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The Effects of Corticosterone Treatment in Long-Evans Rats on Spatial  
Learning, Synaptic Plasticity and Hippocampal Neuropathology

Shari Ruth Bodnoff

A Thesis  
in  
The Department  
of  
Psychology

Presented in Partial Fulfillment of the Requirements for the Degree of  
Doctor of Philosophy at  
Concordia University  
Montreal, Quebec, Canada

March 1993

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## Abstract

### The Effects of Corticosterone Treatment in Long-Evans Rats on Spatial Learning, Synaptic Plasticity and Hippocampal Neuropathology.

Shari Ruth Bodnoff, PhD.  
Concordia University, 1993.

It has been previously demonstrated that three months exposure to high physiological levels of corticosterone in young rats resulted in a pattern of hippocampal neuron loss similar to that observed in aged rats (Sapolsky, Krey and McEwen, 1985). The purpose of the present experiments was to examine the behavioral consequences of this treatment. In a series of experiments, young adult or mid-aged Long-Evans rats were treated for either one or three months with corticosterone, sufficient to mimic the elevated corticosterone levels seen in aged rats. Following the termination of the treatment period, the rats were tested in the Morris water maze (a task that is sensitive to hippocampal damage) to assess spatial learning. There was no observable behavioral deficit in young adult rats treated for either one or three months with corticosterone. A one-month treatment period also had no behavioral effect in mid-aged rats. However, after three months of exposure to elevated corticosterone levels, mid-aged



rats took significantly longer to successfully navigate the maze, although their performance eventually approximated that of the control animals.

A second experiment with mid-aged rats treated with either p.m. diurnal (Medium-B:  $\approx 12-15 \mu\text{g/dl}$ ) or stress (High-B  $\approx 25-30 \mu\text{g/dl}$ ) levels of corticosterone demonstrated that only the performance of the High-B rats was impaired on the Morris maze, relative to the Medium-B and controls. In addition, a threshold model of long-term potentiation (primed burst potentiation) suggested significantly reduced hippocampal synaptic plasticity in both the Medium- and High-B rats, relative to the controls. Finally, hippocampal neuropathology was assessed in two of the experiments and the cell counting data suggested that there was no significant neuron loss in either the CA1 or CA3 pyramidal cell layer of the hippocampus in the corticosterone-treated rats compared with age-matched controls.

Finally, mid-aged rats were exposed to a six-month social stress regimen that elevated corticosterone levels ( $\approx 12-18 \mu\text{g/dl}$ ) throughout the diurnal cycle. A second group of mid-aged rats was adrenalectomized and given basal levels of corticosterone replacement prior to the commencement of the social stress regimen. Acquisition of the Morris swim task was impaired in the socially-stressed animals relative to both the adrenalectomized, stressed animals and age-matched controls.

The data presented here suggested that long-term exposure to elevated corticosterone levels, either by exogenous treatment or social stress, resulted in spatial learning deficits, but only in mid-aged rats. Further, these impairments appeared to be related to reduced synaptic plasticity rather than hippocampal neuron loss.

These findings are discussed in terms of the potential mechanisms by which long-term exposure to high glucocorticoid levels produces deficits in both behavior and hippocampal synaptic plasticity.

## Acknowledgements

My years in Montreal somewhat resemble an Anne Tyler novel: A long, but interesting journey, featuring a cast of unusual characters who have provided me with many stories to share with future generations. Allow me to make the introductions.

I should have known what was ahead back on that February day in 1984 when I called Michael Meaney to discuss the possibility of doing graduate work in his lab and our conversation lasted over an hour and a half (during peak hours no less). Michael likes to talk!! I didn't learn much about his research during that discussion, but I was delighted to discover that an Expos game could fit perfectly into the incubation period of a glucocorticoid receptor assay. Well, I enjoyed my fair share of time bending over an open-top freezer, went to Olympic Stadium just twice during my time in Montreal, and spent many an hour listening to Michael tell (and retell) his favorite anecdotes. You cannot spend almost one-third of your life with a person without them having some influence - I've learned to find something else to do when Michael is introducing a new graduate student to his tales. All that aside, probably the greatest gift I received from my supervisor was independence. As is often the case with most "teacher-student" relationships, we never really appreciate such a gift when it is offered to us. Instead of revelling in such an opportunity, we

are often disappointed or frustrated with that teacher who has chosen to let us learn for ourselves. In addition, Michael has always given his students the opportunity to do the best science money could buy and I consider myself fortunate to leave his lab with a c.v. that has afforded me the opportunity to make choices for my future. For this Michael, I say thank you - every graduate student should be so lucky.

The three months I spent in Denver in the lab of Greg Rose was crucial for reigniting that fire inside me (it's been a long time since I was at work by 7:15 am). Too often we grow complacent and become too comfortable in a safe and familiar environment. It is only when we are challenged by the unknown that we must confront our worst enemy, self-doubt. This experience was the true test of my confidence and abilities and I truly appreciate the opportunity I was given by both Michael and Greg. There was a lot of bureaucratic crap and genuine bad luck that had to be dealt with throughout this project and Greg always managed to handle the situations with his own brand of humor. He has always been generous with his time and his sarcasm and surprisingly, both were appreciated. I'd also like to thank Aaron Humphreys, my teacher and my valium - I could never have done this without you. I'm glad you were better at electrophysiology than you were at picking the NCAA tournament champ. My thanks as well to Karen Stevens (for both her car and her early-morning conversations),

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Well Ma, we did it!! I could never imagine a more supportive fan than Doreen Armoni. She has always shared two pieces of wisdom with me that remain my motto to this day: "They will never ask you a question to which you don't know the answer" and if, by some cruel twist of fate such a situation should arise, then "Baffle them with Bullshit". Ah, words to live by! Thanks for all the years of telling me (and anyone who couldn't run away fast enough) that I was the most brilliant child on earth. I don't believe that your words ever went to my head, but they have always remained in my heart. Thank you, and I love you.

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## Introduction

### The Hypothalamic-Pituitary-Adrenal Axis

Hans Selye (1946) described a stressor as any of a wide range of insults (e.g. hypo- or hyperthermia, physical injury, or emotional distress) that results in a perturbation of homeostasis and the stress response as the organism's effort to re-establish homeostasis. Adrenocortical secretion of glucocorticoids has been viewed as one of the basic endocrine adaptations to stress.

The hypothalamic-pituitary-adrenal (HPA) axis is activated in response to a perturbation of homeostasis. Corticotropin-releasing hormone (CRH) and arginine vasopressin (AVP) are released from the axon terminals in the median eminence of the hypothalamus into the hypophyseal portal blood where they stimulate the synthesis of a large precursor molecule, proopiomelanocortin (POMC) by the corticotrophs of the anterior pituitary. POMC undergoes several cleavages, resulting in the formation of adrenocorticotrophic hormone (ACTH) or other peptides such as  $\beta$ -endorphin and  $\beta$ -lipotropin which are released into circulation. The resulting increase in plasma levels of ACTH stimulate adrenocortical secretion of glucocorticoids from the adrenal cortex (Baxter and Tyrell, 1987).

CRH and some co-secretagogues such as AVP are synthesized in the parvocellular component of the paraventricular nucleus of the hypothalamus (PVN) and released via axonal projections from the PVN to the median eminence of the hypothalamus (Antoni, 1986; Plotsky and Vale, 1984; Rivier and Vale, 1983). It has also been demonstrated that CRH-secreting terminals at the level of the median eminence may contain AVP. Indeed, following adrenalectomy, >70% of the CRH-containing parvocellular neurons are co-localized with AVP (Sawchenko, Swanson and Vale, 1984). Moreover, Wolfson, Manning, Davins, Arentzen and Baldino (1985) demonstrated a significant increase in AVP mRNA within CRH-immunoreactive neurons, suggesting that in the absence of inhibitory HPA signals (as observed following adrenalectomy), the synthesis of AVP within the parvocellular neurons is increased. Under conditions of chronic intermittent stress, AVP synthesis in the CRH-producing parvocellular neurons in the PVN is markedly enhanced (DeGoeij, Kvetnansky, Withnall, Jezova, Berkenbosch and Tilders, 1991). In contrast, the colocalization of CRH and AVP is not apparent in intact, unstressed animals (Plotsky and Sawchenko, 1987).

The assumption that a small fraction of AVP is associated with CRH cells within the median eminence and thus the regulation of ACTH, underestimates the importance of the secretagogue in HPA function.

Although AVP has been shown to be a weak stimulant of ACTH release in vitro, it has been shown to potentiate the corticotroph response to CRH in vivo (Watanabe and Orth, 1988). Indeed, passive immunization of AVP dampens the subsequent ACTH and corticosterone responses to a variety of stressors (Gibbs, 1986). Thus, the magnitude of the corticosterone response to stress is mediated centrally via the synergistic actions of several ACTH co-secretagogues.

High plasma levels of corticosterone are critical for initiating the peripheral catabolic and anabolic processes that are required for maintaining the carbohydrate reserves necessary for meeting the metabolic demands of the stressor (Baxter, 1979; Cahill, 1971; Exton, 1979). For example, glucocorticoids stimulate the process of hepatic glycolysis, thereby permitting the conversion of glycogen into the useable energy substrate, glucose (Baxter and Tyrell, 1987). However, the hepatic production of glucose is not sufficient to account for the high plasma glucose levels observed following glucocorticoid activation. Thus, the ability of glucocorticoids to inhibit glucose uptake by adipose, skin and lymphoid tissue (Munck, 1971) provides an additional mechanism for producing energy substrates. In addition, the inhibition of glucose uptake by extrahepatic tissue eventually results in the catabolism of proteins and nucleic acid substrates in these tissues, thereby providing necessary amino

acids for gluconeogenesis. The lypolysis of adipose tissue by glucocorticoids also results in the efflux of glycerol and free fatty acids which provide further energy substrates and further fuel gluconeogenesis (Baxter and Tyrell, 1987). Together with the direct and indirect effects upon hepatic glyconeogenesis, glucocorticoids increase the overall production of glucose to be used by muscle and liver, the tissues most demanding of energy substrates in response to a stressor.

In response to a stressor, the glucocorticoids rapidly suppress unnecessary anabolic processes that would sequester energy sources away from "fight or flight" responses. Along with the inhibition of growth, immunological function is also suppressed (Munck, Guyre and Holbrook, 1984), thereby assuring that the inflammatory response to tissue damage is prevented at a time when mobility might be necessary for survival. Indeed, glucocorticoid effects upon various mediators of the immune and inflammation responses include the inhibition of prostaglandins and lymphokines (Fahey, Guyre and Munck, 1981), the inhibition of release of histamine from mast cells (Daeron, Sterk, Hirata and Ishizaka, 1982) and suppressed production of interferon, interleukin-1 and interleukin-2 (Gillis, Crabtree and Smith, 1979).

To summarize, the acute effects of glucocorticoids involve the mobilization of energy reserves that are required for meeting the metabolic

demands of the stressor. Moreover, the suppression of immunological and inflammatory responses by glucocorticoids also ensure the ability to physically respond to the threat or challenge.

Although short-term corticosterone responses to stress are adaptive, chronic glucocorticoid elevation results in a range of pathological conditions. In addition to an overall inhibition of anabolic processes, there is an increased resistance to insulin, which creates the risk of steroid-induced diabetes, hypertension, hypercholesterolemia, arteriosclerosis, amenorrhea, impotence, the inhibition of growth and immunosuppression (Baxter and Tyrrell, 1987; Munck et al., 1984; Krieger, 1982). Thus, the ability to terminate the glucocorticoid response is a critical feature of the HPA axis.

### Negative Feedback Mechanisms

Termination of stress-related HPA activity is achieved via a negative feedback loop in which high circulating levels of glucocorticoids serve to inhibit HPA activity. Indeed, removal of the endogenous source of glucocorticoids (via adrenalectomy) results in an exaggerated elevation of ACTH, which can be prevented by corticosterone replacement (Dallman and Jones, 1973; Dallman, Jones, Vernikos-Danellis and Ganong, 1972; Keller-Wood and Dallman, 1984). Glucocorticoids inhibit the release of ACTH by three feedback processes that have been defined by their

temporal characteristics. They include a fast-feedback mechanism that is apparent within seconds to minutes, intermediate feedback which is evident within minutes to hours and a delayed mechanism which regulates ACTH over days (Dallman, Akana, Cascio, Darlington, Jacobsen and Levin, 1987; Keller-Wood and Dallman, 1984).

The fast negative-feedback mechanism operates within a specific time frame defined by Dallman and Yates (1969). The authors found that the inhibitory effects of glucocorticoids upon the corticosterone response to stress (histamine injection) were most potent when the glucocorticoids were administered between 15 seconds and five minutes prior to the stressor, but not when administered either 15 minutes prior to or two minutes following the stressor. Moreover, these fast feedback effects are sensitive to the rate of increase of plasma glucocorticoid levels. Jones, Brush and Neeme (1972) and Abe and Critchlow (1977) found that the rapid inhibition of ACTH secretion occurs during that period when plasma levels of corticosterone are still rising. The rapid inhibition of ACTH release by glucocorticoids, in response to synthetic ovine CRH, has been demonstrated in in vitro preparations of isolated pituitary cell preparations (Mahmoud, Scaccianoce, Scraggs, Nicholson, Gillham and Jones, 1984; Sayers and Portanova, 1973; Smelik, 1977). These findings suggest that a glucocorticoid-responsive fast feedback mechanism exists at the level of the

pituitary corticotrophs. Further, fast feedback involves a suppression of hormone release, with no effect on synthesis. Thus, pretreatment with cyclohexamide, which inhibits protein synthesis, has no effect on the fast-feedback actions of glucocorticoids upon CRH (Vermes, Mulder and Smerik (1977) and ACTH (Vale and Rivier, 1977) release.

There is also a period of quiescence, approximately 15 minutes to two hours following the stressor, in which any inhibition of the HPA axis by glucocorticoids is absent (Dallman and Yates, 1969). Following this period, an intermediate feedback mechanism is activated and this mechanism is dependent upon the level of steroid achieved in response to the stressor. Thus, the degree and duration of glucocorticoid-induced inhibition of CRH and ACTH increase occurs as a function of increasing doses of corticosterone (Jones, Tiptaft, Brush, Fergusson and Neame, 1974; Takebe, Kunita, Sakamura, Horiuchi and Machimo, 1971). In contrast to fast feedback processes, the glucocorticoid-inhibition of ACTH release involves protein synthesis. Indeed, Phillips and Tashjian (1982) demonstrated that in the presence of cyclohexamide, dexamethasone has no effect upon the stimulated release of ACTH in the pituitary tumor cell line, AtT20 (a cell line which produces substantial quantities of ACTH and  $\beta$ -endorphin).

Prolonged exposure to glucocorticoids is associated with reduced synthesis and release of CRH and ACTH. Indeed, in response to glucocorticoid administration, CRH tissue content decreases (Jones and Hillhouse, 1976) and POMC mRNA in the pituitary is inhibited (Roberts, Budarf, Baxter and Herbert, 1979a). Schacter, Johnson, Baxter and Roberts (1982) found that long-term adrenalectomy results in a significant increase in the level of pituitary mRNA for POMC. Moreover, Roberts, Johnson, Baxter, Budarf, Allen and Herbert (1979b) found that the glucocorticoids had specific inhibitory effects upon POMC mRNA transcription without altering the synthesis of other proteins.

Inhibition of CRH synthesis clearly implicates the hypothalamus as an important site in the mediation of delayed feedback effects of glucocorticoids. For example, hypothalamic CRH-like bioactivity is suppressed by corticosterone (Buckingham, 1979; Jones et al., 1972) and stress (Sato, Shinsako and Dallman, 1975). Following adrenalectomy, there is an increase in CRH-like bioactivity (Hillhouse and Jones, 1976; Vernikos-Danellis, 1965) and AVP immunoreactivity in the parvocellular compartment of the PVN (Merchenthaler, Vigh, Petrusz and Schally, 1983). Beyer, Matta and Sharp (1988) demonstrated a near three-fold increase in mRNA for CRH in the PVN seven days following adrenalectomy and this effect could be ameliorated with high levels of



dexamethasone. Plotsky, Otto and Sapolsky (1986) demonstrated that nitroprusside-induced hypotension increased plasma levels of ACTH two-fold and increased immunoreactive CRH (irCRH) almost 1.5-fold. Pretreatment with corticosterone effectively blocked both the rise in ACTH and irCRH, without affecting levels of either irAVP or irOT (Plotsky et al., 1986). These authors also reported the presence of CRH mRNA in extrahypothalamic tissue including the bed nucleus of the stria terminalis, the central nucleus of the amygdala and the supraoptic nucleus. However, mRNA for CRH in these brain regions is unaffected by both adrenalectomy and dexamethasone administration (Beyer et al., 1986).

Taken together, these data demonstrate that the suppression of CRH and ACTH by corticosterone can occur within three separate time frames, with each having differential effects upon the release and synthesis of the peptides. Moreover, the findings offer compelling evidence for both hypothalamic and pituitary mediation of glucocorticoid-mediated negative feedback processes.

#### Hippocampal Regulation of Negative Feedback Processes

The hippocampus has also been implicated in glucocorticoid inhibition of adrenocortical activity. For example, Endroczi, Lissak and Tekeres (1961) demonstrated that injections of corticosterone into the hippocampus suppressed the glucocorticoid response to a surgical stressor.

Electrical stimulation of either the CA<sub>1</sub>, dentate gyrus or subicular region of the hippocampus inhibited the adrenocortical response to urethane anesthesia (Durn and Orr, 1984). Fendler, Karmos and Telegdy (1961) found that hippocampal aspiration in cats resulted in a three-fold increase in adrenal venous corticoid levels that persisted for at least three months. These findings also suggested that the inhibitory action of the hippocampus was not compensated for by another brain structure. Feldman and Conforti (1976, 1980) reported that while neither dorsal fornix transection (which effectively isolated the hypothalamus from the hippocampus; Nauta and Haymaker, 1969) nor dorsal hippocampectomy had any effect upon a.m. adrenocortical activity, dorsal hippocampectomy produced an increase in the adrenocortical response to an ether plus incision stressor (Feldman and Conforti, 1980). Following lesions of the dorsal hippocampus, dexamethasone was less-effective in inhibiting the adrenocortical response, measured four hours following stress, relative to both fornix-lesioned and control rats (Feldman and Conforti, 1976,1980). Herman, Cullinan, Morano and Watson (1992) reported that ibotenic lesions of the ventral hippocampus resulted in significantly elevated levels of corticosterone two hours following the termination of an acute restraint stress relative to controls, although the pattern of recovery did not differ between the groups. These conclusions stood in contrast to the findings of Feldman and

Conforti (1980) in which ventral hippocampectomy had no effect upon dexamethasone inhibition of adrenocortical activity following a stressor.

Likewise, there are some conflicting data on the role of the hippocampus in the regulation of basal HPA activity. Wilson and Critchlow (1973) found that neither fornix transection nor hippocampectomy had any effect upon the circadian rhythmicity of corticosterone when assessed either one or three weeks following surgery. Fischette, Komisurak, Ediner, Feder and Siegel (1980) reported that the transection of the lateral fornix fibres ablated the circadian rhythmicity of corticosterone with a.m. levels as high as the p.m. levels. Wilson, Greer, Greer and Roberts (1980) found no differences in either a.m. ACTH or corticosterone levels between hippocampal-lesioned and cortical-lesioned control animals. In contrast, both p.m. and stress-induced levels of ACTH and corticosterone were significantly higher in the hippocampectomized rats, and these differences were ameliorated following adrenalectomy. These findings suggested that there was a circadian variation in the inhibitory effects of the hippocampus upon adrenocortical activity and that the hippocampus was involved only during the p.m. phase. This finding was consistent with the work of Akana, Cascio, Du, Levin and Dallman (1986) who demonstrated that levels of circulating corticosterone that were effective in suppressing ACTH during the morning were less-effective

during the evening, suggesting that there was a shift in feedback sensitivity during the peak of the diurnal rhythm. Therefore, it was hypothesized that the circulating levels of ACTH during the trough of the diurnal cycle were controlled by the pituitary while regulation of higher levels of ACTH (e.g. during the p.m. peak and following stress) were under the control of both the pituitary and the brain.

Herman, Schafer, Young, Thompson, Douglass, Akil and Watson (1989) reported that either total bilateral hippocampectomy or extirpation of the dorsal hippocampus produced increases in  $\beta$ -endorphin and corticosterone, along with significant increases in mRNA for both CRH and AVP in the PVN. These changes were not observed in animals with large cortical or cerebellar lesions. The finding suggested that the hippocampus exerted a tonic inhibitory role over the synthetic activity of those neurons controlling the release of ACTH secretagogues.

In summary, there is considerable evidence implicating the hippocampus as a regulator of HPA activity, and in particular as a site of glucocorticoid negative feedback. Support for this conclusion arises mostly from lesion studies in which hippocampal lesions have been shown to produce hypersecretion of corticosterone, ACTH, CRH and AVP under basal, stress and post-stress conditions as well as increases in CRH and AVP mRNA.

### Corticosteroid Receptors in the Brain

According to the prevailing theory of steroid hormone action (Muldoon, 1980), circulating steroids that are not bound to plasma binding proteins (e.g. corticosterone binding globulin; CBG) diffuse across the cell membrane and bind to soluble receptor sites that are present either in the cytoplasm or the nucleus. Steroid-receptor binding results in a conformational change of the receptor which increases the binding to specific nuclear sites on the DNA (i.e. translocation). The hormone-receptor complex binds to DNA sites, also known as response elements on target genes and thereby regulates the transcription of messenger RNA (mRNA).

The presence of adrenal steroid receptors in the brain was discovered by McEwen, Weiss and Schwartz (1968, 1969) who injected tracer doses ( $<1 \mu\text{g}$ ) of [ $^3\text{H}$ ] corticosterone into adrenalectomized rats to determine both the selectivity and the duration of retention of the radioactive ligand. Most of the brain regions studied (e.g. cortex, hypothalamus, amygdala and pons/medulla) showed a decline in retention that paralleled the decreasing blood concentrations, suggesting a rapid exchange between radioligand in the brain and the blood. Within the hippocampus and septum, both the increased concentrations of [ $^3\text{H}$ ] corticosterone (relative to the cortex) and the rate of disappearance of the

radioactive tracer relative to plasma concentrations, suggested that these two brain regions were selectively retaining the radiolabelled corticosterone even after it had disappeared from circulation. Moreover, while the hippocampi of intact rats were completely saturated by circulating levels of endogenous corticosterone, the septum retained quantities of [ $^3\text{H}$ ] corticosterone similar to those observed in adrenalectomized rats (McEwen et al., 1969). These data suggested that while the hippocampus and septum preferentially accumulated and retained [ $^3\text{H}$ ] corticosterone, the capacity of the receptors for circulating levels of the steroid was markedly lower in the hippocampus. The implication of this observation will be discussed later.

Autoradiographic studies demonstrated that the dentate gyrus, and the CA1-3 pyramidal neurons of the hippocampus selectively retained [ $^3\text{H}$ ] corticosterone (Gerlach and McEwen, 1972). When purified cell nuclei were assayed, the radiolabelled ligand was again concentrated in the hippocampus and septum (McEwen, DeKloet and Wallach, 1976; McEwen, Weiss and Schwartz, 1970), suggesting a genomic site of action for the glucocorticoids.

In contrast, the concentration of the synthetic glucocorticoid, [ $^3\text{H}$ ] dexamethasone within cell nuclear fractions was highest within the pituitary and more evenly distributed throughout the brain (DeKloet, Wallach and

McEwen, 1975; McEwen et al., 1976). DeKloet and McEwen (1976) suggested that the differential uptake by the pituitary of corticosterone and dexamethasone may be explained by the presence of transcortin, CBG-like molecules (Westphal, 1982) in pituitary cells. These molecules bind and sequester corticosterone (but not dexamethasone) and render the steroid biologically inactive (Ballard, 1979). Indeed, DeKloet and McEwen (1976) found that within the pituitary, a 100-fold excess of unlabelled corticosterone did not interfere with [ $^3\text{H}$ ] dexamethasone binding, while the same dose of unlabelled dexamethasone reduced the binding by more than 90%.

#### Corticosteroid Receptor Subtypes: Differential Localization and Function

While the presence of transcortin may explain the decreased retention of corticosterone relative to dexamethasone within the pituitary, the differential uptake of these ligands within the brain suggested that there may be different receptor subtypes for the glucocorticoids (DeKloet et al., 1975). With the use of highly selective synthetic glucocorticoid compounds, it has been possible to determine two distinct receptor populations that bind corticosterone with different affinities. The type I, mineralocorticoid receptor (the receptor observed by McEwen et al., 1968, 1969) binds both aldosterone and corticosterone with high affinity and is pharmacologically-indistinguishable from the kidney mineralocorticoid

receptor (Beaumont and Fanestil, 1983; Krozowski and Funder, 1983). McEwen et al. (1976) demonstrated that a six-fold increase in the amount of [ $^3\text{H}$ ] corticosterone injected into adrenalectomized rats did not significantly increase the amount of hippocampal retention for the ligand compared with tracer doses, suggesting that the type I receptors were saturated with very low doses of corticosterone. Subsequent studies have shown that the apparent binding affinity ( $K_d$ ) of corticosterone for this receptor was within the 0.5 to 1 nM range (Reul and DeKloet, 1985). The findings were consistent with the earlier work of McEwen et al. (1968, 1969) in which the capacity of corticosteroid receptors for corticosterone was lower in the hippocampus than other brain regions. Indeed, the prevalence of the high-affinity, low capacity type I receptors in the hippocampus would explain why these receptors were saturated by tracer doses of [ $^3\text{H}$ ] corticosterone in the original autoradiographic studies (Gerlach and McEwen, 1972). The type II receptor binds corticosterone with a somewhat lower affinity ( $K_d \approx 2\text{-}5$  nM), while showing preferential binding to either dexamethasone or the synthetic compound RU 28362 ( $K_d \approx 1.5\text{-}2$  nM) and has been distinguished as the glucocorticoid receptor.

The receptor subtypes also demonstrated differential localization with the central nervous system. As mentioned earlier, the type I receptor described by McEwen et al. (1968) was restricted to neurons of the



hippocampus, especially dorsal subiculum and CA<sub>1</sub>, dentate gyrus, CA<sub>3</sub> and lateral septum (Reul and DeKloet, 1985). In contrast, the type II receptors were distributed more widely and were found in both neurons and glial cells of the pituitary (DeKloet et al., 1975), lateral septum, nucleus tractus solitarius, dentate gyrus, central and cortical amygdala, PVN, locus coeruleus and the CA<sub>3</sub> subfield of the hippocampus (Reul and DeKloet, 1985). Within the PVN, type II receptors appear concentrated within the CRH and AVP-containing neurons of the parvocellular division (Fuxe, Harfstrand, Agnati, Yu, Cintra, Wikstrom, Okret, Cantoni and Gustafsson, 1985)

The physiological significance of the type I and II receptors was demonstrated by Meaney, Viau, Aitken and Bhatnagar (1988b), Reul and DeKloet (1985) and Reul, van den Bosch and DeKloet (1987b). They examined the occupancy of both receptor types during the a.m. trough, the p.m. peak of the diurnal phase and following one hour of restraint stress. The authors found that the occupancy of the type I receptor did not change over the diurnal cycle, with approximately 80% of the receptors being occupied. The restraint stress increased the occupancy to 98%. In contrast, the occupancy of the type II receptor better reflected dynamic variations in the level of circulating steroid. The low basal values resulted in < 10% of these receptors being occupied, while both the p.m. and stress

levels of corticosterone resulted in a 67-74% occupancy. Meaney et al. (1988b) reported that the percentage of translocated receptors remained elevated for at least two hours, which was a sufficient time period to activate delayed negative feedback processes (e.g. Dallman and Yates, 1969).

Together, these data suggested that the type I receptor is less sensitive to the dynamic variations in corticosterone observed during both the diurnal cycle and in response to stress. Since the occupancy of type II receptors is sensitive to changes in circulating levels of corticosterone, it could play an important role in the mediation of negative feedback in response to dynamic variations in glucocorticoid levels.

The results of several recent studies suggest that both type I and II receptors play an important role in glucocorticoid negative feedback. Indeed, Dallman and Bradbury (1989) demonstrated that hippocampal implants of either the type I or type II receptor antagonists (RU 28318 or RU 38486 respectively) resulted in increased levels of plasma ACTH. Ratka, Sutanto, Bloemers and DeKloet, (1989) demonstrated that the intracerebroventricular injections of these same antagonists prolonged the adrenocortical activity following exposure to a novel environment, although the type II antagonist had a somewhat more sustained effect. Thus, the occupation of either type I or type II receptors by their

respective antagonists attenuated glucocorticoid negative feedback and resulted in significantly elevated corticosterone levels for two to four hours following the termination of the stressor.

Clearly, these findings were incompatible with Reul and DeKloet (1985) who reported that the occupation of the type I receptor was near-capacity during the a.m. trough. However, a potential confound in that data were the very high p.m. corticosterone values reported. It has been suggested that the use of pentobarbital anesthesia during sacrifice and the collection of plasma in the Reul and DeKloet (1985) study may have contributed to the increased type I occupancy (Jacobson and Sapolsky, 1991). Indeed, Cascio, Jacobson, Akana, Sapolsky and Dallman (1989) reported that only 26% of type I receptors were occupied during the a.m. trough, while Sapolsky, Armanini, Packan, Sutton and Plotsky (1990a) reported that approximately 50% of the receptors were occupied with plasma corticosterone levels of 12 µg/dl (e.g. similar to the pm peak). Together these data are consistent with the idea that both the type I and type II receptors are likely involved in mediating negative feedback processes.

The story is further complicated by recent findings of interactions between type I and type II receptors. Studies from in vitro hippocampal cultures have shown that the type I receptor agonist, aldosterone, decreased

type II receptor concentrations, while the type I antagonist, spironolactone increased the concentrations, suggesting that occupation of type I receptors could regulate the number of type II receptors (O'Donnell and Meaney, 1991). While studying the potential receptor regulation within dispersed hippocampal cells cannot approach the in vivo models of negative feedback, the data do suggest that the understanding of hippocampal regulation of adrenocortical activity may not be as straightforward as originally proposed by Reul and DeKloet (1985).

#### HPA Dysfunction in the Aging Male Rat

Upon exposure to stress, the aged male rat is capable of demonstrating an elevation in plasma corticosterone levels equivalent to those observed in younger animals (Sapolsky, Krey and McEwen, 1983a, 1986a). Moreover, both the time course and maximal secretory capacity of this response remains unchanged in the aged animal (Sapolsky et al., 1986a). Several other features of HPA activity remain intact including a reserve capacity of corticosterone, which allows the rat to mount a subsequent adrenocortical response to a new stress, a normal biological half-life and metabolic clearance rate of corticosterone in the plasma, and an appropriate circadian pattern of corticosterone secretion (Sapolsky et al., 1983a).

However, there are several important changes that do occur within the aging HPA axis. Indeed, in aged rats there is often an increase in circulating levels of both ACTH (Meaney, Aitken, Sharma and Viau, 1992; Tang and Phillips, 1978) and corticosterone (e.g. Brett, Chong, Coyle and Levine, 1983; DeKosky, Scheff and Cotman, 1984; Hess and Riegler, 1970; Landfield, Waymire and Lynch, 1978b; Meaney et al., 1992; Rapaport, Allaire, Bourliere and Girard, 1964; Sapolsky et al., 1983a; Sencar-Cupovic and Milkovic, 1976; Tang and Phillips, 1978) under basal conditions. A more thorough examination of variations in basal HPA functioning was conducted by Meaney et al. (1992) who assessed the effects of early, postnatal handling (in which rat pups were either handled daily or left undisturbed (non-handled) during the first three weeks of life) upon several endocrine measures in both young and aged rats. Plasma levels of corticosterone and ACTH did not differ between the young H and NH animal at any time over the diurnal rhythm. However, with aging, group differences in basal activity became apparent. While there was a slight increase in corticosterone levels during the a.m. phase of the diurnal cycle, regardless of the early intervention, the aged non-handled rats had significantly higher levels of corticosterone during the p.m. phase of the cycle than did aged handled rats. Estimates of free, or biologically-active corticosterone were significantly ( $\approx$  3-4 fold) elevated at three p.m. time

points relative to both groups of young animals and the old handled rats. While ACTH levels during the a.m. did not differ among the four groups, only the old non-handled rats demonstrated significantly elevated plasma levels during the p.m.

In addition to the age-related changes in basal HPA function, senescent animals also exhibit a reduced ability to efficiently terminate the adrenocortical response to restraint stress. Thus, corticosterone levels remain elevated for significantly longer periods of time (two to four hours following the termination of the stressor) relative to young adults (Meaney et al., 1988a; Sapolsky et al., 1983a; 1986a). HPA dysfunction in aged rats has been further demonstrated by the decreased ability of either corticosterone (Sapolsky et al., 1986a) or dexamethasone (Dilman, 1981; Riegle and Hess, 1972) to suppress subsequent corticosterone release, thereby suggesting impaired negative feedback in these animals.

In summary, it is apparent that not all aspects of HPA functioning are impaired during senescence. Indeed, aged rats are capable of demonstrating both an appropriate circadian rhythm of corticosterone and a normal adrenocortical response to a stressor. However, the degree of HPA activation under both basal and post-stress conditions is increased with age, suggesting that there is an impaired sensitivity of the aged HPA axis to

negative feedback regulation (although it is important to consider the variation among aged animals).

### Hippocampal Receptor Concentrations and Negative Feedback

The sensitivity of target cells to glucocorticoids is, in part, determined by the density of corticosteroid receptors. Thus, the concentrations of these receptors play an important role in mediating negative feedback processes following HPA axis activation. Indeed, interventions which either increase or decrease the number of hippocampal glucocorticoid receptors enhance or impair the efficacy of the negative feedback process respectively. Sapolsky, Krey and McEwen (1984a,b) observed a 30-40% decrease in hippocampal corticosterone receptors following either a three or one week exposure to intermittent stressors, respectively. The decrease in receptors is due to the persistent elevation in circulating glucocorticoid levels. Increasing the glucocorticoid signal via the chronic administration of corticosterone ( Sapolsky et al., 1984a, 1985; Tornello, Orti, DeNicola, Rainbow and McEwen, 1982) also reduced the number of hippocampal glucocorticoid receptors. The down-regulation of corticosteroid receptors is accompanied by dampened negative feedback efficacy. Thus, one week exposure to various intermittent stressors (e.g. cold, sham adrenalectomy, vibration etc.) resulted in significantly elevated corticosterone levels measured one hour following the termination of a one

hour restraint stressor (Sapolsky et al., 1984b). A one-week recovery period normalized both the receptor concentrations and the corticosterone responses to acute stress (Sapolsky et al., 1984b).

The vasopressin-deficient homozygotic diabetes insipidus or Brattleboro rat (Valtin and Schroeder, 1964) also represents an interesting model for studying the importance of hippocampal corticosteroid receptors in HPA negative feedback. DeKloet and Veldhuis (1980) reported a significant loss of hippocampal type I receptors ( $\approx 35\%$ ) while both Sapolsky et al. (1984b) and Veldhuis and DeKloet (1982) found a 25-35% decrease in hippocampal type II receptors respectively in the Brattleboro rat relative to controls. Moreover, Veldhuis and DeKloet (1982) reported a 40% loss of type II receptors in the pituitary. Elevated plasma corticosterone values observed in the Brattleboro animals one hour following the termination of a one-hour restraint stressor were suggestive of impaired glucocorticoid negative feedback. While the administration of the vasopressin analog des-glycinamide-arginine vasopressin (dGVP) increased hippocampal type I receptor concentrations, Brattleboro rats still had significantly fewer receptors than controls (Veldhuis and DeKloet, 1982). In contrast, dGVP normalized the reduced type II receptor concentrations in both the hippocampus and pituitary and also normalized the level of adrenocortical activity following exposure to a stressor



(Sapolsky et al., 1984b; Veldhuis and DeKloet, 1982). However, the effects of dGVP were temporary. Following the termination of treatment, receptor concentrations returned to previous low levels and re-instated the delayed termination of the adrenocortical response to stress (Sapolsky et al., 1984b).

Variations in the adrenocortical response to a stressor can also be influenced by the early intervention of neonatal handling described earlier (Hess, Denenberg, Zarrow and Pfeifer, 1969; Levine 1957, 1962; Levine, Haltmeyer, Karas and Denenberg, 1967). As adults, handled rats secrete less corticosterone and show a faster return to basal corticosterone levels following either shock (Levine, 1962) or exposure to novelty (Levine et al., 1967) relative to non-handled rats. Further, both the ACTH and corticosterone responses to an acute stress were prolonged in the non-handled relative to the handled rat (Meaney, Aitken, Sharma, Viau and Sarrieau, 1989a; Viau, Sharma, Plotsky and Meaney, in press).

These differences in HPA activity were correlated with changes in hippocampal type II receptor density. Handled animals had 50-60% more hippocampal type II receptors, with no change in binding affinity relative to NH rats (Meaney, Aitken, Bodnoff, Iny, Tatarewicz and Sapolsky, 1985; Sarrieau, Sharma and Meaney, 1988). There were no differences in type II receptor concentrations in the hypothalamus, pituitary or amygdala of H

and NH rats (Meaney et al., 1985; Sarrieau et al., 1988). Further, handling had no effect upon hippocampal type I binding (Meaney et al., 1992; Sarrieau et al., 1988). Handled and non-handled animals also differ in negative-feedback sensitivity to glucocorticoids. There is greater suppression of ACTH responses to stress following exogenous glucocorticoid administration in handled compared with non-handled rats (Meaney et al., 1989a).

