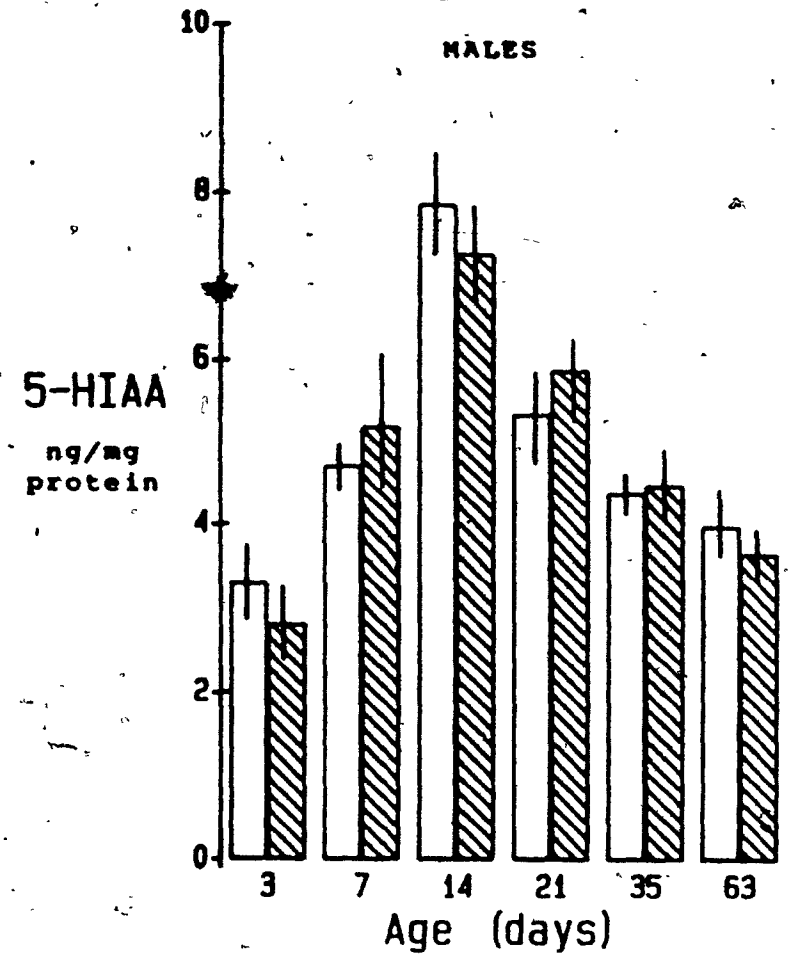


Figure 11b

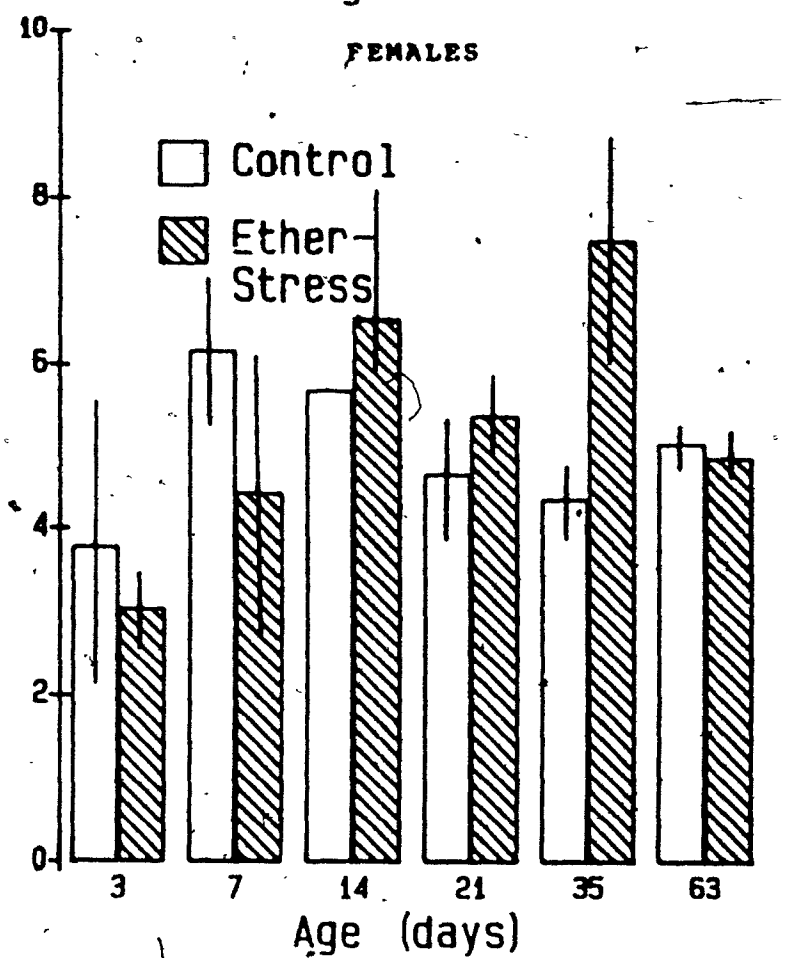


Table 11

Concentrations of 5-HIAA in the hippocampus of male rats

Summary table of the ANOVA

Source	df	Mean Square	F	p
Age	5	27.45	18.75	.001
Stress	1	0.04	0.03	
Age x stress	5	0.67	0.46	
Error	56	1.45		

Table 12

Concentrations of 5-HIAA in the hippocampus of female rats

Summary table of the ANOVA

Source	df	Mean Square	F	p
Age	5	9.33	2.70	.03
Stress	1	2.49	0.72	
Age x stress	5	6.72	1.94	.10
Error	49	3.46		

Table 13

Concentrations of the 5-HIAA/5-HT ratio in the hippocampus of male rats

Summary table of the ANOVA

Source	df	Mean Square	F	p
Age	5	0.67	9.09	.001
Stress	1	0.00	0.00	
Age x stress	5	0.71	0.96	
Error	56	0.74		

Figure 12. Concentrations of the 5-HIAA/5-HT ratio in the hippocampus of control and ether-stressed (12a) male, and (12b) female rats of various ages. Sample sizes are as follows: Males- Day 3, C=4, E=6; Day 7, C=5, E=6; Day 14, C=5, E=6; Day 21, C=6, E=6; Day 35, C=6, E=6; Day 63, C=6, E=6; Female- Day 3, C=2, E=2; Day 7, C=4, E=3; Day 14, C=5, E=6; Day 21, C=6, E=6; Day 35, C=6, E=6; Day 63, C=5, E=6.

Figure 12a

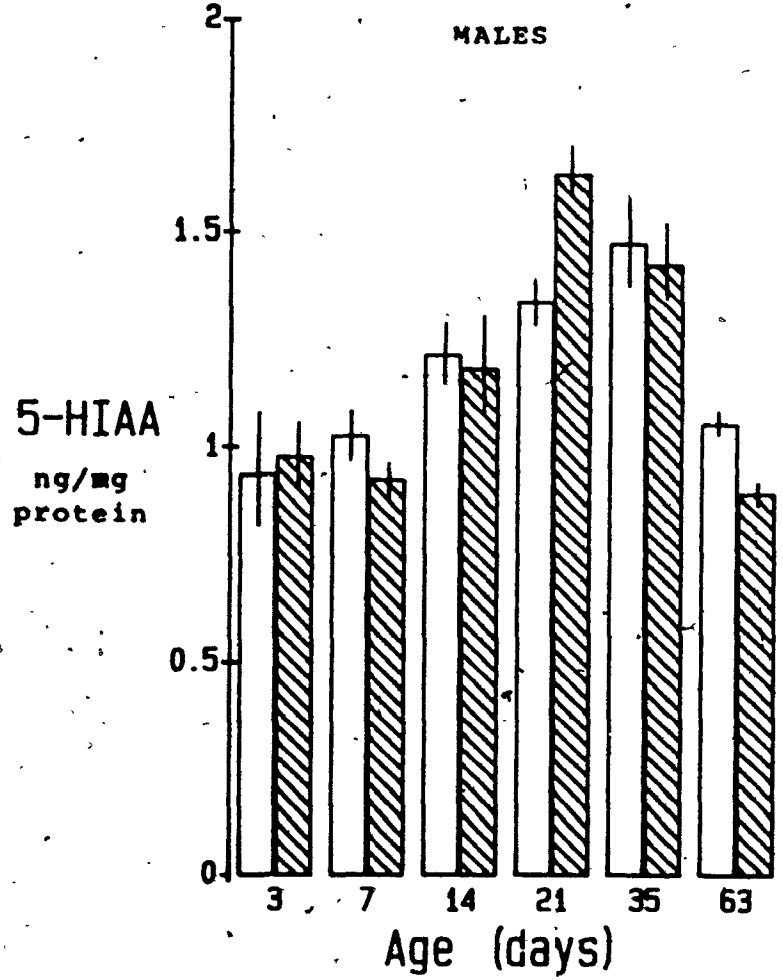


Figure 12b

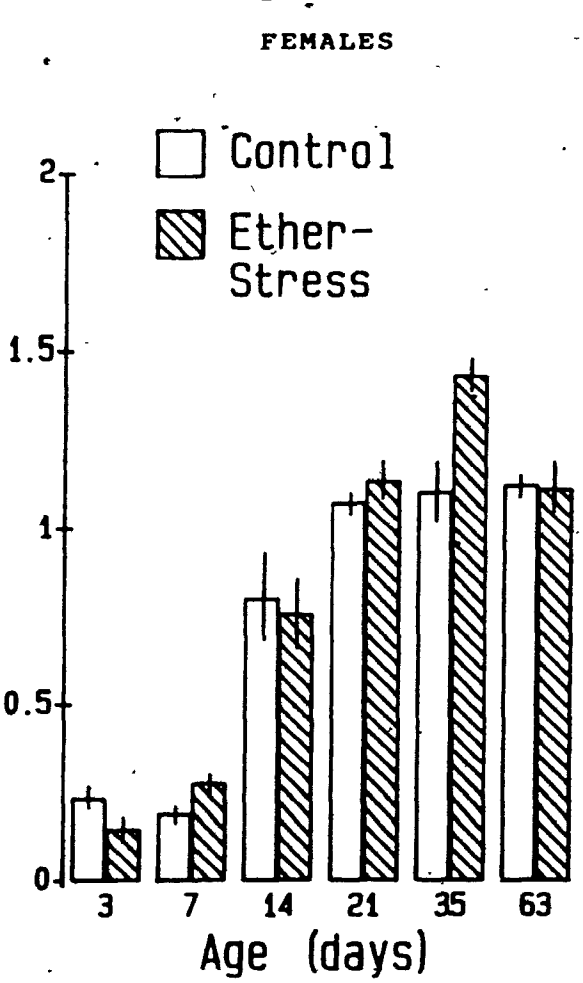


Table 14

Concentrations of the 5-HIAA/5-HT ratio in the hippocampus of female rats

Summary table of the ANOVA

Source	df	Mean Square	F	p
Age	5	1.82	26.70	.001
Stress	1	0.40	0.59	
Age x stress	5	0.44	0.64	
Error	45	0.68		

and females, $F(5, 48) = 25.44$, $p<.001$ (see Tables 15 and 16, respectively). The effect of stress and the age x stress interactions were not significant in either analysis, $F<1$. As may be seen in Figures 13a and 13b, levels of NE in the hypothalamus of both sexes showed a relatively consistent increase with age.

Hypothalamus: Dopamine. Analysis of DA levels in the hypothalamus of male rats revealed a significant main effect for age, $F(3, 39) = 4.15$, $p<.01$, as well as a significant age x stress interaction, $F(3, 39) = 3.64$, $p<.05$. The effect of ether-stress was not significant, $F(1, 39) = 2.34$, n.s. A summary of the ANOVA is presented in Table 17. As shown in Figure 14a, an increase in the concentration of DA in the hypothalamus was present at Day 21 following stress but not in the younger animals, although the alteration did not attain statistical significance.

In female rats, statistical analysis revealed a highly significant age effect, $F(5, 44) = 29.63$, $p<.001$, a significant stress effect, $F(1, 44) = 18.96$, $p<.001$, as well as a significant age x stress interaction, $F(5, 44) = 5.13$, $p<.001$ (see Table 18). A significant increase in DA levels for ether-stressed females at ages 35 ($p<.05$), and 63 ($p<.01$) was also found. Furthermore, as illustrated in Figure 14b, DA levels showed a consistent increase with age: the basal concentration of the catecholamine was significantly greater in mature rats than in pups 3 and 7 days of age ($p<.01$).

Hypothalamus: Serotonin. A summary of the ANOVA is presented in Table 19. Examination of the results for male rats revealed significant main effects of age, $F(3, 39) = 5.34$, $p<.01$, and stress, $F(1, 39) = 6.95$, $p<.01$. The age x stress interaction was not significant, $F<1$. As

Table 15

Concentrations of norepinephrine in the hypothalamus of male rats

Summary table of the ANOVA

Source	df	Mean Square	F	p
Age	3	315.07	16.31	.001
Stress	1	5.42	0.28	
Age x stress	3	20.39	0.96	.38
Error	39	19.32		

Table 16

Concentrations of norepinephrine in the hypothalamus of female rats

Summary table of the ANOVA

Source	df	Mean Square	F	p
Age	5	1,860.20	25.44	.001
Stress	1	1.85	0.03	
Age x stress	5	65.55	0.90	
Error	48	73.12		

Figure 13. Concentrations of NE in the hypothalamus of control (C) and ether-stressed (E) (13a) male, and (13b) female rats of various ages. Sample sizes are as follows: Males- Day 3, C=6, E=5; Day 7, C=6, E=6; Day 14, C=6, E=6; Day 21, C=6, E=6; Females- Day 3, C=5, E=5; Day 7, C=5, E=5; Day 14, C=6, E=5; Day 21, C=5, E=5; Day 35, C=6, E=4; Day 63, C=5, E=4. N/A = not available.

