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**The Effects of Early Postnatal Handling on Stressor-Induced Neurochemical
and Corticosterone Alterations and on Morris Water-Maze Acquisition in
BALB/cByJ and C57BL/6ByJ Mice**

**A thesis presented to the Faculty of Graduate Studies and Research of
Carleton University in partial fulfillment for the degree of Master of Science
with Neuroscience Specialization**

**Marilee D. Zaharia
Department of Psychology
Carleton University**

September, 1994

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Corticosterone Alterations and on Morris Water-Maze Acquisition in BALB/cByJ
and C57BL/6ByJ Mice**

**in partial fulfilment of the requirements
for the degree of Master of Science**

Thesis Supervisor

Chair, Department of Psychology

Carleton University

September 22, 1994

Abstract

Marked differences exist across strains of mice with respect to the neurochemical, endocrine and behavioral responses to stressors. Earlier studies in this laboratory revealed that in comparison to other mouse strains (e.g. C57BL/6ByJ) BALB/cByJ mice are much more reactive to stressors, exhibiting hypersecretion of corticosterone and marked brain catecholamine alterations. Moreover, relative to other strains, BALB/cByJ mice also display behavioral impairments in several paradigms, and these effects are more accentuated following stressor exposure. We have recently observed that BALB/cByJ mice fail to acquire a spatial learning response in a Morris water-maze paradigm, a pattern of behavior which has been shown to be correlated with hippocampal cell loss, as well as with aging. Moreover, it is thought that this cell loss may be a consequence of excessive activation of hippocampal glucocorticoid receptors. Given that the BALB/cByJ mouse displays high reactivity to stressors coupled with glucocorticoid hypersecretion, it was of interest to determine whether this performance deficit (a) became progressively more pronounced with aging, (b) paralleled the effects of hippocampal damage wherein the deficits were not apparent when visual cues were present, and (c) like that associated with aging in rats, could be modifiable by early-life stimulation, such as early-life handling of mouse pups. It was observed that among BALB/cByJ mice, the performance deficit, which was evident in mice as early as 30 days of age, did not exist when intramaze cues were present. Daily handling (handling of pups with brief separation periods from the dam over the course of 21 days postpartum) among BALB/cByJ, but not C57BL/6ByJ mice prevented these performance deficits. Furthermore, nonhandled BALB/cByJ mice cross-fostered with a C57BL/6ByJ dam for 21 days also prevented this spatial learning deficit, suggesting that early-life handling and cross-fostering may have involved similar mechanisms. Additionally,

early-life handling was found to limit the excessive glucocorticoid secretion and hippocampal MHPG variations ordinarily induced by stressors in BALB/cByJ mice. The possibility was raised that the BALB/cByJ mice, owing to their hyperreactive response to stressors, may be useful in modeling behaviors associated with hippocampal disturbances.

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- Figure 28b: Mean (+/-SEM) plasma ACTH concentrations (pg/ml) in handled and nonhandled BALB/cByJ and C57BL/6ByJ mice immediately following exposure to either 360 footshocks or no treatment

The Effects of Early Postnatal Handling on Stressor-Induced Neurochemical and Corticosterone Alterations and on Morris Water Maze Acquisition in BALB/cByJ and C57BL/6ByJ Mice

Numerous investigations have demonstrated a relationship between glucocorticoids and hippocampal functioning, as well as hippocampal-related influences on cognitive processes, including spatial learning and memory (Jacobson & Sapolsky, 1991; Kloet, 1991; Meaney, Aitken, Berkel, Bhatnager, & Sapolsky, 1988; Sapolsky, 1992). The hippocampus, a neural target for circulating glucocorticoids (mainly corticosterone in animals), contains high concentrations of endogenous mineralcorticoid and glucocorticoid receptors, as well as high glucocorticoid receptor binding capacity (Reul & Kloet, 1985). It appears that the hippocampus plays a major role in the regulating hypothalamic-pituitary-adrenal (HPA) functioning. In particular, it has been shown that the release of corticotropin releasing factor (CRF) from the paraventricular nucleus of the hypothalamus gives rise to pituitary adrenocorticotropin hormone (ACTH) secretion, which in turn, provokes release of adrenal glucocorticoids. Circulating glucocorticoids stimulate type I and type II corticoid receptors on the hippocampus, which result in the inhibition of further CRF release (Checkley, 1992; Dantzer & Kelley, 1989; Jacobson & Sapolsky, 1991; Sapolsky, Krey, & McEwen, 1984a). However, excessive stimulation of hippocampal corticoid receptors may culminate in hippocampal cell loss. It has been suggested that factors such as stressor-induced corticosterone hypersecretion may be instrumental in provoking the cell loss and the consequent behavioral disturbances (Jacobson & Sapolsky, 1991; Sapolsky, 1987; Sapolsky, Krey, & McEwen, 1984b; Sapolsky, Krey, & McEwen, 1985; Sapolsky, Krey, & McEwen, 1986).