Treating handled animals with stress-like levels of corticosterone decreased hippocampal type II receptor concentrations to the level of the non-handled animals, without changing receptor density in the hypothalamus or pituitary. Following the termination of a 20-minute restraint stressor, both non-handled and handled, corticosterone-treated animals showed significantly elevated levels of corticosterone both during and following restraint stress compared with untreated handled animals (Meaney et al., 1989a). These findings suggest that reduced concentrations of hippocampal type II receptors were an integral feature of the impaired efficacy of the inhibition of the adrenocortical response to stress observed in non-handled animals.

In summary, the data from chronically-stressed, Brattleboro and handled/non-handled rats suggest that the impaired ability to terminate the adrenocortical response to a stressor is related to the concentration of

hippocampal type II receptors. In each case, reversing the differences in hippocampal receptor concentrations resulted in the elimination of differences in HPA responses to stress.

#### Corticosteroid Receptors in the Aged Rat.

Many authors have also observed an impaired negative feedback response in aged animals. For example, Riegle and Hess (1972) found that dexamethasone was much less effective in inhibiting the adrenocortical response to ether vapors in the aged Long-Evans rat. With Fischer-344 rats, Sapolsky et al. (1983a) found no age-related increase in the speed and magnitude of the stress response or in the ability to manifest a subsequent stress response following three weeks of exposure to intermittent stressors. However, both a.m. and p.m. values of corticosterone increased as a function of age as did corticosterone levels measured 90 minutes following the termination of a one-hour restraint stress. Sapolsky et al. (1984b) showed that young and aged F-344 rats exhibited similar basal and peak stress responses, while the aged animals continued to show elevated corticosterone levels three hours following the termination of the stressor. Further evidence for impaired negative feedback in the aged rat was reported by Sapolsky et al. (1986a) who found that exogenous corticosterone pre-treatment was less effective in suppressing stress-induced adrenocortical activity compared to young rats.

The aged animal also exhibited a reduction (e.g.  $\approx 30\text{-}40\%$ ) in hippocampal type I and type II corticosteroid receptors (Issa, Rowe, Gauthier and Meaney, 1990; Meaney et al., 1988a; Meaney et al., 1992; Reul, Tonnaer and DeKloet, 1988; Ritger, Veldhuis and DeKloet, 1984; Sapolsky et al., 1983a,b; Sapolsky et al., 1984b; Sapolsky, Krey, McEwen and Rainbow, 1984c). Peiffer, Barden and Meaney (1991) reported a significant decrease in hippocampal type II receptor concentrations as a function of age, occurring as early as twelve months of age. In contrast, there were no age-related changes in receptor concentrations in other brain regions (i.e. cortex, amygdala and hypothalamus) and the pituitary. Moreover, aged animals exhibited a corresponding decrease in mRNA for the hippocampal type II receptor. This decrease in hippocampal corticosteroid receptor concentrations provide a plausible mechanism for the exaggerated glucocorticoid response to stress in the aged rat (Sapolsky et al. 1986a).

Sapolsky et al. (1984c) demonstrated that aged F-344 rats had significantly fewer hippocampal corticosteroid receptors as measured in the cytosolic fraction and quantitative autoradiography revealed that these depletions were most apparent in the pyramidal layer of CA3a. In addition to a decrease in the number of silver grains overlying these corticosterone-concentrating pyramidal neurons (e.g. fewer receptors/cell), aged animals

also showed significantly fewer corticosterone-concentrating neurons.

In addition to changes in receptor number in the aged rats, there are also changes in receptor plasticity. Eldridge, Brodish, Kute and Landfield (1989a) reported that six months of chronic escape training, which significantly elevated levels of plasma corticosterone, failed to down-regulate hippocampal type II receptors in both late mid-aged (18 months) and old (24 months) rats. In contrast, young mature rats (10 months) demonstrated a 21% decrease in type II receptor concentration one day following the termination of the six-month stress regimen, although the levels returned to normal by three weeks. Furthermore, Eldridge, Fleenor, Kerr and Landfield (1989b) reported impaired adrenalectomy-induced upregulation of hippocampal type II receptors in aged animals. While a significant increase in the type II receptors was apparent in young animals within two days post-adrenalectomy, no increase was observed over a ten day post-adrenalectomy period in aged rats. These findings reflect the reduced plasticity in corticosteroid receptor systems within the aged hippocampus. In the aged rat, type II receptor density does not appear to be sensitive to changing levels of circulating corticosterone.

#### HPA Activity and Hippocampal Neuron Loss

HPA dysfunction and glucocorticoid hypersecretion have also been implicated in the loss of hippocampal neurons that is generally observed

during aging (reviewed by Coleman and Flood, 1987). Landfield et al. (1978b) examined 4-, 13-, and 25-month old male Fischer-344 rats to address how endocrine and neural measures are related during the aging process. Specifically, they focussed on the 13-month old animals to determine if the etiology of age-related neural changes could be linked to endocrine dysfunction commencing at middle age. Although plasma corticosterone values appeared to increase as a function of age, the authors noted that if two extreme values from the old animals were excluded, their median corticosterone levels did not differ from the young rats. With respect to adrenal weight, there was a slight increase by middle age and a significant increase in the old animals. Landfield et al. (1978b) suggested that adrenal weight may be a more sensitive measure of HPA dysfunction as it may reflect the cumulative exposure of the adrenal gland to ACTH. The authors also found a significant increase in hypertrophied (reactive) astrocytes in the CA3 region of the hippocampus as a function of age. Importantly, there was a significant correlation between the number of reactive astrocytes and both corticosterone levels ( $r = +0.76$ ) and adrenal weights ( $r = +0.72$ ), suggesting that elevated glucocorticoids may play a role (either direct or indirect) in many of the neural changes associated with aging. Eliminating the elevated glucocorticoid levels ameliorated many of the morphological changes normally associated with aging.

Landfield, Baskin and Pitler (1981a) examined several morphological variables in young (8 months) and old (27 months) rats that were either untreated or adrenalectomized at 18 months of age and given basal corticosterone replacement. The authors found that the aged, adrenalectomized animals were remarkably similar to young rats on measures of hippocampal neuronal density, nuclear roundness, reactive astrocytes, glial clusters and a brain aging index which incorporated all the variables measured. Perhaps a more important issue was whether adrenalectomy could also ameliorate the cognitive deficits that were observed in aged animals. The authors found that in contrast to the impressive absence of morphological changes, adrenalectomy did not significantly improve performance on reversal trials in an escape/avoidance task relative to age-matched controls, although their performance was more similar to that of the young animals. Thus, the absence of elevated glucocorticoids during the latter third of the rat's life ameliorated many of the neural changes that are generally observed in aging, although further behavioral analysis seems necessary to evaluate the functional consequences of this treatment.

While the extensive literature presented above offers evidence linking elevated glucocorticoid levels with neuropathology, there have been relatively few studies examining the direct effects of corticosterone upon

hippocampal damage. Aus der Muhlen and Ockenfels (1969) demonstrated that pharmacological doses of glucocorticoids resulted in the degeneration of hippocampal neurons in the guinea pig. However, the effects of the cumulative exposure to physiological levels of glucocorticoids upon hippocampal pathology have not been examined extensively.

Probably the most-cited reference in this area was a study by Sapolsky, Krey and McEwen (1985) who exposed young Fischer-344 rats to stress-like levels of corticosterone for either two weeks (acute) or three months (chronic), and compared these animals to adult (8 months) and old (28 months) untreated controls. Receptor binding experiments, completed immediately following treatment, indicated that both acute and chronic exposure to corticosterone significantly reduced receptor binding capacity (approximately 55%) without affecting the binding affinity. While receptor concentrations returned to control values as early as one week following the acute treatment regimen, even a four-month recovery period did not allow receptor concentrations to normalize in the chronically-treated animals. The permanent receptor down-regulation in the chronic corticosterone rats was explained, in part, by the significant loss of [ $^3\text{H}$ ] corticosterone-concentrating neurons, especially in the CA3 subfield of the hippocampus. In contrast, acute corticosterone treatment resulted in a



reduction in the number of glucocorticoid receptors per neuron, without a reduction in the number of neurons.

Sapolsky et al. (1985) found impressive similarities between chronic corticosterone-treated animals and aged controls including a significant loss of large cell bodies ( $> 140\mu\text{m}^2$ ) and significant increase in the density of small neurons ( $< 70\mu\text{m}^2$ ) or microglia, which infiltrate following tissue damage. While these changes were restricted to the CA3 subfield in corticosterone-treated animals, aged animals showed similar pathological changes in CA1 (Sapolsky et al., 1985). These data suggested that prolonged exposure to high physiological doses of glucocorticoids in young rats produced a pattern of hippocampal damage similar, but not identical to aging. Further, the authors suggested that chronic corticosterone treatment may serve as a model for studying the potential contribution of hippocampal neuron loss to age- and pathology-related HPA dysfunction.

#### Hippocampal Damage and Learning Deficits

The occurrence of cognitive impairments in aged animals with hippocampal neuron loss is consistent with the vast clinical and experimental research implicating the hippocampus in learning and memory. Early neuropsychological evidence provided by Scoville and Milner (1957) and Milner, Corkin and Teuber (1968) suggested that the hippocampus played an important role in learning and memory. Although

their patient, HM, underwent removal of the medial temporal cortex, it was the extensive damage to the hippocampus proper that was believed responsible for his memory deficits (Milner, 1972). This proposal has been reinforced by the studies of patient RB (Zola-Morgan, Squire and Amaral, 1986), who suffered bilateral ischemic damage to the CA<sub>1</sub> subfield of the hippocampus. RB, like HM, showed specific deficits in tasks involving new learning. In general, most researchers in the field agreed that the hippocampus is not the structure for permanent memory representations as patients who have suffered damage to this region usually retain early memories (Squire, 1983; Scoville and Milner, 1957). However, there appears to be consensus that the hippocampus does play an important role in processing information that will eventually be stored in memory (Milner, 1968).

The ability to draw conclusions of the importance of the hippocampus in information processing and storage from clinical studies is restricted. The case of isolated hippocampal damage, as described in RB is rare. Usually, a greater portion of the temporal cortex, along with other structures have been damaged (Scoville and Milner, 1957; Milner et al., 1968). Understanding of the functions ascribed to the hippocampus has been furthered considerably by examining the effects of hippocampal lesions upon learning in the rat and monkey. Deficits in learning following

hippocampal damage are apparent using a variety of behavioral tasks including the radial arm maze (Olton, Becker and Handelmann, 1979), the Hebb-Williams maze (Kimble, 1962), the circular hole-board task (Barnes, 1988), passive avoidance (Blanchard and Fial, 1968), delayed alternation (Racine and Kimble, 1965), delayed matching to sample (Gaffan, 1974), and recognition for complex pictures (Gaffan, 1977). However, for the purpose of this discussion, the focus will be upon spatial memory and specifically, the Morris water maze.

The Morris water maze is a recent and popular task designed to assess intact hippocampal function (Morris, 1984; Morris, Garrud, Rawlins and O'Keefe, 1982). In this task, animals are required to find and climb onto a submerged (2 cm) escape platform in a 1.6-m-diameter pool of opaque water using only distal, spatial cues available within the testing room. It has been demonstrated that intact rats acquire this task rapidly and swim a very direct route to the hidden platform. In contrast, animals who have undergone total (Morris et al., 1982) or selective (Sutherland, Whishaw and Kolb, 1983) hippocampal lesions, or hippocampal stimulation, which disrupts normal hippocampal function (Parker and Walley, 1988) show significantly longer latencies to locate the platform over trials, relative to controls. However, the performance of lesioned animals is unimpaired on a non-spatial task when the platform is raised

above the water level and thus visible, suggesting that the poor performance of these rats is not due to motoric or perceptual deficits (Morris et al., 1982).

The Morris maze is also effective in assessing the learning deficits of aged animals (Gage, Kelly and Bjorklund, 1984; Gallagher and Burwell, 1989; Issa et al., 1990; Meaney et al., 1988). However, in contrast to the early findings of Landfield et al. (1981a) with Fischer-344 rats, it has become apparent that cognitive impairments and neuropathology are not a necessary consequence of aging. Just as in humans (Rowe and Kahn, 1987), there is incredible variability in the aging process of rats (Markowska, Stone, Ingram, Reynolds, Gold, Conti, Pontecorvo, Wenk and Olton, 1989). Gage et al. (1984) have shown that only 30-40% of aged Sprague-Dawley rats show cognitive impairments in the Morris maze (e.g. latencies of more than two standard deviations from the mean), while approximately 35% of the aged population do not differ from young controls (e.g. less than one standard deviation from the mean).

Gallagher and Burwell (1989) similarly reported that 35-50% of an aged Long-Evans population were unimpaired in the acquisition of the Morris water maze. Moreover, categorizing aged animals based upon their performance in the swim maze also distinguished their performance on several other tasks. That is, the performance of aged unimpaired rats was

equivalent to those of young animals on a transfer test (i.e. the escape platform was moved to the opposite quadrant), on a test of gustatory neophobia, and the latency to escape in the circle holeboard task (e.g. Barnes, 1979). In contrast, aged impaired rats displayed impaired performance on the transfer test, enhanced neophobia and increased latencies to escape in the holeboard task. These findings suggested that variations within an aged population that have consistently been observed in the Morris water maze were apparent across several tasks (Gallagher and Burwell, 1989).

This variability can also be manipulated experimentally with postnatal handling. As discussed earlier, this intervention resulted in significant increases in hippocampal type II receptors, a more efficient negative feedback response, and a decreased HPA response to stress (Meaney, Aitken and Sapolsky, 1991). Moreover, these effects persisted throughout the lifespan (Meaney et al., 1988a). Twenty-four month old handled animals looked remarkably like young animals with respect to both their basal corticosterone values and corticosterone levels following a 20-minute period of restraint stress. In contrast, both 12- and 24-month old non-handled rats hypersecreted corticosterone during both the peak of the diurnal phase and following a stressor.

These findings suggest that cumulative exposure to glucocorticoids is greater in the aged, non-handled rats. Consistent with the findings of Landfield et al. (1978b, 1981b), Meaney et al. (1988a) found that old, non-handled rats had also incurred significant neuron loss in the CA<sub>1</sub> and CA<sub>3</sub> subfields of the hippocampus. In contrast, there was no evidence of hippocampal neuron loss in aged handled rats. It is important to note that these differences in neuron density were not apparent in young, non-handled animals. Rather, the differences emerged with age.

The performance of the aged handled animals in the Morris water maze was identical to that of young controls. In contrast, aged non-handled animals took significantly longer to locate the submerged platform, although their performance was unimpaired in the visually-cued task with the visible platform (Meaney et al., 1988a).

The above experiments suggest that cognitive impairments are not a necessary consequence of aging, rather they emerge with HPA dysfunction. Thus, one would predict that within an aged population, measures of HPA function could be used to characterize a sub-population of aged, cognitively-impaired rats. In order to study this question, Issa et al. (1990) examined HPA activity in aged rats screened on the Morris water maze and separated into aged cognitively-impaired (ACI) and aged cognitively-unimpaired (ACU) groups (28% and 34% of the population

respectively) based upon their performance on this task. Indices of HPA activity showed that ACI rats had increased levels of p.m. basal ACTH and corticosterone and took significantly longer to terminate an HPA response to a restraint stressor compared with both ACU and young controls.

Moreover, the ACU and young control rats did not differ on any measures of HPA function. The impaired inhibition of HPA function in the ACI rats could be explained by the significant decrease in hippocampal type II receptor concentrations. No such changes were apparent in ACU rats. Further, although both the ACU and ACI rats showed neuron loss in CA1 and CA3 compared with the young controls, the loss was significantly greater in ACI animals. The results of this study confirm that individual differences in HPA function are associated with hippocampal pathology in later life.

#### Elevated Glucocorticoids and Neuropathology in Aged Humans

Similar to the Long-Evans rat, neither basal levels of ACTH nor cortisol increase as a function of age in humans (Cartlidge, Black, Hall and Hall, 1970; West, Brown, Simons, Carter, Kumagai and Engelbert, 1961). Normal circadian rhythmicity (Touitou, Sulon and Bogdan, 1983), adrenocortical response to surgical stress (Blichert-Toft and Hummer, 1976) and the suppression of adrenocortical activity following dexamethasone administration (Tourigny-Rivard, Raskind and Rivard,

1981) have also been reported in aged humans. However, while HPA dysfunction and glucocorticoid hypersecretion do not appear as a function of normal aging, these features do emerge when aging is coupled with a pathological condition (e.g. senile dementia of the Alzheimer's type - SDAT). For example, both 24-hour urinary free cortisol levels (Maeda, Tanimoto, Terada, Shintani and Kakig, 1991) and nocturnal and 9:00 a.m. plasma cortisol levels (Davis, Davis, Greenwald, Mohs, Mathe, Johns and Horvath (1986) were significantly elevated in SDAT patients relative to age-matched controls. The patients with the most severe dementia had the highest basal cortisol concentrations (Davis et al., 1986). Perhaps more importantly, the inability of dexamethasone to suppress basal adrenocortical function or the early release from suppression was observed in approximately 40-80% of a given SDAT population (Greenwald, Mathe, Mohs, Levy, Johns and Davis, 1986; Maeda et al., 1991; Pomara, Oxenkrug, McIntyre, Block, Stanley and Gershon, 1984). Pomara et al. (1984) and Georgotas, McCue, Kim, Hapworth, Reisberg, Stoll, Sinaiko, Fanelli and Stokes (1986) reported that those patients with the greatest degree of dementia also had the highest post-dexamethasone cortisol levels. Greenwald et al. (1987) reported that within their population of non-depressed SDAT subjects, all of the non-suppressors were older than those



with normal dexamethasone suppression (68 vs 60 years respectively), suggesting that the degree of dexamethasone resistance increased with age.

The intact basal HPA functioning and normal dexamethasone suppression observed in healthy aged individuals is consistent with the general findings that the effects of aging upon neural integrity are apparent, but subtle. Terry, DeTeresa and Hansen (1987) examined the brains of 51 clinically and neuropathologically normal individuals ranging from 24 to 100 years of age. The authors reported that with aging, there was a small, but significant loss of the large neurons. However, the losses were region-specific, principally targeting neurons of the frontal and temporal lobes. There was a concomitant increase in the number of small neurons without a change in the total number of neurons, suggesting that the shrinkage of the large neurons accounted for the increase in the number of small neurons. Coleman and Flood (1987) reviewed the vast number of studies that examined neuron loss in aging and dementia and concluded that while healthy humans generally lost neurons during the aging process, pathological aging was associated with a significantly greater loss, especially in the frontal and temporal lobe regions.

Consistent with the animal literature, the significant degeneration of the hippocampus and neocortex that is a hallmark of Alzheimer's disease (Mountjoy, Roth, Evans and Evans, 1983; Terry and Katzman, 1986) may

explain the high prevalence of dexamethasone non-suppressors in this population. While healthy aged individuals experience neuron loss, the degree is likely not sufficient to provoke HPA dysfunction. Moreover, the increased incidence of dexamethasone non-suppression as a function of age suggests that the pathology of aging may synergize with the pathology of SDAT to result in abnormal HPA functioning.

#### Elevated Glucocorticoids and Neuropathology in Other Populations.

In addition to the HPA dysfunction observed in SDAT, elevated levels of circulating glucocorticoids are a primary symptom of Cushing's Syndrome, or the consequence of steroidal administration for the treatment of arthritis and autoimmune diseases. What are the effects of long-term glucocorticoid exposure upon cognitive functioning and neuropathology?

Cushing's Syndrome patients have been categorized into two distinct subgroups (Krieger, 1982). ACTH-dependent Cushing's Syndrome is associated with pituitary hyperfunction, with or without detectable paraendocrine tumors, and results in the hypersecretion of both ACTH and cortisol. In cases of ACTH-independent Cushing's Syndrome, elevated cortisol levels are the result of either adrenal tumors or non-tumorous adrenocortical hyperfunction. Both disease subtypes result in the increased release of cortisol. Momose, Kjellberg and Kliman (1971) examined the consequences of hypercortisolemia in 31 Cushing's Syndrome patients.

The pneumoencephalograms revealed a 90% and 74% prevalence of cerebral and cerebellar atrophy, respectively. Further, the degree of atrophy could be related to the severity of the disease in each case, but did not correlate with the duration of the disease.

Starkman, Schteingart and Schork (1981) examined 35 patients with Cushing's Syndrome prior to treatment and reported that cortisol levels lacked the normal circadian rhythm and failed to suppress adrenocortical function with 2 mg of dexamethasone. The neuropsychiatric assessment of all patients, independent of etiology suggested that there was a constellation of symptoms that included impairments in affect, concentration, memory and vegetative function. The nature of the memory problems were found to include difficulty in remembering names and the location of objects as well as forgetting important personal or medical histories. However, the relationships between the changes in HPA activity and the memory impairments were not examined.

Bentson, Reza, Winter and Wilson (1978) examined over 7000 CT scans to determine a common denominator that could account for the exaggerated degree of cerebral atrophy in young individuals. One distinguishing feature was the long-term steroid therapy (e.g. prednisone) of individuals suffering from autoimmune diseases. Young patients in the steroid group had both ventricular and cortical sulcal enlargement

proportionally similar to that observed in healthy aged individuals. Moreover, the authors suggested that the daily dosage, and not the cumulative dose or duration of steroid use was a good predictor of damage.

Hall, Popkin, Stickney and Gardner (1979) reported that the high doses of prednisone used in the treatment of a range of autoimmune diseases resulted in an increased prevalence of anxiety, depression, auditory hallucinations and intermittent memory impairments. Okuno, Ito, Konishi, Yoshioka and Nakano (1980) reported that the treatment of children with infantile spasms with ACTH resulted in moderate to marked ventricular and sulcal enlargement.

Together, these data appear to implicate the glucocorticoids in both neuropathology and neuropsychiatric dysfunction in several patient populations, although the studies did not examine all of the relationships between endocrine dysfunction, cognition and pathology. However, piecing together the evidence suggests that elevated glucocorticoids impaired cognitive function and increased the extent of cerebral and cerebellar atrophy. Moreover, long-term exposure to high levels of prednisone also produced changes in cognition and neuropathology, and the severity of these changes were dependent on the level of glucocorticoids.

Probably the most important observation made by both Bentson et al. (1978) and Okuno et al. (1980) was that the steroid-induced atrophy was

reversible either by lowering the dosage or withdrawal of treatment altogether. This was consistent with the report that the corresponding cognitive disturbances associated with long-term corticosteroid therapy were also ameliorated by reducing the dosage (Ling, Perry and Tsuang, 1981). These findings suggested that the neural changes associated with high dosages of steroids represent a phenomenon distinctly different from the permanent changes associated with other pathologies such as SDAT.

Moreover, short-term glucocorticoid exposure appears to compromise cognitive functioning via a mechanism that is independent of permanent hippocampal pathology. Indeed, Newcomer, Craft and Askins (1992) demonstrated that a five-day treatment regimen with dexamethasone produced a significant decrement in declarative memory (e.g. immediate and delayed paragraph recall) in young healthy subjects. This finding offers strong evidence to suggest that in young, healthy individuals, the short-term exposure to physiological levels of glucocorticoids can impair cognitive functioning.

### Glucocorticoids and Hippocampal Function

That high circulating levels of glucocorticoids might alter some physiological mechanism related to learning, independent of cell loss, was suggested by the observation that adrenal steroids generally influenced the excitability of hippocampal neurons. Although the results of these early

experiments were highly variable, most single dose studies indicated the glucocorticoids reduced the excitability of hippocampal neurons. Indeed, both corticosterone (Pfaff, Silva and Weiss, 1971) and dexamethasone (Michal, 1974; Segal, 1976) decreased the discharge rate of hippocampal neurons. In contrast, Ben Barak, Gutnick and Feldman (1977) found that the iontophoretic application of either cortisol or corticosterone had no effect on the firing rates of pyramidal neurons.

Another measure of hippocampal excitability was assessed by examining changes in the superimposed population spike (PS) amplitude component of the excitatory post-synaptic potential (EPSP). The population spike represented the summed action potentials of a number of pyramidal cells (Andersen, Bliss and Skrede, 1971). Reiheld, Teyler and Vardaris (1984) found that levels of corticosterone characteristic of the a.m. trough or the p.m. peak increased CA1 PS amplitudes, while stress-like concentrations of corticosterone required much higher stimulus intensities to induce a similar effect. These findings suggested that there was a range of corticosterone concentrations that increased the excitability of the hippocampus. In contrast, high physiological concentrations of corticosterone reduced the excitability of hippocampal neurons. This latter finding was consistent with the observation that high concentrations of

corticosterone reduced the CA<sub>1</sub> PS amplitude (Vidal, Jordan and Zieglansberger, 1986).

The potential mechanism underlying these differential responses was elucidated by Joels and DeKloet (1989, 1990) who examined the effects of glucocorticoids upon the duration and amplitude of calcium-dependent slow afterhyperpolarizations (AHP). The AHP represents the hyperpolarizing phase evident after a brief depolarizing current and is a potent regulator of neuronal excitability (Alger and Nicoll, 1980; Schwartzkroin and Stafstrom, 1980). Indeed, increased Ca<sup>2+</sup> influx through voltage-sensitive channels results in larger and longer-duration AHPs (Kerr, Campbell, Hao and Landfield, 1989; Landfield and Pitler, 1984), thereby decreasing excitability. Joels and DeKloet (1989) reported that adrenalectomized animals displayed a significant reduction in the amplitude of the AHP. The administration of either high concentrations of corticosterone or the type II receptor agonist RU 28362 increased the amplitude of the AHP while the type II antagonist, RU 38486 blocked this increase. Moreover, both corticosterone and RU 28362 blocked the increase in responsiveness (seen as an increase in the number of action potentials) of the cells to an application of norepinephrine. In contrast, low doses of corticosterone (1 nM) or aldosterone (the endogenous ligand of the type I receptor)

decreased the AHP, while the type I antagonist, spironolactone reversed this effect.

Together, the trend of these studies is towards a complex interaction between level of glucocorticoids and hippocampal activity. The data suggest that low levels of corticosterone, which are associated with the near-full occupation of type I receptors (Reul and DeKloet, 1985) increase the excitability of hippocampal neurons to subsequent excitatory stimulation while the occupation of type II receptors with stress-like levels of corticosterone reduce their excitability.

#### Long-Term Potentiation: A Physiological Model of Learning

A model of learning based upon synaptic plasticity was originally proposed by Hebb (1949) who suggested that information processing within the central nervous system involved long-term changes in the communication between cells (e.g. at the synapses):

When an axon of cell A is near enough to excite cell B or repeatedly or consistently takes part in firing it, some growth process or metabolic change takes place in one or both cells such that A's efficiency, as one of the cells firing B, is increased (p. 62).

During this phase, information was stored as a specific distribution of modifiable synaptic weighting functions. Hebb suggested that a process of heterosynaptic facilitation occurred whereby synaptic inputs that were weak could be paired with strong inputs and become strengthened. Thus, a



phenomenon of coincident activation of pre- and post-synaptic elements could serve as an underlying mechanism of memory formation and storage. Moreover, the ability to artificially induce long-term synaptic modification in brain structures associated with learning and memory was critical in beginning to understand how information was processed and stored.

The physiological existence of a Hebbian synapse which exhibited the long-lasting capability for plasticity that would be necessary for learning and retention was first demonstrated experimentally in rabbits by Bliss and Gardner-Medwin (1973) and Bliss and Lomo (1973). They reported that following brief, repetitive high frequency stimulation (100 Hz for 1 sec) of the entorhinal cortex/dentate gyrus pathway, there was an up to five-fold increase in amplitude of the post-synaptic response (post-tetanic potentiation; PTP), which decayed to a level of approximately 150-200% of baseline within several minutes. This latter increase in the post-synaptic response was labelled long-term potentiation (LTP) has been defined as a use-dependent strengthening of synapses that can be induced by brief increases in activity and persists for long periods of time (Madison, Malenka and Nicoll, 1991).

The two most common measures of the post-synaptic response are changes in the excitatory post-synaptic potential (EPSP) and the population spike (PS). Stimulation of afferent fibres results in the activation of a large

number of synapses and a significant depolarization of the dendrites of the granule cells of the dentate gyrus (EPSP). Superimposed upon the EPSP waveform is a population spike, which represents the summed action potentials of the individual discharges from recorded cells or simply, an index of the number of discharging cells (Andersen et al., 1971).

Following tetanic stimulation, increased synaptic strength may be assessed as an increase in the slope of the EPSP and/or an increase in the PS amplitude. LTP has been demonstrated to last as long as sixteen weeks in the unanesthetized animal (Bliss and Gardner-Medwin, 1973). Douglas and Goddard (1975) originally demonstrated a similar phenomenon of long-lasting potentiation in the rat hippocampus.

There are three excitatory pathways that have demonstrated LTP within the trisynaptic circuit of the hippocampus: the perforant path, originating in the entorhinal cortex and projecting to the granule cells of the dentate gyrus, the Schaeffer collaterals/hippocampal commissure, originating in the CA3 region and projecting to the pyramidal cells of CA1, and the mossy fiber pathway from the dentate gyrus granule cells and projecting to the pyramidal cells of CA3 (Andersen, 1975). For the purpose of this discussion, the perforant path and Schaeffer collateral LTP will be discussed as only these two pathways demonstrate associative properties.

Associative LTP displays the properties of cooperativity, association and specificity. Cooperativity refers to the observation that the tetanizing stimulus must be delivered at high frequency (Dunwiddie and Lynch, 1978) suggesting that the stimulation must be of sufficient strength to activate a minimum number of afferent fibers (McNaughton, Douglas and Goddard, 1978). The cooperativity between pre-synaptic fibers is also associative in nature (as suggested by Hebb). Thus, by placing two separate stimulating electrodes in two separate subpopulations of Schaeffer collaterals and recording from the CA1 post-synaptic cell, one observes that if the weak stimulation of one afferent pathway (which does not induce LTP) is paired (as in classical conditioning) with the strong tetanization of another pathway, both will demonstrate LTP (Gustafsson and Wigstrom, 1986; Larson and Lynch, 1986). As in classical conditioning, their activation must be temporally contiguous. If the two tetanizations are separated by more than 100 msec, the weak synapse will not potentiate (Gustafsson and Wigstrom, 1986). Finally, while many afferent pathways impinge on any given post-synaptic cell, only those afferents that were tetanized will demonstrate LTP. Such synapse specificity is defined by the observation that no LTP is measurable in the non-tetanized afferents (Gustafsson and Wigstrom, 1986; Larson and Lynch, 1986).

LTP has been distinguished from the post-tetanic potentiation (PTP) originally described at the neuromuscular junction (Magleby and Zengel, 1975, 1976). In the case of PTP, there was a relatively short-lasting potentiation ( $< 2$  minutes) that was characteristic of a stimulation-induced increase in the release of neurotransmitter from the pre-synaptic terminal. Moreover, this increase was due to transient increases in the accumulation of free calcium ions in the pre-synaptic terminal (Delaney, Zucker and Tank, 1989; Zucker, 1989). Evidence to suggest a further distinction between LTP and PTP was provided by Humphreys, Engstrom, Young and Rose (1992) who found that animals anesthetized with secobarbital, but not pentobarbital, did not demonstrate the early decremental component characteristic of PTP following a tetanic stimulation, although similar LTP was observed in both groups. This finding suggests that the diminished PTP response did not prevent the induction of a normal LTP response. Finally, McNaughton (1982) demonstrated that PTP was observed following either low- or high-intensity pulse trains, while LTP occurred only following high-intensity stimulation.

Gustafsson, Wigstrom, Abraham and Huang (1987) demonstrated that the depolarization of the post-synaptic membrane together with pre-synaptic activation were the critical features for the induction of LTP. Malinow and Miller (1986) applied current to the post-synaptic membrane,

resulting in hyperpolarization. The subsequent tetanization of afferents did not result in LTP, suggesting the importance of a voltage-dependent post-synaptic mechanism. However, the depolarization of the post-synaptic cell by current application alone was not sufficient to induce LTP, indicating that the activation of pre-synaptic afferents contributed to the development of LTP (Gustafsson et al., 1987; Wigstrom, Gustafsson, Huang and Abraham, 1986).

The induction and maintenance of LTP are believed to involve several pre- and post-synaptic processes. The major excitatory neurotransmitter used by hippocampal neurons is glutamate (Fagg and Foster, 1983; Fonnum, 1984) which binds to both N-methyl-D-aspartate (NMDA) and non-NMDA (kainate and quisqualate) receptors located in close proximity on the dendritic spines (Cotman and Ganong, 1988; Mayer and Westbrook, 1987; Watkins and Olverman, 1987). During normal synaptic transmission, the release of glutamate from pre-synaptic terminals results in binding to and activation of only the non-NMDA sites (Collingridge, 1985), thereby resulting in the flow of  $\text{Na}^+$  and  $\text{K}^+$  through the channel. In contrast, the NMDA ionophores appear to be blocked, in a voltage-dependent manner, by extracellular  $\text{Mg}^{2+}$  (Asher and Nowak, 1988; Mayer and Westbrook, 1987). The activation of these receptors requires adequate depolarization of non-NMDA sites in order to remove

the blockade, thereby allowing the influx of  $\text{Na}^+$  and  $\text{Ca}^{2+}$  (Mayer and Westbrook, 1987; Mayer, Westbrook and Guthrie, 1984). Thus, the NMDA receptor is a double-gated channel, requiring both its activation by the neurotransmitter glutamate, and depolarization to remove the  $\text{Mg}^{2+}$  blockade. As mentioned earlier, McNaughton (1982) reported that while low intensity stimulation could induce PTP, only a high-intensity stimulation resulted in LTP, suggesting that the activation of the voltage-gated NMDA receptors was required for the induction of LTP.

Recently, Tocco, Maren, Shors, Baudry and Thompson (1992) reported that hippocampal  $^3\text{H}$ -AMPA binding (especially in  $\text{CA}_1$  and the dentate gyrus) was significantly increased in animals that demonstrated an increased post-synaptic response (population spike amplitude) to high-frequency, theta-like stimulation. In contrast, no increase in  $^3\text{H}$ -AMPA binding was observed in those animals which received high-frequency stimulation, but did not exhibit LTP. While there were no changes observed in NMDA receptors following stimulation, the administration of ketamine, a non-competitive NMDA receptor antagonist blocked both the induction of LTP and the increase in  $^3\text{H}$ -AMPA binding following tetanic stimulation (Tocco et al., 1992). These findings suggested that increased AMPA binding represented a biochemical correlate of LTP, and was also dependent upon NMDA receptor activation.

To summarize briefly, it is believed that the induction of LTP requires tetanic stimulation (and subsequent release of glutamate) to depolarize the non-NMDA receptors (a phase which is likely aided by the up-regulation of AMPA receptors) which subsequently removes the voltage-sensitive  $Mg^{2+}$  block on the NMDA receptors. Activation of these NMDA sites is the critical step in triggering a series of biochemical events which lead to the induction of LTP. Indeed, blocking the NMDA receptor with the antagonist D-2-amino-5-phosphonovaleric acid (APV), which prevented  $Ca^{2+}$  influx, also blocked the development of LTP (Bliss and Lømo, 1973). Harris, Ganong and Cotman (1984) demonstrated that similar compounds, AP5 and AP7 blocked NMDA depolarizations without affecting normal synaptic transmission (which appeared to be gated through the non-NMDA receptors). Moreover, blockade of the NMDA ionophore itself by the antagonist MK-801 (Wong, Kemp, Priestley, Knight, Woodruff and Iversen, 1986) also prevented the induction of LTP (Coan, Saywood and Collingridge, 1987).

A necessary and potentially sufficient trigger for induction of LTP is the calcium influx through the NMDA ionophores (Malenka, Kauer, Zucker and Nicoll, 1988). Injections of the calcium chelator, EGTA, prevent the development of LTP (Lynch, Larson, Kelso, Barrionuevo and Schottler, 1983). The increased calcium concentrations within the

dendritic spine triggers the activation of two  $\text{Ca}^{2+}$ -dependent second messengers,  $\text{Ca}^{2+}$ /calmodulin kinase and protein kinase C (PKC), thereby resulting in the induction of LTP. Indeed, the intracellular injection of H-7, a PKC inhibitor, into CA1 pyramidal cells prevents the induction of LTP without affecting normal synaptic transmission (Malenka, Kauer, Perkel, Mauk, Kelly, Nicoll and Waxham, 1989). Antagonism of calmodulin also blocks the generation of LTP (Malenka et al., 1989; Malinow, Schulman and Tsien, 1989).

While the induction of LTP is believed to involve both pre- and post-synaptic events, the maintenance of LTP is considered to be a function of the enhanced release of pre-synaptic glutamate. Bliss, Douglass, Errington and Lynch (1986) and Malinow and Tsien (1990) demonstrated that following the induction of LTP, there was an increase in glutamate release without a change in the sensitivity of the post-synaptic membrane. Moreover, Foster and McNaughton (1991) demonstrated that tetanization of the Schaeffer collaterals resulted in an increase in the amount of glutamate released per pre-synaptic impulse. Thus, it was necessary to postulate the existence of a retrograde messenger, released from post-synaptic sites (potentially stimulated by the activated protein kinases) which could diffuse across the synaptic cleft and act upon stimulated pre-synaptic



terminals to somehow increase transmitter release over an extended period of time (Barinaga, 1991).

The most recent candidate for this retrograde messenger is nitric oxide (NO), a highly-reactive gas which is synthesized by the  $\text{Ca}^{2+}$ /calmodulin-dependent enzyme, nitric oxide synthetase (NOS; Bredt and Snyder, 1990). It has been demonstrated that the activation of NMDA receptors in the cerebellum resulted in the formation of NO (Garthwaite, Charles and Chess-Williams, 1988). More importantly, the functional consequence of NMDA receptor activation (i.e. LTP) was blocked by NOS inhibitors (Bohme, Bon, Stutzmann, Doble and Blanchard, 1991; Haley, Wilcox and Chapman, 1992; Schuman and Madison, 1991), although PTP was unaffected (Haley et al., 1992). Sodium nitroprusside, a stimulator of NO, mimicked the effect of high frequency stimulation upon the long-term post-synaptic response (Bohme et al., 1991). Moreover, the level of enhancement was not further potentiated by high frequency stimulation, suggesting that NO-induced and stimulation-induced LTP shared a common mechanism (Bohme et al., 1991).