Figure 13a

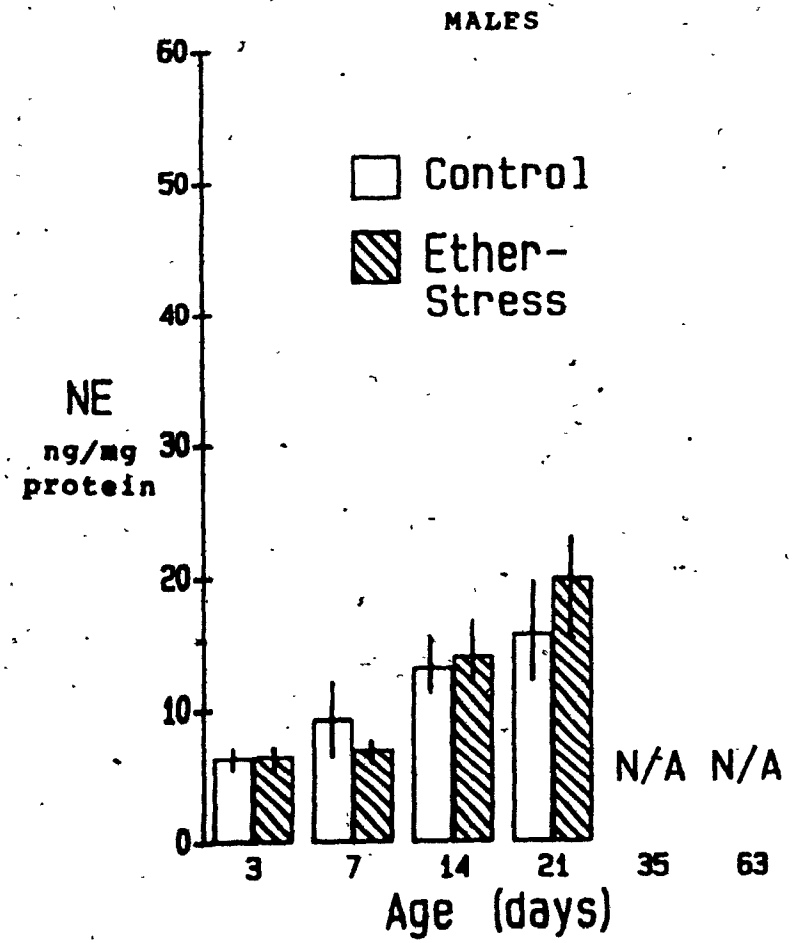


Figure 13b

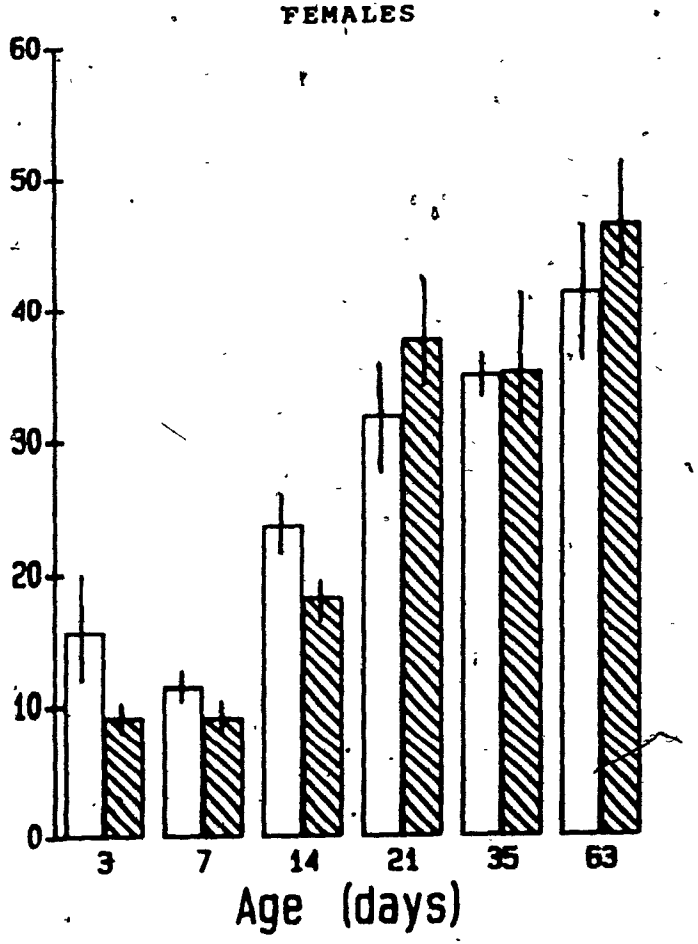


Table 17

Concentrations of dopamine in the hypothalamus of male rats

Summary table of the ANOVA

Source	df	Mean Square	F	p
Age	3	7.39	4.15	.01
Stress	1	4.17	2.34	.13
Age x stress	3	6.47	3.64	.05
Error	39	1.78		

Figure 14. Concentrations of DA in the hypothalamus of control (C) and ether-stressed (E) (14a) male, and (14b) female rats of various ages. Statistically reliable differences at * $p<.05$, ** $p<.01$. Sample sizes are as follows: Males- Day 3, C=6, E=5; Day 7, C=6, E=6; Day 14, C=6, E=6, Day 21, C=6, E=6; Females- Day 3, C=2, E=4; Day 7, C=5, E=5; Day 14, C=5, E=5; Day 21, C=6, E=5; Day 35, C=6, E=4; Day 63, C=4, E=5. N/A = not available.

Figure 14a

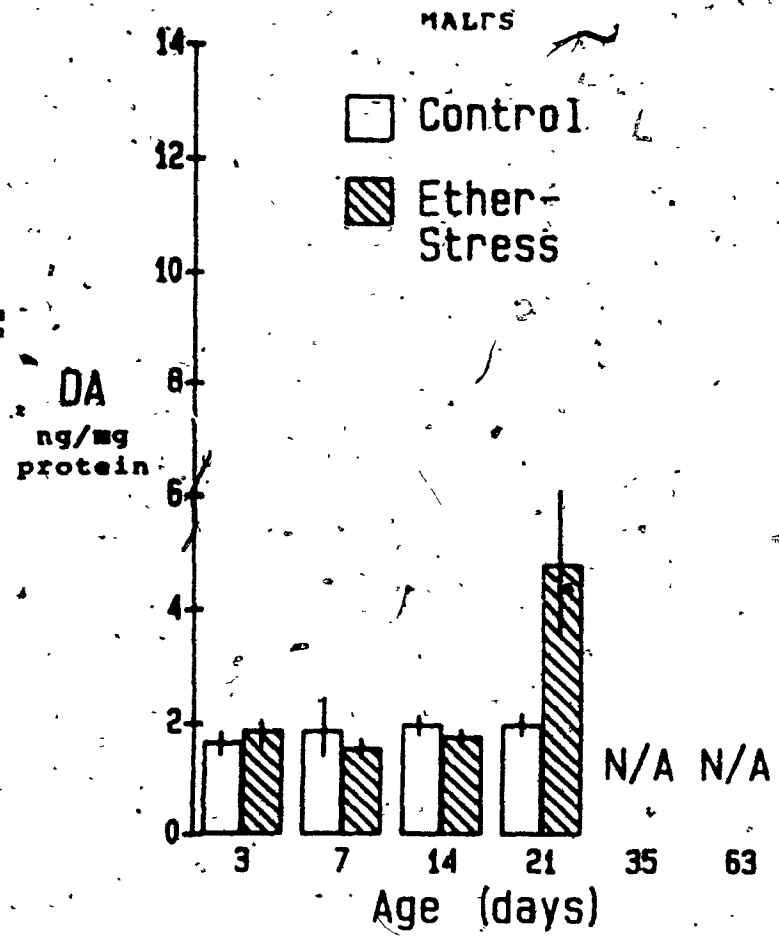


Figure 14b

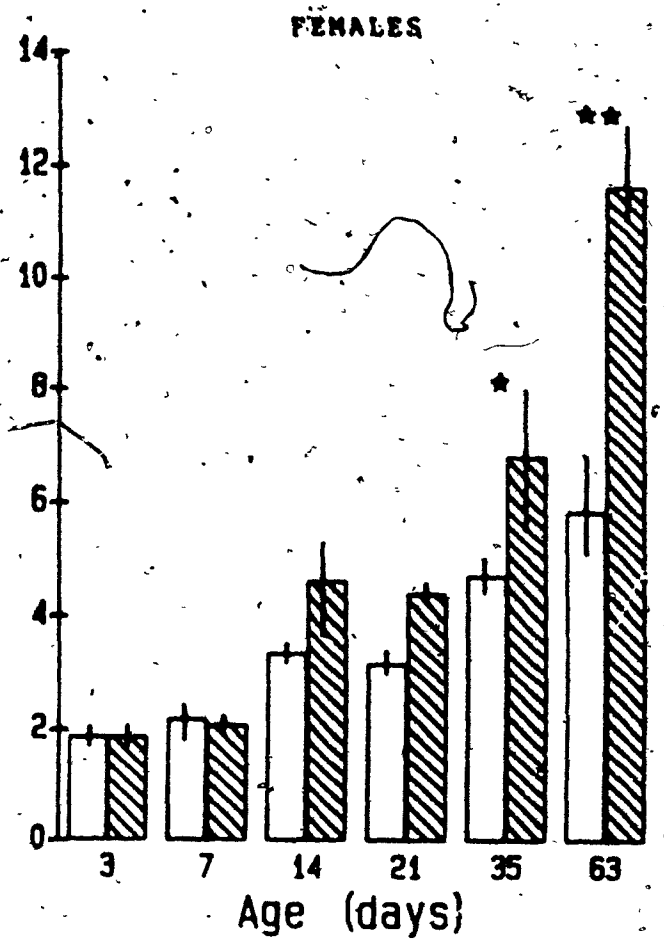


Table 18

Concentrations of dopamine in the hypothalamus of female rats

Summary table of the ANOVA

Source	df	Mean Square	F	p
Age	5	56.93	29.63	.001
Stress	1	36.42	18.96	.001
Age x stress	5	9.85	5.13	.001
Error	44	1.92		

Table 19

Concentrations of serotonin in the hypothalamus of male rats

Summary table of the ANOVA

Source	df	Mean Square	F	p
Age	3	12.28	5.34	.01
Stress	1	15.97	6.95	.01
Age x stress	3	1.26	0.55	
Error	39	2.30		

illustrated in Figure 15a, basal levels of 5-HT in the hypothalamus of Day 21 males were significantly greater than in pups 3 days of age ($p<.01$), although the age-related trend was not as impressive as in females. Although the concentration of 5-HT in the hypothalamus was observed to decrease following exposure to stress in animals 7, 14, and 21 days of age, post-hoc analysis with Tukey tests did not detect a significant difference.

In female rats, the ANOVA yielded a significant main effect for age, $F(5, 52) = 20.11$, $p<.001$. The effect of stress and the age x stress interaction were not significant, $F<1$ (see Table 20). As may be seen in Figure 15b, greater concentrations of 5-HT were detected in the older animals than in pups 3 and 7 days old.

Hypothalamus: 5-Hydroxyindoleactic acid. Analysis of 5-HIAA levels in males revealed a significant main effect for age, $F(3, 39) = 18.43$, $p<.001$. The effect of stress $F(1, 39) = 1.25$, n.s., and the age x stress interaction, $F(3, 39) = 1.23$, n.s., were not significant. A summary of the ANOVA is presented in Table 21. As illustrated in Figure 16a, the concentration of 5-HIAA was found to increase with age: levels of 5-HIAA were lower in Day 3 pups than in animals 21 days of age.

The ANOVA conducted on the data for females also revealed a significant effect of age, $F(5, 50) = 11.24$, $p<.001$, while the main effect of stress and the age x stress interaction were not significant, $F<1$ (see Table 22). As illustrated in Figure 16b, higher concentrations of the metabolite were observed in pups 14 and 21 days of age.

Hypothalamus: 5-HIAA/5-HT. Analysis of the 5-HIAA/5-HT ratio yielded a significant main effect for age in males, $F(3, 39) = 4.05$,

Figure 15. Concentrations of 5-HT in the hypothalamus of control (C) and ether-stressed (E) (15a) male, and (15b) female rats of various ages. Sample sizes are as follows: Males- Day 3, C=6, E=5; Day 7, C=6, E=6; Day 14, C=6, E=6; Day 21, C=6, E=6; Females- Day 3, C=4, E=5; Day 7, C=6, E=6; Day 14, C=6, E=6; Day 21, C=5, E=5; Day 35, C=6, E=5; Day 63, C=5, E=5. N/A.= not available.

Figure 15a

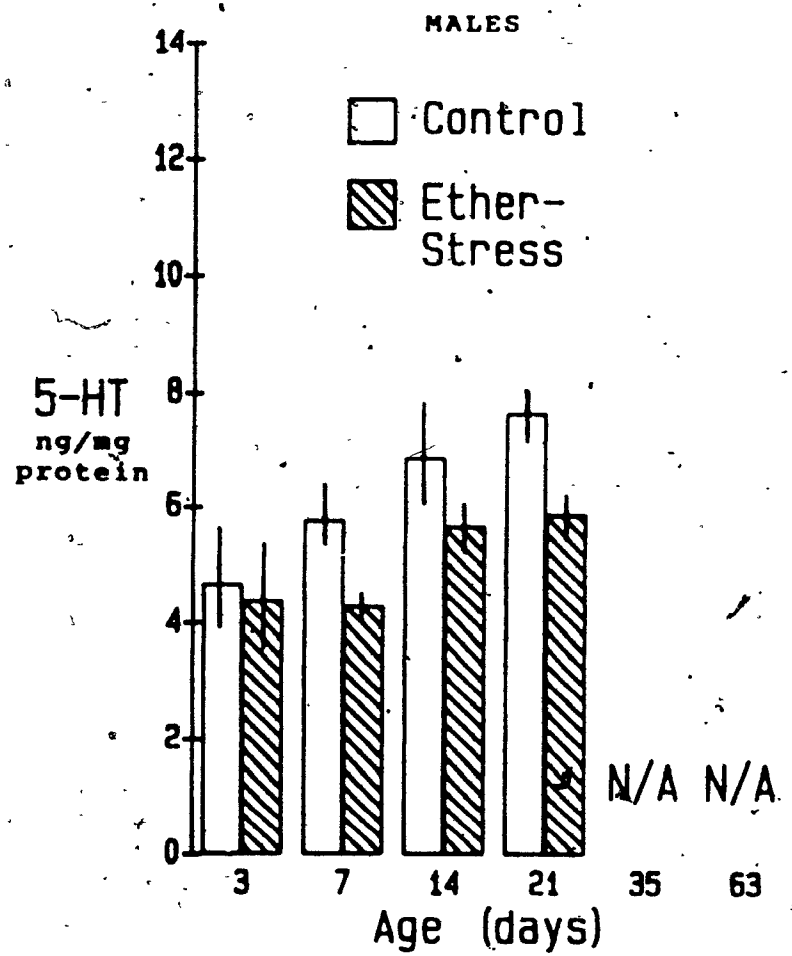


Figure 15b

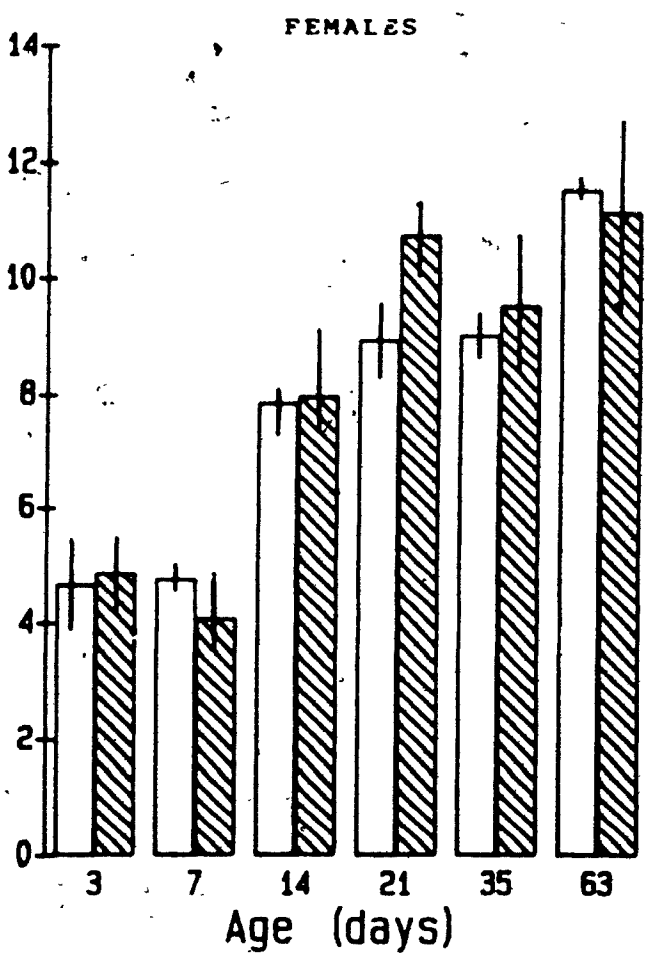


Table 20

Concentrations of serotonin in the hypothalamus of female rats

Summary table of the ANOVA

Source	df	Mean Square	F	p
Age	5	83.15	20.11	.001
Stress	1	1.83	0.44	
Age x stress	5	1.97	0.48	
Error	52	4.14		

Table 21

Concentrations of 5-hydroxyindoleacetic acid in the hypothalamus of male rats

Summary table of the ANOVA

Source	df	Mean Square	F	p
Age	3	53.36	18.43	.001
Stress	1	3.61	1.25	.27
Age x stress	3	3.56	1.23	.31
Error	39	2.90		

Figure 16: Concentrations of 5-HIAA in control (C) and ether-stressed (E) (16a) male, and (16b) female rats of various ages. Sample sizes are as follows: Males- Day 3, C=6, E=5; Day 7, C=6, E=6; Day 14, C=6, E=6; Day 21, C=6, E=6; Females- Day 3, C=4, E=4; Day 7, C=6, E=6; Day 14, C=6, E=6; Day 21, C=5, E=6; Day 35, C=6, E=5; Day 63, C=5, E=3. N/A = not available.