As mentioned earlier, the maintenance of LTP is believed to involve the enhancement of glutamate release. If NO is indeed the retrograde messenger, it must enhance glutamate levels, and to date there is a single study that has examined this issue in vitro. O'Dell, Hawkins, Kandel and

Arancio (1991) reported that the exogenous application of NO to cultured hippocampal neurons increased pre-synaptic glutamate release.

To summarize, it has been hypothesized that the induction and maintenance of LTP involve both pre-and post-synaptic events. While the induction of LTP is dependent upon the activation of voltage-gated NMDA receptors by the activation of pre-synaptic neurons, the maintenance of LTP is dependent upon the activity of the calcium-dependent second messengers in facilitating pre-synaptic events.

#### Does LTP Correlate with Learning?

Since LTP can be elicited by repeated electrical stimulation, both in vivo (awake or anesthetized preparations) and in vitro slices, it is apparent that hippocampal neurons have the potential to support long-term plasticity. However, it is important to determine whether these changes can occur in the absence of experimental intervention. Does a similar LTP phenomenon occur while an animal is learning? This so-called "behavioral LTP" was described by Berger and Thompson (1978) who examined changes in post-synaptic efficacy during classical conditioning of the nictating membrane, whereby a tone (the conditioned stimulus) paired with an air-puff (the unconditioned stimulus) elicited closure of the nictating membrane. Electrodes were implanted in the perforant path/ dentate gyrus pathway, and changes in the post-synaptic response were monitored during

conditioning. In the absence of any tetanic stimulation, the population spike amplitude was enhanced in parallel with the acquisition of the response, suggesting that an LTP-like phenomenon can occur naturally within the hippocampus.

Another method of examining the importance of the LTP in learning was to correlate NMDA receptor activation and ongoing behavior. Morris, Anderson, Lynch and Baudry (1986) demonstrated that the intraventricular infusion of the NMDA receptor blocker 2-amino-5-phosphopentanoate (AP5) blocked the induction of LTP responses (as assessed by a change in the EPSP slope) in the perforant path/dentate gyrus synapse, without affecting pre-tetanic potentials (e.g. normal synaptic transmission was unaffected). In a separate group of animals, AP5 impaired acquisition in the Morris water maze. Here, the deficit was subtle as the rats appeared to have acquired some non-specific learning (e.g. to swim away from the sidewalls of the pool). In contrast, their performance on a probe trial (whereby the escape platform was removed) indicated that the treated animals had no spatial bias for the original training quadrant. However, their performance on a non-spatial version of the task which utilized two visually-distinct platforms that were moved in-between training trials was unimpaired. These data suggested an interesting relationship between the induction of LTP and learning, as a drug that blocked the development of

LTP also impaired spatial, but not discrimination learning. Moreover, the data paralleled the finding that hippocampal lesions disrupt performance in the place, but not visual learning paradigm (Morris et al., 1982). Although these findings suggest that NMDA-dependent LTP may be one of the physiological mechanisms involved in learning, it does not subserve all forms of learning.

#### Primed-Burst Potentiation

If LTP is the endogenous physiological mechanism which underlies learning, why are the stimulus patterns necessary to induce the phenomenon well beyond the normal firing pattern of hippocampal neurons (e.g. multiple tetanic stimuli, delivered at frequencies of more than 100 Hz; reviewed by Teyler and DiScenna, 1987). Larson and Lynch (1986) and Rose and Dunwiddie (1986) described similar phenomena in which the threshold for the induction of LTP was markedly reduced by the administration of physiologically-patterned stimuli that mimicked the normal firing patterns within the hippocampus. This phenomenon, described as primed-burst (PB) potentiation, involved the presentation of a single priming stimulus given 170 msec ( $\approx$  6 Hz) prior to the presentation of a burst of four stimuli at 100 Hz. The PB phenomenon takes advantage of two characteristics of hippocampal activity: complex spike activity (rapid, high frequency bursts of 2-5 spikes; Fox and Ranck, 1981) and

theta rhythm (a field potential, EEG, of summed synaptic potential in the range of 3-12 Hz; Winson, 1974, 1976). Both LTP-like and PB stimulation induce a rapid increase in population spike amplitude within the first minute following stimulation (e.g. the PTP was 331% and 243% of baseline respectively) and a significantly elevated response at the end of a ten-minute recording session (250% and 150% respectively). In a subsequent study, Diamond, Dunwiddie and Rose (1988) determined both the priming interval (140-170 msec) and number of burst stimuli ( $> 2$ ) necessary to induce PB potentiation. The durability of PB potentiation has been demonstrated in several experiments to last at least several hours in awake animals (Diamond et al., 1988).

Is PB potentiation an LTP-like phenomenon? Despite the observation that post-synaptic responses following PB stimulation were not as great or as long-lasting as LTP, there was considerable evidence to suggest that both forms of potentiation were subserved by a similar mechanism. For example, AP5, the NMDA receptor antagonist which prevented the development of LTP (Morris et al., 1986) also blocked the development of PB potentiation (Diamond et al., 1988). Second, the amount of LTP that could be induced in any given synapse was inversely related to the number of PB trains that were delivered prior to LTP (Diamond et al., 1988). This suggested that the saturation of one process

(e.g. multiple PB trains) would prevent the induction of the other (e.g. LTP). Taken together, these data suggested that physiologically-patterned stimulation of hippocampal afferents could result in a long-lasting enhancement of post-synaptic events, that apparently mimicked the LTP phenomenon.

### Glucocorticoids and LTP

Yerkes and Dodson (1908) described an inverted U-shaped function to explain the relationship between arousal and performance. Specifically, they examined how the intensity of shock (arousal) influenced the rate at which mice learned to avoid shock (performance). Avoidance of intermediate levels of shock were learned most rapidly while acquisition of the avoidance response was considerably slower with low or high shock intensities. Although the authors did not measure adrenocortical activity, their data suggested that glucocorticoids may modulate learning and memory. Indeed, corticosterone and dexamethasone facilitate the extinction of a well-learned conditioned avoidance response (Bohus and Lissak, 1968; deWeid, 1967). Based upon these findings, glucocorticoid modulation of the physiological processes which underlie learning would not be unexpected.

Just as the effects of adrenal steroids on hippocampal activity are complex, so too are its effects upon LTP. For example, Dubrowsky,

Liquorik, Noble and Gijssbers (1987) found that a single dose of 5 alpha-dihydrocorticosterone (DHB), a reduced metabolite of corticosterone, decreased the population spike amplitude in the dentate gyrus following low-frequency stimulation and prevented the induction of LTP following high-frequency stimulation for approximately one hour. However, DHB did not suppress the increase in EPSP slope following the LTP stimulation. Filipini, Gijssbers, Birmingham, and Dubrovsky (1991) extended these findings to include corticosterone and the mineralocorticoid-type hormones, 18-hydroxycorticosterone (18-OH-DOC) and aldosterone. Consistent with Dubrovsky et al. (1987), corticosterone inhibited the PS amplitude for at least thirty minutes following the LTP train, while leaving the increase in EPSP slope intact. In contrast, the mineralocorticoids had a greater effect upon the EPSP slope than PS amplitude. That is, 18-OH-DOC and aldosterone inhibited increases in the EPSP slope for as long as sixty minutes following LTP stimulation while they had no inhibitory effect on PS amplitude.

The effects of elevated glucocorticoids on LTP have also been examined in animals exposed to stress. Foy, Stanton, Levine and Thompson (1987) demonstrated that both restraint stress and restraint plus shock for thirty minutes prior to sacrifice significantly reduced the in vitro (hippocampal slice preparation) CA1 PS amplitude relative to controls.

Moreover, plasma corticosterone levels taken at the time of sacrifice (5 to 35  $\mu\text{g/dl}$ ) were negatively correlated with LTP measured thirty minutes after stimulation ( $r = -0.65$ ,  $p < 0.05$ ).

Shors, Seib, Levine and Thompson (1989) exposed rats to seven days of either escapable or inescapable footshock and then sacrificed the animals for in vitro CA1 recordings. Compared with controls, the animals exposed to escapable shock exhibited less potentiation (as measured by PS amplitude), while inescapable shock prevented potentiation altogether (189, 156 and 111% of baseline respectively). Moreover, the PTP was significantly reduced only in those animals receiving inescapable shock. Finally, the authors reported that both groups of animals receiving shock had equally-elevated levels of plasma corticosterone (59 to 63  $\mu\text{g/dl}$ ), suggesting that in this experiment, circulating levels of glucocorticoids did not accurately predict the degree of LTP.

The importance of glucocorticoids in mediating the inhibition of LTP observed in stressed animals was directly examined by Foy, Foy, Levine and Thompson (1990). Forty-eight hours prior to experimentation, the animals received either a dose of the type II receptor agonist, dexamethasone (DEX), that significantly reduced their plasma corticosterone levels ( $< 2 \mu\text{g/dl}$ ) or vehicle in their drinking water. On the testing day, animals were either restrained and tail-shocked (stress) for



thirty minutes or sacrificed directly from the home cage (non-stressed). In vitro LTP in the CA1 was significantly reduced in both the DEX + stress and vehicle + stress groups thirty minutes following the stimulus train, relative to vehicle + unstressed controls, suggesting that prior exposure to stress, even in the absence of elevated glucocorticoids, inhibited LTP. However, dexamethasone pretreatment did prevent the stress-induced inhibition of the PTP response (see Shors et al., 1989 above).

Shors, Levine and Thompson (1990a) demonstrated that both thirty minutes of restraint + shock or adrenalectomy + shock suppressed in vitro CA1 LTP relative to intact controls. Therefore, stress significantly reduced hippocampal LTP via a mechanism that was independent of adrenal gland secretions. These authors also examined the effects of long-term adrenal demedullation upon LTP to determine whether sympathetic adrenomedullary secretions (i.e. catecholamines and enkephalins) released in response to stress, contributed to the suppressive effects of stress upon LTP. Demedullated rats were either exposed to thirty minutes of restraint and tail shock (stress) or left undisturbed (unstressed) prior to recordings. Demedullation did not impair the adrenocortical response to stress (e.g. the mean plasma corticosterone levels were 2 and 64 µg/dl for the unstressed and stressed animals respectively). Both demedullated groups demonstrated significantly reduced LTP relative to control rats, suggesting

that the adrenal medulla, and not circulating levels of corticosterone, was the major contributing factor in the reduction of LTP observed following exposure to a stressor (Shors et al., 1990a).

### Glucocorticoids and PB Potentiation

The effects of glucocorticoids upon primed-burst potentiation have been the exclusive domain of a single research group and offer considerably different findings to those described above. Bennett, Diamond, Fleshner and Rose (1991) reported that urethane anesthesia in intact rats resulted in a wide range of corticosterone levels (25 to 93  $\mu\text{g/dl}$ ) which manifested itself in a significant level-dependent inhibition of in vivo CA1 PB potentiation ( $r = -0.76$ ,  $p < 0.001$ ). Similarly, activation of the HPA axis during exposure to a novel testing apparatus significantly reduced the incidence of PB potentiation, while acclimation increased the incidence of observing synaptic enhancement. (Diamond, Bennett, Stevens, Wilson and Rose, 1990).

In contrast to the findings of Shors et al. (1990a), adrenalectomized rats did exhibit potentiation (e.g.  $\approx 73\%$  above baseline). Moreover, the threshold necessary to induce PB potentiation following urethane anesthesia was significantly reduced in adrenalectomized rats relative to intact controls (Diamond, Bennett, Engstrom, Fleshner and Rose, 1989). It is important to note that in control rats under urethane anesthesia, plasma

corticosterone levels are in the high physiological to pharmacological range (Bennett et al., 1991). In their most recent report, Diamond, Bennett, Fleshner and Rose (1992) described an inverted U-shaped function between the level of plasma corticosterone and the magnitude of PB potentiation, suggesting that there was a range of HPA activation that both enhanced and suppressed these physiological processes. While basal levels of corticosterone associated with the a.m. trough and p.m. peak of the diurnal cycle were associated with an enhanced PB response, stress-like levels of corticosterone ( $> 25\mu\text{g/dl}$ ) decreased the post-synaptic response to PB stimulation. These findings were consistent with Joels and DeKloet (1989) in which lower concentration of corticosterone enhanced while high concentrations of corticosterone suppressed hippocampal excitability.

#### Contrasting the Effects of Glucocorticoids in LTP Versus PB Paradigms

Much of the variance with regards to the role of glucocorticoids in hippocampal synaptic plasticity (e.g. Diamond et al., 1992; Shors et al., 1990a) may be related to differences in methodology. For example, the majority of work on stress and LTP was performed in vitro. The animals were exposed to stress prior to sacrifice and the electrophysiological recordings were conducted on deafferented hippocampal slices. In contrast, the effects of stress or glucocorticoids on PB potentiation have been examined either in the anesthetized or freely-moving animal. Thus,

measures of plasticity were assessed in the intact (with respect to afferent and efferent connections) hippocampus while the animal was exposed to stress. This in vivo preparation clearly offers a more realistic picture of glucocorticoid mediation of hippocampal plasticity.

Chronic stress also appears to have a different effect upon synaptic plasticity than either corticosterone or dexamethasone administration. For example, Diamond et al., (1992) reported that while elevated corticosterone levels had significant effects upon PB potentiation, it did not effect PTP, while stress (exposure to novelty) blocked both PTP and PB (Diamond et al., 1990).

Adrenal steroids and chronic stress also appear to have differential effects upon the receptors believed to mediate synaptic plasticity. Although Halpain and McEwen (1988) reported that exogenous administration of corticosterone resulted in a small reduction of hippocampal [ $^3\text{H}$ ] glutamate binding (as assessed by in vitro binding techniques), a more thorough autoradiographic study of the NMDA, kainate and quisqualate receptors following adrenalectomy or glucocorticoid administrations revealed no effects of adrenal hormone manipulation on these receptor subtypes (Clark and Cotman, 1992). In contrast, Tocco, Shors, Baudry and Thompson (1991) found that exposure to restraint and tailshock increased the concentration of [ $^3\text{H}$ ] AMPA (a quisqualate receptor agonist) binding.

Moreover, these increases were observed in both intact and adrenalectomized rats (Shors, Tocco, Patel, Baudry and Thompson, 1991), suggesting that stress affected the non-NMDA, glutamate receptors concentrations, even in the absence of circulating corticosterone. However, these findings contrast with those described earlier in which increases in [ $^3\text{H}$ ] AMPA binding were observed only in those animals expressing an enhanced post-synaptic response (Tocco et al., 1992). Clearly the modification of NMDA and non-NMDA receptors following adrenocortical modulation and stress needs further examination.

Finally, the type of stressor used may play a critical role in determining the effects of glucocorticoids on plasticity. For example, the disruption of LTP following restraint and tailshock was observed in animals pretreated with dexamethasone (Foy et al., 1990) or in animals adrenalectomized (Shors et al., 1990a) prior to stress. In contrast, when adrenalectomized rats were exposed to novelty, they displayed a normal potentiation response (D. Diamond, personal communication). Together, these data suggest that elevated glucocorticoids can mediate plasticity, but are not the only signals involved. Shors, Levine and Thompson (1990b) reported that the opioid antagonist, naltrexone was effective in attenuating the stress-induced inhibition of LTP, thereby implicating both the adrenocortical and opioid systems in the modulation of their model of

hippocampal plasticity. Based upon this hypothesis of a synergy between the opioids and glucocorticoids, it would be important to determine whether naltrexone could also block the inhibitory effects of either glucocorticoid administration or exposure to novelty.

#### Long-Term Potentiation and Aging

Based upon the findings that glucocorticoid levels increase as a function of age (e.g. Issa et al., 1990; Landfield et al., 1978b; Meaney et al., 1988a) and that elevated levels of glucocorticoids impair LTP (Bennett et al., 1991; Diamond et al., 1992), one would anticipate that aged rats would exhibit diminished LTP. Further, if hippocampal synaptic plasticity is related to learning and memory, one would hypothesize that only aged, cognitively-impaired rats would demonstrate decreased LTP. Barnes (1979) correlated hippocampal LTP with the performance of mature adult (10-16 months) and old (28-34 months) rats in the hole board task, a "dry land" version of the Morris water maze. Here, the rats were required to find an escape tunnel, with the use of distal spatial cues, located in one of the eighteen holes that circled a well-lit platform. The latency to enter the tunnel, the distance covered and the number of incorrect choices made prior to escape were the dependent measures of interest. While performance on the first three trials did not differ between groups, on subsequent trials the aged rats spent more time, covered a greater distance

and made more errors when locating the platform than did the younger animals. There was no age-related decrement in spontaneous alternation. Electrophysiological data suggested that high-frequency, perforant path stimulation produced equivalent granule cell synaptic responses (with respect to the rise and decline of the EPSP slope) in both age groups. However, three repetitions of this stimulation indicated a significant decrease in the durability of the synaptic enhancement in the aged rats (14 days versus 1 test session for adult and old animals respectively). These data suggested that while the induction of LTP was not impaired in old rats, there was an effect on the maintenance of synaptic efficacy.

In a subsequent study, Barnes and McNaughton (1985) found that old animals took significantly more trials to reach asymptotic performance in the hole board task and that their retention of the spatial location was poorer than the younger rats. These behavioral findings correlated with the electrophysiological recording whereby the asymptotic level of LTP occurred more slowly, while decaying much faster in older rats.

Both Landfield and Lynch (1977) and Landfield, McGaugh and Lynch (1978a) examined in vitro hippocampal LTP in aged, F-344 rats that exhibited a significant deficit in the retention of a passive avoidance response (Gold and McGaugh, 1975). While synaptic responses to low frequency stimulation did not differ between young and old animals, PTP

following high frequency stimulation was significantly reduced in the old animals. Moreover, the development of LTP was markedly decreased in the aged animals (Landfield et al., 1978a).

Deupree, Turner and Watters (1991) reported that aged, cognitively-impaired (as determined by performance in the Morris water maze) rats exhibited a significantly reduced post-tetanic response to low-intensity stimulation relative to aged, cognitively-unimpaired and young controls (who did not differ). Further, the normal decaying PTP response was absent only in the aged impaired animals. These findings were consistent with those described above and suggested that impairments in the acquisition of the Morris water maze were strongly correlated with impairments in an electrophysiological model of learning.

To summarize, these studies suggested that although aged animals were capable of displaying some degree of hippocampal plasticity, the long-term post-synaptic response to excitatory stimulation was significantly reduced compared with younger animals. Moreover, this reduced level of plasticity correlated well with the performance deficits of aged rats on tasks dependent upon intact hippocampal function (e.g. Barnes et al. 1979; Turner et al., 1991).



### PB Potentiation and Aging

To date there has been only one published study examining the ability of PB stimulation to induce long-lasting plasticity in aged animals. Moore, Browning and Rose (1992) found that while LTP-like stimulation could produce an equivalent increase in population spike amplitude in both young and aged F-344 rats, a threshold form of stimulation (e.g. PB potentiation) produced an enhanced post-synaptic response only in the young animals. These findings suggested that the capacity to maintain durable synaptic efficacy was not impaired in aging if the stimulation was of sufficient intensity. Similarly, Deupree et al. (1991) demonstrated that high, but not low intensity stimulation resulted in an enhancement of the post-synaptic response in aged, cognitively-impaired rats while aged, cognitively-unimpaired rats did not differ from young controls under either stimulation condition. The authors concluded that impairments in acquisition of the Morris water maze task correlated with the post-synaptic response to low, but not high intensity stimulation. These findings suggested that physiologically-patterned stimulation might offer a more sensitive measure of hippocampal functioning, and one that is more relevant to behavioral/cognitive phenomenon. Thus, primed-burst potentiation may offer an alternative to LTP-like stimulation parameters in

elucidating the variations that exist within a given population with respect to cognitive impairments.

### The Glucocorticoid Cascade Hypothesis

Sapolsky, Krey and McEwen (1986b) attempted to integrate both experimental and clinical research into a unifying hypothesis of how the cumulative exposure to stress and/or the inability to adapt to stress might contribute to the pathology of aging. The glucocorticoid cascade hypothesis represents an updated and extended version of the early hypothesis of Hans Selye (1946). Selye and Tuchweber (1976) suggested that glucocorticoids conferred resistance to stress, but that the body was equipped with only a limited amount of this "adaptational energy". Repeated and prolonged exposure to stress throughout the lifespan depleted these finite reserves, resulting in the loss of the ability to mobilize a response to stress and thus accelerated the onset of senescence.

A critical problem with Selye's hypothesis is that there is no age-related decline in the ability of the HPA axis to respond to stress (Sapolsky et al., 1983a, 1986a). Indeed, aged rats hypersecrete corticosterone following the termination of the stress (Issa et al., 1990; Meaney et al., 1988a; Sapolsky et al., 1983b, 1985) and it has been demonstrated that both the decrease in hippocampal type II receptors and hippocampal neuron loss may account for the impairments in negative feedback that underlie this

effect (Issa et al., 1990; Meaney et al., 1988a; Sapolsky et al., 1983b, 1985).

It would appear that the loss of corticosteroid receptors is secondary to the loss of neurons. Issa et al. (1990) examined both hippocampal type I and type II receptor densities and neurons in aged, cognitively-impaired (ACI) and cognitively-unimpaired (ACU) rats. While both groups of aged rats had fewer receptors and neurons than young controls, the loss was greater in the ACI rats. Indeed, although correlations were not performed, the loss of receptors mapped closely onto the loss of neurons. Sarrieau, Rowe, O'Donnell, LaRocque, Nair, Levin, Seckl and Meaney (1992), using in situ hybridization examined mRNA levels for hippocampal type I and type II receptors in both ACU and ACI rats. They found that the expression of mRNA on a per neuron basis (grains/cell) for the type I receptor was reduced in both aged groups compared with young controls. In contrast, there was no decrease in the expression of mRNA per neuron of type II receptors in either aged group compared with young rats. Together with the findings of Issa et al. (1990), these data suggest that the loss of hippocampal type II receptors is due to the loss of hippocampal neurons.

Sapolsky et al. (1986b) addressed the interrelationships amongst glucocorticoid hypersecretion, receptor reduction and neuron loss by

proposing an hypothesis of a degenerative glucocorticoid "cascade". Based upon the experimental evidence, age-related changes in both basal and stimulated HPA activity are likely due to the loss of the corticosteroid receptor-concentrating neurons. While the number of type II receptors/neuron appear to remain stable (Sarrieau et al., 1992), the total receptor population on a per tissue basis has diminished (Issa et al., 1990; Meaney et al., 1988a; Ritger et al., 1984; Sapolsky et al., 1983a,b, 1984b), and this decrease of receptors is due to the initial loss of hippocampal neurons. This decrease in corticosteroid receptors appears to result in the desensitization of the hippocampus to circulating levels of corticosterone. Indeed, reduced receptor concentrations are associated with elevated post-stress corticosterone secretion (Sapolsky et al., 1984a; 1984b; 1985). Moreover, Eldridge et al. (1989a,b) reported that up- and down-regulation of type II receptors in response to either a diminished or enhanced glucocorticoid signal respectively, was not apparent in aged rats. This may be interpreted as an adaptive mechanism, preventing further down-regulation in response to the elevated glucocorticoid signal. Instead, it would appear that the hippocampal neurons themselves have become targets of the neurotoxic effects of corticosterone (Sapolsky et al., 1985).

It seems that as the animal ages, both neurons and their receptors are lost, while the remaining receptors do not down-regulate in the presence of

high levels of corticosterone. This loss of inhibition of HPA function, together with the diminished ability to regulate the remaining receptor populations in the presence of the elevated corticosterone levels, results in a greater impact of glucocorticoids on hippocampal neurons (see next section for a discussion of the neurotoxicity of glucocorticoids).

The findings of Meaney et al (1988a) appear to offer some support for the cascade hypothesis. Neonatal handling during the first three weeks of life (which results in an increase in hippocampal type II receptors; Meaney et al., 1989a) resulted in a dramatic enhancement of the efficacy of HPA regulation during senescence (Meaney et al., 1988a). In an effort to explain the long-term beneficial effects of these elevated receptor levels, Meaney et al. (1989a) examined handled and non-handled animals as adults and demonstrated that handled animals exhibited a greater suppression of corticosterone following the administration of either corticosterone or dexamethasone compared with non-handled animals. Moreover, handled animals showed a reduced secretion of both ACTH and corticosterone compared with non-handled animals both during and following the termination of a stress. Together, these findings suggested enhanced negative feedback sensitivity in the handled animals. Indeed, Meaney et al. (1989a) reported that the  $IC_{50}$  of both corticosterone and dexamethasone for the suppression of ACTH was lower in young, handled rats.

The differences in hippocampal type II receptors between handled and non-handled animals persists throughout life (Meaney et al., 1988a) and may explain the variations in cognition and neuropathology observed in aged animals (Issa et al., 1990; Meaney et al., 1988a). That is, as non-handled animals age, basal levels of ACTH and corticosterone increase, a change that is not observed in old handled rats (Meaney et al., 1988a; Meaney et al., 1992). This observation suggests that the cumulative exposure to corticosterone over the lifespan is higher in the non-handled animals and consistent with the cascade hypothesis (Sapolsky et al., 1986b), aged non-handled animals demonstrate cognitive impairments and greater neuron loss relative to old handled animals (Meaney et al., 1988a). The diminished rate of neuron loss in aged handled animals likely reflects a reduced cumulative lifetime exposure to corticosterone. Although aged handled animals have elevated levels of type II receptors, the benefit of enhanced negative feedback appears to outweigh the potential risk of increased neurotoxicity by elevated glucocorticoids by ensuring that prolonged exposure to corticosterone does not occur.

How much hippocampal damage is required to manifest HPA dysfunction? Both rats and humans lose neurons as they age, but only a percentage demonstrate any pathology (Coleman and Flood, 1987). Issa et al. (1990) reported that although ACU rats lost approximately 25% of the

hippocampal neurons, they did not exhibit HPA dysfunction. In contrast, HPA dysfunction was evident in ACI rats who had suffered 60% neuron loss (Issa et al., 1990).

Sapolsky et al. (1986b) postulated that in healthy subjects, the observed hippocampal damage attributed to aging was below the threshold necessary to observe HPA dysfunction. Indeed, Greenwald et al. (1987) demonstrated that within a population of Alzheimer's Disease patients, the efficacy of a single dose of dexamethasone in suppressing adrenocortical activity declined as a function of age. Together, these findings suggested that neither the neuropathology attributable to aging nor to the disease state (e.g. SDAT) alone were sufficient to produce glucocorticoid hypersecretion. However, with advancing age, these effects summate to produce a greater prevalence of HPA dysfunction (Sapolsky et al., 1986b).

To summarize, the glucocorticoid cascade hypothesis offered by Sapolsky et al. (1986b) suggested that hippocampal neuron loss is associated with pathological aging. Coincident with the loss of neurons is the loss of type II receptors, which results in the impaired inhibition of ongoing HPA activity. Cumulative exposure to these elevated corticosterone levels would eventually exacerbate the degree of pathology and thus accelerate neuron loss. The critical features of the glucocorticoid cascade are the cumulative

degeneration of the hippocampus and the progressive emergence of corticosterone hypersecretion, with each exacerbating the other.

### Potential Mechanism of Glucocorticoid Toxicity

At doses which do not in themselves cause hippocampal damage, corticosterone does potentiate the damage induced by a variety of toxins and insults. For example, Sapolsky (1985) demonstrated that hippocampal infusion of the neurotoxin kainic acid (Coyle, 1983) induced a dose-dependent increase in damage to the CA3 neurons. The presence of high glucocorticoids for one week prior to lesioning significantly increased the degree of damage incurred compared with either kainic acid-treated controls or adrenalectomized rats. The potentiating effects of glucocorticoids were also observed following a variety of challenges including 3-acetylpyridine (3-AP; Sapolsky, 1985) and hypoxia-ischemia (Sapolsky and Pulsinelli, 1985) which target dentate gyrus and CA1 neurons respectively. In each case, adrenalectomy decreased and glucocorticoids increased the degree of damage relative to non-glucocorticoid-treated controls.

It has been demonstrated that these diverse metabolic insults each induce hippocampal damage via their influence on energy utilization. Kainic acid hyperexcites hippocampal neurons, likely through its interaction with glutamate receptors (Ben-Ari, 1985), placing a



pathological demand on energy substrate utilization. Both the anti-metabolite 3-AP (which disrupts the electron transport chain and inhibits the synthesis of adenosine triphosphate; ATP) and hypoxia-ischemia (which depletes ATP) impair the capacity of neurons to generate energy (Hicks, 1955). As discussed earlier, glucocorticoids inhibit glucose uptake in certain peripheral tissue for utilization by the liver and muscles (Munck, 1971), while Phillips, Berger and Rottenberg (1987) demonstrated that high doses of dexamethasone reduced hippocampal glucose utilization. Moreover, Virgin, Ha, Packan, Yang, Horner and Sapolsky (1991) reported that glucocorticoids inhibit glucose transport in hippocampal astrocytes. Thus, the presence of glucocorticoids may divert energy substrates away from the highly-susceptible neurons, making them more vulnerable to a variety of metabolic insults. This hypothesis was supported by the finding that the exacerbation of neuronal damage of glucocorticoids following either kainic acid or 3-AP lesions were reversed by the administration of a variety of alternative energy sources including glucose and mannose (Sapolsky, 1986). That neither of these fuels could prevent the damage induced by the lesions alone suggested that they did not minimize the impact of the toxins, but rather ameliorated the metabolic challenge established by the glucocorticoids.

Recently Packan and Sapolsky (1990) demonstrated a synergy between kainic acid and glucocorticoids (including corticosterone, dexamethasone and cortisol) in primary cell cultures of dispersed hippocampal cells. While the neuron loss observed following corticosterone administration was no greater than that observed following the addition of kainic acid alone, a synergistic action between the two was apparent. Moreover, the damage was not exacerbated by the presence of other steroids including aldosterone, estrogen, progesterone or testosterone. While the effects of dexamethasone suggested a type II receptor involvement, the prevention of the glucocorticoid synergy of kainic acid damage by the type II antagonist, RU 38486 offered direct evidence. In contrast, the type I antagonist, RU 28318 had no effect. Finally, the specificity of this synergy was limited to the hippocampus as no damage was evident in either hypothalamic or cerebellar cultures.

Together, these data suggest that while glucocorticoids do not directly influence neuron survival themselves, they do act synergistically to increase the toxicity of various metabolic insults. This exacerbation appears to arise from the ability of glucocorticoids to disrupt glucose utilization as the administration of several usable energy sources prevents the endangerment of the neurons. Finally, the hippocampus appears to be a near-exclusive target for this synergy, due likely in part to the high

concentrations of both glutamate (Monaghan, Holets, Toy and Cotman, 1983) and type II receptors (Reul and DeKloet, 1986) in this brain structure.

Recently, another mechanism of the glucocorticoid-exacerbation of lesion-induced damage was elucidated by Armanini, Hutchins, Stein and Sapolsky (1990). They demonstrated that the synergy between 3-AP and corticosterone in damaging neurons of the dentate gyrus was prevented by the NMDA receptor antagonist APV, suggesting that activation of these receptors somehow mediated neuron death.

It has been demonstrated that the neurotoxicity arising from the synergy between metabolic insults and glucocorticoids likely involves glutamate. Glucocorticoids inhibit glutamate uptake by glial cells (Virgin et. al, 1991), a major route for the elimination of glutamate from the synapse (Hertz, Kvamme, McGeer and Schousboe, 1983) by depleting ATP, the energy substrate necessary for its reuptake (Benveniste, Drejer, Schousboe and Diemer, 1984). Therefore, the presence of elevated levels of glucocorticoids, which increase the extracellular levels of glutamate (by preventing its reuptake), persistently activate the NMDA receptor, resulting in an increased influx of  $\text{Ca}^{2+}$ , which initiates the process of neuron death through a mechanism not yet understood.

That increased levels of glutamate and calcium are associated with cell death has been demonstrated indirectly in cerebellar cultures.

Rothman, Thurston and Haubart (1987) demonstrated that glutamate-induced cell death was ameliorated by the NMDA antagonist APV, suggesting that the blockade of the  $\text{Ca}^{2+}$  influx was effective in preventing neuronal damage. Moreover, Cox, Lysko and Henneberry (1989) found that while glutamate, in the presence of both  $\text{Mg}^{2+}$  and glucose did not induce damage within primary rat cerebellar cultures, the removal of glucose from the medium resulted in significant neurotoxicity.

To summarize, it appears that the glucocorticoid-exacerbation of neurotoxic damage to hippocampal neuron may involve two separate processes that initiate an NMDA cascade. The activation of hippocampal neurons (and subsequent release of glutamate) by hypoxic/ischemic insult or the injection of the glutamate analog, kainic acid into the hippocampus results in elevated levels of excitatory amino acids. Moreover, the additional presence of glucocorticoids depletes the energy substrates within the vulnerable neurons. This state of depletion prevents the reuptake of glutamate, thereby increasing the synaptic and extracellular glutamate levels. The reduction of energy substrates also removes the  $\text{Mg}^{2+}$  block on the NMDA receptor, thereby resulting in a persistent influx of  $\text{Ca}^{2+}$  which initiates the process of neuron death.

Finally, a recent study by Kerr, Campbell, Thibault and Landfield (1992) offered the first evidence to suggest that glucocorticoids enhance calcium influx in the absence of metabolic insults (e.g. as might be observed during pathological aging). Kerr et al. (1992) demonstrated that a two-hour exposure of CA1 neurons in hippocampal slices to the glucocorticoid type II agonist RU 28362 increased the voltage-activated  $\text{Ca}^{2+}$  influx relative to controls. Thus, these authors have provided the first direct evidence implicating the glucocorticoids in exacerbating  $\text{Ca}^{2+}$  activity and in the absence of any apparent metabolic insult, may offer another potential link between enhanced glucocorticoid activity and neuron loss.

### Experimental Rationale

Sapolsky et al. (1985) demonstrated that the chronic exposure to high glucocorticoid levels resulted in selective hippocampal damage to CA3 neurons. There is data to suggest that extensive bilateral damage of these neurons following kainic acid infusion results in behavioral impairments in the Morris maze (Sutherland et al., 1983). Thus, it is entirely possible that behavioral deficits in this task could occur in response to glucocorticoid-produced neuron loss.

To date, the long-term behavioral effects resulting from chronic exposure to exogenously-administered glucocorticoids have not been

examined extensively. Does the treatment regimen produce sufficient neuron loss to induce the types of learning deficits that are observed in a sub-population of aged, cognitively-impaired animals (e.g. Issa et al., 1990; Meaney et al., 1988a)? Clearly, the presence of a functional deficit in the corticosterone-treated rats would offer another dimension of similarity between a model of adrenocortical hypersecretion and aging.

In a series of studies, we examined the implications of such elevated corticosterone levels for both spatial learning deficits and hippocampal plasticity under two conditions: exogenous glucocorticoid administration and chronic stress. In contrast to many of the studies in this general research area, the dependent variables were assessed several weeks (Morris water maze) to several months (PB potentiation) following the termination of the treatment. Thus, any observed changes would be the long-term effects of glucocorticoid treatment rather than the result of elevated glucocorticoid levels at the time of testing. Moreover, such delayed testing would offer some insight as to whether the glucocorticoid-induced changes were permanent. Finally, we were interested in replicating the pattern of glucocorticoid-induced hippocampal neuron loss observed by Sapolsky et al. (1985).

## Experiment 1

Sapolsky et al. (1985) demonstrated that a three-, but not one-month exposure to high physiological levels of corticosterone produced significant hippocampal neuron loss. Thus, the purpose of the present experiment was to assess the functional consequences of these treatments. Young Long-Evans rats were implanted with corticosterone pellets for either one or three months and following the termination of the treatment, acquisition of a spatial learning task was assessed in the Morris water maze. If hippocampal neuron loss underlies the spatial learning deficit, it was hypothesized that only those animals receiving the three month treatment would demonstrate an impairment in this task.

### Methods

Subjects. Male, Long-Evans hooded rats, born into the Douglas Hospital Research Centre animal colony to dams derived from the Charles River stock (St. Constant, Quebec), were used in this experiment. Following weaning, the animals were housed in groups of three to four in 20 x 19 x 10 cm cages, maintained on a 12:12 light:dark cycle (lights on at 08:00 h), and provided with free access to food (Purina Lab Chow) and water. The health of all animals was monitored regularly, and any rats with overt signs of respiratory distress, infection or tumors were either removed from the study or sacrificed. In addition, the Douglas Hospital

Research Centre maintains a "sentinel" program overseen by the veterinary staff at McGill University. Under this program, specific animals caged in the same location and under the same conditions as the experimental animals were blood tested every three months and killed at six months in order to monitor the health of the animals in the facility and to screen for the presence of viruses or other forms of infection.

At four to five months of age, one-half of the animals were randomly assigned to receive either corticosterone ( $n = 10$ ) or cholesterol ( $n = 10$ ) pellets for a three-month interval. Two months later, the remaining animals were designated to receive the corticosterone ( $n = 10$ ) or cholesterol ( $n = 10$ ) pellets for one month. This allowed the experimenter to assess the behavior of all treatment conditions simultaneously.

Steroids. Corticosterone (4-pregnene,  $11\beta$ , $-21$ -diol- $3,20$ -dione; Sigma, St. Louis, MO) and cholesterol (5-cholestene- $3\beta$ -ol; Sigma, St. Louis, MO) pellets were formed by slowly heating the powders in a shallow beaker over a low gas flame to the point when the liquified steroids could be poured into embedding molds (Catalogue # 106: Ted Pella Inc., Redding, CA) to solidify. The pellets weighed approximately 100 mg each.