Figure 16a

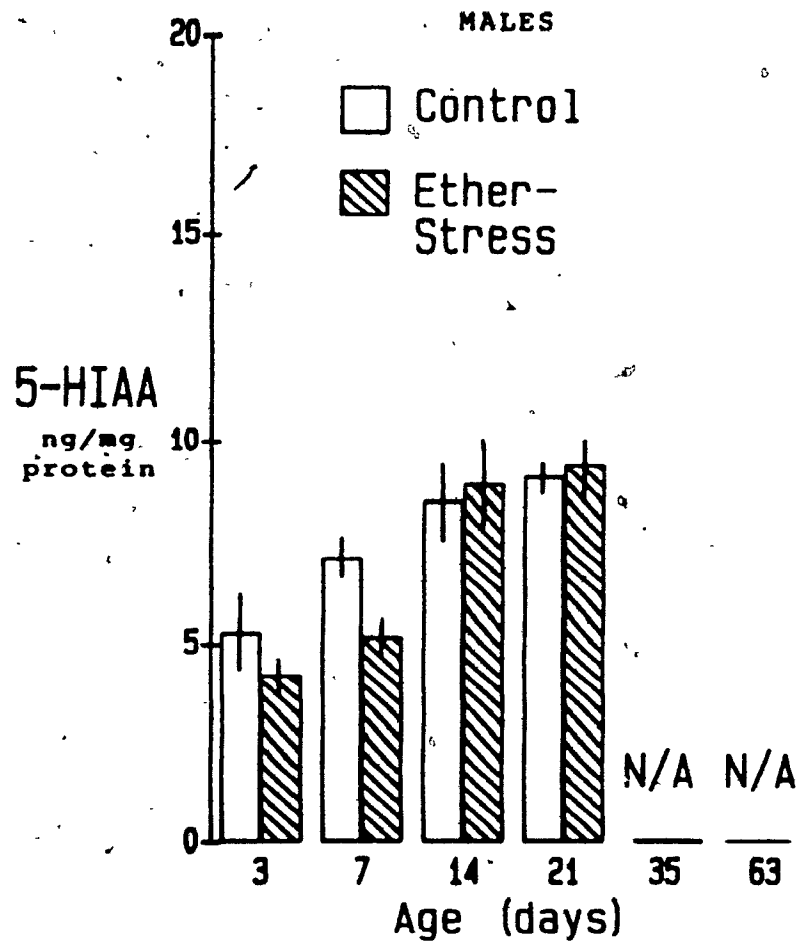


Figure 16b

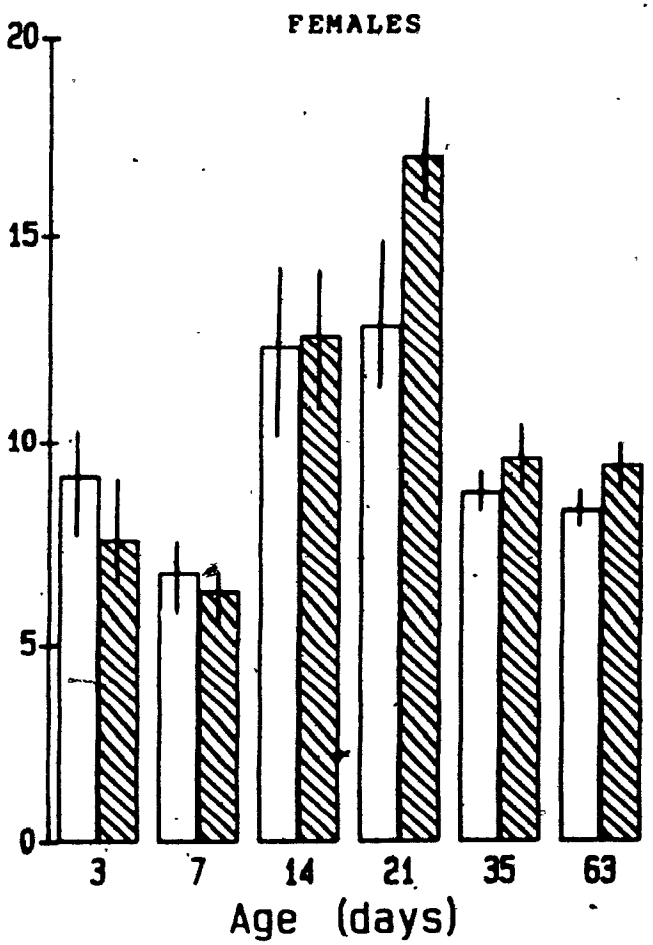


Table 22

Concentrations of 5-hydroxyindoleacetic acid in the hypothalamus of female rats

Summary table of the ANOVA

Source	df	Mean Square	F	p
Age	5	95.78	11.24	.001
Stress	1	8.45	0.99	
Age x stress	5	9.12	1.07	.39
Error	50	8.52		

$p<.05$, and females $F(5, 51) = 10.53$, $p<.001$. In male rats, neither the stress nor the interaction effects were significant, $F(1, 39) = 2.11$, n.s., and $F(3, 39) = 2.16$, n.s., respectively (see Table 23). As shown in Figure 17a, the age-related difference in serotonergic activity observed for male pups could be attributed to greater 5-HT turnover in ether-stressed animals 14 and 21 days of age than in 3 day-old pups similarly treated ($p<.05$). As illustrated in Figure 17b, females also demonstrated a decrease in the 5-HIAA/5-HT ratio with age, as levels in animals 35 and 63 days old were lower than in females at younger ages. The effect of stress and the age x stress interaction were not significant, $F<1$ (see Table 24).

Frontal cortex: Norepinephrine. Examination of the results for male pups revealed a significant main effect for age, $F(5, 57) = 8.27$, $p<.001$. A summary of the ANOVA is presented in Table 25. As may be seen in Figure 18a, exposure to stress had no effect on NE levels in the frontal cortex of males, $F<1$, and the age x stress interaction was not significant, $F(5, 57) = 1.17$, n.s.

Monoamine levels for female pups 3, 7 and 14 days old in the frontal cortex are not available. Results of the ANOVA conducted on NE levels in this region for females 21, 35 and 63 days old are presented in Table 26. Examination of the analysis yielded no change in the concentration of NE in the frontal cortex due to the age of the animal, $F(2, 25) = 1.87$, n.s., or to stress, $F(1, 25) = 1.11$, n.s., and the age x stress interaction was not significant, $F<1$ (see Figure 18b).

Frontal cortex: Dopamine. Peak heights for DA were predominantly below the detectable limit in samples from both male and female animals.

Table 23

Concentrations of 5-HIAA/5-HT in the hypothalamus of male rats

Summary table of the ANOVA

Source	df	Mean Square	F	p
Age	3	0.38	4.05	.05
Stress	1	0.20	2.11	.15
Age x stress	3	0.20	2.16	.11
Error	39	0.09		

Figure 17. Concentrations of the 5-HIAA/5-HT ratio in the hypothalamus of control (C) and ether-stressed (E) (17a) male, and (17b) female rats of various ages. Sample sizes are as follows: Males- Day 3, C=6, E=5; Day 7, C=6, E=6; Day 14, C=6, E=6; Day 21, C=6, E=6; Females- Day 3, C=4, E=4; Day 7, C=6, E=6; Day 14, C=6, E=6; Day 21, C=6, E=6; Day 35, C=6, E=5; Day 63, C=5, E=3. N/A = not available.

Figure 17a

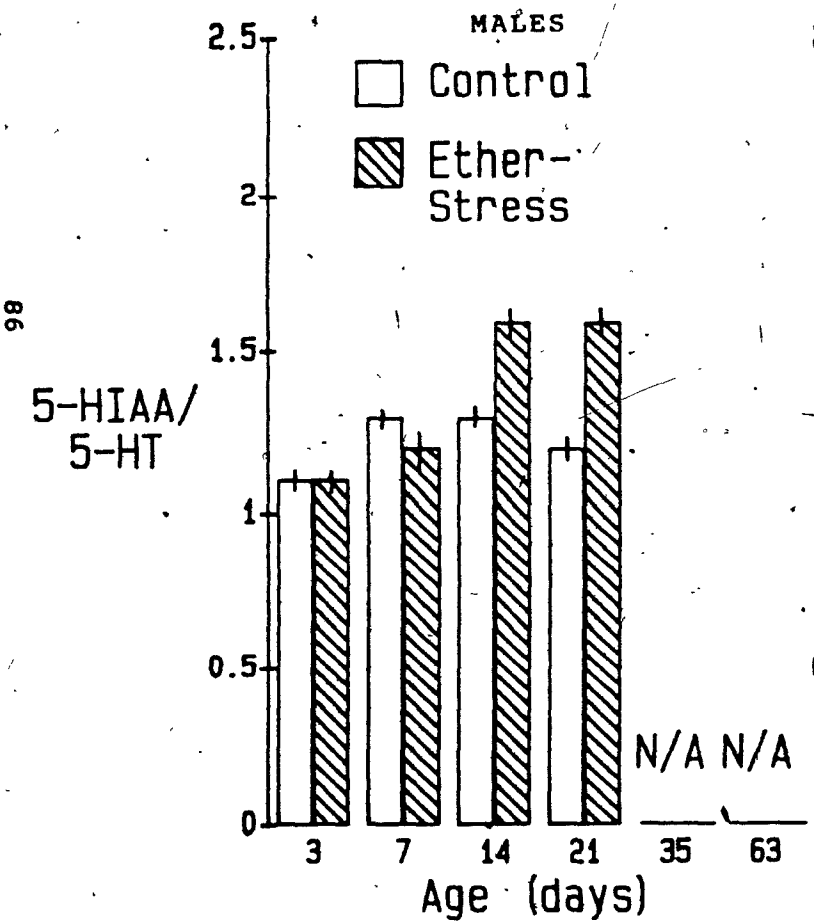


Figure 17b

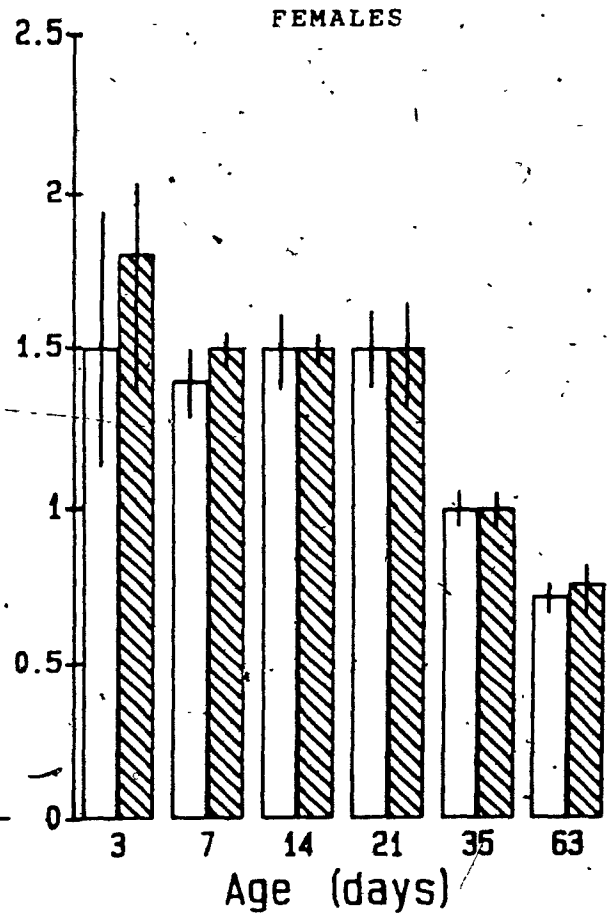


Table 24

Concentrations of 5-HIAA/5-HT in the hypothalamus of female rats

Summary table of the ANOVA

Source	df	Mean Square	F	p
Age	5	1.20	10.53	.001
Stress	1	0.11	0.98	
Age x stress	5	0.03	0.30	
Error	51	0.11		

Table 25

Concentrations of norepinephrine in the frontal cortex of male rats

Summary table of the ANOVA

Source	df	Mean Square	F	p
Age	5	5.52	8.27	.001
Stress	1	0.02	0.03	
Age x stress	5	0.78	1.17	.33
Error	57	0.67		

Figure 18. Concentrations of NE in the frontal cortex of control (C) and ether-stressed (E) (18a) male, and (18b) female rats of various ages. Sample sizes are as follows: Males- Day 3, C=7, E=6; Day 7, C=5, E=6; Day 14, C=6, E=4; Day 21, C=6, E=6; Day 35, C=6, E=6; Day 63, C=6, E=5; Female- Day 21, C=4, E=4; Day 35, C=6, E=6; Day 63, C=5, E=6. N/A = not available.

Figure 18a

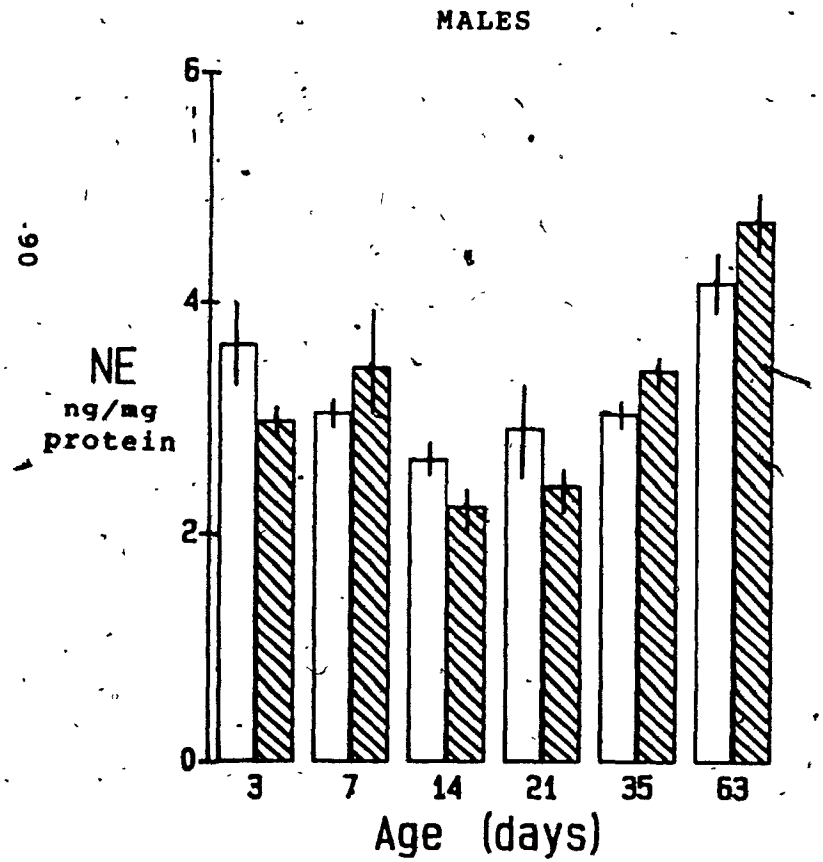


Figure 18b

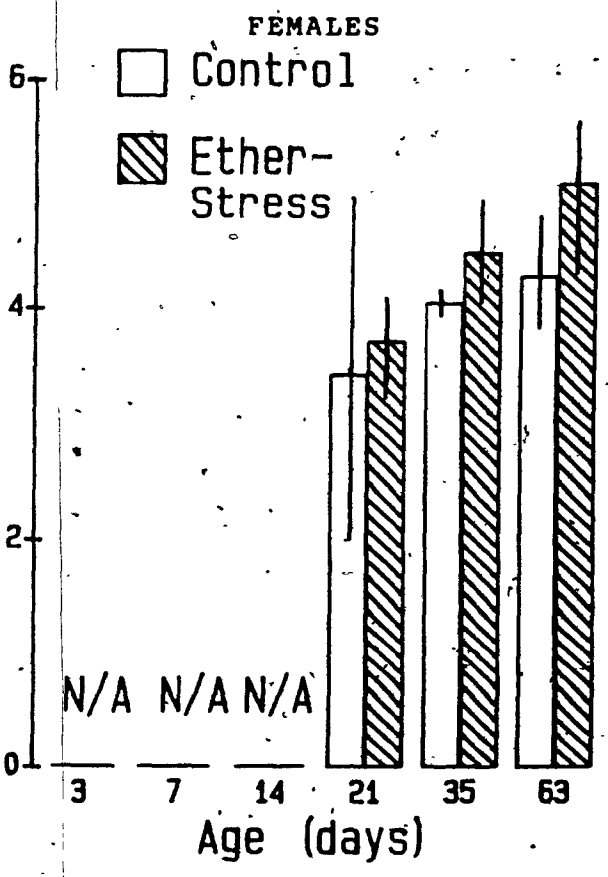


Table 26

Concentrations of norepinephrine in the frontal cortex of female rats

Summary table of the ANOVA

Source	df	Mean Square	F	p
Age	2	3.39	1.87	.17
Stress	1	2.02	1.11	.30
Age x stress	2	0.21	0.11	
Error	25	1.81		

Frontal cortex: Serotonin. Analysis of the concentration of 5-HT in this region revealed a significant age-related difference among male pups, $F(5, 59) = 24.21$, $p<.001$ (see Table 27). The elevated concentrations of 5-HT in animals 63 days old appeared to be the source of the effect, as concentrations of 5-HT at this age were higher than those found in pups at the younger ages examined. The data are illustrated in Figure 19a. The main effect of stress, $F(1, 59) = 2.49$, n.s., and the age x stress interaction, $F<1$, were not significant.

As shown in Figure 19b, a significant increase in frontal cortex 5-HT was observed in females following exposure to ether fumes, $F(1, 25) = 5.16$, $p<.05$. A significant effect of age was also found, $F(2, 25) = 11.67$, $p<.001$. A summary of the ANOVA may be found in Table 28. The age difference could be attributed to levels of 5-HT being significantly lower in Day 21 pups than in the older animals examined. No significant age x stress interaction was detected, $F<1$.

Frontal cortex: 5-Hydroxyindoleacetic acid. Analysis of 5-HIAA concentrations in the frontal cortex of male animals yielded a significant effect of age, $F(5, 58) = 14.43$, $p<.001$ (see Table 29). As may be seen in Figure 20a, comparison of the means suggested that the effect may be attributed to a lower rate of 5-HT metabolism in the frontal cortex of pups 3, 7 and 14 days old than in the older animals. No differences in 5-HIAA levels were found in rats following exposure to stress, and the age x stress interaction was not significant, $F(5, 58) = 1.31$, n.s.

A significant main effect of stress was detected in females, $F(1, 25) = 14.49$, $p<.001$, which may be attributed to an elevation following

Table 27

Concentrations of serotonin in the frontal cortex of male rats

Summary table of the ANOVA

Source	df	Mean Square	F	p
Age	5	15.02	24.21	.001
Stress	1	1.54	2.49	.12
Age x stress	5	0.60	0.97	
Error	59	0.62		

Figure 19. Concentrations of 5-HT in the frontal cortex of control (C) and ether-stressed (E) (19a) male, and (19b) female rats of various ages. Sample sizes are as follows: Males- Day 3, C=7, E=6; Day 7, C=5, E=6; Day 14, C=6, E=5; Day 21, C=6, E=6; Day 35, C=6, E=6; Day 63, C=6, E=6; Females- Day 21, C=4, E=4; Day 35, C=6, E=6; Day 63, C=5, E=6. N/A= not available.

Figure 19a

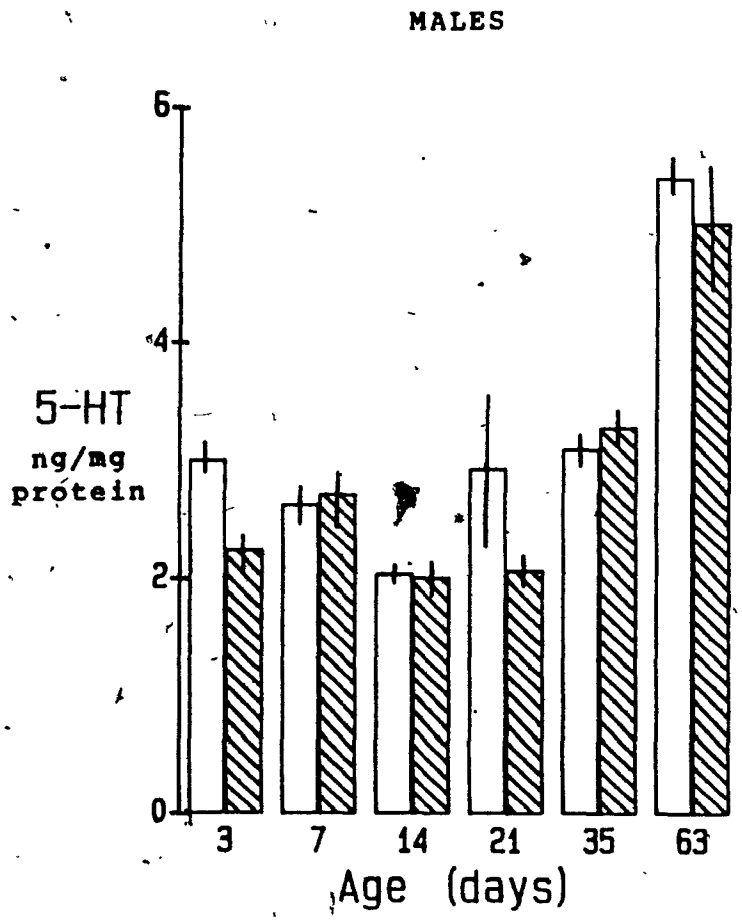


Figure 19b

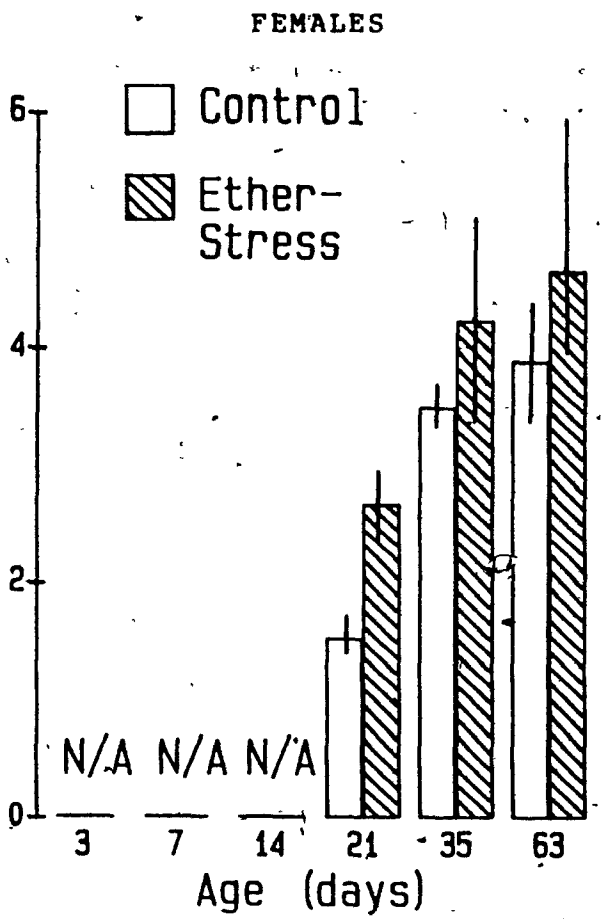


Table 28

Concentrations of serotonin in the frontal cortex of female rats

Summary table of the ANOVA

Source	df	Mean Square	F	p
Age	2	13.89	11.67	.001
Stress	1	6.14	5.16	.03
Age x stress	2	0.16	0.13	
Error	25	1.19		

Table 29

Concentrations of 5-hydroxyindoleacetic acid in the frontal cortex of male rats

Summary table of the ANOVA

Source	df	Mean Square	F	p
Age	5	6.93	14.43	.001
Stress	1	0.02	0.04	
Age x stress	5	0.63	1.31	.27
Error	58	0.48		

Figure 20. Concentrations of 5-HIAA in the frontal cortex of control (C) and ether-stressed (E) (20a) male, and (20b) female rats of various ages. Sample sizes are as follows:- Males- Day 3, C=7, E=6; Day 7, C=5, E=6; Day 14, C=6, E=4; Day 21, C=6, E=6; Day 35, C=6, E=6; Day 63, C=6, E=6; Females- Day 21, C=4, E=4; Day 35, C=6, E=6; Day 63, C=5, E=6. N/A= not available.

Figure 20a

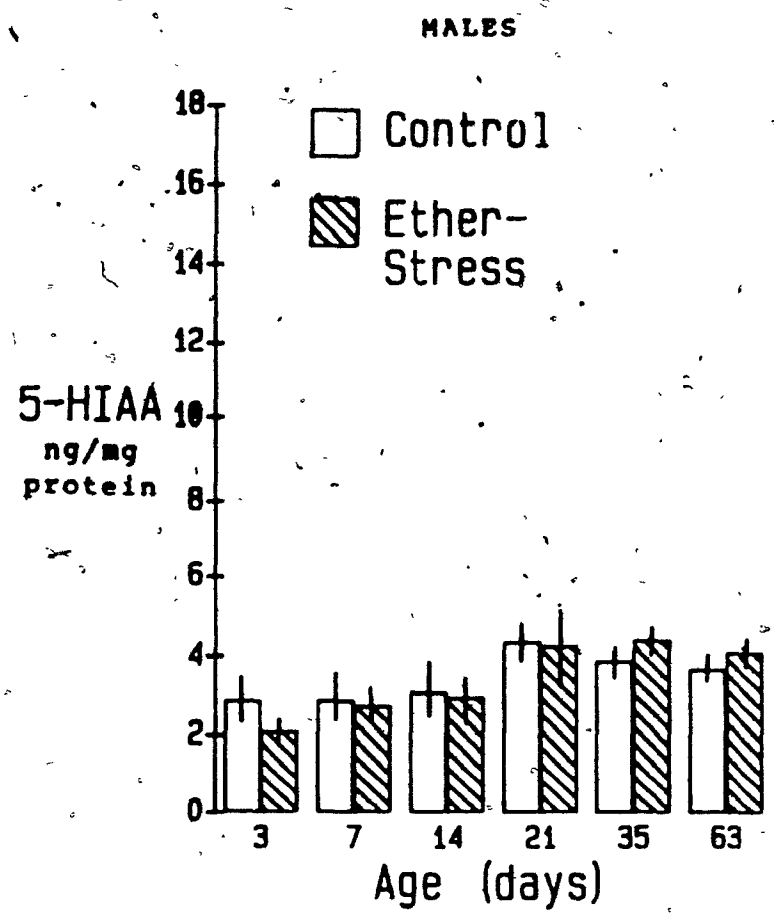
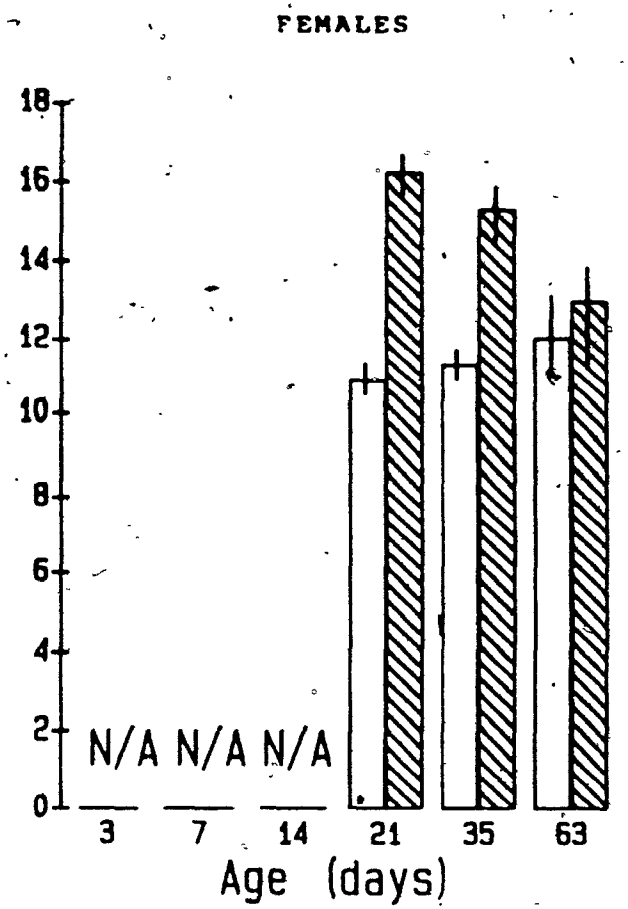


Figure 20b



exposure to ether in pups 21 days of age (see Figure 20b). The effect of age, and the age x stress interaction were not significant, $F<1$, and (2, 25) = 0.14, n.s., respectively. A summary of the ANOVA is presented in Table 30.

Frontal cortex: 5-HIAA/5-HT. A significant main effect of age on the 5-HIAA/5-HT ratio was found in males, $F(5, 58) = 17.16$, $p<.001$. As illustrated in Figure 21a, the effect appears to be due to a greater rate of serotonin turnover in males 21 days of age than at the other ages examined. The effect of stress, and the age x stress interaction, were not significant, $F<1$. A summary of the ANOVA is shown in Table 31.

A significant age-related change was also observed in the 5-HIAA/5-HT ratio in the frontal cortex of females, $F(2, 25) = 26.07$, $p<.001$ (see Table 32). As may be seen in Figure 21b, the effect appears to be due to a higher rate of serotonin turnover in Day 21 animals than at the other ages examined. The effect of stress and the interaction term were not significant, $F<1$.

Discussion

In the present investigation, exposure to stress resulted in different response patterns, depending on the system examined. The concentrations of plasma B-EPLIR observed in rats of various ages indicated that pups respond to stressors with increased levels of B-END during the early postnatal period, a time when the H-P-A system is hyporesponsive to stress. In contrast, no increase in a-MSH was detected during this period, suggesting the quiescence of the neurointermediate lobe in response to stress at this time. Furthermore, an increase in the concentration of hypothalamic dopamine was detected following

Table 30

Concentrations of 5-hydroxyindoleactic acid in the frontal cortex of female rats

Summary table of the ANOVA

Source	df	Mean Square	F	p
Age	2	2.86	0.47	
Stress	1	88.48	14.49	.001
Age x stress	2	12.78	2.09	.14
Error	25	6.11		

Figure 21. Concentrations of 5-HIAA/5-HT in the frontal cortex of control (C) and ether-stress (E) (21a) male, and (21b) female rats of various ages. Sample sizes are as follows: Males- Day 3, C=7, E=6; Day 7, C=5, E=6; Day 14, C=6, E=4; Day 21, C=6, E=6; Day 35, C=6, E=6; Day 63, C=6, E=6; Females- Day 21, C=4, E=4; Day 35, C=6, E=6; Day 63, C=5, E=6. N/A = not available.