Pellet Implantation. Using the protocol described by Meyer, Micco, Stephenson, Krey and McEwen (1979), the animals were lightly



anesthetized with Metofane (methoxyflurane: Pitman Moore, Washington Crossing, New Jersey) and the skin around the nape of the neck was shaved and cleaned with isopropyl alcohol. A small incision was made and the underlying fascia was spread and separated with a hemostat inserted into the opening. Two, 100 mg pellets were placed under the skin at least two cm caudal to the incision, which was subsequently closed with one or two wound clips. The corticosterone pellets produced elevated corticosterone levels for approximately two weeks, in the range of 18-22  $\mu\text{g/dl}$  as determined by corticosterone radioimmunoassay (see below). The cholesterol pellets did not alter basal corticosterone levels (i.e. a.m values were  $< 5 \mu\text{g/dl}$ ). The pellets were removed and new ones were replaced in various locations around the nape of the neck according to this schedule. While there was considerable lesioning at the site of implantation, the animals were otherwise in good health.

Blood Sampling and Collection. To assess basal circulating levels of corticosterone produced by the pellets, tail-vein blood samples were taken from restrained rats within two minutes following their removal from the home cage. Sampling occurred between two and three days following pellet implantation and plasma was taken two hours into the onset of the light cycle. The samples were collected in heparinized tubes placed on ice,

and centrifuged at 3000 rpm for ten minutes in a refrigerated Beckman centrifuge (Model J-6). The plasma was stored at -30°C until assayed.

Corticosterone Radioimmunoassay. Plasma corticosterone was measured using the radioimmunoassay of Krey, Lu, Butler, Hotchkiss, Piva and Knobil (1975), with a corticosterone antiserum (F3-163; Endocrine Science, Tarzana, CA) and [<sup>3</sup>H] corticosterone (101.0 µCi/nmol; New England Nuclear, Boston, MA) as tracer. The antiserum cross-reacts < 4% with deoxycorticosterone and < 1% for the other steroids tested including aldosterone, cortisol, estradiol, prednisone, prednisolone, progesterone and testosterone. Intra- and inter-assay coefficients of variability were 10 and 12% respectively. The sensitivity of the assay was approximately 50 pg/ml. The assay procedure is outlined in detail in Appendix A.

Apparatus. The Morris water maze was a circular tank, 1.6 m in diameter and 60 cm in depth, located in a large room with low-intensity overhead lighting. There were many stable extramaze cues throughout the room including a sink, counters, computer equipment, ceiling tiles and the experimenter. The pool was filled with water (approximately 26 °C) to a level sufficient to cover the plexiglas escape platform (10 x 10 x 30 cm) by approximately two cm. The water was made opaque by the addition of 0.5 kg of powdered milk. The platform was hidden in one of four quadrants (i.e. NE, NW, SE, SW) halfway between the sidewalls and the center of the

pool and was maintained in a constant position for each animal. The swim paths, distances and latencies to locate the escape platform were monitored with a digitized television system mounted over the centre of the pool and connected to a Videomex-V Image Motion Computer (Columbus Instruments, Columbus, OH).

Procedure. Approximately seven days prior to testing, all pellets were removed to allow for clearance of the steroids and healing of the incision wounds. Tail blood samples indicated that a.m. levels of plasma corticosterone ranged from 2 to 6  $\mu\text{g}/\text{dl}$  in both the cholesterol- and corticosterone-treated animals. Behavioral testing began approximately two to three hours into the light cycle with the animals being removed from the home cage, placed into plexiglas cages containing several diapers and transported to the testing room. Each trial began with the animal being placed into the pool facing the sidewalls at either the north, south, east, or west positions in a quasi-random sequence, thereby requiring the animals to swim in different directions on successive trials. Each trial ended when the animal found the platform and remained there for ten seconds. If the animal did not find the platform within two minutes, the trial was terminated and it was placed on the platform by the experimenter. The animals were given twelve trials over three days (4 trials/day) with the platform submerged. The critical measures were the time elapsed from the

start of the trial until the animal found the platform (latency) and the swimming distance travelled before the animal located and climbed onto the platform. Measures of distance travelled were important to ensure that increased swimming latencies were the result of greater distances travelled rather than any changes in swimming speeds.

Statistics. For all experiments described in this thesis, the data were analyzed over days, by collapsing across blocks of trials within days, using a repeated measures analysis of variance and Tukey HSD post-hoc tests where appropriate. A single-factor ANOVA of the Trial 1 data was also performed to determine whether the groups differed upon their initial exposure to the Morris water maze. In the case of experiments with two groups, a single t-test was performed on the Day 1 data. All of the summary ANOVA tables for this and subsequent experiments appear in order in Appendix B.

### Results and Discussion

Both the latency and distance data for the one- and three-month treatment period were analyzed separately. Figure 1 shows the mean latency (sec) for animals to locate the submerged platform over days (collapsed across blocks of four trials within days) following a one-month treatment regimen. While there was a significant effect of days, ( $F = 21.8$ ,  $df = 2,36$ ,  $p < 0.0001$ ) neither the effect of corticosterone treatment ( $F =$

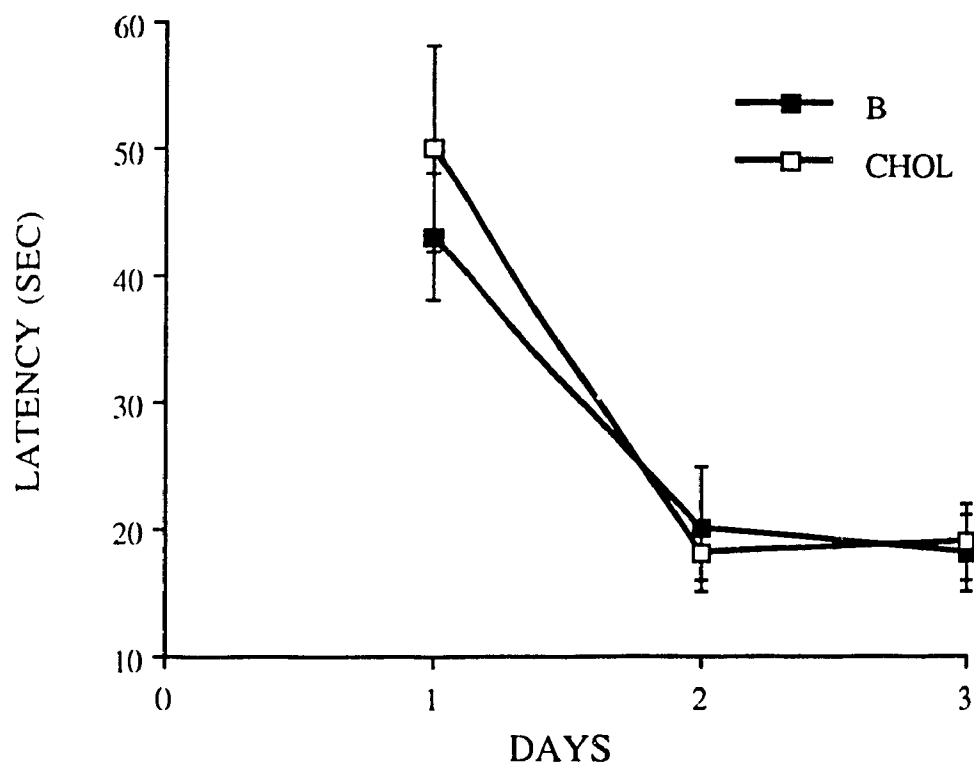


Figure 1. Mean latency (sec) over days (collapsed across blocks of four trials) to locate a submerged platform in rats treated with either corticosterone (B) or cholesterol (CHOL) for one month.

0.26,  $df = 1,18$ ,  $p > 0.6$ ), nor the treatment by days interaction ( $F = 0.4$ ,  $df = 2,36$ ,  $p > 0.6$ ) was significant. With respect to the latency data, the groups did not differ on Trial 1 ( $t = 0.31$ ,  $df = 18$ ,  $p > 0.7$ ). The analysis of the distance data (see Figure 2) revealed an identical pattern (Days:  $F = 21.06$ ,  $df = 2,36$ ,  $p < 0.0001$ ; Treatment:  $F = 0.04$ ,  $df = 1,18$ ,  $p > 0.8$ ; Treatment x Days:  $F = 0.21$ ,  $df = 2,36$ ,  $p > 0.8$ ). The groups did not differ on Trial 1 with respect to the distance data ( $t = 0.04$ ,  $df = 18$ ,  $p > 0.9$ )

Figure 3 represents the mean latency (sec) over days (collapsed across blocks of four trials within days) for animals to locate the platform following a three-month treatment regimen. There was a significant effect of days ( $F = 82.09$ ,  $df = 2,36$ ,  $p < 0.0001$ ), corticosterone treatment ( $F = 23.32$ ,  $df = 1,18$ ,  $p < 0.0001$ ) and a significant treatment by days interaction ( $F = 9.24$ ,  $df = 2,36$ ,  $p < 0.0006$ ). Post-hoc tests revealed that the corticosterone-treated animals took significantly longer ( $p < .01$ ) to locate the platform on Days 1 and 2 versus the cholesterol-treated controls. Moreover, the corticosterone-treated animals took significant longer to locate the platform of Trial 1 versus the cholesterol-treated controls ( $t = 5.23$ ,  $df = 18$ ,  $p < 0.0001$ ). The mean latency for Trial 1 was  $60.5 \pm 11$  and  $119.6 \pm 0.3$  sec for cholesterol- and corticosterone-treated animals respectively.

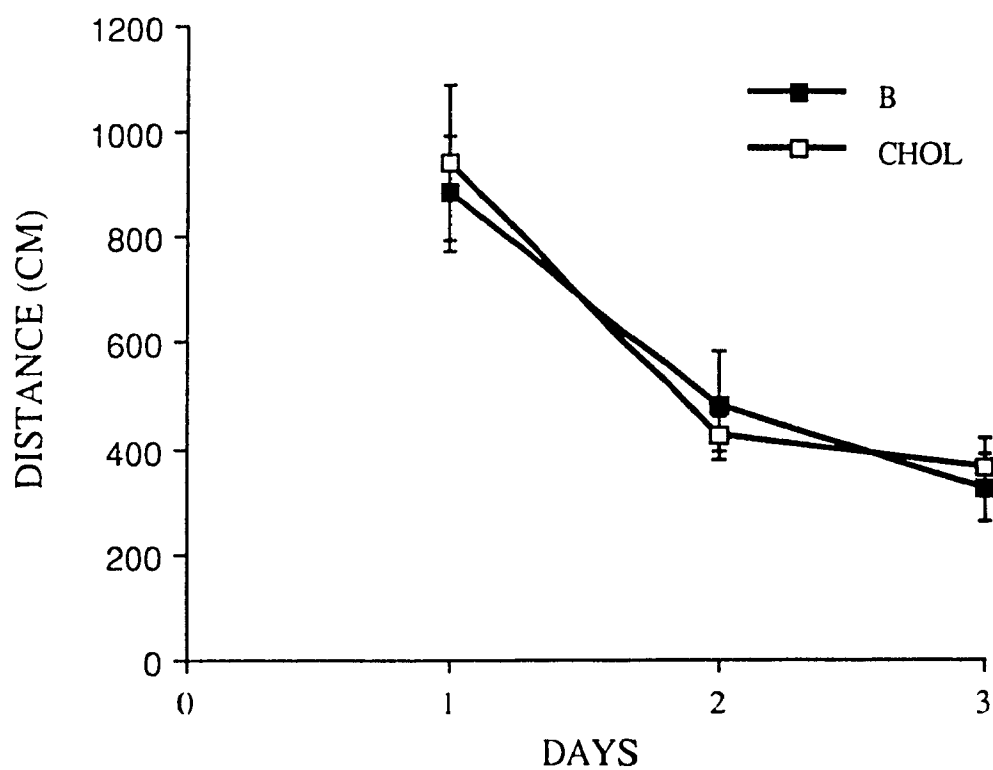


Figure 2. Mean distance travelled (cm) over days (collapsed across blocks of four trials), to locate a submerged platform in rats treated with either corticosterone (B) or cholesterol (CHOL) for one month.

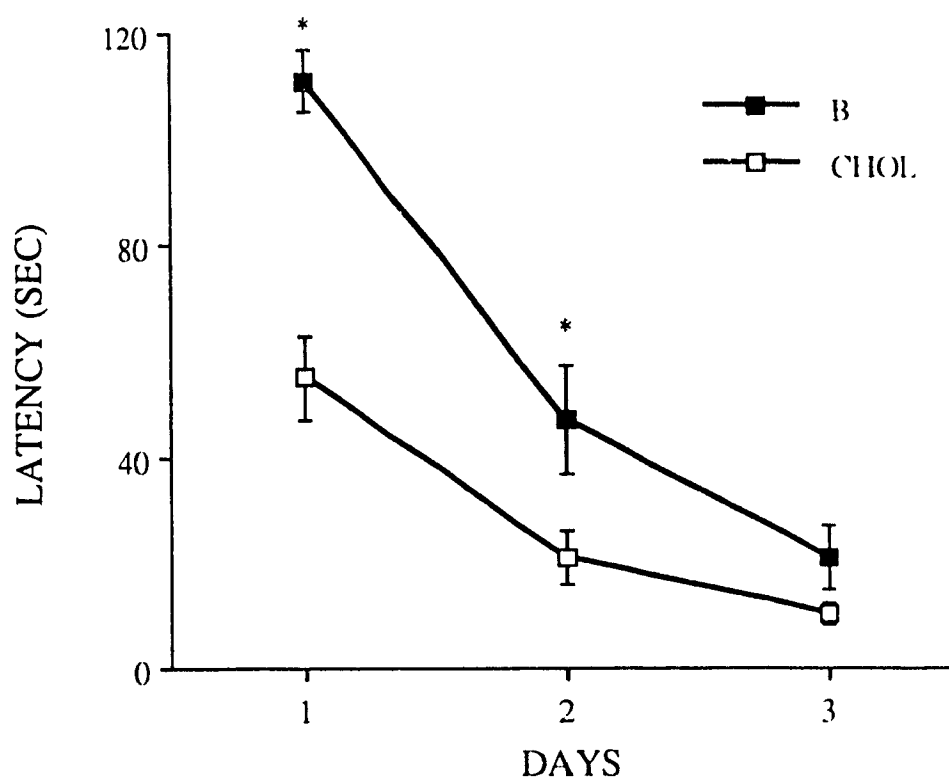


Figure 3. Mean latency (sec) over days (collapsed across blocks of four trials) to locate a submerged platform, in rats treated with either corticosterone (B) or cholesterol (CHOL) for three months. An asterisk indicates a mean that is significantly different from cholesterol-treated animals ( $p < 0.01$ ).



A similar pattern was observed with the distance data (see Figure 4). There was a significant effect of days ( $F = 160.04$ ,  $df = 2,36$ ,  $p < 0.0001$ ), corticosterone treatment ( $F = 20.1$ ,  $df = 1,18$ ,  $p < 0.0002$ ) and a significant treatment by days interaction ( $F = 27.9$ ,  $df = 2,36$ ,  $p < 0.0001$ ). Post-hoc analyses revealed that the corticosterone-treated animals travelled significantly farther ( $p < 0.01$ ) in locating the platform on Day 1. Again, the corticosterone-treated animals swam farther in locating the platform on Trial 1 compared with cholesterol-treated controls ( $t = 3.89$ ,  $df = 18$ ,  $p < 0.001$ ). The mean distance travelled was  $1085 \pm 179$  and  $1862 \pm 90$  cm for cholesterol- and corticosterone-treated animals respectively.

The findings from Experiment 1 revealed that a one-month corticosterone treatment regimen had no effect upon behavior in the Morris water maze. In contrast, there were significant differences in both latency and distance travelled in the three-month corticosterone-treated rats, although a treatment group difference on Trial 1 made the findings difficult to interpret. By examining the maps of swimming patterns from the corticosterone- and cholesterol-treated rat (see Figure 5 for representative maps), it was apparent that the corticosterone-treated animals spent considerable time circling the perimeter of the pool on the first three trials, a pattern of behavior generally restricted to the initial exposure to the task (Morris, 1984). That is, the continuous perimeter

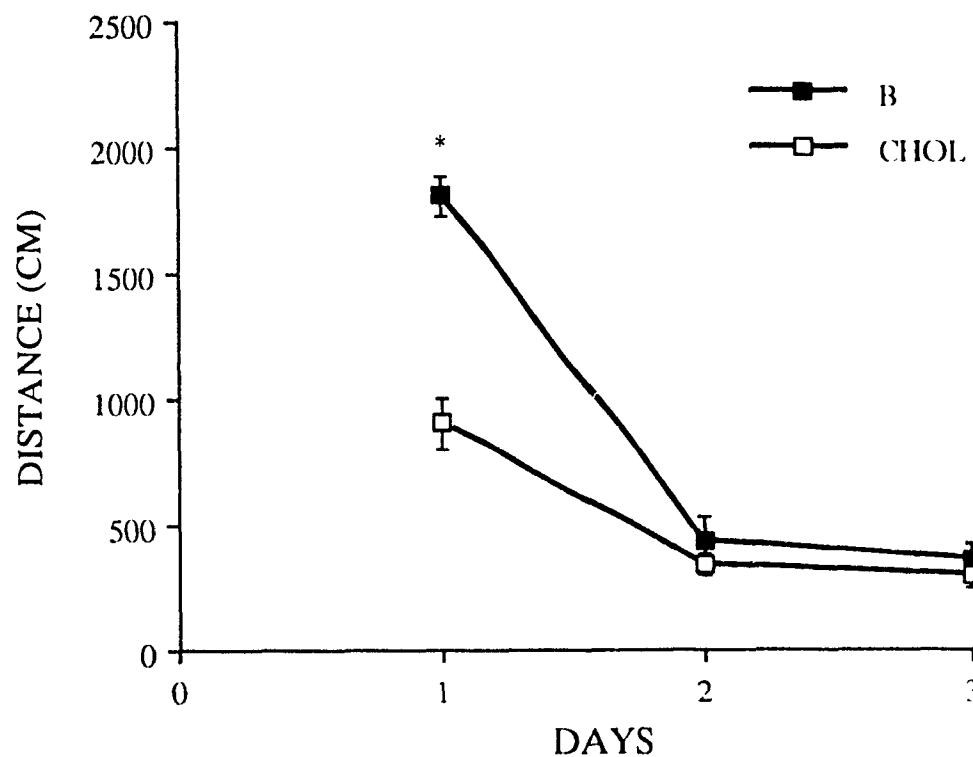


Figure 4. Mean distance travelled (cm) over days (collapsed across blocks of four trials) to locate a submerged platform, in rats treated with either corticosterone (B) or cholesterol (CHOL) for three months. An asterisk indicates a mean that is significantly different from cholesterol-treated animals ( $p < 0.01$ ).

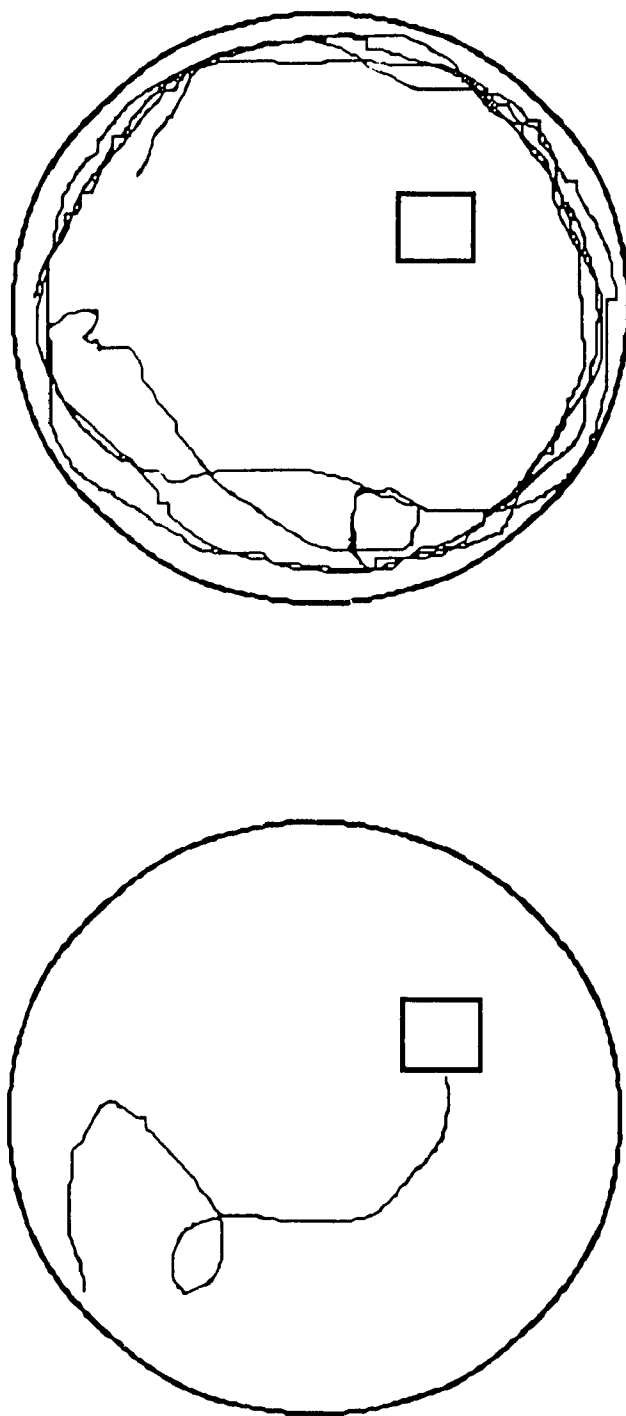


Figure 5. Representative swimming patterns travelled in locating the hidden platform in the Morris water maze on Trial 3, from a rat treated with corticosterone (top) or cholesterol (bottom) for three months.

swimming was believed to reflect a heightened fear response to the testing apparatus that was slower to habituate in these animals. However, by the second day of training, the performance of the corticosterone-treated animals began to approximate that of controls. It was hypothesized that the behavioral differences during the first training day observed in corticosterone-treated rats may reflect an enhanced response to novelty as opposed to any true learning deficit and this issue was addressed directly in Experiment 2.

## Experiment 2

The results of Experiment 1 suggested that a three-month corticosterone treatment regimen induced no real cognitive deficit, but rather a heightened response to novelty. Thus, in replicating the experiment, it was critical to assess the effects of long-term glucocorticoid exposure upon both spatial learning and neophobia.

In previous experiments, the latency of food-deprived rats to begin eating in an unfamiliar environment was a reliable measure of an animal's response to novelty (Britton and Thatcher-Britton, 1981; Bodnoff, Suranyi-Cadotte, Quirion and Meaney, 1989b). Indeed, under conditions of fear or novelty, consummatory behaviors such as eating or drinking are suppressed (see review by Gray, 1982) while ambulation (Whimbey and

Denenberg, 1967) or freezing (Bolles, 1970) are increased. Thus, placing a food-deprived rat into an unfamiliar open-field containing food would result in a conflict between two opposing incentive motivational states: one related to novelty and the other to hunger. Novelty-related behaviors would predominate until the fear-related state had diminished. Only then would the animal begin to eat. We have demonstrated that drugs which have been effective in relieving a range of anxiety disorders in humans (e.g. diazepam and tricyclic anti-depressants) decrease both the novelty-related behaviors and the latency for animals to begin eating in the novel environment (Bodnoff, Suranyi-Cadotte, Aitken, Quirion and Meaney, 1988; Bodnoff, Suranyi-Cadotte, Quirion and Meaney, 1989a) relative to controls. Moreover, pre-exposure to the novel environment decreased, while the administration of the anxiogenic analogue, FG 7142, to pre-exposed animals, increased the latencies for animals to begin eating (Bodnoff et al., 1989b). Together, these findings suggest that if long-term glucocorticoid treatment enhanced neophobia, the animals should take significantly longer to begin eating in the novel environment relative to controls.

The purpose of Experiment 2 was to assess the nature of the performance deficit in the Morris water maze following a three-month corticosterone treatment period: Does the change in performance

represent a true learning deficit or an enhanced neophobic response. Only the three-month glucocorticoid treatment was replicated, using the methodology of daily injections described by Sapolsky et al. (1985). It was hypothesized that if the behavioral impairment observed in Experiment 1 following long-term glucocorticoid exposure was the result of a heightened response to novelty rather than a learning deficit, animals in the present study would exhibit poor performance on the early training trials and enhanced neophobia in the conflict test.

### Methods

Animals. Male, Long-Evans hooded rats were obtained from Charles River (St. Constant, Que.) at four to five months of age and weighed between 450-500 g. The housing conditions were identical to Experiment 1. The sample sizes for the corticosterone and cholesterol-treated animals were 11 and 9 respectively.

Injections. Corticosterone (20 mg/kg) was prepared daily in a peanut oil vehicle and sonicated until dissolved. The injections, which were administered approximately one to two hours following the onset of the light cycle, were given at different locations around the nape of the neck. Blood sampling from selected animals taken approximately two hours following the injection indicated a range of plasma corticosterone between 20-25  $\mu\text{g/dl}$  and remained elevated for approximately 12 hours. This

regimen continued for three months and the injections were discontinued seven days prior to behavioral testing to allow for clearance of the steroid and healing of the injection sites.

Procedure. The Morris water maze was described in Experiment 1. The animals were given a total of sixteen trials (4 trials/day for 4 days) with the platform submerged. On the day following the completion of the training trials, rats were given a single 30-sec retention (probe) trial in which the platform was absent. The percentage of time spent in the original training quadrant was the dependent measure of interest.

Conflict Test: Assessment of Neophobia. Following the completion of all swimming trials, the corticosterone- ( $n = 10$ ) and cholesterol-treated ( $n = 8$ ) animals were tested in the conflict paradigm. All food was removed from the home cage 48 hours prior to testing, although water was readily available. The testing apparatus consisted of individual plexiglas cages, 54 x 28 x 21 cm, with stainless steel grid lids. The floor of each cage was covered with approximately 1.5 cm of beta chips and ten lab chow pellets were piled in the center of the cage. The animals were placed into a plastic bucket and transported to a small testing room with overhead fluorescent lighting. Each subject was placed into a separate cage, the lids were closed and a stopwatch was started immediately. The dependent measure of interest was the latency for animals to begin eating and this was

defined as chewing of the food, not simply sniffing or playing with a pellet. If animals had not eaten within ten minutes, the test was terminated.

### Results and Discussion

Morris Swim Maze. Figure 6 depicts the mean latency (sec) over days (collapsed across blocks of three trials within days) for animals to locate the platform. There was a significant effect of Days ( $F = 37.99$ ,  $df = 3,54$ ,  $p < 0.0001$ ) while neither the effect of corticosterone treatment ( $F = 0.29$ ,  $df = 1,18$ ,  $p > 0.6$ ) nor the treatment by days interaction ( $F = 0.35$ ,  $df = 3,54$ ,  $p > 0.7$ ) was significant. The groups did not differ significantly on Trial 1 with respect to the latency data ( $t = 0.87$ ,  $df = 18$ ,  $p > 0.39$ ).

Analysis of the distance data revealed an identical pattern (Days:  $F = 32.38$ ,  $df = 3,54$ ,  $p < 0.0001$ ; Treatment:  $F = 0.23$ ,  $df = 1,18$ ,  $p > 0.6$ ; Treatment x Days:  $F = 0.3$ ,  $df = 3,54$ ,  $p > 0.8$ ; see Figure 7). The distance travelled on Trial 1 did not differ between the groups ( $F = 0.81$ ,  $df = 18$ ,  $p > 0.42$ ).

The 30-sec probe trial revealed no significant effect of treatment ( $t = 0.4$ ,  $df = 18$ ,  $p > 0.7$ ) with the corticosterone-treated and control animals spending equivalent percentages of time in the original training quadrant ( $33.27 \pm 4.5$  vs  $30.56 \pm 5.1$  sec respectively).

Conflict Test. Figure 8 shows that corticosterone-treated animals took significantly longer to begin eating in the novel environment relative



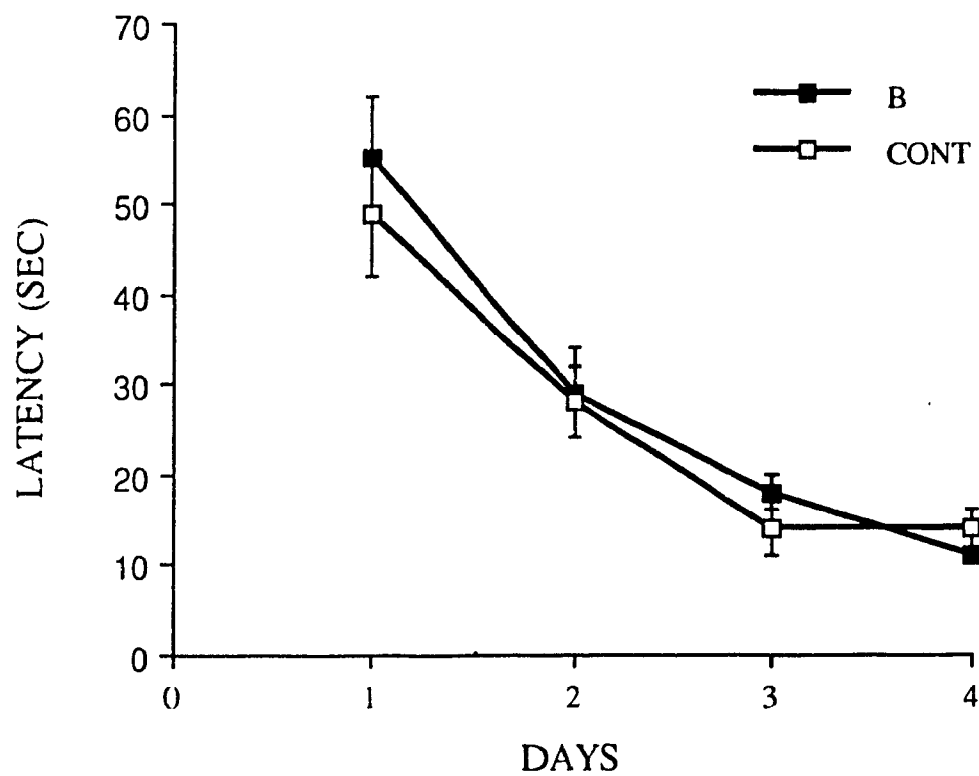


Figure 6. Mean latency (sec) over days (collapsed across blocks of three trials) to locate a submerged platform, in adult rats injected daily with either corticosterone (B) or a peanut oil vehicle control (CONT) for three month.

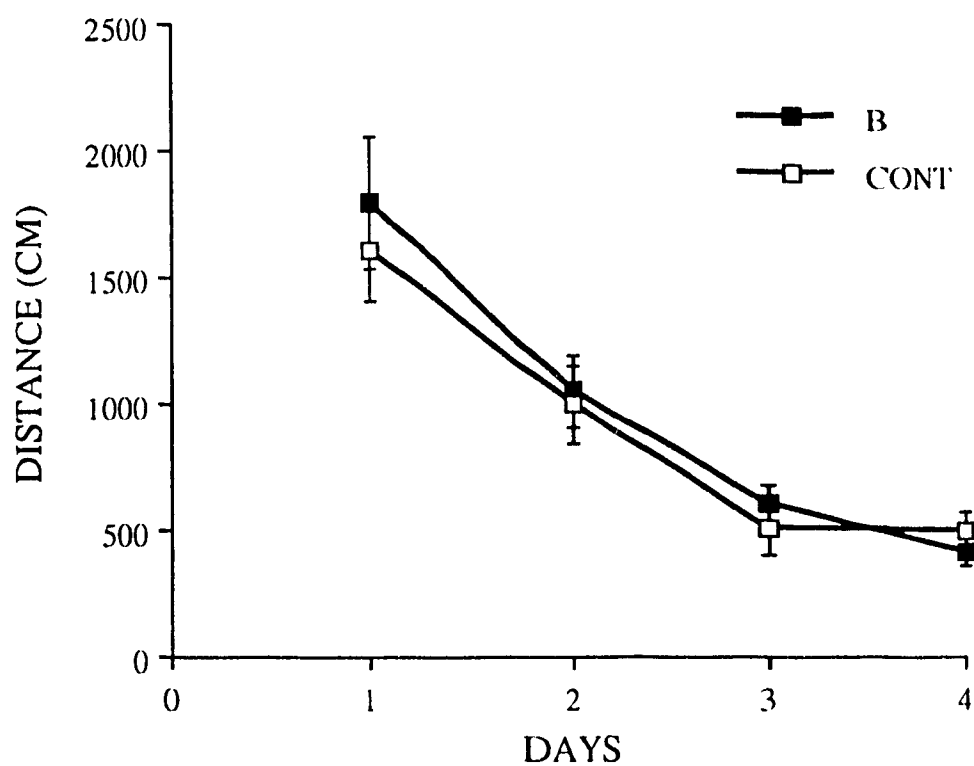


Figure 7. Mean distance travelled (cm) over days (collapsed across blocks of three trials) to locate a submerged platform in rats injected daily with either corticosterone (B) or a peanut oil vehicle control (CONT) for three months.

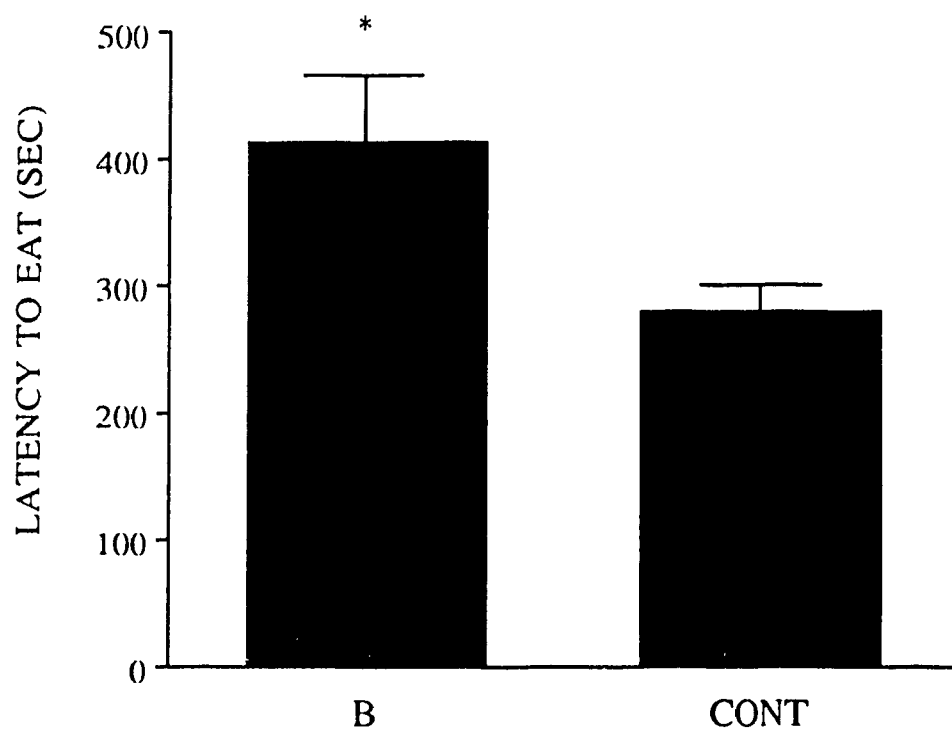


Figure 8. Mean latency (sec) for food-deprived animals to begin eating in a novel environment following a three-month treatment regimen with either corticosterone (B) or a peanut oil vehicle control (CONT). An asterisk indicates a mean that is significantly different from controls ( $p < 0.05$ ).

to controls ( $413 \pm 53$  vs  $278 \pm 22$  sec, respectively:  $t = 2.13$ ,  $df = 16$ ,  $p < 0.05$ ).

The major finding of Experiment 2 was that a three-month corticosterone treatment regimen had no effect upon performance in the Morris water maze relative to control animals. In contrast to the impaired Day 1 performance observed in corticosterone-treated animals in Experiment 1, the treated animals in the present experiment did not exhibit significantly longer latencies to locate the platform relative to controls on either the first training trial or the first training day. However, it is important to note that there were differences between the two experiments with respect to the method of steroid delivery. Although the pellets and injections produced equivalent peak elevations in plasma corticosterone (between 20-25  $\mu\text{g/dl}$ ), the corticosterone levels of those animals receiving corticosterone injections (Experiment 2) had decreased to p.m. values (i.e. 10-15  $\mu\text{g/dl}$ ) approximately 12 hours later. Thus, it would suggest that performance deficits in the Morris maze (i.e. those observed in Experiment 1) were dependent upon both stable (the corticosterone levels remained elevated throughout the diurnal cycle) and high physiological levels of corticosterone. Indeed, this hypothesis is discussed in Experiments 4 and 5.

The conflict data may offer some insight into the performance deficits observed in the Morris maze in Experiment 1. In the absence of

any exogenous corticosterone, corticosterone-treated rats took longer to eat in a novel environment relative to controls, suggestive of an enhanced response to novelty in the former group. In the Morris maze, this response would likely be demonstrated as an increased, non-specific searching pattern (e.g. perimeter swimming, a strategy associated with poor escape performance) during the early training trials. However, habituation occurred relatively quickly as their performance on subsequent exposures to the testing apparatus was identical to control animals.

Taken together, the data from Experiments 1 and 2 suggested that in adult, Long-Evans rats, a three-month exposure period to elevated levels of glucocorticoids did enhance neophobia as assessed in the conflict test, while any meaningful spatial learning deficit was potentially shrouded by these effects upon the heightened response to novelty.