Figure 21a

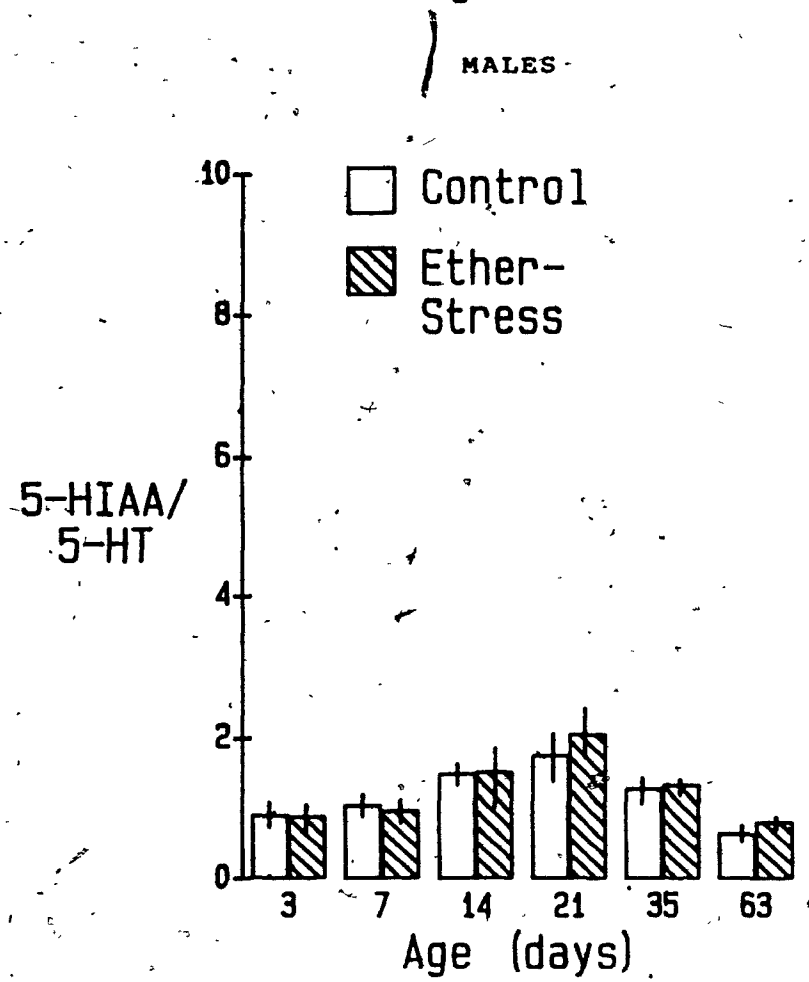


Figure 21b

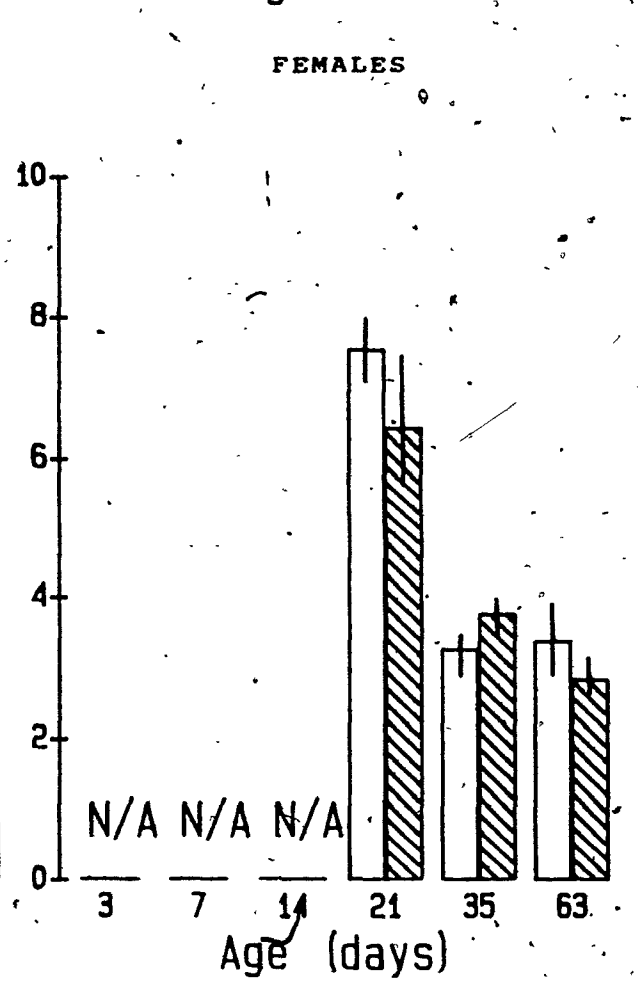


Table 31

Concentrations of 5-HIAA/5-HT in the frontal cortex of male rats

Summary table of the ANOVA

Source	df	Mean Square	F	p
Age	5	2.10	17.16	.001
Stress	1	0.10	0.83	
Age x stress	5	0.06	0.45	
Error	58	0.12		

Table 32

Concentrations of 5-HIAA/5-HT in the frontal cortex of female rats

Summary table of the ANOVA

Source	df	Mean Square	F	p
Age	2	45.72	26.07	.001
Stress	1	1.15	0.65	
Age x stress	2	1.71	0.98	
Error	25	1.75		

stress-inducing manipulations, but not until the third week of life, suggesting that the response to stress of the monoaminergic system in the neonate is different from that of the adult.

The effects of stress on the H-P-A system in postnatal rats observed here are consistent with earlier reports (see Sapolsky and Meaney, 1986). During the first two weeks of life, a period when plasma and anterior pituitary levels of ACTH are low (Seizinger et al., 1984; Walker et al., 1985) and the release of ACTH in response to either CRF or stress is diminished (e.g., Walker, Sapolsky, Meaney, Rivier, and Vale, 1986), no significant increase in the concentration of circulating corticosterone was detected. In Day 14 animals, however, the approximate age at which ACTH release is no longer suppressed, a significant rise in plasma corticosterone was observed following exposure to both handling- and ether-stress.

In contrast to the H-P-A quiescence observed during the first two weeks of life, a stress-induced increase in plasma β-EPLIR was observed in pups throughout this neonatal period, as well as at the older ages. This stress-induced increase in β-EPLIR observed during the early postnatal period in the present study suggests a dissociation between ACTH and β-END responsivity to stress in the neonatal rat, since enhanced ACTH release in response to noxious stimuli does not occur until the third week of life (Walker, Perrin, Vale, and Rivier, 1986; Walker, Sapolsky, Meaney, Vale, and Rivier, 1986). Furthermore, the results suggest that the secretion and regulation of ACTH and β-END in the pup during the first two weeks of life in response to stress are not entirely under the influence of the same factors, as is reported in the older

animal (Govoni et al., 1984; Guillemin et al., 1977; Hollt et al., 1978).

The antiserum used in the present study recognized both the acetylated and non-acetylated forms of β-END, as well as β-END-(1-27), and recent studies suggest that the pituitary of the newborn rat contains both forms of the peptide (Alessi et al., 1983; Sato and Mains, 1985; Seizinger et al., 1984). There is, however, evidence that the β-END measured here was in the biologically active, non-acetylated form. Seizinger et al. (1984) reported that the anterior lobe appears to be a major source of β-EPLIR in the neonate, with concentrations of the peptide twice as high as those found in the adult. In their study, the concentration of β-EPLIR in the anterior pituitary increased rapidly after birth and peaked on Day 3, before declining to adult-like levels during the fourth week of life. Thus, it is interesting that the pattern of age-related changes in plasma β-EPLIR observed in the present study, in which greater levels were detected during the first two weeks of life than in the older animals, closely parallels the changes in the anterior lobe reported by Seizinger et al. (1984). Indeed, positive correlations were found between the values reported by Seizinger et al. in the anterior lobe and the concentrations of β-EPLIR in control, $r=.63$, and ether-stressed animals, $r=.80$, observed in the present study (see Figure 22a). As in the adult animal, the anterior lobe of the neonate has been found to consist predominantly of the non-acetylated form of β-END (Sato and Mains, 1985; Seizinger et al., 1984), an important finding that suggests that the β-END measured here was the biologically active form of the peptide.

The developmental pattern seen in the neurointermediate lobe of the

Figure 22. Pearson correlations between the concentrations of B-EPLIR reported by Seizinger et al., (1984) in the (22a) anterior, and (22b) neurointermediate lobes of the pituitary, and plasma levels of B-EPLIR observed for control and ether-stressed animals of various ages in the present study.

Figure 22a

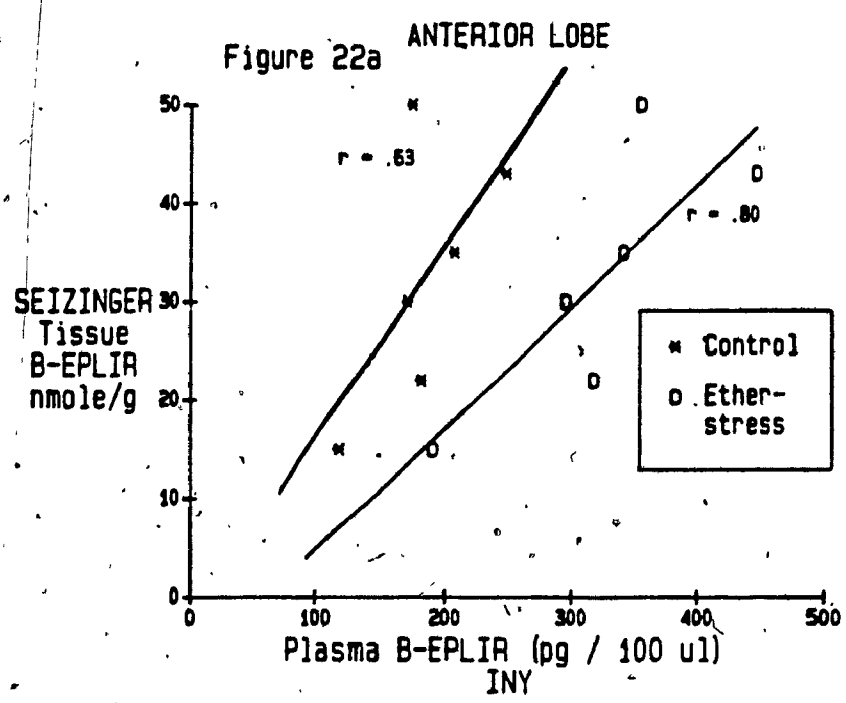
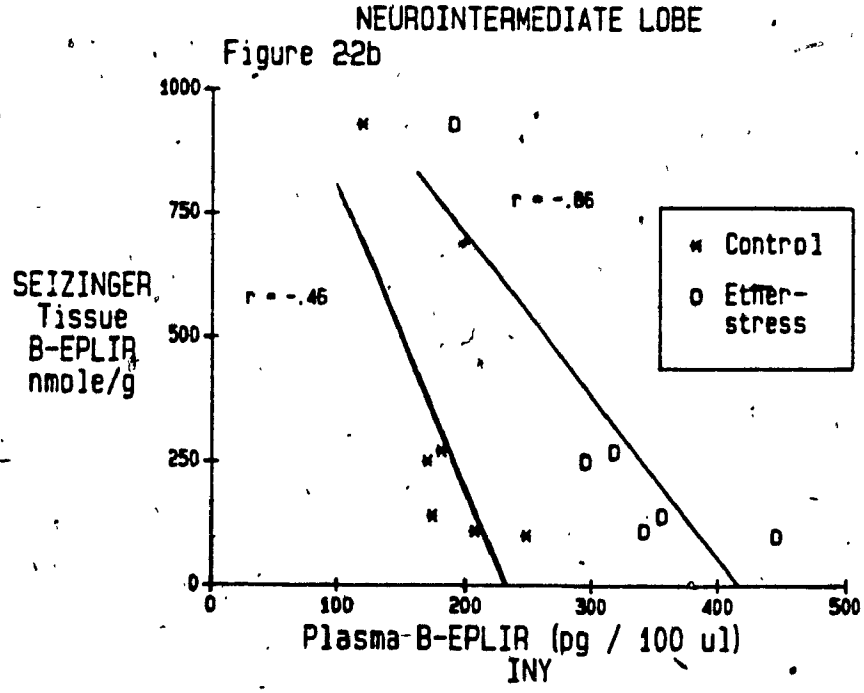


Figure 22b



pituitary is very different from that seen in the anterior pituitary. Concentrations of β-EPLIR in the neurointermediate lobe have been reported to increase dramatically between birth and adulthood (Alessi et al., 1983; Seizinger et al., 1984). Moreover, β-END-like peptides detected in the neurointermediate lobe during this period are predominantly acetylated (Sato and Mains, 1985; Seizinger et al., 1984), and assumed to be inactivate with respect to their opiate-like properties. Interestingly, the concentrations of β-EPLIR observed in the present study correlated strongly in a negative direction with the values reported by Seizinger et al. (1984) for the control, r=-.46, and ether-stressed animals, r=-.86 (see Figure 22b), thereby providing further evidence that the β-END measured here was the non-acetylated form of the peptide released from the anterior lobe of the pituitary. Thus, the developmental pattern in the anterior pituitary best accounts for the data obtained in this study.

The antiserum used in this study also recognizes β-LPH, raising the possibility that the increase in β-EPLIR observed here may have been due to an elevation in the concentration of β-LPH rather than β-END. The ratio of β-LPH/β-END has been reported as being similar in the anterior lobe of newborn and adult rats (Seizinger et al., 1984), however, and equal amounts of β-END and β-LPH have been found in the plasma of adult rats following stressful manipulations (de Souza and Van Loon, 1985). Thus, the elevation in β-LPH/β-END plasma concentrations seems to be at least partially due to the increased release of β-END-sized molecules.

These findings suggest that a major portion of the β-EPLIR measured here is in the biologically-active, non-acetylated form of β-END-(1-31)

released from the anterior pituitary. We are currently examining this question by investigating the exact nature of the B-EPLIR material in the plasma of the neonate. Separation of B-LPH- from the B-END-like material in plasma of control and stressed animals is being conducted using G-75 columns, following Sep-Pak extraction. Radioimmunoassays will then be conducted on the endorphin fraction using antibodies for either total B-EPLIR or acetylated B-END. By subtraction, the results will determine the proportion of acetylated and non-acetylated forms of B-END released in response to stress during development in the rat pup. Following the above discussion, one would expect that the concentration of total B-EPLIR would increase following stress-inducing manipulations, as observed in the present study, while the radioimmunoassay for the acetylated forms of B-END would show little or no increase.

In the mature rat, a-MSH has been reported to be released into the circulation in response to stress (Akil et al., 1985; Khorram et al., 1985). Since the neurointermediate lobe of the pituitary is a major source of this peptide in both the neonatal and adult rat (Sato and Mains, 1985; Usategui et al., 1976), the observation that the plasma concentration of the peptide in 7 day-old pups was not altered following exposure to ether indicates a lack of responsiveness to stress by this region of the pituitary during the early postnatal period. Moreover, since a-MSH is reported to be secreted concomitantly from the neurointermediate lobe with B-END following stress (Akil et al., 1985), this finding provides further evidence that the stress-induced increase in plasma B-EPLIR found here in young pups was indeed of anterior pituitary origin, and not from the neurointermediate lobe.

In the present study, the absence of an a-MSH response to stress by pups occurred despite evidence that control of the neural and intermediate lobes of the pituitary by hypothalamic dopamine neurons is present during the first week of life (Davis, Lichtensteiger, Schlumpf, and Bruinink, 1984). Since projections to the median eminence also originate from cell bodies in the arcuate nucleus, the developmental profile of prolactin (PRL) might also be expected to resemble that of a-MSH. Indeed, similar levels of serum prolactin have been reported during early development in the rat (Dohler and Wuttke, 1975), and no alteration in levels of the peptide has been found in response to stress before the third week of life (Fenske and Wuttke, 1977; Johnston and Negro-Vilar, 1986; Negro-Vilar, 1983; Ojeda, Jameson, and McCann, 1976). The inhibitory dopaminergic tone on PRL secretion has been reported to be present in the neonatal animal (Negro-Vilar, 1983), and DA receptor blockers have been observed to cause PRL release as early as postnatal Day 1 (Becu and Libertun, 1982).