### Experiment 3

The results from the first two experiments led to several questions. Was the absence of any observable spatial learning deficit due to the inability of the treatment to produce sufficiently high levels of corticosterone to affect neuron loss? This explanation was unlikely since the treatment was comparable to that of Sapolsky et al. (1985) in which neuron loss was observed. Was the Morris water maze sensitive to the

pattern of selective hippocampal neuron loss that would have been predicted by the treatment? Here, we can cite several examples to suggest that the task was indeed sensitive to this pattern of neuron loss (Issa et al., 1990; Meaney et al., 1988a; Sutherland et al., 1983). Thus, it was apparent that in young, Long-Evans rats, long-term corticosterone exposure had no effect on learning a spatial location in the Morris maze.

The purpose of Experiment 3 was to compare the effects of glucocorticoids in both adult and mid-aged Long-Evans rats. The rationale for this study was based upon two independent lines of research. Meaney et al. (1988a) found that mid-age (12 months) was a critical time for the loss of both hippocampal type II receptors and neurons and the corresponding onset of adrenocortical hypersecretion in non-handled rats. According to the glucocorticoid cascade hypothesis (Sapolsky et al., 1986b), this sub-threshold pathology would be exacerbated by glucocorticoid treatment, eventually resulting in significant neuropathology. Indeed, aged non-handled rats display both cognitive impairments and hippocampal neuron loss. Second, there is evidence to suggest that hippocampal glutamate levels increase as a function of age (R. Sapolsky, personal communication; but see Zhang, Mundy, Thai, Hudson, Gallagher, Tilson and Hong, 1991). Based upon the importance of increased glutamate levels in initiating the NMDA cascade that eventually

results in cell death, and the synergy between glutamate and glucocorticoids in exacerbating this neuron loss (see introduction), we would expect that the deleterious effects of glucocorticoids would be enhanced in older rather than younger animals.

We also chose to make the task more difficult by giving fewer trials per day. Dekker, Connor, Gage and Thal (1990) reported that subtle learning deficits in animals with nucleus basalis of Meynert lesions became more pronounced when the number of training trials was reduced to two per day.

Thus, in the present experiment we compared the effects of exposure to high levels of corticosterone for either one or three months in young adult and mid-aged rats. Based upon the findings of Experiment 2, a learning deficit was not expected in the adult animals treated for either one or three months with corticosterone. Moreover, no deficit was anticipated following a one-month corticosterone treatment period in the mid-aged group. However, if the effects of long-term exposure to elevated levels of corticosterone were exacerbated by age, it was hypothesized that mid-aged, corticosterone-treated rats would exhibit a behavioral deficit in the Morris maze relative to controls. Moreover, it was expected that only this latter group would exhibit hippocampal neuron loss at the termination of a three-month treatment regimen.

## Methods

Animals. The animals used in this study were obtained from Charles River (Raleigh, NC) at either two or nine months of age (retired breeders) and left undisturbed for three months until the beginning of the experiment. The housing and feeding conditions were identical to those described in Experiment 1. At the beginning of the experiment, the animals were either five (young adult) or twelve (mid-aged) months of age.

Pellet Implantation. Young adult and mid-aged animals were randomly assigned to receive either corticosterone or cholesterol pellets (described in Experiment 1) for either one or three months. Preliminary measures of plasma titers, assessed approximately two hours into the light cycle, indicated that two-100% fused corticosterone pellets (described in Experiment 1) were sufficient to produce elevated corticosterone levels for ten days (e.g.  $41 \pm 2$   $\mu\text{g/dl}$  approximately 16 hours following implantation to  $18 \pm 3$   $\mu\text{g/dl}$  on day 10, with an average of  $30 \pm 3$   $\mu\text{g/dl}$  per day). The formation of scar tissue around the pellets was believed to be the major contributing factor to the decline in plasma corticosterone levels over time. Body weights and plasma corticosterone levels were monitored throughout the experiment and additional 25 mg pellets were implanted to maintain high corticosterone levels. The pellets were replaced eight times for the three-month-treated animals and twice for the one-month-treated animals.



At the completion of the one-month treatment, the sample sizes were: 7, 11, 7 and 8 for the mid-aged cholesterol- and corticosterone-treated rats and young, adult cholesterol- and corticosterone-treated animals, respectively. In the three-month treatment experiment, the sample sizes were: 7, 11, 8 and 8 for the same groups respectively.

Procedure. The animals were given twenty trials over ten days (2 trials per day) in the Morris water maze with the platform submerged. One day following the termination of the testing period, rats were given a 30-sec probe trial in which the platform was removed, to determine a spatial bias for the original platform location. Finally, all animals were given five trials in which the platform was raised two cm above the water level (visually cued condition; see Morris, 1985). This condition was used to ensure that impairments were not related to the animal's inability to perform the motor demands of the task (see Gage et al., 1984).

Hippocampal Cell Counting. A second group of mid-aged rats, treated as above for three months with either cholesterol ( $n = 3$ ) or corticosterone ( $n = 4$ ) were used to quantify hippocampal neuron density. The rats were deeply anesthetized with Metofane and perfused intracardially with 0.9% NaCl, followed by 10% formalin (Fisher Scientific). The brains were removed and stored in 10% formalin for one week and then placed into a 20% sucrose/formalin solution for

cryoprotection. Cell counting was performed blindly on 20  $\mu\text{m}$  cresyl violet-stained sections of dorsal hippocampus. Raw cell counts were obtained using a 250 x 250  $\mu\text{m}$  grid over a 400x magnified section with a light microscope. Three to five sections of dorsal hippocampus [corresponding to Plates 21 or 22 of the rat brain atlas of Paxinos and Watson (1982)] were analyzed for each animal. Raw cell counts of the pyramidal cell fields were transformed into measures of neuron density per 0.1  $\text{mm}^2$  using the method of Abercrombie (1946) to correct for split cell artifacts. Cell size was estimated by measuring the diameter of randomly selected neurons from both CA1 and CA3.

### Results and Discussion

Morris Swim Maze. Figure 9 shows the mean latency (sec) over days (collapsed across blocks of two trials within days) to locate the submerged platform in young adult and mid-aged rats following the one-month corticosterone-treatment regimen. While there was a significant effect of days ( $F = 26.48$ ,  $df = 9,261$ ,  $p < 0.0001$ ), neither the main effects of age ( $F = 2.02$ ,  $df = 1,29$ ,  $p > 0.17$ ) or treatment ( $F = 1.67$ ,  $df = 1,29$ ,  $p > 0.21$ ) nor any interactions (age x treatment:  $F = 0.20$ ,  $df = 1,29$ ,  $p > 0.6$ ; age x days:  $F = 1.65$ ,  $df = 9,261$ ,  $p > 0.1$ ; treatment x days:  $F =$

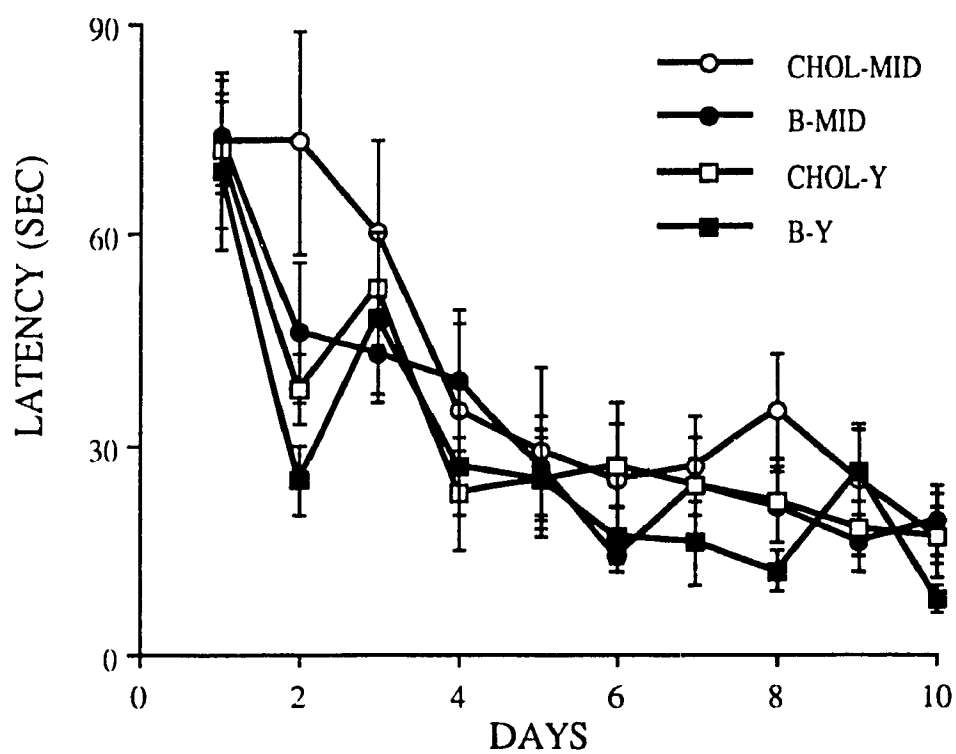


Figure 9. Mean latency (sec) over days (collapsed across blocks of two trials) to locate a submerged platform, following a one-month treatment regimen with either cholesterol (CHOL) or corticosterone (B) in young adult (Y) and mid-aged (MID) rats.

1.02,  $df = 9,261$ ,  $p > 0.4$ ; age x treatment x days:  $F = 0.42$ ,  $df = 9,261$ ,  $p > 0.9$ ) was significant. Overall, the four groups did not differ significantly on Trial 1 ( $F = 0.57$ ,  $df = 3,29$ ,  $p > 0.64$ ).

The distance data revealed an identical pattern (see Figure 10). There was a significant effect of days ( $F = 31.76$ ,  $df = 9,252$ ,  $p < 0.0001$ ) but neither the main effects of age ( $F = 0.38$ ,  $df = 1,28$ ,  $p > 0.5$ ) or treatment ( $F = 2.04$ ,  $df = 1,28$ ,  $p > 0.13$ ) nor any interactions (age x treatment:  $F = 0.51$ ,  $df = 1,28$ ,  $p > 0.4$ ; age x days:  $F = 1.39$ ,  $df = 9,252$ ,  $p > 0.19$ ; treatment x days:  $F = 1.34$ ,  $df = 9,252$ ,  $p > 0.2$ ; age x treatment x days:  $F = 0.33$ ,  $df = 9,252$ ,  $p > 0.9$ ) were significant. Overall, the distance travelled on the first trial did not differ significantly between the four groups ( $F = 0.30$ ,  $df = 3,29$ ,  $p > 0.83$ ).

The single 30-sec probe trial revealed no significant differences (age:  $F = 1.65$ ,  $df = 1,29$ ,  $p > 0.2$ ; corticosterone treatment:  $F = 0.27$ ,  $df = 1,29$ ,  $p > 0.6$ ; age x treatment:  $F = 0.01$ ,  $df = 1,29$ ,  $p > 0.9$ ) in the percentage of time spent in the original training quadrant for the one-month treated animals (young control:  $31 \pm 4$ ; young corticosterone:  $35.1 \pm 6$ ; mid-aged control:  $40 \pm 6$ ; mid-aged corticosterone:  $42 \pm 6\%$ ).

In contrast, the mean latency (sec) over days (collapsed across blocks of two trials within days) following a three-month treatment regimen (see Figure 11) revealed a significant effect of corticosterone treatment ( $F =$

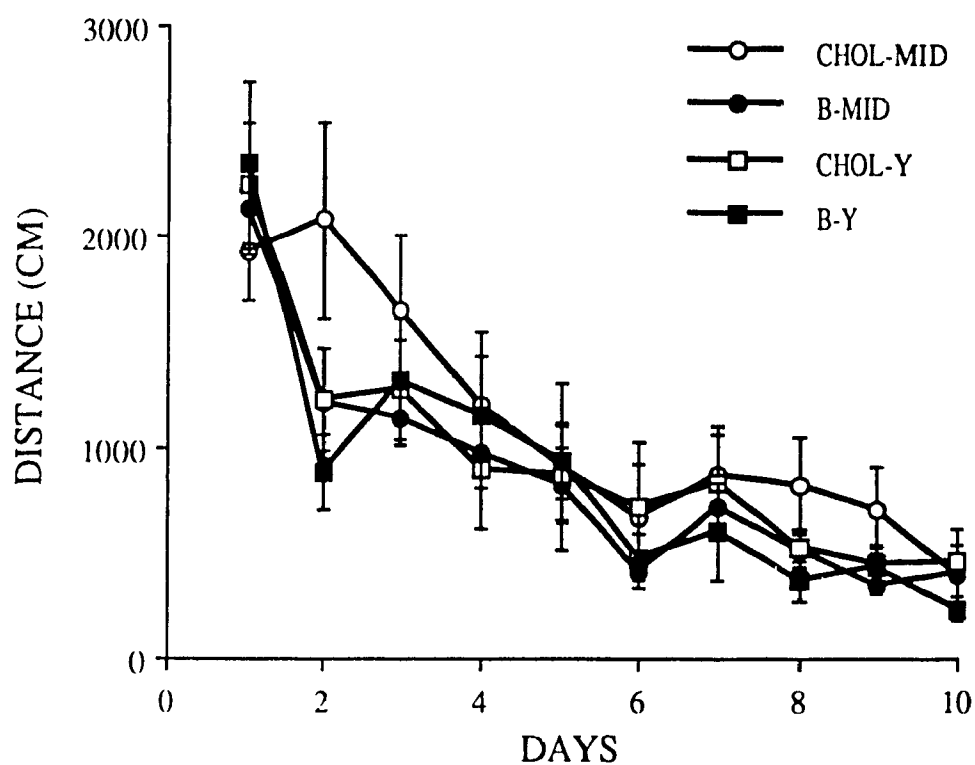


Figure 10. Mean distance travelled (cm) over days (collapsed across blocks of two trials) to locate a submerged platform following a one-month treatment regimen with either cholesterol (CHOL) or corticosterone (B) in young adult (Y) and mid-aged (MID) rats.

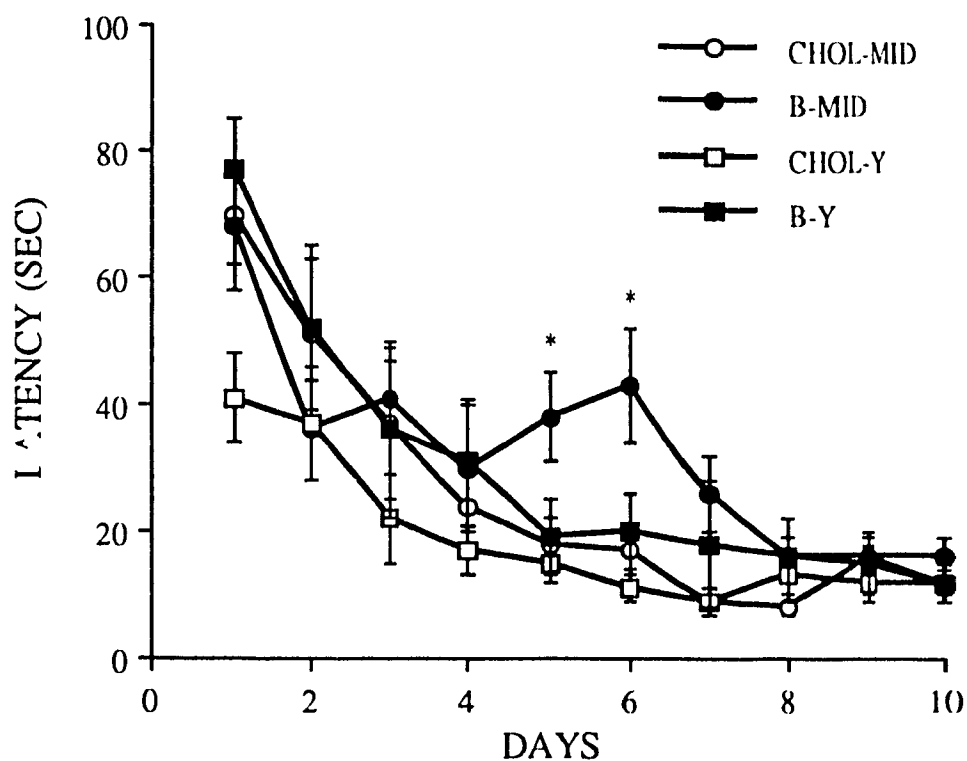


Figure 11. Mean latency (sec) over days (collapsed across blocks of two trials) to locate a submerged platform following a three-month treatment regimen with either cholesterol (CHOL) or corticosterone (B) in young adult (Y) and mid-aged (MID) rats. An asterisk indicates a B-MID mean that is significantly different from the CHOL-MID mean ( $p < 0.05$ ) at that timepoint.

8.41,  $df = 1,30$ ,  $p < 0.007$ ), days ( $F = 22.82$ ,  $df = 9,270$ ,  $p < 0.0001$ ) and an age by treatment by days interaction ( $F=1.95$ ,  $df = 9,270$ ,  $p < 0.04$ ). There was no significant effect of age ( $F = 3.32$ ,  $df = 1,30$ ,  $p > 0.1$ ) and none of the simple interactions was significant (age x treatment:  $F = 0.06$ ,  $df = 1,30$ ,  $p > 0.8$ ; age x days:  $F = 0.83$ ,  $df = 9,270$ ,  $p > 0.5$ ; treatment x days:  $F = 0.6$ ,  $df = 9,270$ ,  $p > 0.8$ ). Post-hoc analyses revealed increased latencies ( $p < 0.05$ ) of mid-aged corticosterone-treated rats on Days 5 and 6 relative to the mid-aged, cholesterol-treated controls, while the adult, corticosterone-treated rats differed from their age-matched controls on Day 1. The four groups did not differ on Trial 1 ( $F = 1.89$ ,  $df = 3,30$ ,  $p > 0.15$ ).

The distance data (see Figure 12) revealed a significant effect of corticosterone treatment ( $F = 6.86$ ,  $df = 1,30$ ,  $p < 0.01$ ), days ( $F = 23.88$ ,  $df = 9,270$ ,  $p < 0.0001$ ) and an age x treatment x days interaction ( $F = 2.07$ ,  $df = 9,270$ ,  $p < 0.03$ ). Again, there was no significant effect of age ( $F = 2.38$ ,  $df = 1,30$ ,  $p > 0.1$ ) and none of the simple interactions was significant (age x treatment:  $F = 0.03$ ,  $df = 1,30$ ,  $p > 0.8$ ; age x days:  $F = 1.32$ ,  $df = 9,270$ ,  $p > 0.2$ ; treatment x days:  $F = 0.67$ ,  $df = 9,270$ ,  $p > 0.7$ ). Post-hoc tests revealed that mid-aged corticosterone-treated animals swam farther in locating the platform ( $p < 0.05$ ) relative to their age-matched controls on Days 5 and 6, while the adult, corticosterone- and

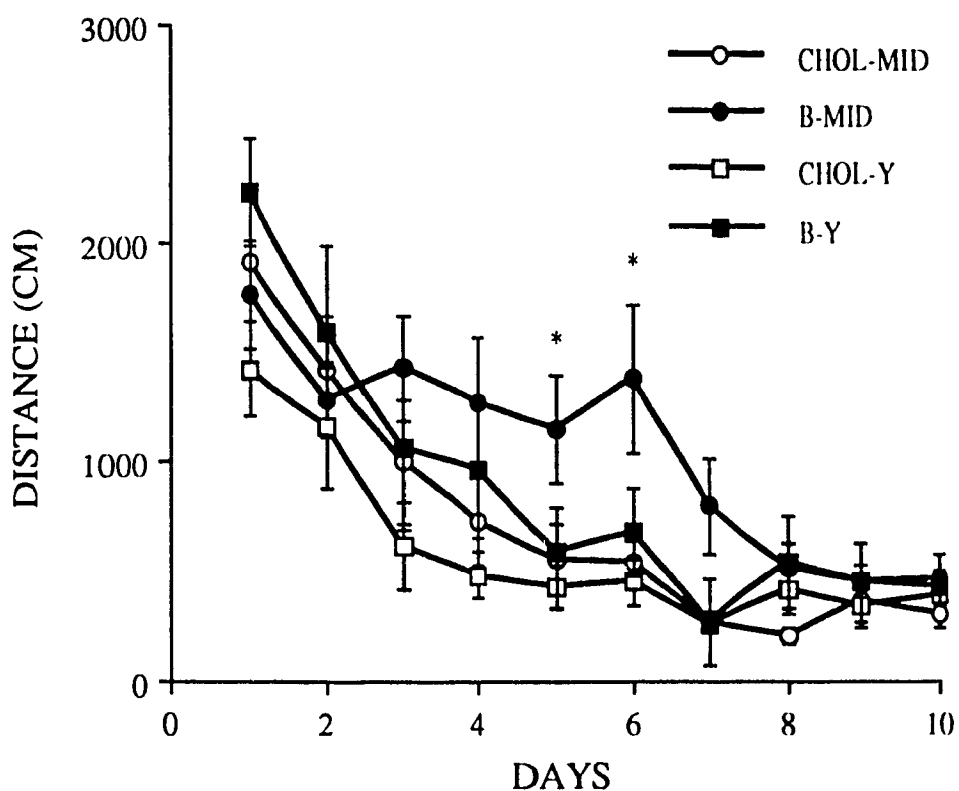


Figure 12. Mean distance travelled (cm) over days (collapsed across blocks of two trials) to locate a submerged platform, following a three month treatment regimen with either cholesterol (CHOL) or corticosterone (B) in young adult (Y) and mid-aged (MID) rats. An asterisk indicates a B-MID mean that is significantly different from the CHOL-MID mean ( $p < 0.05$ ) at that timepoint.



cholesterol-treated animals differed only on Day 1. Once again, the four groups did not differ on Trial 1 ( $F = 1.56$ ,  $df = 3,30$ ,  $p > 0.22$ ).

The single 30-sec probe trial revealed no significant differences between groups in the percentage of time spent in the original training quadrant (young control:  $39 \pm 5$ ; young corticosterone:  $29 \pm 6$ ; mid-aged control:  $31 \pm 5$ ; mid-aged corticosterone:  $31 \pm 4\%$ ). The ANOVA revealed that neither the main effects of age ( $F = 1.01$ ,  $df = 1,30$ ,  $p > 0.3$ ) or treatment ( $F = 0.35$ ,  $df = 1,30$ ,  $p > 0.6$ ) nor the age by treatment interactions ( $F = 1.59$ ,  $df = 1,30$ ,  $p > 0.2$ ) was significant. The five trials in which the platform was elevated produced swim latencies of under ten seconds in all groups.

Hippocampal Cell Counting. The mean number of corrected CA1 and CA3 neurons per  $0.1 \text{ mm}^2$  from a separate group of mid-aged animals treated for three months with either corticosterone ( $n = 4$ ) or cholesterol ( $n = 3$ ), but were not assessed in the Morris maze is shown in Table 1. There were no significant differences between the groups with respect to neuron density in either CA1 ( $t = -1.85$ ,  $df = 12$ ,  $p > 0.08$ ) or CA3 ( $t = 0.67$ ,  $df = 12$ ,  $p > 0.5$ ). Moreover, there were no group differences with respect to cell size (CA1:  $t = 1.63$ ,  $df = 5$ ,  $p > 0.2$ ; CA3:  $t = -0.63$ ,  $df = 5$ ,  $p = 0.5$ ). Indeed the mean diameter ( $\pm$  s.e.m.) of the CA1 neurons was  $12.4 \pm 0.8$  and  $11 \pm 0.6 \text{ }\mu\text{m}$  for the cholesterol- and corticosterone-treated

Table 1

Neuron Density (per 0.1 mm<sup>2</sup>) in CA<sub>1</sub> and CA<sub>3</sub> Hippocampal Neurons in  
Mid-Aged Cholesterol (CHOL)- and Corticosterone-Treated (B) Animals  
Following a Three-Month Treatment Regimen

	CA <sub>1</sub>	CA <sub>3</sub>
CHOL (n = 3)	29.7 ± 2.2	20.8 ± 1.2
B (n = 4)	34.2 ± 1.4	19.7 ± 1.0

animals respectively. The mean diameter ( $\pm$  s.e.m.) of the CA3 neurons was  $19.7 \pm 0.3$  and  $20.18 \pm 0.6 \mu\text{m}$  for the cholesterol- and corticosterone-treated animals respectively.

As expected, a one-month corticosterone treatment regimen had no effect upon performance in the Morris maze in either adult or mid-aged rats. The three-month treatment period produced an interesting pattern of results. Once again, there was no overall performance deficits in young rats. In contrast, mid-aged corticosterone-treated rats showed a significant deficit in the acquisition of a spatial task, taking longer to reach asymptotic performance compared to their age-matched controls, although their performance did not differ during the first four training days. The mid-aged corticosterone-treated animals took longer and swam farther in successfully locating the platform on Days 5 and 6 although their performance had reached the same asymptotic level as controls by Day 7. Moreover, the absence of group differences on the probe trial again indicated that all of the animals retained the spatial location of the original training quadrant equally by the end of the ten training days.

With respect to hippocampal integrity, there was no observable neuron loss in either CA1 or CA3 in mid-aged animals following a three-month corticosterone treatment regimen relative to age-matched controls.

Moreover, there was no apparent neuron shrinkage associated with long-term corticosterone administration.

Together, these findings indicated that in the Long-Evans rat, a three-month corticosterone treatment impaired the performance in the Morris water maze, but only in mid-aged animals. These results suggest a synergy between the effects of age and corticosterone treatment upon acquisition of a spatial task. Indeed, the corticosterone treatment had no effect (with respect to acquisition of the task) in young adult rats, a finding consistent with the first two experiments. It is important to consider that this behavioral deficit occurred following the termination of treatment, once normal basal values had been re-established in the corticosterone-treated rats. Moreover, the spatial learning impairments of the mid-aged rats occurred in the absence of any change in hippocampal morphology, suggesting that neuron loss was not the mechanism underlying this deficit.

#### Experiment 4

The findings of Experiment 3 suggested that in the Long-Evans strain, hippocampal neuron loss does not appear to be the mechanism underlying the acquisition impairments in the Morris water maze. Although cognitive deficits and neuron loss might be the eventual endpoint of a lifetime exposure to elevated glucocorticoid levels (as observed in the

aged, cognitively-impaired rat; Issa et al., 1990), this was not observed following a relatively shorter treatment period (i.e. three months). However, a more sensitive measure of hippocampal function might be related to the apparent spatial learning deficit observed in the mid-aged animals.

As described in the introduction, the most extensively-studied mechanism believed to underly learning and memory is long-term potentiation (LTP) and there are several reports that hippocampal plasticity is significantly reduced in the presence of high levels of glucocorticoids (see Bennett et al., 1991; Diamond et al., 1992; Foy et al., 1987) and in aged, cognitively-impaired animals (Barnes and McNaughton, 1985; Landfield and Lynch, 1977; Landfield et al., 1978a). The question addressed in the present experiment concerned the effects of glucocorticoids on LTP from a different perspective. Does long-term exposure to corticosterone, which results in spatial learning deficits in mid-aged rats (see Experiment 3), have long-term effects on hippocampal plasticity when assessed two to three months following the removal of the steroid?

In Experiment 4, the effects of a three-month exposure period to either high p.m. diurnal (Medium-B) or stress-like (High-B) levels of glucocorticoids upon performance in the Morris maze and synaptic

plasticity (using the threshold model of primed burst potentiation described by Rose and Dunwiddie, 1986) were examined. This two-dose study was conducted to determine whether physiological levels of corticosterone achieved during the diurnal cycle (Medium-B) had effects upon behavior and hippocampal synaptic plasticity similar to those observed following exposure to stress-like levels of corticosterone (High-B). (The High-B rats received a level of steroid similar to that observed in our laboratory following acute exposure to a 20-minute restraint stressor.)

Based upon the findings of Experiment 3, it was hypothesized that a three-month exposure of mid-aged rats to stress-like levels of corticosterone would result in significant impairments in the acquisition of a spatial location. Moreover, if this treatment induced a permanent change in the functioning of the hippocampal synapses, the long-term post-synaptic response to PB stimulation should be reduced. If a three-month exposure to high physiological levels of corticosterone is necessary to produce behavioral deficits in the Morris water maze (a point addressed in the discussion of Experiment 2), those rats receiving the Medium-B level of corticosterone would not be expected to demonstrate a learning impairment. However, the long-term effects of this treatment upon a more sensitive measure of hippocampal function (i.e. synaptic plasticity) were unknown, although we expected that the absence of a behavioral deficit

would be associated with no decrement in synaptic plasticity. Finally, based upon the findings of Experiment 3, no changes in hippocampal morphology were anticipated following either of the three-month corticosterone treatments.

### Methods

Subjects. The animals used in this study were obtained from Charles River (Raleigh, NC) at nine months of age (retired breeders) and left undisturbed for three months until the beginning of the experiment. The detail of the housing and feeding conditions were identical to those described in Experiment 1. Due to the limited number of retired breeders available, five young rats (3-4 months of age) were delivered to the animal colony approximately one month prior to behavioral testing and were used to increase the sample size of the control group.

Steroid Pellets. The pellets were purchased from Innovative Research of America (Toledo, Ohio). Three-month release corticosterone (100 mg each: Catalogue # NG-111) were shown to deliver a constant levels of steroid for approximately three months while the cholesterol pellets had no effect upon basal circulating levels of corticosterone. A.M. and p.m. tail blood samplings taken approximately 2.5 months following implantation indicated that two pellets produced a range of plasma corticosterone between 12-17  $\mu\text{g/dl}$  (Medium-B), while four pellets

produced levels in the range of 23-32  $\mu\text{g/dl}$  (High-B). Control pellets (# NC-111) did not alter basal corticosterone levels (see Figure 13).

Pellet Implantation. At twelve months of age, the animals were randomly assigned to receive either one of two levels of corticosterone (Medium-B, High-B) or cholesterol (Chol) for three months. The sample sizes were: 16, 14 and 7 respectively. The animals were implanted with the pellets as described in Experiment 1. The weights and plasma corticosterone levels of the animals were monitored, and additional 10 mg four-week release pellets (# G-111) were implanted to calibrate for every 10% increase in body weight.

Morris Swim Maze. The behavioral testing paradigm was identical to Experiment 3 (2 trials per day for 10 days).

Conflict Test. Approximately two weeks following completion of the swim maze testing, all animals were food-deprived for 24 hours and exposed to the conflict test described in Experiment 2. The sample sizes for this experiment were 14, 14 and 8 for the High-B, Medium-B and cholesterol controls, respectively.

Electrophysiology. Following the completion of behavioral testing, the rats were shipped in insulated packing crates to the V.A. Medical Center in Denver, Colorado for the electrophysiology recordings. The animals were given at least two to three weeks to acclimate to altitude and



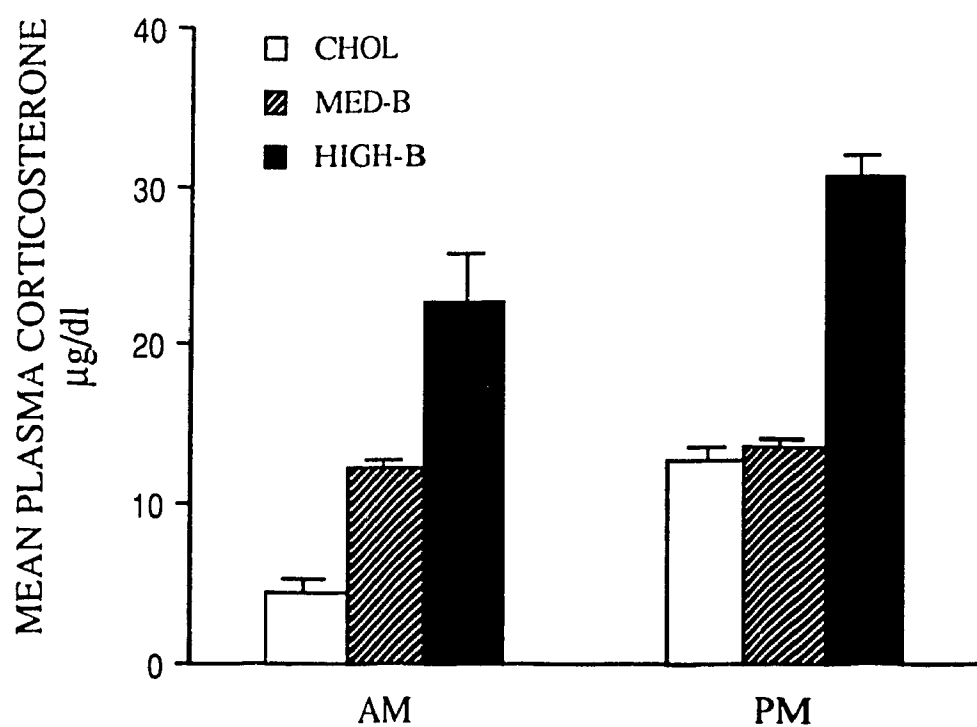


Figure 13. Mean plasma corticosterone levels (µg/dl), taken at 1000h (AM) and 2200h (PM) in mid-aged rats treated with either cholesterol (CHOL) or a medium (MED-B) or high (HIGH-B) level of corticosterone for three months.

the new colony. The day before recording, animals were taken from the vivarium and brought to the testing room. Food and water was removed to prevent fluid accumulation in the lungs while under anesthesia. On the recording day, atropine methyl nitrate was administered (0.2 mg/kg, i.p.) and rats were anesthetized with intraperitoneal injections of sodium pentobarbital (50 mg/ml in 0.9% NaCl). Supplemental injections of anesthetic were given pro re nata throughout the experiment to maintain a stable surgical plane. The rats were placed in a stereotaxic apparatus with the skull oriented in the horizontal plane. The skin overlying the skull was retracted, and the bone and dura above the right dorsal hippocampus and the left side of the ventral hippocampal commissure were removed.

Extracellular recordings of stimulus evoked field potentials in the right dorsal hippocampus were made using capillary glass micropipettes filled with 2M saline (0.75 - 1.50 M $\Omega$ ), which were lowered into the CA1 pyramidal cell layer (Coordinates from bregma: A.P., -4.0 mm; M.L., 2.5 mm; D.V., 1.8 - 2.3 mm). A stimulating electrode, consisting of a 125  $\mu$ m diameter stainless steel wire insulated with Teflon except at the tip, was lowered into the ventral hippocampal commissure (A.P., -2.0 to -2.5 mm; M.L., -1.0 mm; D.V., 2.8 - 3.2 mm) to activate commissural afferents to the hippocampus. Stimulus pulses were 150  $\mu$ sec duration, cathodal constant current pulses delivered with respect to an indifferent electrode

wire located over the posterior cortex (A.P., -8.0 mm; M.L., -4.0 mm). A separate recording reference wire, connected to ground, was placed over the frontal cortex (A.P., 4.0 mm; M.L., 3.0 mm). Electrode positions were optimized electrophysiologically to record the maximal positive field excitatory postsynaptic potential (EPSP) with superimposed population spike (Anderson et al., 1971). Population spike amplitude, measured at the midpoint of a tangent connecting the preceding and following positivity of the field EPSP, was automatically recorded by a microcomputer.

The stability of baseline recording was monitored for a least ten minutes before initiating data acquisition procedures. Following this period, population spike measurements were taken from responses evoked by single pulse stimuli given at 30-second intervals for five minutes. After the five-minute period of baseline recording, a patterned stimulus train was delivered with the aim of inducing long-lasting synaptic plasticity. This treatment, termed Primed Burst (PB) stimulation, consisted of a single "priming" stimulus followed 170 msec later by four pulses at 200 Hz (5 pulses total). PB stimulation is designed to mimic the complex spike and theta rhythm activity which occurs in the hippocampus (Diamond et al., 1988). In all cases, PB stimulation was applied using the same pulse duration and stimulation intensity as was used to evoke baseline responses.

Population spike amplitude was monitored for an additional twenty minutes following the PB train.

At the conclusion of the recording session, a tail sample of plasma was taken for the subsequent analysis of plasma corticosterone levels (see Experiment 1).

Hippocampal Cell Counting. Following the electrophysiological recordings, deeply anesthetized rats were perfused intracardially with 0.9% NaCl, followed by 10% formalin (Fisher Scientific). The brains were removed and stored in 10% formalin for one week and then placed into a 20% sucrose/formalin solution for cryoprotection. Slicing was performed on a microtome, with 50  $\mu$ m sections being thaw-mounted onto the gelatin-coated slides. Cell counting was performed as described in Experiment 3.