The suppression of ACTH during the early postnatal period appears to be due to exaggerated glucocorticoid negative feedback on pituitary corticotrophes (Sakly and Koch, 1981; Sakly and Koch, 1983). This has been suggested to result from the developmental pattern of the glucocorticoid receptor systems in the pituitary of the young pup (Sapolsky and Meaney, 1986; Walker, Sapolsky, Meaney, Vale, and Rivier, 1986). Throughout the postnatal period, adult-like concentrations of the cytosolic receptor, the predominant glucocorticoid receptor in the rat, exist in the pituitary (Olpe and McEwen, 1976). In contrast, concentrations of pituitary CBG and CBG-like receptors during this period

are low (Sakly and Koch, 1981). In the absence of these secondary receptors, which serve to buffer the cell from corticosterone, the steroid that is present is more likely to be transported into the cell. The result is an amplified corticosterone signal on the pituitary, which in turn serves to suppress ACTH secretion from the gland. Thus, in adrenalectomized neonates, where corticosterone has been removed, there is a profound increase in plasma ACTH in response to stress (Walker, Sapolsky, Meaney, Vale, and Rivier, 1986). Again, it is interesting that a stress-induced increase in B-endorphin is observed during this period of exaggerated glucocorticoid negative feedback, thus underscoring the dissociation in the control over the release of these POMC-derived peptides during the neonatal period.

Glucocorticoids are a central feature of the stress response, as they play an important role in helping the organism to cope with noxious stimuli and assist in the re-establishment of homeostatis. Therefore, the period shortly after birth, during which the H-P-A system of the rat is hyporesponsive to stressors, may appear to be counterintuitive. However, studies on the effects of glucocorticoid administration during the first week of life have pointed to the adaptive value of this mechanism. Although low levels of corticosterone are necessary for the normal development of the animal, high levels of the steroid in the neonate have been found to have growth-inhibiting effects. Thus, glucocorticoid treatment in pups has been reported to lead to permanent reductions in body size (Cotterrell, Balazs, and Johnson, 1972; Howard, 1968; Howard and Benjamins, 1975; Olton, Johnson, and Howard, 1974; Schapiro, 1971), brain weight (Cotterrell et al., 1972; Gumbinas, Oda,

and Huttenlocher, 1973; Howard, 1968; Howard and Benjamins, 1975) and cerebral DNA (Howard, 1968; Howard and Benjamins, 1975). Glucocorticoids have also been found to cause a reduction in brain gangliosides and sulfatides (Howard and Benjamins, 1975), leading to a decrease in neuronal myelination. Suppression of mitotic activity (Cotterrell et al., 1972; Howard, 1968) and impaired immunological response (Schapiro and Huppert, 1967) have also been reported. These biochemical effects have been found to have behavioral consequences, as glucocorticoid-treated rats have been reported to display decreased motor coordination (Howard and Granoff, 1968), as well as delayed startle reflex and swimming development (Schapiro, 1971). Therefore, the stress hyporesponsive period appears to serve an important function by helping to protect the animal against the possible deleterious effects of changing hormone levels during this period of rapid CNS growth and development.

High levels of B-END during the perinatal period have been proposed to serve the function of rendering the animal better able to cope with the trauma of delivery, and helping it adjust to the change of environment by regulating physiological functions such as temperature and respiration (Gianoulakis and Chretien, 1985; Moss et al., 1982). The endorphins have also been proposed to serve a trophic function, although to date the evidence is mostly circumstantial. The strongest support is for an influence of B-END on the development of the CNS, as B-END immunoreactivity has been found within the spinal cord and motor nerves of fetal and postnatal rats and is undetectable beyond the fourth week of life (Haynes, Smith, and Zakarian, 1982), while at the same time

adult-like concentrations of opioid receptors in this region exist (Kirby, 1981). Haynes and Smith (1984) have described the biochemical mechanism for a neurotrophic effect of B-END at the neuromuscular junction, that involves an enhancement of cholinergic transmission through the suppression of acetylcholinesterase activity by B-END. High levels of B-END immunoreactivity and μ-type opioid receptors have also been found in other central and peripheral tissue in the rat pup (Bayon, Shoemaker, Bloom, Moss, and Guillemin, 1979; Kent, Pert, and Herkenham, 1982; Unnerstall, Molliver, Kuhar, and Palacios, 1983). Moreover, the rate of appearance of opiate binding sites in the rat is higher between the mid-fetal stage and postnatal Day 21 (Clendennin, Petraitis, and Simon, 1976; Spain, Roth, and Coscia, 1985), a period during which much neurological development takes place.

Interestingly, there is evidence suggesting that the unperturbable levels of a-MSH during the postnatal period observed in the present study may serve an adaptive function. Since a-MSH has been found to stimulate glucocorticoid secretion during development (Dupouy, 1982; Challis and Torosis, 1977; Llanos et al., 1979), the lack of a-MSH responsiveness to stress may protect the pup from the catabolic effects of high levels of circulating glucocorticoids during a period of accelerated growth and development of the CNS.

Taken together, these findings suggest that selective processing of pituitary hormones occurs that favours the production and release of potentially trophic peptides and suppresses those considered to be neurotoxins to the developing animal. Thus, during the early postnatal period, release of B-END occurs in response to stress, while no

alteration in levels of circulating corticosterone or a-MSH are seen.

Although a neurotrophic function for B-END appears likely, there are also reports that endogenous opiates in the brain may have deleterious effects during development such as inhibition of neural growth, and the delayed appearance of behaviors such as eye-opening (Zagon and McLaughlin, 1983). Moreover, high levels of B-END in the neonate have been reported to depress respiration (Chernick and Craig, 1982), and have been implicated in the etiology of apnea in the human neonate (Hindmarsh and Sankaran, 1985). It should be emphasized, however, that the high concentrations of B-END observed in the present study represent peripheral levels of the peptide, and may therefore not exert such catabolic effects on brain tissue. Clearly, further investigation is needed to determine the function of the stress-induced elevations in B-END during the postnatal period, as well as to determine if B-END cells in the brain are responsive to stress.

Following stress, a nonresponsive period was found with respect to hypothalamic dopamine activity, that corresponds to the quiescence observed in the H-P-A axis. Although exposure to ether vapors resulted in an increase in the concentration of DA in hypothalamic nuclei in animals 21 days of age and older, no significant response was detected during the early postnatal period. Some support for this finding comes from a study by Johnston and Negro-Vilar (1986), who found no change in neuronal dopamine synthesis in the median eminence of Day 13 rats following a 5-minute exposure to ether fumes, although DA metabolism in the area was increased. The lack of stress-responsiveness is adaptive, since DA has been reported to suppress cell mitotic activity in the

pituitary during development (Rychter and Stepien, 1977). This provides further support for the notion that mechanisms exist during development which favour the growth of the organism, by providing an optimal environment within which anabolic processes may take place.

No other consistent stress-induced changes in brain monoamines were found, in spite of the sensitive assay procedure employed, and the age-related changes detected. Alterations in monoamine levels following stress are difficult to detect, as evidenced by the lack of consistency in the literature discussed earlier. Investigations on stress and monoamines have used different stressors, and employed a range of techniques in the analysis of samples. The time course of alterations in levels of the monoamines following stress is also an important factor, and one that varies considerably between investigations. In the present investigation, a period of 5 minutes following the onset of stress was selected, similar to the interval reported in previous studies. In the future, however, systematic investigation should be conducted on the course of stress-induced changes in the monoamine systems, especially in the neonate, if a clearer picture of these systems is to be obtained. It is also possible that the lack of a significant aminergic response to stress in the present study, especially the more robust alterations such as those reported in the noradrenergic system, may be due to a rapid increase in the synthesis of the amines as a result of decapitation, which would serve to mask stress-induced changes (T. Slotkin, personal communication, November 14, 1986). Thus, in future studies, animals shall be injected with a catecholamine-synthesis blocker, such as α-methyl peratyrosine, prior to blood sampling.

The amount of 5-HT in the hippocampus of female pups during the first week of life was found to be almost ten-fold greater than levels seen in older animals of the same sex. Furthermore, a significant decrease in 5-HT levels following ether-stress was seen during this period in females, suggesting increased release of the monoamine. A sex difference in brain 5-HT was also reported by Ladosky and Gaziri (1970), although the time period of the effect differed. They found the amount of 5-HT in whole rat brain was comparable between the sexes until Day 8, rising significantly in females on Day 12. In the present study, levels of 5-HT in the female pups dropped dramatically by Day 14, at which point they approximated the concentration observed in the males. A very different pattern of 5-HT was seen in developing male pups. Serotonin levels were significantly higher on Day 14 as compared to pups at the other ages, although the effect was not as dramatic as that seen for the females.

The striking elevation in 5-HT levels observed in females during the first week of life occurred despite a report by Holets and Cotman (1984) of a lower density of serotonergic fibers innervating the developing hippocampus at this age than in the older animals. Female rats appear to have greater 5-hydroxytryptophan decarboxylase activity than do males neonatally, however, thus possibly accounting for the higher concentration of 5-HT (Hardin, 1973). It is important to note, however, that the concentrations of 5-HIAA in the hippocampus were within the same range for male and female rats, suggesting that although female neonates exhibit higher levels of brain 5-HT than males, the elevated concentrations may not be functionally important.

No sex differences in the concentrations of 5-HT were found in the hypothalamus, which is consistent with the investigation of Watts and Stanley (1984) demonstrating no such differences in 5-HT or 5-HIAA within the hypothalamic-preoptic area and midbrain raphe region of male and female rats throughout development. Concentrations of frontal cortex 5-HT were similar in male and female pups examined, and were found to increase with age.

Some investigators (Chumasov, Konovalov, and Chubakov, 1978; Lauder and Krebs, 1976, 1978a, 1978b) have reported that 5-HT stimulates cell growth and differentiation, and accelerates the formation of neuronal-glial interrelations, myelinization of the axons, and the formation of interneuronal synapses. Depletion is associated with a retardation in brain growth (Hole, 1972; Patel, Bendek, Balazs, and Lewis, 1977), while injection of 5-HT directly into the albumen of incubating eggs has been found to increase the rate of protein synthesis in the brain (Ahmed and Zamenhof, 1978). Interestingly, central mechanisms have been found to favour tryptophan accumulation in the brain during the early life period (Bourgoin et al., 1974), and are thus consistent with the trophic effects of 5-HT.

Thus, in the present study, evidence was found suggesting that the developmental course of monoamines and peptides favour the growth of the animal. Consistent with several earlier reports, no change in plasma corticosterone was observed following stress prior to the second week of life. In contrast, a significant, stress-related increase in plasma β-EPLIR was found in pups during the early postnatal period. These results suggest a dissociation between the adrenocortical and β-END

response to stress during the first week of life. It is further suggested that the β-END measured here was the opioid-active form of the peptide secreted from the anterior pituitary. Support for this hypothesis was provided by the finding that α-MSH, a peptide secreted predominantly from the neurointermediate lobe of the pituitary, was not altered in the plasma of neonates following stress. Examination of monoamines in various brain regions following exposure to ether-stress revealed a suppression of hypothalamic dopamine until the third week of life, when an elevation in dopamine content was observed. Furthermore, age- and sex-related differences in cerebral monoamines were detected, particularly evidence of elevated concentrations of serotonin in the hippocampus of female pups during the first two weeks of life.

The results of this study support the notion that concentrations of peptides and monoamines in the neonate are different from those observed in the adult, and that the neonate responds differently to stressors than animals at older ages. This suggests that the effects of early experience on functioning in later life may not be assumed to be a result of mechanisms similar to those operating in the mature animal. Rather, effects of exposure to stressors during the early postnatal period may be the result of a different pattern of alterations, one that is adaptive in the context of the needs of the growing organism. Further studies are needed in order to elucidate the exact nature of stress on various systems during development.

References

Agrawal, H. C., Glisson, S. N., & Himwich, H. A. (1966). Changes in monomines of rat brain during postnatal ontogeny. Biophysica Acta, 130, 511-513.

Agrawal, H. C., Glisson, S. N., & Himwich, W. A. (1968). Developmental changes in monoamines of mouse brain. International Journal of Neuropharmacology, 7, 97-101.

Akil, H., Watson, S. J., Young, E., Lewis, M. E., Khachaturian, H., & Walker, J.M. (1984). Endogenous opioids: biology and function. Annual Reviews of Neuroscience, 7, 223-255.

Akil, H., Young, E., Watson, S. J. & Coy, D. (1981). Opiate binding properties of naturally occurring N- and C-terminus modified β-endorphin. Peptides, 2, 289-292.

Alessi, N. E., Khachaturian, H., Watson, S., & Akil, H. (1983). Postnatal ontogeny of acetylated and non-acetylated β-endorphin in rat pituitary. Life Sciences, 33, 57-60.

Anisman, H. (1978). Neurochemical changes elicited by stress. In H. Anisman and G. Bignami (Eds.), Psychopharmacology of Aversively Motivated Behavior (pp. 119-172). New York, NY: Plenum Press.

Azmitia, E.C. & McEwen, B.S. (1969). Corticosterone regulation of tryptophan hydroxylase in midbrain of the rat. Science, 166, 1274-1276.

Bartova, A. (1968). Functioning of the hypothalamic-pituitary-adrenal system during postnatal development in rats. General and Comparative Endocrinology, 10, 235-239.

Becu, D. & Libertun, C. (1982) Comparative maturation of the regulation of prolactin and thyrotropin by serotonin and thyrotropin-releasing hormone in male and female rats. Endocrinology, 10, 1879-1885.

Bloom, F., Batterberg, E., Rossier, J., Ling, N., Leppalveto, J., Vargo, T. M., & Guillemin, R. (1977). Endorphins are located in the intermediate and anterior lobes of the pituitary gland, not in the neurohypophysis, Life Science, 20, 43-48.

Bourgoin, S., Faivre-Bauman, A., Benda, P. Glowinski, J., & Hamon, M. (1974). Plasma tryptophan and 5-HT metabolism in the central nervous system of the newborn rat. Journal of Neurochemistry, 23, 319-328.

Bower, A., Hadley, M. E., and Hruby, V. J. (1974). Biogenic amines and control of melanophore-stimulating hormone release. Science, 184, 70-72.

Bradford, M. M. (1976). A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry, 72, 248-254.

Briski, K. P., Quigley, K., & Meites, J. (1984). Counteraction by morphine of stress-induced inhibition of growth hormone release in the rat. Proceedings of the Society for Experimental Biology and Medicine, 177, 137-142.

Butte, C., Kakihana, R., Farnham, M. L., & Noble, E. P. (1973). The relationship between brain and plasma corticosterone stress in developing rats, Endocrinology, 92, 1775-1779.

Chatelain, A., Dupouy, J. P., & Dubois, M. P. (1979). Ontogenesis of cells producing polypeptide hormones (ACTH, MSH, LH, GH, prolactin) in the fetal hypophysis of the rat: influence of the hypothalamus. Cell and Tissue Research, 196, 409-427.

Chen, C. L., Dionne, F. T., & Roberts, J. L. (1983). Regulation of the pro-opiomelanocortin mRNA levels in rat pituitary by dopaminegic compounds. Proceeding of the National Academy of Sciences, U.S.A., 80, 2211-2215.

Chumasov, E. L., Chubakov, A. R., Konovalov, G. V., and Gromova, E. A. (1984). Effect of noradrenaline on growth and differentiation of explanted hippocampal cells. Neuroscience, Behavior and Physiology, 14, 7-14.

Clendennin, N.J., Petraitis, J. M., & Simon, E. J. (1976). Ontological development of opiate receptors in rodent brain. Brain Research, 118, 157-160.

Cohen, A. (1976). Adrenal and plasma corticosterone levels in the pregnant, foetal, and neonatal rat in the perinatal period. Hormone and Metabolic Research, 8, 474-478.

Cote, T. E., & Yasumura, S. (1975). Effect of ACTH and histamine stress on serum corticosterone and adrenal cyclic AMP levels in immature rats. Endocrinology, 96, 1044-1047.

Cotterrell, M., Balazs, R., & Johnsson, A. L. (1972). Effects of corticosteroids on the biochemical maturation of rat brain: Postnatal cell formation. Journal of Neurochemisty, 19, 2151-2167.

Coyle, J. T. & Axelrod, J. (1971). Development of the uptake and storage of L-[^{3}H] NE in the rat brain. Journal ofNeurochemistry, 18, 2061-2075.

Coyle, J. T. & Axelrod, J. (1972). Tyrosine hydroxylase in rat brain: Developmental characteristics. Journal of Neurochemistry, 19, 1117-1123.