### Results and Discussion

Morris Swim Maze. For the purpose of this analysis, the untreated young adult and mid-aged cholesterol-treated animals (whose performance did not differ,  $F = 1.04$ ,  $df = 1,10$ ,  $p > 0.3$ ) were combined to increase the sample size. Figure 14 represents the mean latency (sec) over days (collapsed across blocks of two trials within days) for rats to locate the hidden platform in controls, Medium-B and High-B animals following a three-month treatment regimen. There was a significant effect of corticosterone treatment ( $F = 5.97$ ,  $df = 2,38$ ,  $p < 0.006$ ) and days ( $F =$

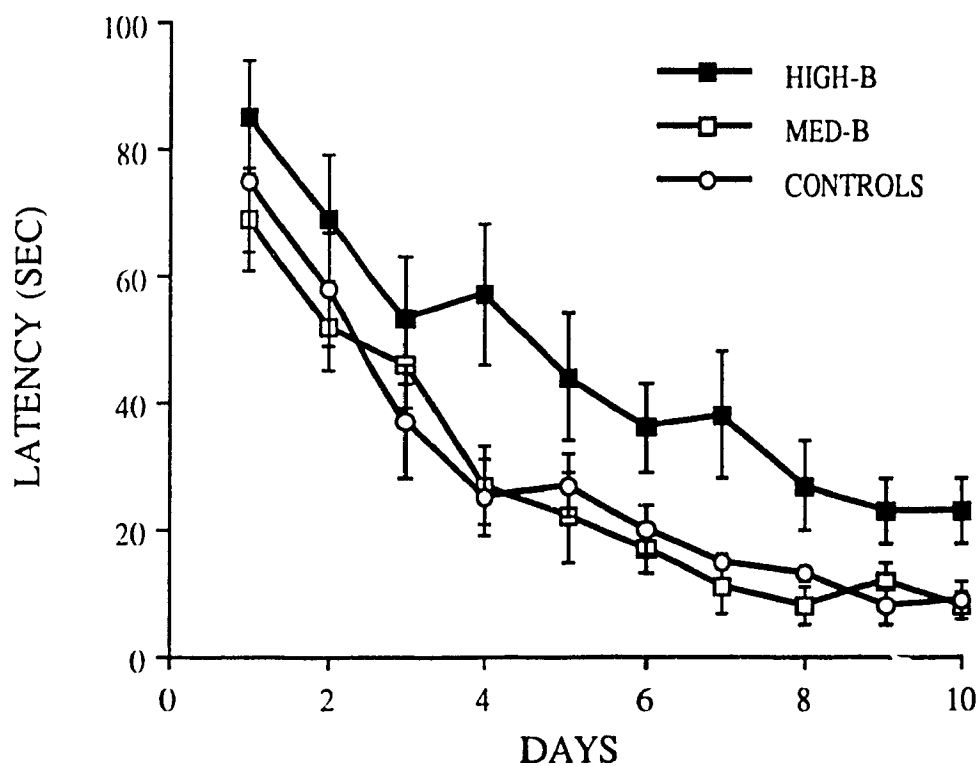


Figure 14. Mean latency (sec) over days (collapsed across blocks of two trials) to locate a submerged platform, in a combined group of untreated young adults and mid-aged rats treated with cholesterol (CONTROL, see text for explanation) or a medium (MED-B) or high (HIGH-B) level of corticosterone for three months.

38.02,  $df = 9,342$ ,  $p < 0.0001$ ) but no treatment by days interaction ( $F = 0.55$ ,  $df = 18,342$ ,  $p > .9$ ). Analysis of the simple main effect of treatment indicated that the High-B rats were significantly different from both Medium-B and Controls ( $p < 0.05$ ) although the differences were more pronounced (i.e. no overlapping standard error bars) from Days 4 to 10. There was no group difference on Trial 1 ( $F = 2.42$ ,  $df = 2,38$ ,  $p > 0.1$ ).

Figure 15 shows an identical pattern for the distance data (Treatment:  $F = 4.37$ ,  $df = 2,38$ ,  $p < 0.02$ ; Days:  $F = 34.14$ ,  $df = 9,342$ ,  $p < 0.0001$ ; Treatment  $\times$  Days:  $F = 0.63$ ,  $df = 18,342$ ,  $p > 0.8$ ), although the groups did not differ on Trial 1 ( $F = 1.66$ ,  $df = 2,38$ ,  $p > 0.20$ ). Analysis of the simple main effect of treatment indicated that the High-B rats were significantly different from both the Medium-B and Controls ( $p < 0.05$ ), although the differences were most pronounced from Day 4 to Day 10.

The probe trial data demonstrated a significant effect of treatment ( $F = 8.19$ ,  $df = 2,38$ ,  $p < 0.001$ ). The analysis of the simple main effect indicated that High-B rats spent significantly less time ( $p < 0.01$ ) in the original training quadrant relative to both Medium-B and control animals ( $22 \pm 3$ ,  $40 \pm 4$  and  $41 \pm 4\%$  respectively). Performance on the visually-cued platform did not differ between the groups as all animals located the elevated platform with latencies of less than 12 seconds.

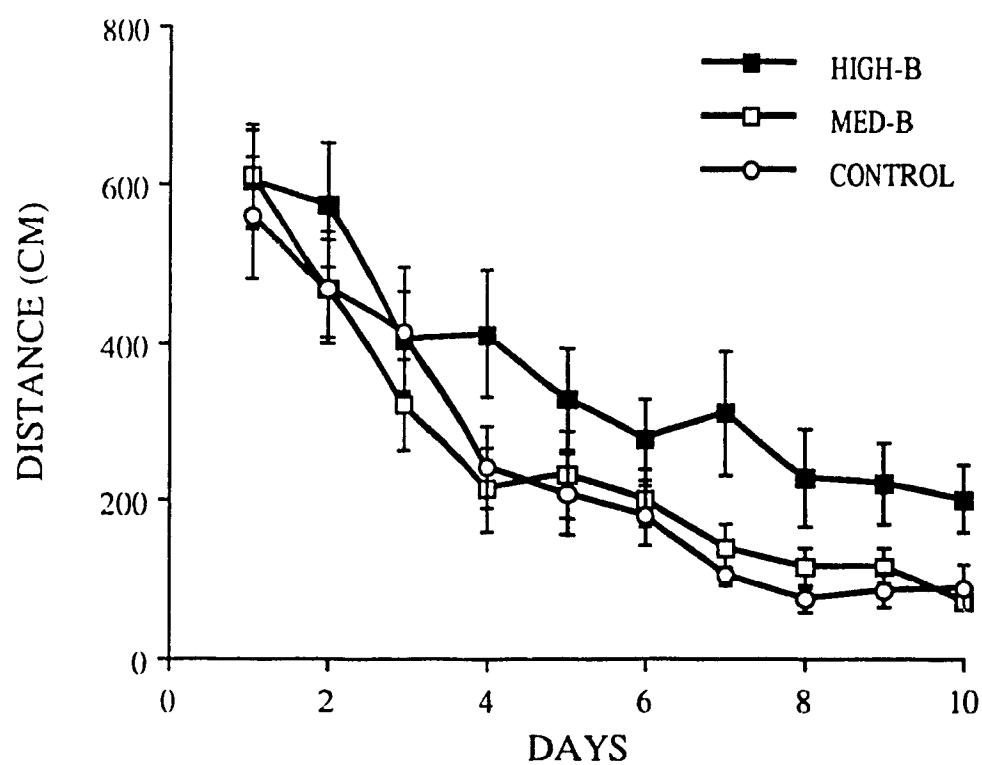


Figure 15. Mean distance travelled (cm) over days (collapsed across blocks of two trials) to locate a submerged platform in a combined group of untreated young adults and mid-aged rats treated with cholesterol (CONTROL) or a medium (MED-B) or high (HIGH-B) dose of corticosterone for three months.

Conflict Testing. Figure 16 represents the mean latency (sec) for animals to begin eating in the novel environment. Although the control animals took longer to eat relative to High-B and Medium-B rats ( $205 \pm 29$ ,  $141 \pm 7$ ,  $143 \pm 29$  sec respectively) this difference was not statistically significant ( $F = 1.56$ ,  $df = 2,33$ ,  $p > 0.2$ ).

Electrophysiology. Recordings were obtained from 11 control (4 untreated young adults and 7 mid-aged, cholesterol-treated controls), 7 Medium-B and 9 High-B animals. For all the rats, the response to commissural stimulation recorded in the CA1 pyramidal cell layer consisted of a typical positive field EPSP with superimposed population spike. Neither the baseline population spike amplitude (mean  $\pm$  s.e.m in mV) (Control:  $2.52 \pm 0.16$ ; Medium-B:  $2.21 \pm 0.22$ ; High-B:  $2.55 \pm 0.11$ ;  $F = 1.5$ ,  $df = 2,24$ ,  $p > 0.2$ ) nor the stimulus intensity necessary to evoke the baseline population spike (mean  $\pm$  s.e.m. in  $\mu A$ ) (Control:  $134 \pm 12$ ; Medium-B:  $130 \pm 17$ ; High-B:  $107 \pm 7$ ;  $F = 1.5$ ,  $df = 2,24$ ,  $p > 0.2$ ) were different between any of the groups.

The time course of the change in population spike amplitude following PB stimulation in Controls, Medium- and High-B rats is shown in Figure 17. The incidence of enhancement for individual rats was determined by comparing the response obtained in the last five minutes of the baseline period with those recorded during the interval 16-20 minutes



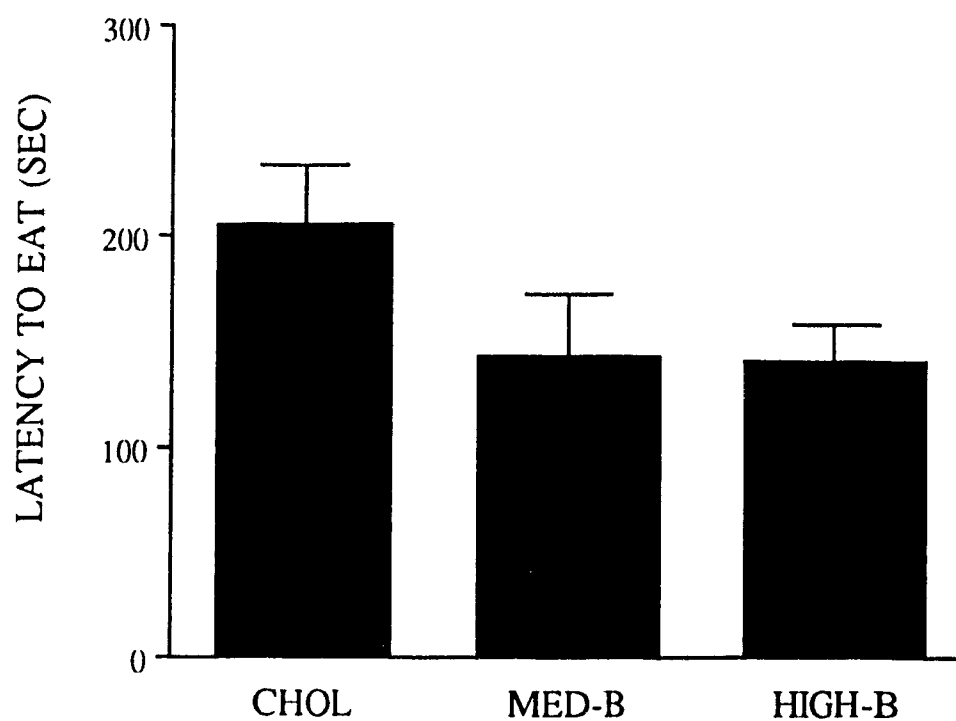


Figure 16. Mean latency (sec) for food-deprived animals to begin eating in a novel environment following a three-month treatment regimen with either cholesterol (CHOL) or medium (MED-B) or HIGH (HIGH-B) levels of corticosterone.

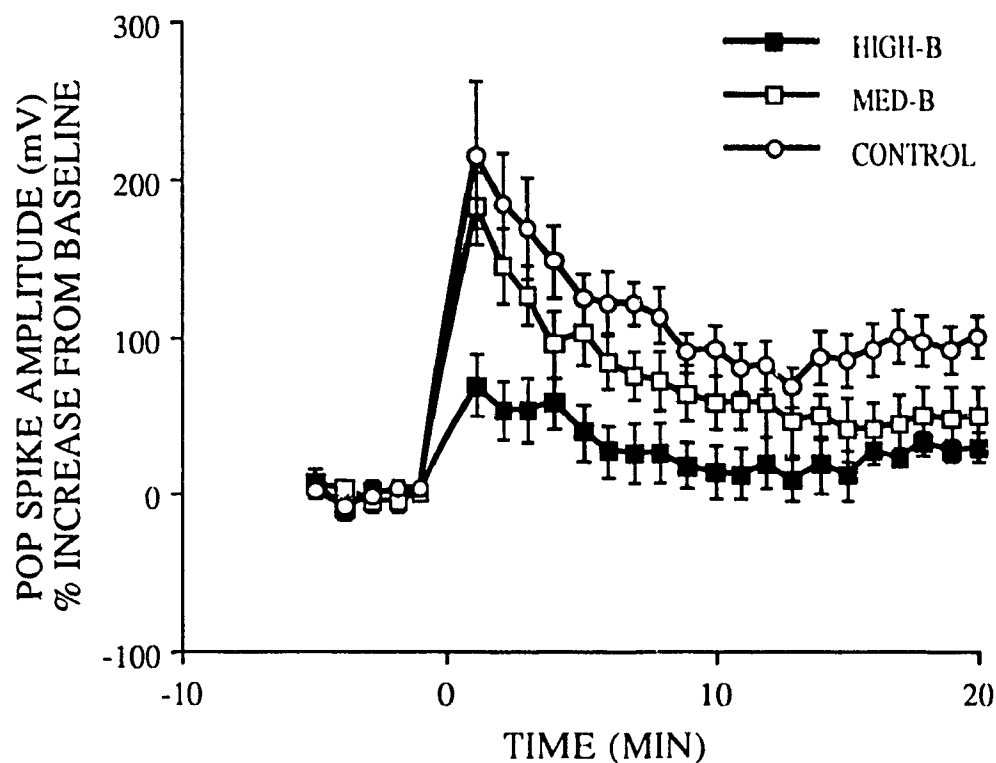


Figure 17. Mean percent increase over baseline in population spike amplitude (mV) following a primed burst stimulation, over a 20-minute recording period in a combined group of untreated young adults and mid-aged rats treated for three months with cholesterol (CONTROL) or either a medium (MED-B) or high (HIGH-B) levels of corticosterone.

after the PB train had been delivered (Student's t-test). Using this analysis, the incidence of enhancement following PB stimulation was: Control - 11 of 11 cases; Medium-B - 5 of 7 cases; High-B - 6 of 9 cases. Figure 18 shows the percent increase in the population spike amplitude during the interval 16-20 minutes following tetanic stimulation relative to the baseline measure (-5 to 0 minutes). Overall, there was a significant effect of treatment ( $F = 6.99$ ,  $df = 2,24$ ,  $p < 0.004$ ) and a treatment by time interaction ( $F = 4.75$ ,  $df = 18,216$ ,  $p < 0.001$ ). Post-hoc analyses showed that Control animals exhibited significantly more enhancement than both Medium-B ( $p < 0.05$ ) and High-B ( $p < 0.01$ ) animals, which did not differ significantly from each other ( $p > 0.1$ ).

High-B animals showed significantly less increase in population spike amplitude (i.e. PTP) measured one minute following PB stimulation (High-B:  $58 \pm 19$ , Medium-B:  $182 \pm 26$ ,  $215 \pm 47$  mV;  $F = 4.45$ ,  $df = 2,24$ ,  $p < 0.02$  vs Controls and Medium-B).

The Pearson Product Moment Correlation coefficient calculated across all animals between the percentage of time spent in the original training quadrant during the probe trial and the mean population spike amplitude one minute following stimulation (PTP) was significant ( $r_{21} = 0.57$ ,  $p < 0.05$ ; see Figure 19).

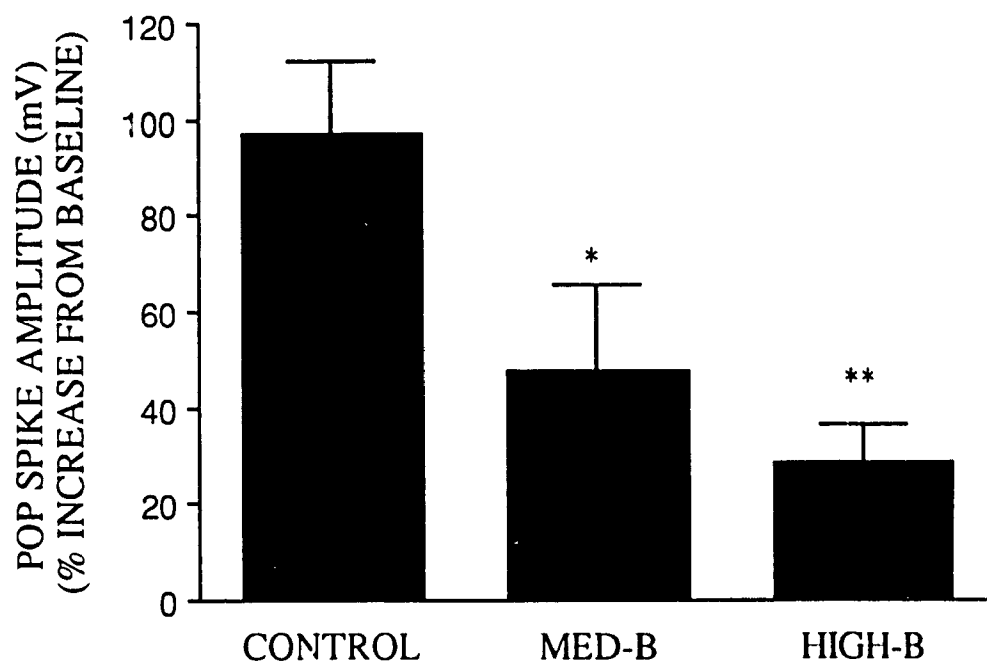


Figure 18. Mean percent enhancement over baseline of population spike amplitude (mV) during the interval 16-20 minutes following a primed burst stimulation, in a combined group of untreated adult rats and mid-aged rats treated with cholesterol (CONTROL) or either a medium (MED-B) or high (HIGH-B) level of corticosterone for three months. (\*  $p < .05$  vs CONTROL; \*\*  $p < 0.01$  vs CONTROL).

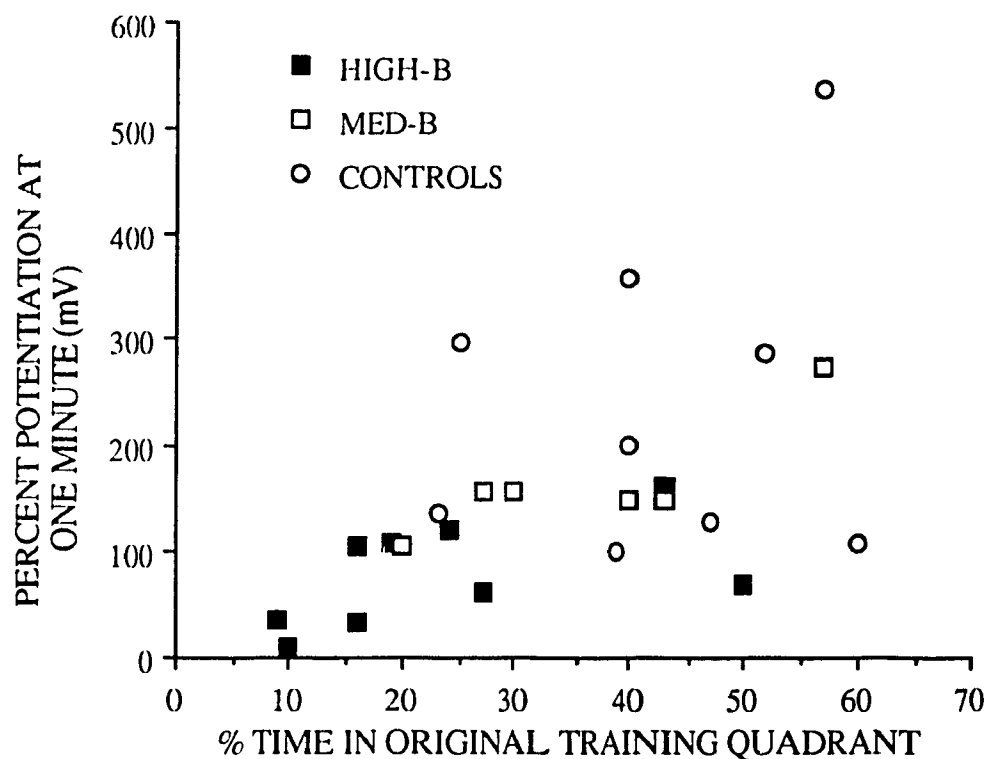


Figure 19. Scattergram of the percent potentiation (above baseline) of the mean population spike amplitude (mV) recorded one minute following the PB stimulation (i.e. PTP) versus the mean percent of time spent in the original training quadrant during a 30-second probe trial from Control, Medium-B and High-B animals. The equation of the line of best fit is:  $y = 8.97 + 4.37x$ ,  $r^2 = 0.31$ .

Plasma corticosterone levels, taken from tail blood following the termination of the electrophysiological recording, revealed no significant differences between the groups ( $F = 0.90$ ,  $df = 2,23$ ,  $p > 0.4$ ). The control, Medium-B and High-B rats had corticosterone levels of  $31 \pm 12$ ,  $24 \pm 3$  and  $26 \pm 4$   $\mu\text{g/dl}$  respectively.

Hippocampal Cell Counting. Table 2 depicts the mean number of corrected CA1 and CA3 neurons ( $\pm$  s.e.m.) per  $0.1 \text{ mm}^2$  in mid-aged cholesterol-treated, Medium-B and High-B rats ( $n = 5$ ). There were no significant differences between the treatment groups with respect to neuron density in either CA1 ( $F = 0.60$ ,  $df = 2,12$ ,  $p > 0.5$ ) or CA3 ( $F = 2.21$ ,  $df = 2,12$ ,  $p > 0.15$ ). Moreover, there were no group differences with respect to cell size (CA1:  $F = 0.20$ ,  $df = 2,12$ ,  $p > 0.8$ ; CA3:  $F = 0.01$ ,  $df = 2,12$ ,  $p > 0.9$ ). The mean diameter ( $\pm$  s.e.m.) of the CA1 neurons was  $11.5 \pm 0.8$ ,  $11.6 \pm 0.5$  and  $12.0 \pm 0.5$   $\mu\text{m}$  for the cholesterol-treated, Medium-B and High-B animals respectively. The mean diameter ( $\pm$  s.e.m.) of the CA3 neurons was  $19.4 \pm 0.7$ ,  $19.5 \pm 0.5$  and  $19.5 \pm 0.6$   $\mu\text{m}$  for the cholesterol-treated, Medium-B and High-B animals respectively.

Once again, there was a clear impairment in the acquisition of a spatial task following a three-month corticosterone treatment regimen in mid-aged rats, but only in those animals treated with stress-like levels of the steroid. As was evident in both the latency and distance data, High-B

Table 2

Neuron Density (per 0.1 mm<sup>2</sup>) in CA<sub>1</sub> and CA<sub>3</sub> Hippocampal Neurons in  
Mid-Aged Cholesterol-Treated (Chol), Medium-B and High-B Rats  
Following a Three-Month Treatment Regimen

	CA <sub>1</sub>	CA <sub>3</sub>
Chol (n = 5)	40.0 ± 0.9	22.5 ± 0.8
Medium-B (n = 5)	38.0 ± 2.4	24.5 ± 1.1
High-B (n = 5)	40.1 ± 0.9	22.3 ± 0.6

rats did not achieve the same asymptotic performance of the Medium-B or control animals. This difference was further reflected by the decreased percentage of time spent in the original training quadrant during the probe trial. However, when the escape platform was visible, the High-B animals had no difficulty locating the platform quickly.

These behavioral deficits were accompanied by a significant reduction in hippocampal synaptic plasticity observed following tetanic stimulation, a pattern that has been previously-reported in aged, cognitively-impaired animals (Barnes and McNaughton, 1985; Landfield and Lynch, 1977; Landfield et al., 1978a).

The performance of the Medium-B rats in both the acquisition trials and the retention probe test did not differ from Controls. While these data may appear inconsistent with the findings of Experiment 3, it must be noted that the level of steroid between the two studies did differ. Indeed, the corticosterone-treated animals in Experiment 3 received average daily doses equivalent to those of the High-B rats. These findings suggested that the long-term consequences of glucocorticoid administration upon behavior were dependent upon high, stress-like levels of corticosterone. Persistently-elevated glucocorticoid levels within the normal p.m. circadian range did not affect behavior.



While the performance of Medium-B rats in the swim maze and the conflict test did not differ from controls, the electrophysiological data suggested that the chronic administration of a lower level of corticosterone did influence some aspect of hippocampal function. Indeed, while Medium-B rats displayed similar acquisition and retention (probe trial) to young and age-match controls, they exhibited significantly reduced PB potentiation (assessed 16-20 minutes following stimulation) relative to age-matched controls.

While the difference was not significant, Medium-B rats displayed a greater degree of PB enhancement relative to High-B animals (48 vs 28% increase over baseline respectively). This finding led to the speculation that there was a threshold of plasticity necessary to preserve intact performance in the Morris maze and that the High-B rats did not achieve this.

There was also a qualitative difference in the topography of the response to the PB stimulation between Medium- and High-B animals. While the early-phase, PTP response of the Medium-B rats was equivalent to controls, High-B rats demonstrated a non-decaying (i.e. flat) response to the tetanic stimulation. The latter finding was similar to those described by Deupree et al. (1991). They reported that old F-344 rats were significantly impaired in the Morris maze (i.e. the animals showed no learning curve),

and in vitro LTP within the CA1 region indicated a significantly smaller increase in population spike amplitude at the one-minute mark following stimulation, and significantly reduced LTP. Moreover, retention scores on a single probe trial correlated best with changes in population spike amplitude one minute following tetanic stimulation, suggesting that the PTP response was the best predictor of performance in the Morris maze, with a correlation of  $r = +0.63$ , between these variables. Indeed, in the present experiment, the correlation coefficient was similar ( $r = +0.57$ ). Thus, based upon the "normal" PTP response observed in the Medium-B animals, a behavioral deficit in the Morris maze would not be expected, while the absence of an enhanced PTP response in the High-B animals would predict impaired performance in the task.

It was important to note that the differences in PB potentiation between the controls, Medium- and High-B rats were not due to differences in circulating plasma levels of corticosterone at the time of recording. As discussed earlier, the degree of hippocampal plasticity following PB stimulation is reduced in the presence of high glucocorticoid levels (Bennett et al., 1991; Diamond et al., 1992). In the present experiment, plasma corticosterone levels at the time of the recording were high, but did not differ between the groups. Thus, the significant reductions in plasticity observed between the control and corticosterone-treated groups were due

to the earlier glucocorticoid intervention rather than differences in the adrenocortical response to anesthesia and the stress of surgery. Indeed, one may speculate that a three-month exposure to stress-like levels of corticosterone did induce long-term changes in the functioning of the CA<sub>1</sub> synapses.

Behavior in the conflict task did not differentiate between the groups and these findings were unexpected based upon the apparent anxiogenic effects of long-term glucocorticoid exposure in the younger animals described in Experiment 2. Based upon the data from the conflict paradigm, it would appear that the effect of corticosterone on neophobia diminished as a function of age. However, these findings stand in contrast to others who have reported an increase in gustatory neophobia in aged animals (Spangler, Chachich, Curtis and Ingram, 1989). Gallagher and Burwell (1989) found that while equivalent suppression of drinking a novel solution was evident in both young and aged animals during the initial presentation, the former group exhibited a more rapid recovery from the neophobia.

It was very difficult to address why a glucocorticoid-enhancement of neophobia would be present in younger but not older rats, although it was important to note a procedural difference between the two experiments. Prior to conflict testing, the mid-aged animals were used in another

experiment that required a 23 hour/day food-deprivation schedule that continued for approximately ten days. In contrast, the animals tested in the conflict task described in Experiment 2 were given an acute 48-hour food-deprivation period. It has been previously demonstrated that the number of hours of food-deprivation do not influence behavior in this conflict test (Bodnoff, unpublished findings). Indeed, a 72-hour acute deprivation period is as effective as a 24-hour period. Thus, the degree of hunger is not an important variable in this paradigm. However, it has been suggested that animals maintained on a feeding schedule whereby food is available only during a restricted period (e.g. a 23 hour/day food-deprivation schedule) are "primed" to eat, and approach and ingest food when it is made available (Bolles, 1975). Therefore, it would appear that in the Medium- and High-B animals, any effects of the corticosterone treatment upon performance in the conflict task were confounded by the effects of their food-deprivation schedule upon feeding behavior.

The cell counting data once again revealed that long-term glucocorticoid treatment did not result in hippocampal cell loss or shrinkage in mid-aged animals. Thus, the spatial learning impairments occurred independently of hippocampal pathology. We would suggest that a decrease in hippocampal synaptic plasticity, and especially the PTP response following PB stimulation, was a more sensitive predictor of

impaired performance in the Morris maze than gross hippocampal pathology.

### Experiment 5

It has been suggested that the pattern of changes observed in pathological aging (e.g. HPA dysfunction, cognitive impairments and neuropathology) may be the eventual consequence of persistent adrenocortical activation. That is, chronic stress may accelerate the aging process (see introduction for the discussion of stress and the glucocorticoid cascade hypothesis of aging; Sapolsky et al., 1986b). Indeed, O'Steen and Brodish (1985) and O'Steen, Sweatt, Eldridge and Brodish (1987) reported that long-term exposure to chronic stress resulted in patterns of retinal degeneration similar to those observed in aged animals. Foy et al. (1987) and Shors et al. (1989, 1990a) reported that three weeks of inescapable shock significantly reduced in vitro hippocampal LTP in young rats. Based on the findings of previous experiments, it was important to determine whether physiological elevations of endogenous corticosterone resulted in similar deficits as those observed in the mid-aged animals treated with equivalent levels of corticosterone. Is the long-term exposure to elevated levels of glucocorticoids in younger animals an appropriate

model for understanding the effects of chronic stress upon many of the biological endpoints affected in pathological aging?

The selection of a chronic stress paradigm involved several considerations. Although many chronic stress regimens employed highly-invasive, painful techniques (e.g. electric shock), the adrenocortical response to the stressor may attenuate over a long-term experiment (Pollard, Bassett and Cairncross, 1976). In contrast to such invasive, experimentally-induced stressors, there are naturalistic or psychosocial models that attempt to mimic the elements that occur within a species, and allow glucocorticoid levels to remain elevated throughout the manipulation. For example, Levine (1992) described a paradigm in which squirrel monkeys lived in colonies that placed differential emphasis on food-gathering strategies. The low demand (LD) condition made food readily available while the high demand (HD) condition required constant searching for food. While both groups received adequate nutrition (e.g. the amount consumed in the HD group was approximately 125% of baseline values), the HD animals exhibited significantly elevated cortisol levels throughout the course of the experiment (8 weeks). Further, this group showed a marked reduction in social behavior as a consequence of this stressful environment. However, neither the cognitive nor neuropathological consequences of this manipulation has yet been studied.

Kerr, Campbell, Applegate, Brodish and Landfield (1991) demonstrated that six-months exposure to a psychological stressor (i.e. a two-way shuttle escape task in which animals received very little shock) accelerated electrophysiological markers of hippocampal aging in young and mid-aged rats. What was important in this study was that these changes in hippocampal activity were assessed several weeks following the termination of the stressor, suggesting that the intervention had induced some long-term modification in hippocampal functioning. Moreover, the authors reported that chronic stress had no effect in young or mid-aged rats upon morphological markers of aging, such as hippocampal neuron loss. In contrast, the identical treatment regimen in old rats markedly accelerated pyramidal cell loss in the CA1 region relative to age-matched controls, again supporting the hypothesis that sub-threshold pathology associated with aging was exacerbated by increased glucocorticoids (Sapolsky et al., 1986b).

The purpose of Experiment 5 was to examine the effects of a chronic stress regimen in mid-aged rats upon acquisition of the Morris water maze. The results presented by Kerr et al. (1991) suggested that a similar regimen had long-lasting effects upon hippocampal electrophysiology. The issue addressed in the present experiment was whether these changes in

hippocampal electrophysiology also had a behavioral correlate (a finding previously demonstrated in the High-B animals of Experiment 4).

The paradigm selected was a manipulation of the home cage setting that has previously been described by Mormede, Lemaire, Castanon, Dulluc, Laval and LeMoal (1990). They reported that co-habitation with females, in a disrupted social group, elevated basal levels of corticosterone in the males during the a.m. nadir of the circadian cycle and increased adrenal weight. Mormede et al. (1990) reported that there was relatively little habituation of the adrenocortical response to the social stressor over the four-week testing period. In the present experiment, the stressor was continued for a period of six months as it has been suggested that this was the minimum duration necessary to observe subtle changes in the markers of hippocampal function (Landfield, 1988).

It is important to consider that one obvious difference between chronic stress and chronic corticosterone administration is that the former intervention would activate the HPA axis and increase ACTH while the latter would, via negative feedback, suppress ACTH. These differential effects upon ACTH may be important as Landfield et al. (1981a) suggested that the neuron loss observed during aging was less the effect of elevated levels of corticosterone, but rather the eventual result of significantly reduced levels of ACTH. They demonstrated that the administration of



ACTH analogues prevented the behavioral and neuropathological changes observed during aging (Landfield et al., 1981a).

It was hypothesized that if long-term exposure to corticosterone does contribute to disrupted performance in the Morris water maze, animals exposed to the social stress regimen for six months would demonstrate impairments in the acquisition of this task compared with controls. When assessing the role of corticosterone in the long-term effects of chronic stress, it is imperative to include a stressed group that is adrenalectomized and replaced with basal levels of corticosterone, to conclude that adrenocortical secretions are the major contributing factor to those deficits. Therefore it was hypothesized that exposure to chronic stress, in the absence of elevated levels of corticosterone would not produce decrements in learning relative to controls.

### Methods

Animals. The male animals used in this study were obtained from Charles River (Portage, Michigan) at ten months of age (retired breeders). Virgin females arrived from the same breeder at approximately three months of age. The housing and feeding conditions were identical to those described in Experiment 1. The chronically-adrenalectomized rats (see below) received water bottles containing 0.9% saline and 20% sucrose by volume. The health of the animals was monitored regularly, and any

animals with overt signs of chronic respiratory distress, or tumors were removed from the study. In order to prevent pregnancy during the interactions between some males and females, all male rats were vasectomized under Metofane anesthesia. Approximately three weeks of recovery was allowed prior to the initiation of the chronic stress paradigm.

Social Stress Regimen: All males were randomly assigned to one of the three conditions. Control (n = 10): For the duration of the experiment, the same four males were housed in a single cage. Stress Condition (n = 9): Each cage contained two females and two vasectomized males. Three times weekly, the males were rotated according to a quasi-random schedule, such that the same two males were never paired together during consecutive rotations. ADX + B + Stress Condition (n = 7): Each cage contained two females and two vasectomized males and this group was rotated as above. However, prior to implementing the rotation schedule, all males were bilaterally-adrenalectomized and received low levels of corticosterone (e.g. 5-8  $\mu\text{g}/\text{dl}$ ), delivered via a single, three-month release, 100 mg pellet described in Experiment 4. The pellets were replaced three months into the stress regimen. This group was included to determine whether the changes observed in the Stress animals were due to high circulating levels of corticosterone. Furthermore, basal concentrations of corticosterone were necessary to maintain levels of ACTH within an

appropriate range (Dallman and Jones, 1973; Keller-Wood and Dallman, 1984). The social stress paradigm continued for six months.

Measurement of Diurnal Corticosterone Levels. Approximately five months into the experiment, plasma corticosterone values were determined (see Experiment 1 for procedure) over the diurnal cycle. Tail blood was taken from restrained animals within two minutes following their removal from the home cage at six consecutive four-hour intervals, beginning at 8:00 a.m. and concluding at 4:00 a.m.

Morris Swim Maze. At the termination of the social stress regimen, all females were removed from the study and the males were housed either four per cage (in the Control group) or two per cage (in the Stress and Adx + B + Stress groups) until the swim maze testing began three weeks later. The procedure was described in Experiment 3 (2 trials/day for 10 days).

## Results and Discussion

Diurnal Corticosterone Levels. Figure 20 shows the mean plasma corticosterone levels ( $\mu\text{g/dl}$ ) over the diurnal cycle. There was a significant effect of group ( $F = 38.52$ ,  $df = 2,19$ ,  $p < 0.0001$ ) and time of day ( $F = 12.97$ ,  $df = 5,95$ ,  $p < 0.0001$ ) while there was a trend for a significant group by time interaction ( $F = 1.55$ ,  $df = 10,95$ ,  $p < 0.13$ ). Analysis of the simple main effect of group indicated that the Stress group

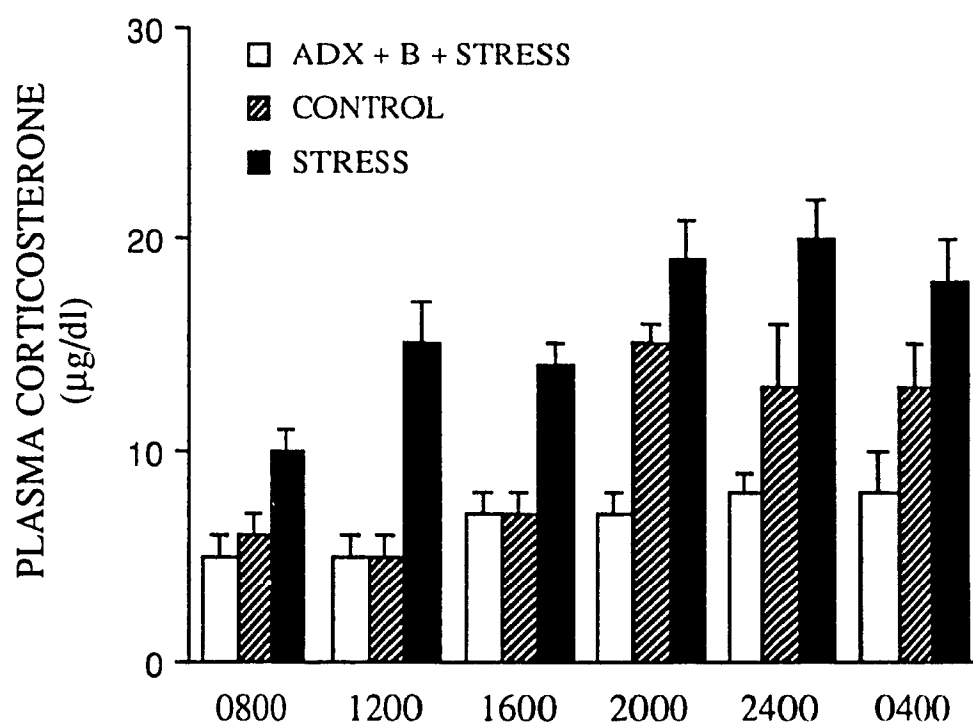


Figure 20. Mean plasma corticosterone levels ( $\mu\text{g/dl}$ ), taken at six consecutive four-hour intervals over the diurnal cycle, following a six-month social stress regimen, in adrenalectomized + corticosterone + social stress (ADX + B + STRESS), controls (CONTROL) and social stressed (STRESS) rats.

had higher plasma corticosterone levels relative to both the Control and the ADX + B + Stress groups ( $p < 0.05$ ). Although it is inappropriate to conduct post-hoc analyses of the group effect at each time point (i.e. the group by time interaction was not significant), it is apparent from Figure 20 that the Stress group had higher corticosterone levels than the ADX + B + Stress group throughout the entire cycle and had higher levels compared with the Controls at 0800, 1200 and 1600h.

Morris Swim Maze. Figure 21 represents the mean latency (sec) over days (collapsed across blocks of two trials within days) for rats to locate the escape platform. There was a significant effect of days ( $F = 40.37$ ,  $df = 9,18$ ,  $p < 0.0001$ ) and group by days interaction ( $F = 2.58$ ,  $df = 18,207$ ,  $p < 0.0007$ ) and although non-significant, there was a trend for an effect of group ( $F = 2.52$ ,  $df = 2,23$ ,  $p < 0.12$ ). Post-hoc analyses revealed that the Stress group differed ( $p < 0.05$ ) from the Controls on Days 2 and 4 and differed from the ADX + B + Stress on days 2 and 3. The groups did not differ on Trial 1 ( $F = 1.7$ ,  $df = 2,23$ ,  $p > 0.20$ ).

The mean distance travelled (cm) over days (collapsing across blocks of two trials within days) in locating the platform is shown in Figure 22. There was a significant effect of days ( $F = 32.36$ ,  $df = 9,18$ ,  $p < 0.0001$ ), a near-significant group by days interaction ( $F = 1.59$ ,  $df = 18,207$ ,  $p <$

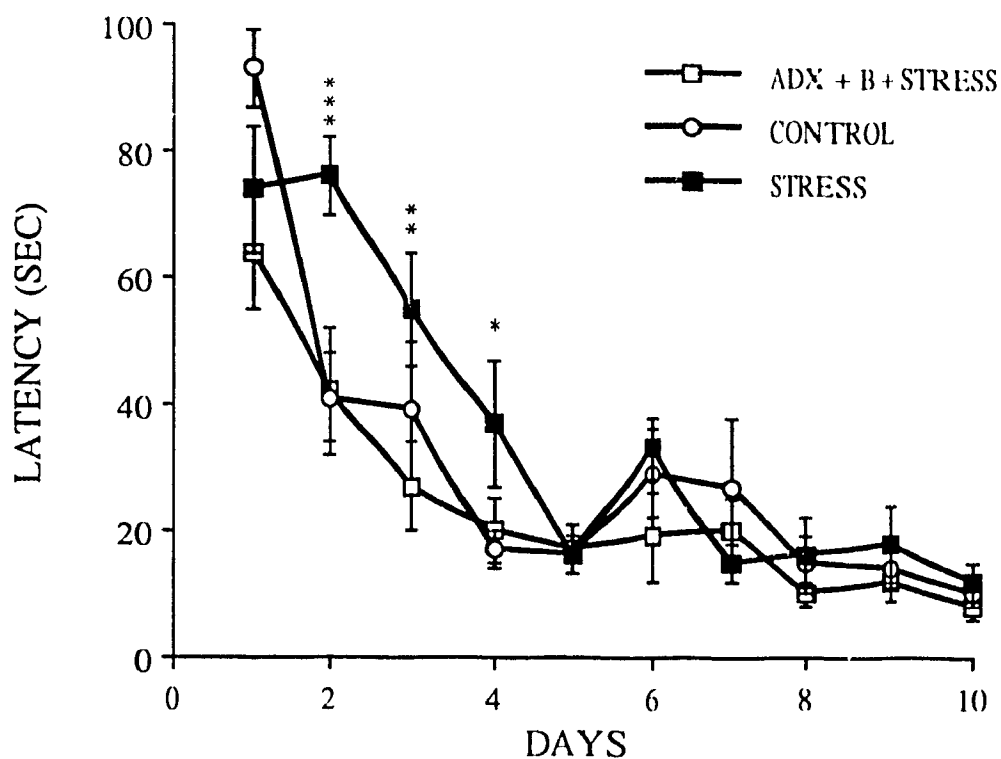


Figure 21. Mean latency (sec) over days (collapsed across blocks of two trials) to locate a submerged platform following a six-month social stress regimen, in adrenalectomized + corticosterone + social stress (ADX + B + STRESS), controls (CONTROL) and social stressed (STRESS) rats. (\*  $p < 0.05$  vs CONTROL; \*\*  $p < 0.05$  vs ADX + B + STRESS; \*\*\*  $p < 0.05$  vs ADX + B + STRESS and CONTROL).

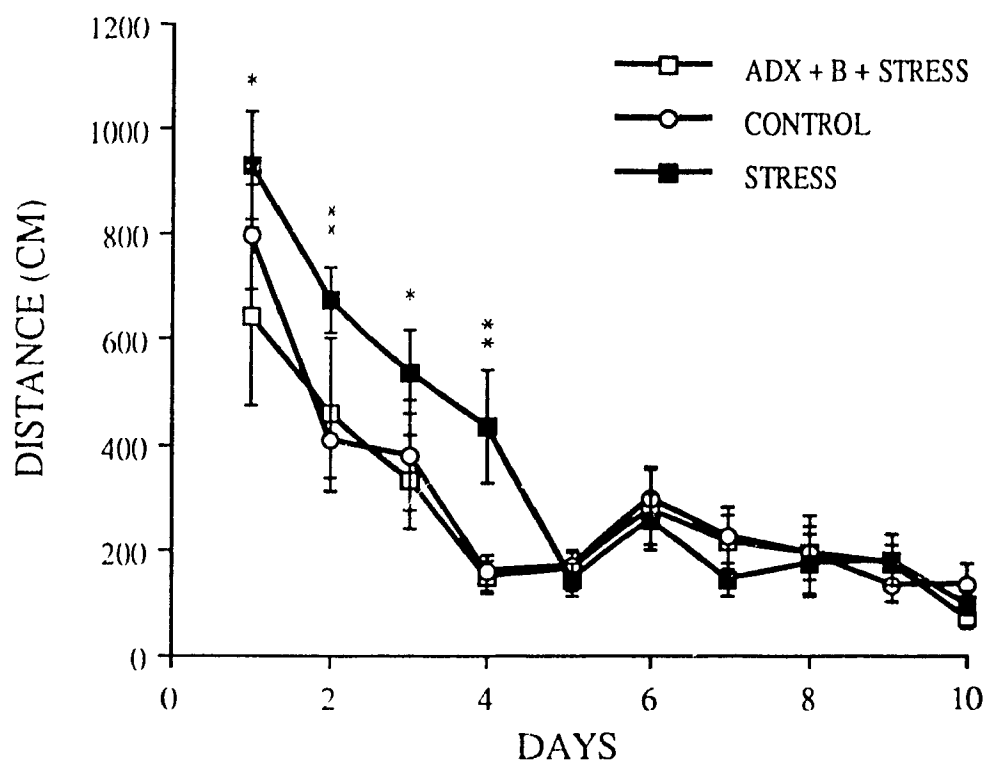


Figure 22. Mean distance travelled (cm) over days (collapsed across blocks of two trials) to locate a submerged platform following a six-month social stress regimen, in adrenalectomized + corticosterone + social stress (ADX + B + STRESS), controls (CONTROL) and social stressed (STRESS) rats. (\*  $p < 0.05$  vs ADX + B + STRESS; \*\*  $p < 0.05$  vs ADX + B + STRESS and CONTROL).

0.06) and although non-significant, there was a trend for a significant effect of group ( $F = 1.94$ ,  $df = 2,23$ ,  $p < 0.17$ ). The post-hoc analyses indicated that the Stress group differed from the ADX + B + Stress on Days 1 through 4 and differed from Controls on Days 2 and 4. The groups did not differ on Trial 1 ( $F = 1.86$ ,  $df = 2,14$ ,  $p > 0.20$ ).

The diurnal corticosterone levels indicated that the Stress group did not show an appropriate circadian pattern of a morning nadir and evening peak. Indeed, these animals had higher plasma levels of corticosterone than the controls during each of the three early time points (i.e. 0800, 1200 and 1600h). Moreover, the observation that the levels were elevated five months following the initiation of the chronic stress regimen suggested that the adrenocortical response to the mixed-sex, disrupted housing conditions did not diminish. As expected, the adrenalectomized, corticosterone-replaced stressed group exhibited stable levels of corticosterone throughout the cycle.

Assessment of performance in the Morris maze indicated that the stress group exhibited subtle, but statistically significant acquisition deficits during the early training days relative to controls. Moreover, this deficit was absent in stressed animals that had been adrenalectomized and replaced with lower, stable levels of corticosterone. These findings suggest that the deleterious effects of stress upon acquisition in the Morris maze are likely



the result of elevated levels of corticosterone. Moreover, the present data would offer experimental support for the hypothesis that the long-term effects of corticosterone, whether via exogenous administration or stress, are somehow disruptive to learning.

The deleterious effects of a six-month chronic stress regimen upon acquisition were not as severe as a three-month treatment with high levels of corticosterone, but were readily apparent compared with rats treated with the Medium-B rats (who were treated with equivalent levels of glucocorticoids for three months). The argument was made earlier that level of steroid was an important contributor to the observation of a behavioral deficit in the Morris maze. The present experiment would suggest that the deleterious effects of lower doses of corticosterone may be observed if the treatment is continued for a longer period of time. Indeed, while Medium-B rats were unimpaired in the swim maze, the PB potentiation data indicated that these animals did exhibit reduced synaptic efficacy, suggesting that the behavioral task was not sensitive to the subtle electrophysiological changes that had occurred as a result of the treatment. It would be interesting to replicate the Medium-B experiment for six months to determine whether the behavioral deficit becomes evident.

## Experiment 6

The previous experiments demonstrated that a three-month exposure to high levels of exogenous corticosterone in young Long-Evans rats had no effects upon spatial learning in the Morris water maze. Was the absence of a deficit due to the inability of the treatment to affect neuron loss?

Although cell counting was not performed in young corticosterone-treated Long-Evans rats, the absence of changes in hippocampal morphology in mid-aged, corticosterone-treated rats would suggest that the treatment does not induce hippocampal pathology in this strain. These findings are in contrast to those of Sapolsky et al (1985) who reported significant hippocampal neuron loss in young F-344 rats following a three-month exposure to elevated corticosterone levels. However, in the F-344 strain, the treatment also resulted in significant mortality (approximately 50% of the animals had died by the termination of the treatment). In contrast, we have lost fewer than 3% of all Long-Evans subjects during the course of seven experiments. It may be suggested that the hippocampal neuron loss observed in the young, corticosterone-treated F-344 rats may be an artifact of the apparent toxicity of the steroid in this strain.

The purpose of Experiment 6 was to compare the effects of a three-month corticosterone treatment regimen in young F-344 and Long-Evans animals on acquisition in the Morris water maze. Would spatial learning

deficits, that have never been observed in young, corticosterone-treated Long-Evans animals become apparent in young, corticosterone-treated F-344 rats? It was hypothesized that if the levels of corticosterone remained well-below the LD50, no impairments in performance would be observed in either strain relative to their strain-matched controls.

### Methods

Animals. Male, Fisher-344 or Long-Evans rats, two to three months of age (Charles River, St. Constant, Quebec) were housed in groups of two in 20 x 19 x 10 cm cages, maintained on a 12:12 light dark cycle (lights on a 08:00 h), and provided with free access to food (Purina Lab Chow) and water. The F-344 animals arrived at a weight of 275-300 g while the Long-Evans rats were between 300-350 g. Because of the toxicity of corticosterone in the F-344 rats reported by Sapolsky et al. (1985), all animals were monitored carefully by both the animal care technicians and the veterinary staff of McGill University.

Pellet Implantation. The F-344 and Long-Evans animals were implanted subcutaneously with either two or three, 100 mg corticosterone pellets respectively (see Experiment 4) in anticipation of the differences in weight achieved by the two strains. The a.m. plasma levels of corticosterone, taken approximately one week following implantation ranged between 18-23 µg/dl. Control animals from the two strains were

implanted with either two or three-100 mg cholesterol pellets. During the study, over 50% of the corticosterone-treated F-344 rats showed serious signs of infection at the site of implantation and had to have their pellets replaced. This was not observed in any of the Long-Evans rats. At the termination of the study, the sample sizes were 9, 7, 8 and 7 for the F-344 corticosterone- and cholesterol-treated and Long-Evans corticosterone- and cholesterol-treated animals respectively.

Morris Swim Maze. After removal of the pellets, the rats were given three weeks to recover from the treatment. The F-344 rats responded very poorly to the treatment (see discussion) but did recover well. The animals were not assessed in the swim maze until the veterinarian consented to the testing. The procedure was identical to that described in Experiment 3 (2 trials per day), and continued for eight days. A single 30-second probe trial was given on Day 9 along with five trials with the platform visible.

### Results and Discussion

The mean latency (sec) over days (collapsed across blocks of trials within days), for animals to locate the hidden platform is shown in Figure 23. The analyses revealed a significant effect of strain ( $F = 9.33$ ,  $df = 1,27$ ,  $p < 0.005$ ), strain by days ( $F = 2.25$ ,  $df = 7,189$ ,  $p < 0.03$ ), strain by treatment ( $F = 9.34$ ,  $df = 1,27$ ,  $p < 0.005$ ) and strain by treatment by days

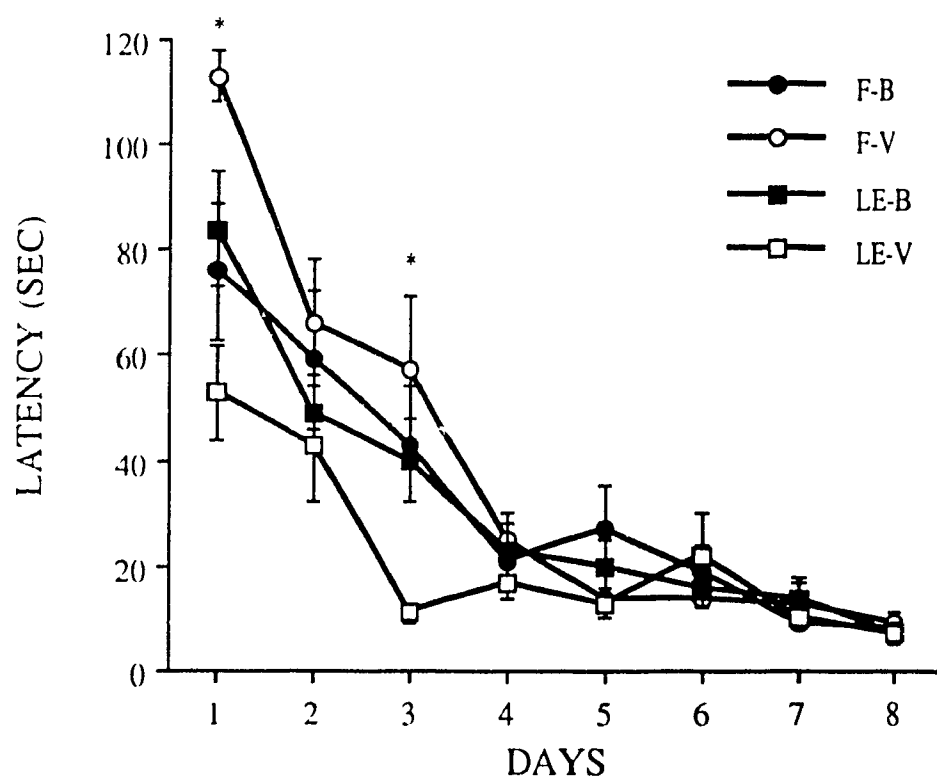


Figure 23. Mean latency (sec) over days (collapsed across blocks of two trials) to locate a submerged platform in young Fischer-344 (F-344) and Long-Evans (L-E) rats treated with either cholesterol (CHOL) or corticosterone (B) for three months. An asterisk indicates an L-E-B mean that is significantly different from the L-E-V ( $p < 0.05$ ) at that timepoint.

( $F = 3.71$ ,  $df = 7,189$ ,  $p < 0.0009$ ). Neither the main effect of treatment ( $F = 0.57$ ,  $df = 1,27$ ,  $p > 0.4$ ) nor the treatment by days interaction ( $F = 0.35$ ,  $df = 7,189$ ,  $p > 0.9$ ) was significant. Post-hoc analyses revealed that while the four groups did not differ on the first trial ( $F = 1.42$ ,  $df = 3,27$ ,  $p > 0.26$ ), the Long-Evans corticosterone-treated animals performed more poorly ( $p < 0.05$ ) than their controls on Day 1 and 3. In contrast, the performance of the F-344 corticosterone-treated animals was better than their controls, but the difference was significant only on Day 1.

A single 30-sec probe trial revealed that the groups did not differ in the percentage of time spent in the preferred quadrant ( $51 \pm 4$ ,  $44 \pm 6$ ,  $34 \pm 5$  and  $49 \pm 8\%$  for the F-344-B, F-344-Chol, L-E-B and LE-Chol rats respectively). The analysis revealed no significant effects of strain ( $F = 1.3$ ,  $df = 1,27$ ,  $p > 0.25$ ), treatment ( $F = 0.49$ ,  $df = 1,27$ ,  $p > 0.4$ ) or strain by treatment interaction ( $F = 3.45$ ,  $df = 1,27$ ,  $p > 0.1$ ). Finally, all rats found the raised platform in under 15 sec.

Although there was no mortality as a result of the corticosterone treatment, the two strains did differ in their physical response to the treatment. Approximately 50% of corticosterone-treated F-344 animals showed considerable signs of infection at the site of implantation. Indeed, the pellets had to be replaced and the infections treated throughout the experiment. Moreover, these animals sustained extensive hair loss across

their backs, away from the site of pellet implantation. The veterinarian described the phenomenon as looking like "excessive grooming" but could give no official diagnosis. Young Long-Evans rats showed none of these signs.

Decreased food and water intake has been demonstrated as a consequence of elevated levels of corticosterone (Simpson, DiCara and Wolf, 1974; Stevenson and Franklin, 1970) and the present treatment reduced the weights of animals in both strains relative to their respective controls. While the treated Long-Evans rats were 7% lighter than their controls ( $672 \pm 14$  vs  $723 \pm 9$ g, respectively:  $t = -2.87$ ,  $df = 14$ ,  $p < 0.01$ ), the weights of the corticosterone-treated F-344 rats were approximately 23% lower than their controls ( $365 \pm 11$  vs  $474 \pm 5$ g, respectively:  $t = -7.87$ ,  $df = 14$ ,  $p < 0.0001$ ).

A direct comparison of the effects of chronic corticosterone administration in F-344 and Long-Evans rats revealed that although the former strain showed clear adverse reactions to the treatment, there was no significant spatial learning deficit induced in these animals. Moreover, the performance of corticosterone-treated F-344 rats was superior (although not significantly) to their controls.

## General Discussion

### Long-Term Corticosterone Treatment in Adult Rats

The findings from three separate experiments (Experiments 2, 3 and 6) showed that in young adult, Long-Evans rats, a three-month treatment regimen with high, stress-like levels of corticosterone had no effect upon the acquisition of a spatial learning task relative to controls. In Experiment 1 we observed significantly longer latencies over the first four trials in the corticosterone-treated rats. However, the results of the conflict test in Experiment 2 suggest that these differences may be related to an apparent enhanced response to novelty.

To date, there have been two contradictory behavioral findings in young glucocorticoid-treated rats. Bardgett, Newcomer, Csernansky, Nock and Taylor (1992) reported that a two-month treatment with stress-like levels of corticosterone in young Long-Evans rats impaired spontaneous alternation relative to controls. However, in this study, the authors continued the glucocorticoid treatment throughout the behavioral testing period. Based upon the findings discussed earlier, acutely-elevated levels of glucocorticoids appear to interfere with both behavioral (Bohus and Lissak, 1968; deWeid, 1967) and electrophysiological (Bennett et al., 1991; Diamond et al., 1992) correlates of learning. Therefore it is unclear whether the glucocorticoid-mediated changes in behavior described



by Bardgett et al. (1992) would be apparent in the absence of these elevated levels of corticosterone. Clearly, it is important to assess the behavioral effects of glucocorticoids once the intervention has been terminated, as was done in the present experiments.

Levy and Dachir (1991) reported subtle deficits (i.e. first entry error) in the radial arm maze in young F-344 rats following the termination of a three-month corticosterone treatment. However, there was no difference in the rate of acquisition of the task between corticosterone-treated and control animals.

Therefore, based upon these studies and the findings from the present series of experiments, the interpretation of long-term glucocorticoid-induced behavioral deficits in young animals remains equivocal. It is possible that impairments would become apparent if a more difficult task was selected. Thus, it would be interesting to examine the effects of long-term glucocorticoid exposure upon performance in a variety of tests that are sensitive to either hippocampal damage (see Gray, 1982 for a review) or age-related decline (see Gallagher and Burwell, 1989).

Although cell counting was not completed in the present experiments with young adult animals, the study by Sapolsky et al. (1985) suggested that a similar glucocorticoid treatment resulted in significant neuron loss in the

CA3 cell field of the hippocampus, a pattern of pathology that has previously been demonstrated to manifest in performance deficits in the Morris water maze: Near-total ablation of CA3 neurons resulted in slower acquisition in this task (Auer, Jensen and Whishaw, 1989).

However, it is also apparent that the extent of such selective damage is an important factor. That is, no deficits in acquisition in the Morris maze were observed in rats that incurred a near total unilateral destruction of CA3 neurons (Auer et al., 1989). Issa et al. (1990) demonstrated that although aged, cognitively-unimpaired rats did lose approximately 20-25% CA3 neurons relative to young controls, their performances in the Morris maze did not differ. In contrast, the 45-60% neuronal loss observed in the aged, cognitively-impaired rats (Issa et al., 1990) and aged, non-handled rats (Meaney et al., 1988a) did manifest in a significant behavioral impairment. It appears that a behavioral deficit in the Morris maze can be observed only if sufficient hippocampal neuropathology is incurred.

To date, there are only two studies other than Sapolsky et al. (1985) which examined hippocampal neuron loss following long-term corticosterone treatment. Bardgett et al. (1992) reported that a two-month corticosterone treatment regimen did not induce neuron loss within the CA1, CA3 or dentate gyrus cell fields of the hippocampus of young Long-Evans animals. In contrast, Levy and Dachir (1991) reported that a three-

month corticosterone treatment in young F-344 rats treatment produced pyramidal cell loss in CA3 and granule cell loss within the dentate gyrus. This latter finding was unexpected as the dentate gyrus neurons were not targeted by the treatment described in Sapolsky et al. (1985). Indeed, these neurons appear to be sensitive only to the absence of a glucocorticoid signal. Slovitar, Valiquette, Abrams, Ronk, Sollas, Paul and Neubort (1989) reported a near total loss of granule cells in the adult animal following adrenalectomy, while the pyramidal neurons of Ammon's horn were spared. Moreover, replacement with low levels of either corticosterone (Gould, Woolley and McEwen, 1990) or aldosterone (Woolley, Gould, Sakai, Spencer and McEwen, 1991) protected against the effects of long-term adrenalectomy. Finally, the endogenous elevation of corticosterone (i.e. during six months of shuttle escape training) failed to produce hippocampal neuron loss in either young or mid-aged F-344 rats (Kerr et al., 1991).

To date, the pattern of neuropathology described by Sapolsky et al. (1985) following long-term glucocorticoid treatment in young F-344 animals has not been completely replicated. Indeed, the findings from several independent laboratories suggest that young animals do not exhibit hippocampal neuron loss following either chronic corticosterone treatment

(Bardgett et al., 1992) or chronic stress (Kerr et al., 1991), although Levy and Dahir (1991) did report both pyramidal and granule cell loss.

Are the effects of long-term corticosterone treatment strain-dependent? Using F-344 animals, Sapolsky reported a 50% mortality rate in the corticosterone-treated group by the end of the three month period, and their highly fragile state following adrenalectomy. With the Long-Evans strain, corticosterone treatment resulted in weight loss over time and some infection at the site of pellet implantation (but only in the mid-aged rats), and these factors were normalized following the termination of the treatment. More importantly, we observed no mortality due to the treatment itself.

In a direct comparison of Fischer-344 and Long-Evans rats (Experiment 6), the side-effects of high physiological levels of corticosterone differed in both degree and kind between the strains. Indeed, while both strains of corticosterone-treated animals exhibited weight loss, the F-344 rats lost a considerably larger percentage of weight compared with their controls. Moreover, only the F-344 rats exhibited infection at the site of pellet implantation and considerable hair loss along their hindquarters. Thus, the glucocorticoids appeared to be more noxious to the Fischer-344 strain.

Of the four studies which examined the effects of long-term corticosterone exposure upon hippocampal neuropathology, the two experiments with positive findings used the F-344 strain while the negative findings were observed in the Long-Evans strain. What accounts for this apparent increased susceptibility to glucocorticoid-induced neurotoxicity in F-344 rats? A potential contributor to the apparent strain difference in neurotoxicity may be related to calcium. Calcium homeostasis is of interest considering the evidence demonstrating the cytotoxic effects of elevated intracellular calcium (Choi, 1988; Farber, 1981). An interesting study by McBroom and Weiss (1973) examined the levels of soft tissue calcium longitudinally in the F-344 (a short-lived strain) and an A x C, Irish line (a long-lived strain). The authors reported an average lifespan of 21 and 28 months respectively. During aging, calcium content declined within skeletal muscle and rose within the cerebral cortex of both strains. Although calcium from bone catabolism was eventually taken up by the cerebral cortex, the buildup was more gradual in the A x C strain and never reached the same level attained by the Fischer rats. In contrast, the F-344 strain exhibited a rapid and marked increase relatively late in life (19 months), suggesting a breakdown in calcium equilibrium. These strain differences in calcium, which the authors suggested contributed to the

reduced lifespan of the F-344 rats, may also influence potential strain difference observed in glucocorticoid-induced neuropathology.

The neurotoxicity induced by an interaction between elevated glucocorticoids and increased extracellular calcium levels appears to be related to the activation of NMDA receptors by glutamate. Glucocorticoids inhibit glutamate uptake by glial cells (Virgin et al., 1991), the major route for the elimination of glutamate from the synapse (Hertz et al., 1983) by depleting ATP, the energy substrate necessary for reuptake (Benveniste et al., 1984). Therefore, the presence of elevated levels of glucocorticoids, which increase the extracellular levels of glutamate (by preventing its reuptake), results in the persistent activation of the NMDA receptor and an increased influx of  $\text{Ca}^{2+}$ . Sufficiently high levels of calcium can initiate the process of neuron death through a mechanism not yet understood. This suggestion is supported indirectly by the observation that NMDA antagonists also block the endangerment of hippocampal neurons by glucocorticoids (Armanini et al., 1990).

Although the issue has not been studied directly, one might hypothesize that extracellular calcium levels within the hippocampus may be significantly higher in F-344 than in the Long-Evans strain. Thus, long-term treatment with elevated levels of corticosterone would have greater pathological consequences for hippocampal neurons in the Fischer strain.

Indeed, with respect to the aging literature, it appears that the F-344 strain as a whole is more susceptible to glucocorticoid-induced changes than other strains. Landfield et al. (1978b,1981), in reporting the correlations between adrenocortical activity and neuropathology in aged F-344 rats, made no implication that a sub-population of animals was similar to young controls. In contrast, Gage and Bjorklund (1986), Gage et al. (1984); Issa et al. (1990) and Pelleymounter et al. (1990) reported considerable variability within both the Sprague Dawley and Long-Evans strains with respect to performance in the Morris maze, HPA dysfunction and neuropathology.

In summary, the long-term exposure of young animals to elevated levels of corticosterone results in varying effects upon learning, depending upon the task. The present studies using the Morris water maze indicated no impairments, while the radial arm maze (Levy and Dachir, 1991) and spontaneous alternation task (Bardgett et al., 1992) produced either subtle or significant effects. Similarly, the effects of increased glucocorticoid levels upon hippocampal neuropathology ranged from: no damage (Bardgett et al., 1992), to pyramidal cell loss exclusively (Sapolsky et al., 1985) to both pyramidal and granule cell loss (Levy and Dachir, 1991).

It may be suggested that the original report of glucocorticoid-induced neuropathology (Sapolsky et al., 1985) was confounded by the

toxicity of the treatment. As discussed in the introduction, glucocorticoids compromise the ability of hippocampal neurons to survive a range of metabolic insults including hypoxia/ischemia and hypoglycemia (Sapolsky, 1985; Sapolsky and Pulsinelli, 1985). If the health of the rats was severely compromised (as observed in the 25% decrease in body weight of the corticosterone-treated F-344 rats compared with their controls), a state of hypoglycemia or hypoxia might occur. While neither insult alone may be sufficient to result in significant hippocampal neuron loss, coincident exposure to elevated glucocorticoids would exacerbate the damage.

While both Levy and Dachir (1991) and Sapolsky et al. (1985) reported pyramidal neuron loss, the degree of neuropathology appeared greater in the latter experiment. The important variable here is that the treatment of Levy and Dachir (1991) did not increase mortality relative to controls. Thus, it would appear that the toxicity of the glucocorticoid treatment contributes to the degree of hippocampal damage, at least with respect to the F-344 strain. Indeed, a six-month exposure to a chronic stress regimen (an intervention which increased plasma corticosterone levels but did not induce mortality), did not result in hippocampal neuron loss in either young or mid-aged F-344 rats (Kerr et al., 1991).

In conclusion, the absence of either performance deficits in young adult, corticosterone-treated rats (the present experiments) or hippocampal



neuron loss (Bardgett et al., 1992) would suggest that the significant neuropathology reported by Sapolsky et al. (1985) following a similar treatment may be an artifact of the toxicity of the steroid to the F-344 strain.

#### Long-Term Corticosterone Treatment in Mid-Aged Rats

In contrast to the absence of a behavioral impairment in young, corticosterone-treated rats, the findings of Experiments 3 and 4 indicated that in the mid-aged rat, a three-month treatment regimen with stress-like levels of glucocorticoids had deleterious effects upon performance in the Morris water maze. While both the mid-aged corticosterone-treated (Experiment 3) and the High-B (Experiment 4) animals did exhibit a significant learning curve over days, they differed from aged-matched controls during the middle days of training (i.e. Days 4 through 6). However, the magnitude of the acquisition deficit did differentiate between the two experiments. For example, the glucocorticoid-treated rats in Experiment 3 showed a slower rate of acquisition (relative to controls) but eventually reached an equivalent level of performance. In contrast, the performance of the High-B rats treated with the three-month, slow release pellets failed to reach the same asymptotic level as their controls by the conclusion of the training trials. Moreover, impaired retention of the original spatial location (i.e. the probe trial), was observed only in the

High-B rats that had not achieved equivalent asymptotic escape latencies on the last day of training. These latter findings suggested a qualitative difference in the deficit incurred by the glucocorticoid-treated animals in these separate experiments.

A possible contributing factor to this variability may be the stability of the corticosterone dose achieved in the two experiments. In Experiment 4, the factory-produced pellets resulted in corticosterone levels that remained high and stable ( $\approx 25\text{-}30\text{ }\mu\text{g/dl}$ ) for the duration of the three-month treatment period. Moreover, there were considerably fewer signs of infection at the site of pellet implantation. In contrast, the levels of corticosterone achieved in Experiment 3 were very high on Day 2 ( $> 40\text{ }\mu\text{g/dl}$ ) but were considerably lower nine days later ( $< 20\text{ }\mu\text{g/dl}$ ), although the average corticosterone level per day was equivalent between the two experiments.

That the level of circulating steroid achieved during treatment was an important contributor to the behavioral deficit was demonstrated by the marked absence of an impairment in either acquisition or retention, in mid-aged rats treated with lower, p.m.-like levels of corticosterone ( $\approx 12\text{-}17\text{ }\mu\text{g/dl}$ ). While the three groups of mid-aged rats were not tested in the same experiment, it appeared that the performance of the variable, high corticosterone group (Experiment 3) was intermediate between the

Medium-B and High-B rats (with both groups maintaining stable levels of corticosterone). These findings may suggest that impairments in performance in the Morris maze are tightly regulated by both high and stable levels of glucocorticoids.

However, another important variable influencing these behavioral deficits appears to be duration of the treatment regimen. Indeed, while three months exposure to p.m. diurnal levels of corticosterone (Medium-B) had no effect upon performance in the swim maze, the comparable increases in endogenous corticosterone levels observed during the six-month social stress regimen produced impairments in this task. Moreover, these deficits were absent in stressed animals that had been adrenalectomized and given basal corticosterone replacement to maintain ACTH within a normal range. These findings suggest that the elevation of corticosterone during the intervention was a major contributing factor to the impairments observed in stressed animals. It is suggested that a longer duration of exposure to lower levels of corticosterone is necessary to observe these behavioral changes. Indeed, Landfield (1988) recommended that a minimum treatment duration of six months (i.e. 25-30% of the average lifespan) is necessary to observe subtle changes in biomarkers of aging. These considerations suggest that it is indeed reasonable to speak in terms of cumulative hormone exposure in relation to aging.

Why were performance deficits apparent in mid-aged but not young, corticosterone-treated rats? Based on the glucocorticoid cascade hypothesis (Sapolsky et al., 1986b), one would suggest that there is an absence of ongoing pathology in the younger animals. Therefore, elevated levels of corticosterone would have little effect upon hippocampal-dependent performance. In contrast, the prevalence of sub-clinical pathology is greater in older rats, and concurrent exposure to high glucocorticoid levels would exacerbate this pathology. While neuron loss was not apparent in these animals, we did observe impairments in behavior and LTP, suggesting that hippocampal dysfunction did occur. Furthermore, this age-related difference in the behavioral response to corticosterone may reflect differences in the capacity of recovery following insult or damage in younger versus older rats.

The concept of plasticity, or reactive synaptogenesis has been well-established within the adult CNS. Indeed, there are a variety of compensatory events that are invoked when neurons lose their normal synaptic input following the partial deafferentation of selected regions. Following unilateral damage of the entorhinal cortex, there is a degeneration of more than 90% of the normal synapses in the granule cells of the dentate gyrus (e.g. Steward and Vinsant, 1983). The compensatory mechanisms begin within four days following the lesion and involve the

"sprouting" or proliferation of presynaptic processes, restoration of spine density and length, and the formation of new synaptic contacts to replace those that have degenerated (Matthews, Cotman and Lynch, 1976; Steward and Vinsant, 1983). Although reactive synaptogenesis occurs in aged animals, the process occurs more slowly (Cotman and Scheff, 1979; Hoff et al., 1982) and less completely (Scheff, DeKosky and Cotman, 1980a; Hoff, Scheff, Benardo and Cotman, 1982) than in younger animals.

This reduced synaptogenesis appears to be dependent upon the high circulating levels of glucocorticoids in the aged rat. Indeed, young adrenalectomized rats replaced with the high p.m. levels of corticosterone observed in old animals showed a marked reduction in sprouting following entorhinal cortex lesions, and this effect was dose-dependent (Scheff et al., 1980a; Scheff and DeKosky, 1983). Scheff, DeKosky and Cotman, (1980b) found that hydrocortisone treatment prior to lesioning reduced axonal sprouting and increased astrocyte reactivity relative to untreated-lesioned controls.

Poirier, May, Osterburg, Geddes, Cotman and Finch (1989) reported an increased prevalence of several mRNAs within two week following entorhinal cortex lesions. The increase in glial fibrillary acidic protein (GFAP) mRNA was expected based upon the glial proliferation that accompanies neuronal damage. Moreover, its levels peaked approximately

two day following lesion, implicating GFAP in the early stage of neuronal degeneration and the loss of synapses (Poirier, Bacchichet and Dea, 1991). At a time point corresponding to the early phase of the formation of new synapses (i.e. six days post-lesion), there was an increase in the mRNA for apolipoprotein E (Poirier et al., 1991). Apolipoprotein E binds to a low density lipoprotein (LDL) receptor and facilitates in the scavenging of cholesterol from neuronal debris and its transport to surviving neurons (Mahley, 1988).

The potential mechanism by which glucocorticoids may modulate the synaptogenic response following neuronal damage was elucidated by Poirier, Bacchichet and Dea (1991). Following entorhinal cortex lesions there was an increase in the mRNA for both LDL receptors and GFAP in the hippocampus and this effect was prevented by the administration of corticosterone.

Together, these data suggest that in addition to regulating the extent of tissue damage, glucocorticoids may actively dampen the compensatory response of surviving neurons in the presence of neuropathology. Based upon these findings, one might hypothesize that while the degree of damage incurred in the young and mid-aged rats by a high level of corticosterone was equivalent, the capacity for functional recovery of synapses would be greater in the younger animals once the steroid had been removed.

Moreover, since the effects of glucocorticoids upon the degree of sprouting is dose-dependent (Scheff et al., 1980a; Scheff and DeKosky, 1983) the exposure to lower levels of glucocorticoids (e.g. Medium-B rats) would presumably result in greater recovery of function than in animals exposed to higher levels of corticosterone (e.g. High-B rats). Indeed, the absence of a performance deficit in the swim maze in the Medium-B group would support this hypothesis.

The behavioral deficit in the glucocorticoid-treated mid-aged rats of Experiments 3 and 4 could not be explained by hippocampal neuron loss. Neither the number nor the size of the pyramidal neurons of CA1 or CA3 region was affected by the corticosterone treatment. These findings were consistent with those described by Bardgett et al. (1992) in young Long-Evans animals.