Coyle, J.T. & Henry, D. (1973). Catecholamines in fetal and newborn rat brain. Journal of Neurochemistry, 21, 61-67.

Crine, P., Gianoulakis, C., Seidah, N. Y., Gossard, F., Pezzalla, P. D., Lis, M., & Chretien, M. (1978). Biosynthesis of ß-endorphin from ß-lipotropin and a larger molecular weight precursor in rat pars intermedia. Proceedings of the National Academy of Sciences, U.S.A., 75, 4719-4723.

Dahlstrom, A. & Fuxe, K. (1964). Evidence for the existence of monoamine-containing neurons in the central nervous system. Acta Physiologica Scandinavia, 62, 1-55.

Dallman, M. F., & Yates, F. E. (1969). Dynamic assymmetries in the corticosteroid feedback path, and distribution, metabolism, and binding elements of the adrenal-cortical system. Annals of the New York Academy of Sciences, 156, 696-721.

Davis, M. D., Lichtensteiger, W., Schlumpf, M., & Bruinink, A. (1984). Early postnatal development of pituitary intermediate control in the rat by dopamine neurons. Neuroendocrinology, 39, 1-8.

Deakin, J. F., Dostrovsky, J. O., and Smyth, D. G. (1980). Influence of N-terminal acetylation and C-terminal proteolysis on the analgesic activity of ß-endorphin. Biochemistry Journal, 189, 501-506.

de Souza, E. B. & Van Loon, G. R. (1985). Differential plasma β-endorphrin, β-lipotropin, and adrenocorticotropin responses to stress in rats. Endocrinology, 116, 1577-1586.

de Souza, E. B. & Van Loon, G. R. (1986). Brain serotonin and catecholamine responses to repeated stress in rats. Brain Research, 367, 77-86.

Dohler, K. D., & Wuttke, W. (1975). Changes with age in levels of serum gonadotrapins, prolactin, and gonadal steroids in prepubertal male and female rats. Endocrinology, 97, 898-907.

Dunn, A. J., & File, S. E. (1983). Cold restraint alters dopamine metabolism in frontal cortex, nucleus accumbens and neostriatum. Physiology and Behavoir, 31, 511-513.

Dupouy, J. P. (1980). Differentation of MSH, ACTH, endorphin and LPH-containing cells in the hypophysis during embryonic and fetal development. International Review of Cytology, 68, 197-249.

Dupouy, J.P. (1982). Responses of rat fetal adrenals to synthetic adrenocorticotropic hormone and a-melanocyte-stimulating hormone: In vivo and in vitro studies. Journal of Endocrinology, 92, 23-28.

Eberwine, J. H. & Roberts, J. L. (1984). Glucocorticoid regulation of pro-opiomelanocortin gene transcription in the rat pituitary. Journal of Biological Chemistry, 259, 2166-2170.

Eipper, B. A. & Mains, R. E. (1981). Further analysis of post-translational processing of β-endorphin in rat intermediate pituitary. Journal of Biological Chemistry, 256, 5689-5695.

Fadda, F., Argiolas, A., Melis, M. R., Tissari, A. H., Onali, P. L., & Gessa, G.L. (1978). Stress-induced increase in 3, 4-dihydroxyphenylacetic acid (DOPAC) levels in the cerebral cortex and in neucleus accumbens: Reversal by diazepam. Life Sciences, 23, 2219-2224.

Farah, J. M., Malcolm, D. S., & Mueller, G. P. (1982). Dopaminergic inhibition of pituitary B-endorphin-like immunoreactivity secretion in the rat. Endorcrinology, 110, 657-659.

Fenske, M., & Wuttke, W. (1977). Development of stress-induced pituitary prolactin and TSH release in male rats. Acta Endocrinologia, Copenhagen, 85, 729-735.

Fuxe, K., Andersson, K., Eneroth, P., Siegel, R. A., & Agnati, L. F. (1983). Immobilization stress-induced changes in discrete hypothalamic catecholamine levels and turnover, their modulation by nicotine and relationship to neuroendocrine function. Acta Physiologica Scandinavia, 117, 421-426.

Fuxe, K., Hokfelt, T., & Ungerstedt, U. (1970). Morphological and functional aspects of central monomine neurons. International Reviews in Neurobiology, 13, 93-126.

Fuxe, K. & Johnsson, G. (1974). Further mapping of central hydroxytryptamine neurons: Studies with the neurotoxic dihydrotryptamines. In E. Costa, G. G. Gessa & M. Sandler (Eds.), Advances in Biochemical Psychopharmacology (Vol. 10, pp. 1-12). New York, NY: Raven Press.

Ganong, W. F. (1984). Neurotransmitter mechanisms underlying stress responses. In G.H., Koslow, S. H., & S. Reichlin (Eds.), Neuroendocrinology and Psychiatric Disorder (pp. 133-143). New York: Raven Press.

Gianoulakis, C., Drouin, J. N., Seidah, N. G., Kalant, H. & Chretien, M. (1981). Effect of chronic morphine treatment on B-endorphin biosynthesis by the rat neurointermediate lobe. European Journal of Pharmacology, 72, 313-321.

Gianoulakis, C. & Chretien, M. (1985). Endorphins in fetomaternal physiology. In N. Gleicher (Ed.), Principles of Medical Therapy in Pregnancy (pp. 162-171). New York: Plenum Medical Book Company.

Gianoulakis, C. N., Woo, N., Drouin, J. N., Seidah, N. G., Kalant, H., & Chretien, M. (1981). Effect of chronic morphine treatment of B-endorphin. Journal of Pharmacology, 72, 313-318.

Glavin, G., Tanaka, M., Tsuda, A., Kohno, Y., & Nagasaki, M. (1983). Regional rat brain noradrenaline turnover in response to restraint stress. Pharmacology, Biochemistry, & Behavior, 19, 287-290.

Govoni, S., Pasinetti, G., Inzoli, M. R., Rozzini, R., & Trabucchi, M. (1984). Correlation between beta-endorphin/beta-lipotropin-immunoreactivity and cortisol plasma concentrations. Life Sciences, 35, 2549-2552.

Gray, P. (1971). Pituitary-adrenocortical response to stress in the neonatal rat. Endocrinology, 89, 1126-1128.

Guillemin, R., Vargo, T., Rossier, J., Minick, S., Ling, W., Rivier, C., Vale, W., & Bloom, F. (1977). B-endorphin and adrenocorticotropin are secreted concomitantly by the pituitary gland. Science, 197, 1367-1369.

Guillet, R. & Michaelson, S.M. (1978). Corticotropin responsiveness in the neonatal rat. Neuroendocrinology, 27, 119-125.

Guillet, R., Saffran, M., & Michaelson, S. M. (1980). Pituitary-adrenal response in neonatal rats. Endocrinology, 106, 991-994.

Gumbinas, M., Oda, M., & Huttenlocher, P. (1973). The effects of corticosteroids on myelination of the developing rat brain. Biology of the Neonate, 23, 355-361.

Guttmann, S.T. & Boissonnas, R.A. (1961). Influence of the structure of the N-terminal extremity of a-MSH on the melanophore-stimulating activity of this hormone. Experientia, 17, 265-267.

Hadley, M.E. (1984). Endocrinology. New Jersey: Prentice-Hall.

Hardin, C. M. (1973). Sex differneces and the effects of testosterone injections on biogenic amine levels of neonatal rat brain. Brain Research, 62, 286-290.

Hary, L., Dupouy, J. P., & Chatelain, A. (1981). Pituitary response to bilateral adrenalectomy, metyrapone treatment and other stress in the newborn rat. Biology of the Neonate, 34, 28-36.

Haynes, L. W., & Smith, M.E. (1984). The actions of pro-opiomelanocortin peptides at the developing neuromuscular junction. Trends in Pharmacological Science, 6, 165-168.

Haynes, L. W., Smyth, D. G., and Zakavian, S. (1982). Immunocytochemical localization of β-endorphin (lipotropin C-fragment) in the developing rat spinal cord and hypothalamus. Brain Research, 232, 115-128.

Hedge, G. A., Van Ree, J. M., & Versteeg, D.H. (1976). Correlation between hypothalamic catecholamine synthesis & ether-stress-induced ACTH secretion. Neuroendocrinology, 21, 236-246.

Henning, S. J. (1978). Plasma concentrations of total and free corticosterone during development in the rat. Annual Journal of Physiology, 235, 451-456.

Hindmarsh, K. W. & Sankaran, K. (1985). Endorphins and the neonate (editorial). Canadian Medical Assosication Journal, 132, 331-334.

Hiroshige, T. & Sato, T. (1970). Changes in hypothalamic content of corticotropin-releasing activity following stress during neonatal maturation in the rat. Neuroendocrinology, 7, 257-270.

Hole, K. (1972). The effects of cyproheptadine, methysergide, BC 105 and reserpine on 5-hydroxytryptamine and brain growth. European Journal of Pharmacology, 19, 156-159.

Holets, V. R. & Cotman, C. W. (1984). Postnatal development of the serotonin innervation of the hippocampus and dentate gyrus following raphe implants. Journal of Comparative Neurology, 226, 457-476.

Hollt, V. & Bergmann, M. (1982). Effects of acute and chronic haloperidol treatment on the concentrations of immunoreactive β-endorphin in plasma, pituitary and brain of rats. Neuropharmacology, 21, 147-154.

Howard, E. (1968). Reductions in size and total DNA of cerebrum and cerebellum in adult mice after corticosterone treatment in infancy. Experimental Neurology, 22, 191-208.

Howard, E. & Benjamins, J. A. (1985). DNA, ganglioside and sulfatide in brains of rats given corticosterone in infancy, with an estimate of cell loss during development. Brain Research, 92, 73-87.

Howard, E. & Granoff, D.M. (1968). Increased voluntary running and decreased motor coordination in mice after neonatal corticosterone implantation. Experimental Neurology, 22, 661-673.

Hunter, W. M. & Greenwood, F. C. (1962). Preparation of Iodine-131 labelled human growth hormone of high specific activity. Nature, 194, 495-496.

Iimori, K., Tariaka, M., Kohno, Y., Ida, Y., Nakagawa, R., Hoaki, Y., Tsuda, A., & Nagasaki, N. (1982). Psychological stress enhances noadrenaline turnover in specific brain regions in rats. Pharmacology, Biochemistry, & Behavior, 16, 637-640.

Jackson, S. & Lowry, P.J. (1980). Distribution of adrenocorticotrophic and lipotrophic peptides in the rat. Journal of Endocrinology, 86, 205-219.

Johnston, C. A., Demarest, K. T., & Moore, K. E. (1984). 5-hydroxytryptamine synthesis and metabolism in discrete nuclei of the rat brain during surges of prolactin associated with restraint stress or suckling. Neuroendocrinology, 38, 117-122.

Johnston, C. A., Negro-Vilar, A. (1986). Maturation of the prolactin and proopiomelanocortin-derived peptide responses to other stress and morphine: Neurochemical analysis. Endocrinology, 118, 797-804.

Johnston, C. A., Spinedi, E. J., & Negro-Vilar, A. (1985). Effect of acute ether stress on monoamine metabolism in median eminence and discrete hypothalamic nuclei of the rat brain and on anterieur pituitary hormone secretion. Neuroendocrinology, 41, 83-88.

Jones, M. T., Brush, F. R., & Neame, R. L. B. (1972). Characteristics of fast feedback control of corticotropin release by corticosteroids. Journal of Endocrinology, 55, 489-497.

Karki, N., Kuntzman, R., & Brodie, B. B. (1960). Norepinepherine and serotonin brain levels at various stages of ontogenetic development. Federation Proceedings, 19, 282.

Kartesi, M., Palkovits, M., Kiss, J. Z., Kanyicska, B., Fekete, M. I., & Stark, E. (1981). Lack of correlation between hypothalamic serotonin and the ether-induced ACTH secretion in adrenalectomized rats. Neuroendocrinology, 32, 7-13.

Kastin, A. J., Schally, A. V., Viosca, S., & Miller, C. M. (1969). MSH activity in plasma and pituitary of rats after various treatments. Endocrinology, 84, 20-27.

Keller-Wood, M. E. & Dallman, M. F. (1984). Corticosteroid inhibition of ACTH secretion. Endocrine Reviews, 5, 1-24.

Kennett, G. A. & Joseph, M. J. (1981). The functional importance of increased brain tryptophan in the serotonergic response to restraint stress. Neuroendocrinology, 20, 39-43.

Khachaturian, H., Alessi, N. E., & Lewis, M. E. (1983). Ontogeny of opioid and related peptides in the rat brain and pituitary. Life Sciences, 33, 61-64.

Khachaturian, H., Alessi, N. E., Munfakh, N., & Watson, S. J. (1983). Ontogeny of opioid and related peptides in the rat CNS and pituitary: An immunocytochemical study. Life Sciences, 33, 61-64.

Khorram, O., Bedran De Castro, J. C. & McCann, S. M. (1984). The physiological role of a-melanocyte-stimulating hormone (a-MSH) in modulating the secretion of prolactin (Prl) and luteinizing hormone (LH) in the female rat. Proceedings of National Academy of Science, U.S.A., 81:, 8004-8008.

Kirby, M. L. (1982). Ontogeny of opiate receptors in rat forebrain: Visualization by in vitro autoradiography. Developmental Brain Research, 2, 487-504.

Knott, P. J., Joseph, M. H., & Curzon, G. (1973). Effects of food deprivation and immobilization on tryptophan and other amino acids in rat brain. Journal of Neurochemistry, 20, 249-251.

Kraicer, J., Beraud, G., & Lywood, D. W. (1977). Pars intermedia ACTH and MSH content: Effect of adrenalectomy, gonadectomy, and a neurotropic (noise) stress. Neuroendocrinology, 23, 352-367.

Krey, L., Lu, K., Butler, W., Hotchkiss, J, Piva, F., & Knobil, E. (1975). Surgical disconnections of the medial basal hypothalamus and pituitary function in the rhesus monkey: II. GH and cortisol secretion. Endocrinology, 96, 1088-1096.

Krieger, D. T., Liotta, A. S., Brownstein, M. J., & Zimmerman, E. A. (1980). ACTH, B-lipotropin and related peptides in brain, pituitary and blood. Recent Progress in Hormone Research, 36, 277-336.

Kuypers, H. G. & Marsky, V. A. (1975). Retrograde axonal transport of horseradish peroxidase from spinal cord to brain stem cell groups in the cat. Neuroscience Letters, 1, 9-14.

Ladowsky, W. & Gaziri, L. C. (1970). Brain serotonin and sexual differentiation of the nervous system. Neuroendocrinology, 6, 168-174.

Lauder F. M. & Krebs, H. (1976). Effects of p-chlorophenylalanine on time of neuronal orgin during embryogenesis in the rat. Brain Research, 107, 638-644.

Lauder J. M. & Krebs, H. (1978). Serotonin and early neurogenisis. In A. Vernadakis, E. Giacobini, and G. Filogame (Eds.), Maturation of Neurotransmission (pp. 171-180). Basel: Karger.

Lauder, J. M. & Krebs, H. (1978). Serotonin as a differentiation signal in early neurogenesis. Developmental Neuroscience, 1, 15-30.

Laveille, S., Tassin, J. P., Thierry, A. M., Blanc, G., Herve, D., Barthelemy, C., & Glowinski, J. (1979). Blockade by benzodiazepines of the selective high increase in dopamine turnover induced by stress in mesocortical dopaminergic neurons of the rat. Brain Research, 168, 585-594.

Leenders, H. J., Janssens, J. J., Theunissen, H. J., Jenks, B. G., & Van Overbeeke, A. P. (1986). Acetylation of melanocyte-stimulating hormone and β-endorphin in the pars intermedia of the perinatal pituitary gland in the mouse. Neuroendocrinology, 43, 166-174.

Lehnert, H., Reinstein, D. K., Strowbridge, B. W., & Wurtman, R. J. (1984). Neurochemical and behavioral consequences of acute, uncontrollable stress: Effects of dietary tyrosine. Brain Research, 303, 215-223.