The absence of any gross neuropathology offered a unique opportunity to explore further a potential mechanism underlying the learning deficit. While the suppressive effects of acute increases in corticosterone upon hippocampal synaptic plasticity have been documented (Bennett et al., 1991; Diamond et al., 1992), the consequences of long-term glucocorticoid administration (following the termination of the treatment) upon this measure had never been examined. In the experiment presented here, a three-month treatment in mid-aged rats with either p.m.-

like diurnal or stress-like levels of corticosterone significantly altered hippocampal electrophysiology compared with age-matched controls. More importantly, these effects were observed approximately three months following the termination of glucocorticoid treatment, when circulating levels of glucocorticoids did not differ between the groups.

There was a qualitative difference in the topography of the response between the two corticosterone-treated groups. While the Medium-B rats demonstrated an appropriate PTP response to tetanic stimulation (i.e. equivalent to Controls), the High-B rats exhibited a markedly-reduced PTP response. As outlined earlier, a diminished PTP response to tetanic stimulation does not prevent the induction of LTP (Humphreys et al., 1992). Thus, it is not necessary to postulate different mechanisms for the decrements in LTP observed in the Medium- and High-B animals.

What are the potential mechanisms that might explain the durable effects of glucocorticoids upon synaptic plasticity? Because plasma corticosterone levels at the time of the electrophysiological recording did not differ between the groups, the suppressive effects of acute elevations of glucocorticoid levels can be ruled out. Instead, it is conceivable that prolonged exposure to corticosterone initiates a chain of events that eventually result in the decreased excitability of hippocampal neurons.



Glucocorticoid exposure impairs glutamate reuptake (which is dependent upon sufficient energy sources) in the hippocampus (Virgin et al., 1991) by decreasing glucose transport in hippocampal neurons (Horner et al., 1990) and glial cells (Virgin et al., 1991). These increased levels of glutamate will result in the over-activation of the NMDA receptors, thereby creating a situation of increased influx of calcium ions. While the cytotoxic effects of excessive levels of intracellular calcium have been extensively reviewed by both Choi (1988) and Farber (1981), this explanation is unlikely in the absence of neuropathology reported in Experiments 3 and 4.

Another potential mechanism to explain the reduced plasticity observed in the Medium- and High-B rats may be a glucocorticoid-dependent increase in  $\text{Ca}^{2+}$  influx which would decrease hippocampal excitability by increasing the duration and amplitude of the calcium-dependent AHP (e.g. Joels and DeKloet, 1989; Kerr et al., 1989). Indeed, Kerr et al. (1992) reported that the type II glucocorticoid agonist, RU 28362 directly increased calcium conductance, which they suggest accounts for the increased AHP observed in the presence of elevated levels of glucocorticoids. Decreased hippocampal excitability would disrupt one of the physiological mechanisms (i.e. LTP) known to correlate well with some forms of learning.

While the effects of acute exposure to corticosterone upon hippocampal excitability are well-defined, the effects of prolonged exposure to elevated levels of corticosterone upon the AHP have not been examined directly. However, the issue can be addressed indirectly by the observation that the AHP is significantly increased in aged rats (Landfield and Pitler, 1984; Kerr et al., 1989). These authors suggested that this decrease in hippocampal excitability was the net result of an increased glucocorticoid signal (e.g. Issa et al., 1990; Meaney et al., 1988a) enhancing the influx of calcium (e.g. Kerr et al., 1992).

The importance of calcium homeostasis in aged animals has an additional functional implication unrelated to any permanent changes in morphology. Prevention of the influx of excessive extracellular calcium by the administration of the calcium channel blocker, Nimodipine, enhances the performance of aged rats in the 8-arm radial maze in a dose-dependent fashion (Levere and Walker, 1991). Moreover, the dose-response curve resembled an inverted-U, suggesting that there was a limited range of endogenous calcium required for good performance. Presumably, the blockade of too much or too little calcium impairs performance by influencing hippocampal excitability.

Can a phenomenon of enhanced glutamate and/or enhanced extracellular calcium be described in long-term glucocorticoid-treated rats,

several months following the termination of the treatment regimen?

Clearly, if one can demonstrate that several tetanic stimulations can induce long-lasting enhancement of the post-synaptic response when assessed several months later (Bliss and Gardner-Medwin, 1973), then it is possible to hypothesize that the prolonged exposure to glucocorticoids can also have effects upon glutamate, calcium conductance and hippocampal excitability several months later. Therefore, in future experiments it would be important to measure both extracellular levels and tissue content of glutamate and calcium of the Medium- and High-B-treated rats immediately after, and several months following, the termination of the treatment.

In summary, the information presented above suggests that both the acute administration of corticosterone (Diamond et al., 1992) and the long-term effects of glucocorticoids (Experiment 4) have similar effects upon hippocampal synaptic plasticity as assessed by PB potentiation. However, the potential mechanism(s) to explain the reduced potentiation observed following chronic glucocorticoid treatment have yet to be elucidated. While acute corticosterone has been demonstrated to effect the excitability of the hippocampus (Joels and DeKloet, 1989, 1990), the number of hippocampal glutamate receptors (Halpain and McEwen, 1988), hippocampal glucose transport (Horner et al., 1990; Virgin et al., 1991) and glutamate uptake (Virgin et al., 1991), any, all or even none of these factors may mediate the

diminution in long-term potentiation observed following long-term exposure to elevated levels of corticosterone.

### Conclusions

The original purpose of these experiments was to determine whether long-term corticosterone treatment in young animals had behavioral consequences. The study by Sapolsky et al. (1985) demonstrated that a three-months exposure to high physiological levels of corticosterone in F-344 rats, resulted in patterns of hippocampal neuron loss similar to that observed in aged rats. These findings were a crucial piece of evidence to support the correlations suggesting a possible relationship between HPA dysfunction and neuropathology in aged rats (e.g. Landfield et al., 1978b). However, the absence of hippocampal neuron loss in these and other experiments (e.g. Bardgett et al., 1992; Kerr et al., 1991) suggest that this conclusion is premature.

The only evidence to date to offer some support for a causal relationship between increased corticosterone levels and neuron loss was reported by Kerr et al. (1991) who reported that a six-month exposure to intermittent stress resulted in neuron loss in the CA1 subfield of the hippocampus, but only in old rats. Because the experimenters did not include a chronic stress group that had been adrenalectomized, the exact role of the glucocorticoids in the loss of neurons remains unclear.

While neuron loss was not apparent in either young or mid-aged rats exposed to chronic stress, Kerr et al. (1991) did report impairments in hippocampal electrophysiology in these animals. In the present experiments, we have behavioral evidence to support the hypothesis that exposure to chronic stress results in some hippocampal dysfunction. More importantly, these changes were absent in those stressed animals that had been adrenalectomized. Together, these findings implicate elevated levels of corticosterone in the manifesting the behavioral impairment associated with chronic stress.

The findings from Kerr et al. (1991) also suggest a "transition point" that occurs during the aging process whereby the glucocorticoid enhancement of calcium-induced suppression of hippocampal excitability becomes the glucocorticoid enhancement of calcium-induced neurotoxicity. Indeed, mid-aged corticosterone-treated or chronically-stressed rats exhibited impaired performance on a hippocampal-dependent task, but showed no signs of neuron loss. We hypothesized that these impairments were related to the effects of glucocorticoids upon hippocampal excitability and the AHP. Indeed, Kerr et al. (1991) reported that chronic stress impaired hippocampal electrophysiology in mid-aged rats, a finding which would explain our behavioral impairments.

Eldridge et al. (1989a) demonstrated that six months exposure to intermittent stress failed to down-regulate hippocampal glucocorticoid receptors in both mid-aged and aged rats. However, hippocampal cell counting (restricted to CA1) indicated that only the aged animals lost neurons (Kerr et al., 1991). These findings would suggest that those mechanism responsible for maintaining calcium homeostasis were still intact in the mid-aged rats and although the neurons are exposed to elevated levels of calcium (and thus the changes in hippocampal excitability), a stage has not been reached whereby cell death is observed.

The findings from the present experiments offer some support for the cascade hypothesis of stress and aging (Sapolsky et al., 1986b). Long-term exposure to elevated levels of glucocorticoids either by endogenous (i.e. chronic stress) or exogenous manipulations resulted in both behavioral and electrophysiological deficits similar to those observed in aged rats (e.g. Deupree et al., 1991; Issa et al., 1990; Meaney et al., 1988a; Moore et al., 1992). However, the absence of morphological changes in mid-aged, cognitively-impaired rats suggests that a brief exposure (in relation to the animal's lifespan) to elevated glucocorticoids in otherwise healthy animals is not a sufficient explanation of the relationship between adrenocortical dysfunction and neuropathology in aged rats. Thus, in the absence of ongoing, subclinical pathology, glucocorticoid exposure has no effect upon

neuron loss. However, hippocampal dysfunction is apparent along both the behavioral and electrophysiological domains.

### Future Directions

Can the exposure to elevated glucocorticoid levels for a relatively small percentage of the lifespan result in neuropathology in healthy aged animals? There is considerable evidence to suggest that adrenocortical activity and neuropathology may coexist in the aged rat (Issa et al., 1990; Landfield et al., 1978b; 1981; Meaney et al., 1988a). However, the only data to suggest that elevated levels of corticosterone (i.e. exposure to a chronic intermittent stressor) play a causal role in neuron loss was reported by Kerr et al. (1991). Although cell counting was restricted to the CA1 subfield of the hippocampus, these authors demonstrated that old animals exhibited neuron loss at the termination of the six-month stress regimen. Thus, it would be important to determine whether a short period of exposure (e.g. three months) to elevated glucocorticoid levels in otherwise healthy aged animals results in significant neuron loss in several subregions of the hippocampus and other brain regions that are affected in aging (e.g. the cerebral cortex, locus coeruleus and nucleus basalis of Meynert; see Coleman and Flood, 1987). Moreover, are there other morphological changes occurring including increases in glial clusters, astrocyte reactivity

and lipofuscin accumulation, (Landfield, Braun, Pitler, Lindsey and Lynch, 1981b).

Because this experiment requires the use of otherwise healthy aged animals, and due to the variance in HPA activity, cognition and neuropathology observed in aged rats (e.g. Issa et al., 1990), the study would be facilitated by the use of animals that were handled during the neonatal period. It has been consistently demonstrated that aged, handled animals exhibit similar basal (Meaney et al., 1988a, 1992) and stimulated HPA function (Meaney et al., 1988a), similar performance in the swim maze and hippocampal integrity relative to young animals (Meaney et al., 1988a). What are the effects of varying lengths of exposure to elevated levels of corticosterone upon performance in the swim maze, hippocampal synaptic plasticity and neuropathology? These animals would be compared with both aged-matched handled and non-handled animals.

Based upon the findings of Experiments 3 and 4 in this thesis, we would expect to observe impairments in both performance and synaptic plasticity in addition to significant hippocampal neuron loss. It is also likely that the behavioral deficits will be considerably greater than observed in the present experiments (e.g.  $> 2$  S.D. from controls, as is observed in aged, cognitively-impaired rats; Issa et al., 1990).



Issa et al. (1990) reported that HPA dysfunction in aged animals was associated with both impairments in the Morris water maze and hippocampal neuron loss. However, these findings do not resolve the issue of the role of glucocorticoid elevations in pathological aging. Does HPA dysfunction play a causal role in the development of age-related cognitive impairments and hippocampal pathology or do alterations in these three variables emerge at approximately the same time? One method of approaching this issue would be to propose a longitudinal, within-subjects design to determine whether elevations in HPA activity predict the onset of cognitive dysfunction and neuropathology later in life. Thus, measures of basal, stress and post-stress HPA activity would be taken throughout the lifespan. At two years of age, assessment of cognitive ability and neuropathology would be completed to determine the age when HPA dysfunction becomes apparent and whether these animals demonstrate signs of pathological aging. Further, these findings of these studies may have important implications for the possible therapies selected in perhaps preventing the onset of pathological aging (e.g. chemical adrenalectomy).

How do the effects of glucocorticoid administration upon the process of reactive synaptogenesis change as a function of age? It would be interesting to examine levels of mRNA for LDL and apolipoprotein E following the termination of a three-month treatment regimen with

glucocorticoids in young and aged rats. In the presence of high physiological levels of corticosterone, are the compensatory mechanisms still more effective in younger animals?

Another issue of interest to address directly, especially if differences in the process of new synapse formation becomes evident in aged, glucocorticoid-treated rats, is the mechanism by which long-term glucocorticoid exposure has long-lasting effects upon hippocampal synaptic plasticity. The issue of decreased hippocampal excitability via impaired calcium homeostasis was discussed earlier. In future experiments, it would be interesting to take advantage of both in vivo electrophysiology and in vivo dialysis techniques to examine changes in the behaving corticosterone-treated animal. First, one could implant chronic recording electrodes in the CA1 region of the hippocampus and examine whether enhancement in the post-synaptic response occurs during the learning of the Morris water maze (in the absence of tetanic stimulation). Second, does long-term exposure to glucocorticoids diminish this so-called behavioral LTP? Once again, the animals would be assessed several months following the termination of the treatment to determine whether glucocorticoid-induced learning impairments are associated with a diminished post-synaptic response. Third, how do levels of glutamate and other neurotransmitters such as serotonin and acetylcholine change as a function of the learning

process? Using either in vivo dialysis or in vivo electrochemistry, ongoing measurements of these variables can be assessed as the animal learns the task. How does long-term exposure to glucocorticoids influence these variables? Fourth, how are extracellular levels of calcium influenced by the long-term corticosterone treatment? Finally, how do the effects of corticosterone upon these variables change as a function of age?

While it would be naive to propose that pathological aging is mediated solely by elevated levels of glucocorticoids, it is apparent from the vast array of both in vivo and in vitro studies that corticosterone is an important regulator of many of the processes that become targeted during the aging process. Whether elevated levels of corticosterone prior to senescence play a causative role in pathological forms of human aging has yet to be determined.

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## Appendix A

### Radioimmunoassay Protocol for Corticosterone

The assay procedure involved pipetting 10  $\mu$ l of sample along with 1 ml of absolute ethanol (100%) into 12 x 75 mm borosilicate glass tubes (Fisher Scientific), vortexing for 2 minutes and centrifuging at 3000 rpm for 10 min, after which 100  $\mu$ l of the supernatant from each sample was pipetted in duplicate and dried in a freeze dryer (Labonco). 100  $\mu$ l of the extract from any sample was also taken, in duplicate, as an estimate of non-specific binding (NSB).

A standard curve was also prepared in duplicates of 100  $\mu$ l each corresponding to aliquots of the following concentrations of corticosterone: 10, 20, 50, 100, 200, 500 and 1000 pg in absolute ethanol. B0's were prepared by pipetting 100  $\mu$ l of absolute ethanol, in duplicate. These tubes of B0's were placed, in duplicate, before and after the standard curve points, and subsequently after every 30 samples. The standards, NSBs and B0's were all freeze-dried along with the samples.

The antisera was diluted to 40  $\mu$ l/ml of phosphate-buffered saline (PBS) gel and 100  $\mu$ l was aliquoted into all the tubes with the exception of the totals and NSBs. A 1:10 dilution was made for the tracer mixture after first taking 5  $\mu$ l of the tracer in 5 ml of PBS gel. This dilution was adjusted until counts per minute

(CPM) of between 12,500 and 14,000 per 100  $\mu$ l were obtained. A 100  $\mu$ l aliquot was pipetted into all the tubes. The totals (in duplicate) consisted only of the tracer in PBS gel. All the tubes were then sealed with parafilm and incubated overnight in a cold room (4°C).

The following day, 1 ml of dextran-coated charcoal was added into all the tubes with the exception of the totals, and vortexed to allow for the separation of the antibody from the free corticosterone. This procedure was carried out in the cold room and after a 10-12 minute waiting period, the tubes were centrifuged for 10 minutes at 3000 rpm in a refrigerated centrifuge. The supernatant was then poured and tapped into scintillation vials, after which, 5 ml of liquid scintillation cocktail (Liquiscint; National Diagnostics) was added. All the tubes were counted in a Packard beta scintillation counter.



## Appendix B

Complete Source Tables for the Analyses of Variance Presented in the Order  
Described in the Text.

Source Table for the ANOVA of the Mean Latency Data Over Days (Collapsed Across Blocks of Four Trials) in Young Long-Evans Rats Following a One-Month Cholesterol or Corticosterone Treatment Regimen.

Source of Variance	Degrees of Freedom	Sum of Squares	Mean Square	F	p
Treatment (T)	1	44.20	44.20	0.26	0.62
Error	18	3030.21	168.35		
Days (D)	2	10062.34	5031.17	21.80	$1 \times 10^{-4}$
T x D	2	223.32	111.66	0.40	0.62
Error	36	8299.47	230.54		

Source Table for the ANOVA of the Mean Distance Data Over Days (Collapsed Across Blocks of Four Trials) in Young Long-Evans Rats Following a One-Month Cholesterol or Corticosterone Treatment Regimen.

Source of Variance	Degrees of Freedom	Sum of Squares	Mean Square	F	p
Treatment (T)	1	3132.04	3132.04	0.04	0.85
Error	18	1617853.85	89880.777		
Days (D)	2	3626928.58	1813464.29	21.06	$1 \times 10^{-4}$
T x D	2	35703.01	17851.50	0.21	0.81
Error	36	3100577.33	86127.15		

Source Table for the ANOVA of the Mean Latency Data Over Days (Collapsed Across Blocks of Four Trials) in Young Long-Evans Rats Following a Three-Month Cholesterol or Corticosterone Treatment Regimen.

Source of Variance	Degrees of Freedom	Sum of Squares	Mean Square	F	p
Treatment (T)	1	14789.40	14789.40	23.32	$1 \times 10^{-4}$
Error	18	11414.64	634.15		
Days (D)	2	49147.23	24573.62	82.09	$1 \times 10^{-4}$
T x D	2	5533.81	2766.91	9.24	$6 \times 10^{-4}$
Error	36	10776.36	299.34		

Source Table for the ANOVA of the Mean Distance Data Over Days (Collapsed Across Blocks of Four Trials) in Young Long-Evans Rats Following a Three-Month Cholesterol or Corticosterone Treatment Regimen.

Source of Variance	Degrees of Freedom	Sum of Squares	Mean Square	F	p
Treatment (T)	1	1845032.70	1845032.70	20.10	$2 \times 10^{-4}$
Error	18	1586092.83	88116.27		
Days (D)	2	13155850.92	6577925.46	160.04	$1 \times 10^{-4}$
T x D	2	2293650.48	1146825.24	27.90	$1 \times 10^{-4}$
Error	36	1479704.65	41102.91		

Source Table for the ANOVA of the Mean Latency Data Over Days (Collapsed Across Blocks of Three Trials) in Young Long-Evans Following a Three-Month Vehicle or Corticosterone Injection Regimen.

Source of Variance	Degrees of Freedom	Sum of Squares	Mean Square	F	p
Treatment (T)	1	101.03	101.03	0.29	0.60
Error	18	6338.22	352.12		
Days (D)	3	19206.13	6402.05	37.99	$1 \times 10^{-4}$
T x D	3	175.89	58.63	0.35	0.79
Error	54	9099.35	168.51		

Source Table for the ANOVA of the Mean Distance Data Over Days (Collapsed Across Blocks of Three Trials) in Young Long-Evans Following a Three-Month, Vehicle or Corticosterone Injection Regimen.

Source of Variance	Degrees of Freedom	Sum of Squares	Mean Square	F	p
Treatment (T)	1	78720.13	78720.13	0.23	0.63
Error	18	6048260.41	336014.47		
Days (D)	3	19504454.65	6501484.89	32.38	$1 \times 10^{-4}$
T x D	3	182422.22	60830.74	0.30	0.82
Error	54	10842748.16	200791.63		

Source Table for the ANOVA of the Percentage of Time Spent in the Original Training Quadrant During a 30-Second Probe Trial Following a Three-Month Vehicle or Corticosterone Treatment Regimen in Young Long-Evans Rats.

Source of Variance	Degrees of Freedom	Sum of Squares	Mean Square	F	p
Treatment (T)	1	36.55	36.55	0.16	0.6
Error	18	4140.40	230.02		
Total	19	4176.95			



Source Table for the ANOVA of the Mean Latency Data Over Days (Collapsed  
Across Blocks of Two Trials) Following a One-Month Cholesterol or  
Corticosterone Treatment Regimen in Adult and Mid-Aged Long-Evans Rats.

Source of Variance	Degrees of Freedom	Sum of Squares	Mean Square	F	p
Age (A)	1	3078.34	3078.34	2.02	0.17
Treatment (T)	1	2541.66	2541.66	1.67	0.21
A x T	1	301.33	301.33	0.20	0.66
Error	29	44263.88	1526.34		
Days (D)	9	91261.45	10140.16	26.48	$1 \times 10^{-4}$
A x D	9	5867.22	631.91	1.65	0.10
T x D	9	3528.85	392.10	10.2	0.42
A x T x D	9	1450.05	161.12	0.42	0.92
Error	261	99966.79	383.02		

Source Table For the ANOVA of the Mean Latency to Locate the Platform on  
Trial 1 Following a One-Month Cholesterol or Corticosterone Treatment Regimen  
in Young and Mid-Aged Rats.

Source of Variance	Degrees of Freedom	Sum of Squares	Mean Square	F	p
Treatment (T)	3	2830.24	943.41	0.57	0.64
Error	29	47946.49	1653.33		
Total	32	50776.73			

Source Table for the ANOVA of the Mean Distance Data Over Days (Collapsed Across Blocks of Two Trials) Following a One-Month Cholesterol or Corticosterone Treatment Regimen in Adult and Mid-Aged Long-Evans Rats.

Source of Variance	Degrees of Freedom	Sum of Squares	Mean Square	F	p
Age (A)	1	451508.45	451508.45	0.38	0.54
Treatment (T)	1	2868178.62	2868178.62	2.04	0.13
A x T	1	611686.02	611686.12	0.51	0.48
Error	28	33488264.76	1196045.17		
Days (D)	9	84474216.83	9386024.09	31.76	$1 \times 10^{-4}$
A x D	9	3703085.25	411453.92	1.39	0.19
T x D	9	3549908.38	394434.26	1.34	0.27
A x T x D	9	873599.40	97066.60	0.33	0.97
Error	252	74479262.36	295552.63		

Source Table For the ANOVA of the Mean Distance to Locate the Platform on  
Trial 1 Following a One-Month Cholesterol or Corticosterone Treatment Regimen  
in Young and Mid-Aged Rats.

Source of Variance	Degrees of Freedom	Sum of Squares	Mean Square	F	p
Treatment (T)	3	140648.34	468828.45	0.3	0.83
Error	29	45375006.90	1564655.41		
Total	32	4678142.24			

Source Table for the ANOVA of the Mean Percentage of Time Spent in the  
Original Training Quadrant During a 30-Second Probe Trial Following a One-  
Month Cholesterol or Corticosterone Treatment Regimen in Adult and Mid-Aged  
Long-Evans Rats.

Source of Variance	Degrees of Freedom	Sum of Squares	Mean Square	F	p
Age (A)	1	43.98	43.98	1.65	0.21
Treatment (T)	1	7.20	7.20	0.27	0.61
A x T	1	0.35	0.35	0.01	0.91
Error	29	775.39	26.74		

Source Table for the ANOVA of the Mean Latency Data Over Days (Collapsed Across Blocks of Two Trials) Following a Three-Month Cholesterol or Corticosterone Treatment Regimen in Adult and Mid-Aged Long-Evans Rats.

Source of Variance	Degrees of Freedom	Sum of Squares	Mean Square	F	p
Age (A)	1	3564.63	3564.63	3.32	0.01
Treatment (T)	1	9013.66	9013.66	8.41	0.007
A x T	1	61.02	61.02	0.06	0.81
Error	30	32170.46	1072.35		
Days (D)	9	72365.10	8040.57	22.82	$1 \times 10^{-4}$
A x D	9	2617.15	290.79	0.83	0.59
T x D	9	1802.91	200.32	0.57	0.82
A x T x D	9	6195.29	688.37	1.95	0.04
Error	270	95118.36	352.29		

Source Table For the ANOVA of the Mean Latency to Locate the Platform on  
Trial 1 Following a Three-Month Cholesterol or Corticosterone Treatment  
Regimen in Young and Mid-Aged Rats.

Source of Variance	Degrees of Freedom	Sum of Squares	Mean Square	F	p
Treatment (T)	3	7456.98	2485.66	1.89	0.15
Error	30	39561.14	1318.70		
Total	33	47018.12			

Source Table for the ANOVA of the Mean Distance Data Over Days (Collapsed Across Blocks of Two Trials) Following a Three-Month Cholesterol or Corticosterone Treatment Regimen in Adult and Mid-Aged Long-Evans Rats.

Source of Variance	Degrees of Freedom	Sum of Squares	Mean Square	F	p
Age (A)	1	2508842.44	2508842.44	2.38	0.13
Treatment (T)	1	7219834.49	7219834.49	6.86	0.01
A x T	1	35031.36	35031.36	0.03	0.86
Error	30	31572201.41	1052406.71		
Days (D)	9	69938837.37	7437648.60	23.88	$1 \times 10^{-4}$
A x D	9	369585.99	410665.11	1.32	0.23
T x D	9	1885470.93	209496.77	0.67	0.73
A x T x D	9	5789702.96	643300.33	2.07	0.03
Error	270	84089019.26	311440.81		



Source Table For the ANOVA of the Mean Distance to Locate the Platform on  
Trial 1 Following a Three-Month Cholesterol or Corticosterone Treatment  
Regimen in Young and Mid-Aged Rats.

Source of Variance	Degrees of Freedom	Sum of Squares	Mean Square	F	p
Treatment (T)	3	5983758.58	1994586.19	1.56	0.22
Error	30	38290309.66	1276343.66		
Total	33	44274068.24			

Source Table for the ANOVA of the Percentage of Time Spent in the Original Training Quadrant During a 30-Second Probe Trial Following a Three-Month Cholesterol or Corticosterone Treatment Regimen in Adult and Mid-Aged Long-Evans Rats.

Source of Variance	Degrees of Freedom	Sum of Squares	Mean Square	F	p
Age (A)	1	21.09	21.09	1.01	0.32
Treatment (T)	1	7.39	7.39	0.35	0.56
A x T	1	33.24	33.24	1.59	0.22
Error	30	626.56	20.89		

Source Table for the ANOVA of the Mean Latency Data Over Days (Collapsed Across Blocks of Four Trials) in Untreated Adult Long-Evans Rats and Mid-Aged Cholesterol-Treated Controls

Source of Variance	Degrees of Freedom	Sum of Squares	Mean Square	F	p
Treatment (T)	1	1138.80	1138.80	1.04	0.33
Error	10	10946.12	1094.61		
Days (D)	9	50040.84	5560.09	13.42	$1 \times 10^{-4}$
T x D	9	2092.55	232.51	0.56	0.83
Error	90	37283.61	414.26		

Source Table for the ANOVA of the Mean Latency Data Over Days (Collapsed Across Blocks of Two Trials) Following a Three-Month Treatment Regimen in Mid-Aged Control, Medium-B and High-B Rats.

Source of Variance	Degrees of Freedom	Sum of Squares	Mean Square	F	p
Treatment (T)	2	26554.96	13277.48	5.97	0.006
Error	38	84550.07	2225.00		
Days (D)	9	168278.75	18697.64	38.02	$1 \times 10^{-4}$
T x D	18	4874.33	270.80	0.55	0.93
Error	342	168211.00	491.85		

Source Table For the ANOVA of the Mean Latency to Locate the Platform on  
Trial 1 Following a Three-Month Treatment Regimen in Mid-Aged Rats Controls,  
Medium-B and High-B Rats.

Source of Variance	Degrees of Freedom	Sum of Squares	Mean Square	F	p
Treatment (T)	2	7908.95	3954.47	2.42	0.10
Error	38	61033.83	1606.15		
Total	40	68942.78			

Source Table for the ANOVA of the Mean Distance Data Over Days (Collapsed Across Blocks of Two Trials) Following a Three-Month Treatment Regimen in Mid-Aged Control, Medium-B and High-B Rats.

Source of Variance	Degrees of Freedom	Sum of Squares	Mean Square	F	p
Treatment (T)	2	1097139.59	548569.79	4.37	0.02
Error	38	4772750.67	125598.70		
Days (D)	9	9484196.66	1053799.63	34.14	$1 \times 10^{-4}$
T x D	18	348461.64	19358.98	0.63	0.88
Error	342	10555497.58	30864.03		

Source Table For the ANOVA of the Mean Distance Travelled to Locate the Platform on Trial 1 Following a Three-Month Treatment Regimen in Mid-Aged Control, Medium-B and High-B Rats.

Source of Variance	Degrees of Freedom	Sum of Squares	Mean Square	F	p
Treatment (T)	2	288744.40	144372.20	1.66	0.20
Error	38	3303485.84	86933.84		
Total	40	3592230.24			

Source Table For the ANOVA of the Mean Percentage of Time Spent in The Original Training Quadrant During a 30-Second Probe Trial Following a Three-Month Treatment Regimen in Mid-Aged Control, Medium-B and High-B Rats.

Source of Variance	Degrees of Freedom	Sum of Squares	Mean Square	F	p
Treatment (T)	2	3094.76	1547.38	8.19	0.001
Error	38	7183.29	189.03		
Total	40	10278.05			



Source Table For the ANOVA of the Mean Latency to Begin Eating in the Open-Field in Control, Medium-B and High-B Mid-Aged Rats.

Source of Variance	Degrees of Freedom	Sum of Squares	Mean Square	F	p
Treatment (T)	2	24107.77	12053.89	1.56	0.22
Error	33	254448.79	7710.57		
Total	35	278556.56			

Source Table For the ANOVA of the Mean Baseline Population Spike Amplitude (mV) for the Electrophysiological Recording Following a Three-Month Treatment Regimen in Mid-Aged Controls, Medium-B and High-B Rats.

Source of Variance	Degrees of Freedom	Sum of Squares	Mean Square	F	p
Treatment (T)	2	0.56	0.28	1.15	0.33
Error	24	5.88	0.25		
Total	26	6.45			

Source Table For the ANOVA of the Mean Baseline Stimulus Intensity (mA) for  
the Electrophysiological Recording Following a Three-Month Treatment  
Regimen in Mid-Aged Controls, Medium-B and High-B Rats.

Source of Variance	Degrees of Freedom	Sum of Squares	Mean Square	F	p
Treatment (T)	2	3919.13	1959.56	1.5	0.24
Error	24	31286.89	1303.62		
Total	26	35206.02			

Source Table for the ANOVA of the Enhancement of the Population Spike  
Amplitude Versus Baseline Following PB Stimulation Following a Three-Month  
Corticosterone Treatment Regimen in Mid-Aged Controls, Medium-B and High-B  
Rats.

Source of Variance	Degrees of Freedom	Sum of Squares	Mean Square	F	p
Treatment (T)	2	63237.62	31618.81	6.99	0.004
Error	24	108493.04	4520.54		
time (t)	9	216735.12	24081.68	31.30	$1 \times 10^{-4}$
T x t	18	65846.01	3658.11	4.75	$1 \times 10^{-4}$
Error	216	166187.83	769.39		

Source Table For the ANOVA of the Mean Percent Increase in Population Spike Amplitude, Measured One-Minute Following PB Stimulation (PTP) Following a Three-Month Treatment Regimen in Mid-Aged Controls, Medium-B and High-B Rats.

Source of Variance	Degrees of Freedom	Sum of Squares	Mean Square	F	p
Treatment (T)	2	110651.84	55325.92	4.45	0.02
Error	24	298624.89	12442.70		
Total	26	409276.74			

Source Table For the ANOVA of the Plasma Corticosterone Levels Assessed  
Following the Electrophysiological Recording in Mid-Aged Control, Medium-B  
and High-B Animals.

Source of Variance	Degrees of Freedom	Sum of Squares	Mean Square	F	p
Treatment (T)	2	173.76	86.88	0.90	0.41
Error	23	2229.36	96.93		
Total	25	2403.12			

Source Table For the ANOVA of the Mean Neuron Density (per 0.1mm<sup>2</sup>) in CA<sub>1</sub>  
Following a Three-Month Treatment Regimen from Mid-Aged Control, Medium-  
B and High-B Rats.

Source of Variance	Degrees of Freedom	Sum of Squares	Mean Square	F	p
Treatment (T)	2	14.33	7.17	0.60	0.56
Error	12	142.92	11.9		
Total	14	157.2495			

Source Table For the ANOVA of the Mean Neuron Density (per 0.1mm<sup>2</sup>) in CA<sub>3</sub>  
Following a Three-Month Treatment Regimen from Mid-Aged Control, Medium-  
B and High-B Rats.

Source of Variance	Degrees of Freedom	Sum of Squares	Mean Square	F	p
Treatment (T)	2	15.20	7.60	2.21	0.15
Error	12	41.23	3.44		
Total	14	56.43			



Source Table For the ANOVA of the Mean Cell Size ( $\mu\text{m}$ ) in CA<sub>1</sub> Following a  
Three-Month Treatment Regimen in Mid-Aged Control, Medium-B and High-B  
Rats.

Source of Variance	Degrees of Freedom	Sum of Squares	Mean Square	F	p
Treatment (T)	2	0.76	0.38	0.20	0.81
Error	12	22.4	1.87		
Total	14	23.25			

Source Table For the ANOVA of the Mean Cell Size ( $\mu\text{m}$ ) in CA3 Following a  
Three-Month Cholesterol or Corticosterone Treatment Regimen in Mid-Aged  
Control, Medium-B and High-B Rats

Source of Variance	Degrees of Freedom	Sum of Squares	Mean Square	F	p
Treatment (T)	2	0.03	0.01	.01	0.9
Error	12	22.72	1.98		
Total	14	22.75			

Source Table for the ANOVA of the Corticosterone Levels Mid-Aged Controls,  
Social Stress and Adrenalectomy + Social Stress Rat. Over Six Sampling Points  
During the Diurnal Cycle.

Source of Variance	Degrees of Freedom	Sum of Squares	Mean Square	F	p
Groups (G)	2	1956.64	978.32	38.52	$1 \times 10^{-4}$
Error	19	482.52	25.40		
Hour (H)	5	1067.95	213.59	12.97	$1 \times 10^{-4}$
G x H	10	256.23	25.62	1.55	0.13
Error	95	1569.66	16.5		

Source Table for the ANOVA of the Mean Latency Data Collapsed Across Days  
In Mid-Aged Controls, Social Stressed and Adrenalectomy + Social Stressed Rats.

Source of Variance	Degrees of Freedom	Sum of Squares	Mean Square	F	p
Group (G)	2	5217.16	2608.88	2.52	0.10
Error	23	23841.92	1036.61		
Days (D)	9	111722.05	12413.56	40.37	$1 \times 10^{-4}$
G x D	18	14252.24	791.79	2.58	$7 \times 10^{-4}$
Error	207	63654.04	307.51		

Source Table For the ANOVA of the Mean Latency (sec) to Locate the Platform  
on Trial 1 in Mid Aged Controls, Stressed and Adrenalectomy + Stressed Rats.

Source of Variance	Degrees of Freedom	Sum of Squares	Mean Square	F	p
Group (G)	2	5369.80	2684.90	1.7	0.20
Error	23	36324.05	1579.31		
Total	25	41693.85			

Source Table for the ANOVA of the Mean Distance Data Collapsed Across Days  
In Mid-Aged Controls, Social Stressed and Adrenalectomy + Social Stressed Rats.

Source of Variance	Degrees of Freedom	Sum of Squares	Mean Square	F	p
Group (G)	2	369661.80	184830.89	1.94	0.17
Error	23	2186083.73	95047.12		
Days (D)	9	10811038.36	1201226.5	32.36	$1 \times 10^{-4}$
G x D	18	1061005.66	58944.76	1.59	0.06
Error	207	7684121.65	37121.36		

Source Table For the ANOVA of the Mean Distance to Locate the Platform on  
Trial 1 in Mid-Aged Controls, Stressed and Adrenalectomy + Stressed Rats.

Source of Variance	Degrees of Freedom	Sum of Squares	Mean Square	F	p
Group (G)	2	836912.25	418456.13	1.86	0.20
Error	14	3144771.87	224626.56		
Total	16	3981684.12			

Source Table for the ANOVA of the Latency Data Over Days (Collapsed Across Blocks of Two Trials) Following a Three-Month Cholesterol or Corticosterone Treatment Regimen in Young Fischer-344 and Long-Evans Rats.

Source of Variance	Degrees of Freedom	Sum of Squares	Mean Square	F	p
Strain (S)	1	4437.20	4437.20	9.33	0.005
Treatment (T)	1	272.18	272.18	0.57	0.46
S x T	1	4442.63	4442.63	9.34	0.005
Error	27	12838.16	475.49		
Days (D)	7	142330.23	20332.89	49.05	$1 \times 10^{-4}$
S x D	7	6527.49	932.50	2.25	0.03
T x D	7	1017.28	145.33	0.35	0.93
S x T x D	7	10766.12	1538.02	3.71	0.009
Error	189	78345.00	414.25		



Source Table For the ANOVA of the Mean Latency (sec) to Locate the Platform  
on Trial 1 Following a Three-Month Treatment Regimen in Long-Evans and  
Fischer-344 Rats.

Source of Variance	Degrees of Freedom	Sum of Squares	Mean Square	F	p
Group (G)	3	5757.27	1919.09	1.42	0.26
Error	27	36388.60	1347.73		
Total	30	42145.87			

Source Table for the ANOVA of the Percentage of Time Spent in the Original Training Quadrant During a 30-Second Probe Trial Following a Three-Month Cholesterol or Corticosterone Treatment Regimen in Young Fischer-344 and Long-Evans Rats.

Source of Variance	Degrees of Freedom	Sum of Squares	Mean Square	F	p
Strain (S)	1	334.83	334.83	1.30	0.26
Treatment (T)	1	126.94	126.94	0.49	0.49
S x T	1	886.16	886.16	3.45	0.1
Error	27	6935.82	256.88		