Lim, A.T. & Funder, J.W. (1983). Stress-induced changes in plasma, pituitary and hypothalamic immunoreactive β-endorphin: Effects of diunal variation, adrenalectomy, corticosteroids, and opiate agonists and antagonists. Neuroendocrinology, 36, 225-234.

Lindvall, O. & Bjorklund, A. (1974). The organization of the ascending catecholamine neuron systems in the rat brain as revealed by the glyoxylic acid fluorescence method. Acta Physiologica Scandinavia, 412, 1-48.

Liotta, A. S., Suda, T., & Krieger, D. T. (1978). β-lipotropin is the major opioid-like peptide of human pituitary and rat pars distalis: Lack of significant β-endorphin. Proceedings of the National Academy of Sciences, U.S.A., 75, 2950-2954.

Llanos, A., Ramachadran, J., Creasey, R. K., Rudolf, A. M., Seron-Ferre, M. (1979). a-Melanocyte-stimulating hormone and adrenocorticotropin in the regulation of glucocorticoid secretion during the perinatal period in sheep. Endocrinology, 105, 613-617.

Mains, R. E. & Eipper, B. A. (1979). Synthesis and secretion of corticotropins, melanotropins and endorphins by rat intermediate pituitary cells. Journal of Biological Chemistry, 254, 7885-7894.

Mains, R. E., & Eipper, B.A. (1981). Differences in the post-translational processing of β-endorphin in rat anterior and intermediate pituitary. Journal of Biological Chemistry, 256, 5683-5688.

Mains, R. E., Eipper, B. A., & Ling, N. (1977). Common precursor to corticotropin and endorphins. Proceedings of the National Academy of Sciences, U.S.A., 74, 3014-3018.

Martin, C. E., Cake, M. H., Hartmann, P. E. & Cook, I. F. (1977). Relationship between foetal corticosteroids, maternal progesterone and parturition in the rat. Acta Endocrinologia, 84, 167-176.

Meaney, M. J., Sapolsky, R. M., & McEwen, B. S. (1985) The development of the glucocorticoid receptor system in the rat limbic brain. I. Ontogeny and autoregulation. Developmental Brain Research, 18, 159-164.

Milkovic, K. & Milkovic, S. (1963). Functioning of the pituitary-adrenocortical axis in rats at and after birth. Endocrinology, 73, 535-539.

Milkovic, K. & Milkovic, S. (1967). Studies on the pituitary-adrenocortical system in the fetal rat. Endocrinology, 71, 799-802.

Milkovic, S. amd Milkovic, K. (1969). Responsiveness of the pituitary-adrenocortical system during embryonic and early postnatal periods of life. In S. Bajusz (Ed.), Physiology and Pathology of Adaptation Mechanisms (pp. 28-47). Oxford: Permagon Press.

Moss, I. R., Conner, H., Yee, W. F., Iorio, P. & Scarpelli, E. M. (1982). Human β-endorphin-like immunoreactivity in the perinatal/neonatal period. Clinical and Laboratory Observations, 101, 443-446.

Mueller, G. P. (1981). β-Endorphin immunoreactivity in rat plasma: Variations in response to different physical stimuli. Life Sciences, 29, 1668-1674.

Munck, A., Guyre, P. M., & Holbrook, N. J. (1984). Physiological functions of glucocorticoids in stress and their relation to pharmacological actions. Endocrine Reviews, 5, 25-44.

Negro-Vilar, A. (1983) Maturation of stress-induced prolactin release in male rats: involvement of the tuberohypophyseal dopaminergic system. Neuroendocrinology Letters, 8, 79.

Newman, C. B., Wardlaw, S. L., & Frantz, A. G. (1985). Suppression of basal and stress-induced prolactin release and stimulation of luteinizing hormone secretion by a-MSH. Life Sciences, 36, 1661.

Nygren, L. G. & Olson, L. (1977). A new major projection from locus coeruleus: The main source of noradrenergic nerve terminals in the ventral and dorsal columns of the cord. Brain Research, 132, 85-94.

O'Donohue, T. L., Handelmann, G. E., Chaconas, T., Miller, T. S., & Jacobowitz, D.M. (1981). Evidence that N-acetylation regulates the behavioral activity of a-MSH in the rat and human central nervous systems. Peptides, 2, 333-344.

Ojeda, S. R., Jameson, H. E., & McCann, S. M. (1976). Plasma prolactin levels in maturing intact and cryptorchid male rats: Development of the stress response. Proceedings of the Society for Experimental Biology and Medicine, 151, 310.

Olpe, H., and McEwen, B. S. (1976). Glucocorticoid binding to receptor-like proteins in rat brain and pituitary: ontogenetic and experimentally-induced changes. Brain Research, 105, 121-126.

Olson, L. & Fuxe, K. (1972). Further mapping out of central noradrenaline systems: Projections of the "subcoeruleus" area. Brain Research, 43, 289-295.

Olton, D. S., Johnsson, C. T., & Howard, E. (1974). Impairment of conditioned active avoidance in adult rats given corticosterone in infancy. Developmental Psychology, 8, 55-61.

Palkovits, M., Kobayashi, R. M., Kizer, J. S., Jacobowitz, D. M., & Kopin, I. J. (1975). Effects of stress on catecholamines and tyrosine hydroxylase activity of individual hypothalamic nuclei. Neuroendocrinology, 18, 144-153.

Palkovits, M. (1980). Guide and Map for the Isolated Removal of Rat Brain Areas. Budapest: Academic Press.

Palkovits, M. & Brownstein, M. J. (1983). Microdissection of brain areas by the punch technique. In A. C. Cuello (Ed.), Brain Microdissection Techniques (pp. 1-36). New York: John Wiley and Sons.

Panerai, A. E., Martini, A., Di Giulio, A., Fraioli, F., Vegni, C., Pardi, G., Marini, A., & Mantegazza, P. (1983). Plasma ß-endorphin, ß-lipotropin, and met-enkephalin concentrations during pregnancy in normal and drug-addicted women and their newborn. Journal of Clinical Endocrinology and Metabolism, 57, 537-543.

Patel, A. J., Bendek, G., Balazs, R., and Lewis, P. D. (1977). Effect of reserpine on cell proliferation in the developing rat brain. Neuroscience, 4, 139-143.

Pelletier, G., Leclerc, R., Labrie, F., Cote, J., Chretien, M., & Lis (1977) Immunohistochemical localization of B-lipotropin hormone in the pituitary gland. Endocrinology, 100, 770-776.

Plotsky, P.M. (1985). Hypophysiotropic regulation of adenohypophyseal adrenocoticotropin secretion. Federation Proceedings, 44, 207-213.

Proulx-Ferland, L., Labrie, F., Dumont, D., Cote, J., Coy, D. H., & Sveiraf, J. (1982) Corticotropin-releasing factor stimulates secretion of melanocyte stimulating hormone from the rat ptiuitary. Science, 217, 62-63.

Reinhard, J. F., Bannon, M. J., & Roth, R. H. (1982). Acceleration by stress of dopamine synthesis and metabolism in prefrontal cortex: Antagonism by diazepam. Archives of Pharmacology, 318, 374-377.

Reinstein, D. K., Lehnert, H., Scott, N. A., & Wurtman, R. J. (1984). Tyrosine prevents behavioral and neurocheminal correlates of an acute stress in rats. Life Sciences, 34, 2225-2231.

Renner, K. J. & Luine, V. N. (1984). Determination of monoamines in brain nuclei by high performance liquid chromatography with electrochemical detection: Young versus middle-aged rats. Life Sciences, 34, 2193-2199.

Rosa, P. A., Policastro, P., & Herbert, E. (1980). A cellular basis for the differences in regulation of synthesis and sectretion of ACTH/endorphin peptides in anterior and intermediate lobes of the pituitary. Journal of Experimental Biology, 89, 215-237.

Rudman, D., Chawla, R. K., & Hollins, B. M. (1979). N, o-diacetyl-serine a-melanocyte-stimulating hormone, a naturally occurring melanotropic peptide. Journal of Biological Chemistry, 254, 10102-10108.

Rychter, J. & Stepien, H. (1977). Effects of pimozide and bromocriptine on the proliferation of rat pituitary pars intermedia cells. Journal of Endocrinology, 75, 443-444.

Sakly, M. & Koch, B. (1981). Ontogenesis of glucocorticoid receptors in anterior pituitary gland: Transient dissociation among cytoplasmic receptor density, nuclear uptake, and regulation of corticotropic activity. Endocrinology, 108, 541-596.

Sakly, M. & Koch, B. (1983). Ontogenetical variations of transcortin modulate glucocorticoid receptor function and corticotropic activity in the pituitary gland. Hormone and Metabolic Research, 15, 92-96.

Sapolsky, R. M. & Meaney, M. J. (1986). The maturation of the adrenocortical stress response in the rat. Brain Research Reviews, 11, 65-76.

Sato, S. M. & Mains, R. E. (1985). Post-translational processing of proadrenocorticotropin/endorphin-derived peptides during postnatal development in the rat pituitary. Endocrinology, 117, 773-786.

Sauter, A. M., Baba, Y., Stone, E. A., & Goldstein, M. (1978). Effects of stress and of phenylethanolamine N-methyltransferase inhibition on central norepinephrine levels. Brain Research, 144, 415-419.

Schapiro, S. (1962). Pituitary ACTH and compensatory adrenal hypertrophy in stress non-responsive infant rats. Endocrinology, 71, 986-989.

Schapiro, S. (1971). Influence of hormones and environmental stimulation on brain development. In D. H. Ford (Ed.), Influence of hormones on the nervous system. Proceedings of the International Society of Psychoneuroendocrinology (pp.63-73). Karger: Basal.

Schapiro, S., Geller, E., & Eiduson, S. (1962). Neonatal adrenal cortical response to stress and vasopressin. Proceedings of the Society for Experimental Biology and Medicine, 109, 937-941.

Schapiro, S. & Huppert, M. (1967). Neonatal cortisol administration and immunological impairment in the adult rat. Proceedings of the Society for Experimental Biology and Medicine, 124, 744-746.

Schultzberg, M., Hokfelt, T., Lundberg, J., Terenius, L., Elfirm, L. G., & Elde, R. (1978). Enkephalin-like immunoreactivity in nerve terminals in sympathetic ganglia and adrenal medulla and in adrenal medullary gland cells. Acta Physiologica Scandinavia, 103, 475-477.

Schwartzberg, D. G. & Nakane, D. K. (1982). Ontogenesis of adrenocorticotropin-related peptide determinants in the hypothalamus and pituitary gland of the rat. Endocrinology, 110, 855-864.

Seggie, J. A. & Brown, G. M. (1975). Stress response patterns of plasma corticosterone, prolactin and growth hormone in the rat, following handling or exposure to novel environment. Canadian Journal of Physiology and Pharmacology, 5, 629-637.

Seizinger, B. R., Hollt, V., & Herz, A. (1984). Postnatal development of β-endorphin-related peptides in rat anterior and intermediate pituitary lobes: Evidence for contrasting development of pro-opiomelanocortin processing. Endocrinology, 115, 136-142.

Selye, H. (1950). The Physiology and Pathology of Exposure to Stress. Montreal: Acta.

Smyth, D. G., Massey, D. E., Zakarian, S., & Finnie, M. D. (1979). Endorphins are stored in biologically active and inactive forms: Isolation on alpha-N-acetyl peptides. Nature, 279, 252-254.

Smythe, G. A., Bradshaw, J. E., & Vining, R. F. (1983). Hypothalamic monoamine control of stress-induced ACTH release in the rat. Endocrinology, 113, 1062-1071.

Spain, J. W., Roth, B. L., and Coscia, C. J. (1985). Differential ontogeny of multiple opioid receptors. Journal of Neuroscience, 5, 584-588.

Stone, E.A. (1975). Stress and catecholamines. In A.J. Friedhoff (Ed.), Catecholamines and Behavior (Vol. 2, pp. 31-72). New York, N.Y.: Plenum Press.

Tanaka, M., Kohno, Y., Tsuda, A., Nakagawa, R., Ida, T., Iimori, K., Hoaki, Y., & Nagasaki, N. (1983). Differential effects of morphine on noradrenaline release in brain regions of stressed and non-stressed rats. Brain Research, 275, 105-115.

Unnerstall, J. R., Molliver, M. E., Kichar, M. J., Palacios, J. M. (1983). Ontogeny of opiate binding sites in the hippocampus, olfactory bulb and other regions of the rat forebrain by autoradiographic methods. Brain Research, 283, 159-169.

Usategui, R., Oliver, C., Vaudry, H., Lombardi, G., Rozenburg, I, & Mourre, A. (1976). Immunoreactive a-MSH and ACTH levels in the rat plasma and pituitary. Endocrinology, 98, 189-196.

Vale, W., Rivier, C., Yang, L., Minick, S., & Guillemin, R. (1978). Effects of purified hypothalamic corticostropin-releasing factor and other substances on the secretion of adrenocorticotropin and β-endorphin-like immunoreactivities in vitro. Endocrinology, 103, 1910-1915.

Vale, W., Speiss, J., Rivier, C., & Rivier, J. (1981). Characterization of a 41-residue ovine hypothalamic peptide that stimulates the secretion of corticotropin and β-endorphin. Science, 213, 1394-1397.

Vermes, I., Mulder, G. H., Smelik, P. G., & Tilders, F.J. (1980). Differential control of β-endorphin/β-lipotropin secretion from anterior and intermediate lobes of the rat pituitary gland in vitro. Life Sciences, 27, 1761-1768.

Vinson, G. P., Whitehouse, B. J., & Thody, A. J. (1980). a-MSH at physiological concentrations stimulates "late pathway" steroid products in adrenal zona glomerulosa cells from sodium restricted rats. Peptides, 2, 141-144.

Voight, K. H., Weber, E., Fehm, H. L., & Martin, R. (1984). The concomitant storage and simultaneous release of ACTH and β-endorphin. In W. Wuttke, A. Weindl, K. H. Voight, & R. R. Dries (Eds.), Brain and Pituitary Peptides (pp. 54-67). Basel: Karger.

Walker, C. D., Sapolsky, R. M., Meaney, M. J., Vale, W. W., & Rivier, C.L. (1986) Increased pituitary sensitivity to glucocorticoid feedback during the stress non-responsive period in the neonatal rat. Endocrinology, 119, 1816-1821.

Walker, C. D., Perrin, M., & Rivier, C. (1985). Maturation of the hypothalamic-pituitary-adrenal axis in the neonatal rat. Endocrine Society Abstracts, Baltimore.

Walker, C. D., Perrin, M., Vale, W., & Rivier, C. (1986). Ontogeny of the stress response in the rat: Role of the pituitary and the hypothalamus. Endocrinology, 118, 1445-1451.

Wapnir, R. S., Hawkins, R. L., & Stevenson, J. H. (1971). Ontogenesis of phenylalanine and tryptophan hydroxylation in brain and liver. Biology of the Neonate, 18, 85-93.

Ward, I. L. & Weisz, J. (1984). Differential effects of maternal stress on circulating levels of corticosterone, progesterone, and testosterone in male and female rat fetuses and their mothers. Endocrinology, 114, 1635-1644.

Wardlaw, S. L., Smeal, M. M., & Markowitz, C. E. (1986). Antagonism of β-endorphin-induced prolactin release by melanocyte-stimulating hormone and corticotropin-like intermediate lobe peptide. Endocrinology, 119, 112-118.

Weber, E., Martin, R., & Voigt, K. H. (1979). Corticotropin/endorphin precursor: Concomitant storage of its fragments in the secretory granules of anterior pituitary corticotropin/adrenocorticotropin cells. Life Sciences, 25, 1111-1118.

Young, W. S., Wamsley, J. K., Zarbin, M. A., & Kuhar, M. J. (1980). Opiate receptors undergo axonal flow. Science, 210, 76-77.

Zagon, I. S. & McLaughlin, P. J. (1983). Increased brain size and cellular content in infant rats treated with an opiate antagonist. Science, 221, 1174-1180.