It has been shown that basal levels of glucocorticoids, tend to rise progressively with age (Sapolsky, Krey, & McEwen, 1983a). It is thought that aging is associated with the loss of hippocampal cells, thereby reducing the inhibitory influence on corticosteroid release. The resultant increases of corticoid secretion associated with receptor down-regulation favors further hippocampal cell loss and hence, exacerbation of glucocorticoid secretion. In particular, aged rats show a loss of hippocampal pyramidal neurons within the CA1 and CA3 layers of the hippocampus (Landfield, 1987; Landfield, Baskin, & Pitler, 1981; Landfield, Waymire, & Lynch, 1978; Sapolsky, et al., 1984b) and a decrease in hippocampal type II glucocorticoid receptors (Sapolsky, Krey, & McEwen, 1983b; Sapolsky, et al., 1984a). Moreover, it has been demonstrated that aging-induced corticosterone hypersecretion is positively correlated with hippocampal damage in aging animals (Landfield, et al., 1978). Conversely, the age-related cell loss resulting from glucocorticoid over-exposure can be prevented by an adrenalectomy performed during mid-life (Landfield, et al., 1978; Sapolsky, et al., 1983a). Interestingly, the behavioral deficits associated with hippocampal dysfunction, such as deficits of spatial learning and memory (e.g. in the Morris water-maze task) are also evident in aged rats that exhibit the most pronounced hippocampal cell loss (Landfield, 1987; Landfield, et al., 1981; Landfield, et al., 1978; Sapolsky, et al., 1985). Thus, the proposition was advanced that sustained glucocorticoid release may be instrumental in provoking age-related behavioral disturbances that involve the hippocampus (Sapolsky, 1987; Sapolsky, 1992; Sapolsky, et al., 1986).

Genetic factors may influence hippocampal functioning, glucocorticoid-induced hippocampal degeneration, and the behavioral disruptions due to altered hippocampal functioning. It has been demonstrated that relative to other strains of mice, the BALB/cByJ strain shows hypersecretion of corticosterone following

acute or chronic footshock (Shanks, Griffiths, Zalcmann, & Zacharko, 1990), as well as disturbances in several behavioral paradigms (Shanks & Anisman, 1993). In addition, the BALB/cByJ strain shows significant deficits in Morris water-maze escape performance wherein a significant proportion of subjects fail to acquire the response entirely (Francis, Zaharia, Shanks, & Anisman, 1994; Upchurch & Wehner, 1988). Indeed, the observed Morris water-maze performance deficit was not uniform across individuals within the BALB/cByJ strain. The deficits occurred in approximately 8 out of 10 animals (Francis, Zaharia, Shanks, & Anisman, 1994). In contrast, the C57BL/6J and C57BL/6ByJ strains did not show stress-induced corticosterone hypersecretion, or deficits in shuttle-escape performance (Shanks & Anisman, 1993; Shanks, et al., 1990) and displayed Morris water-maze acquisition, retention and task-reversal learning superior to that of BALB/cByJ mice (Francis, Shanks, & Anisman, 1991; Francis, et al., 1994).

In addition to genetic factors, early-life experiences may influence glucocorticoid degenerative processes and hippocampal-related spatial learning and memory, as well as hippocampal physiology and functioning. Early environmental stimulation such as light-rearing versus dark-rearing (Tees, Buhrmann, & Hanley, 1990), postnatal handling coupled with short periods of maternal separation attenuates the aforementioned physiological and cognitive deficits associated with glucocorticoid-induced hippocampal neurodegeneration (Meaney, et al., 1988; Meaney, Aitken, Bodnoff, Iny, & Sapolsky, 1985a; Meaney, Aitken, Bodnoff, Iny, Tatarewicz, & Sapolsky, 1985b). Throughout aging, handled animals had significantly more type II glucocorticoid receptors in the hippocampus and frontal cortex (Meaney, et al., 1988; Meaney, Mitchell, Aitken, Bhatnagar, Bodnoff, Iny, et al., 1991a; Sarrieau, Sharma, & Meaney, 1988) and showed less cell loss within the CA1 and CA3 fields of the hippocampus (Meaney, et al., 1988). Similarly, handled animals exhibited shorter latencies in

escape performance in the Morris water-maze (reflected by shorter swimming distances) in comparison to nonhandled animals (Meaney, et al., 1988; Meaney, et al., 1991a).

It is thought that early-life handling moderates the corticosterone response to stressors, and that this effect involves alterations in hippocampal glucocorticoid type II receptor binding (Meaney, et al., 1985b; Meaney, Aitken, Sharma, Viau, & Sarriau, 1989). It has been demonstrated that handled and nonhandled animals do not differ in their basal levels of corticosterone during any point in the diurnal cycle. However, in adulthood, animals handled for the first 21 days of life secrete less corticosterone and ACTH in response to various types of stressors and exhibit a faster return to basal corticosterone levels following stressor termination than nonhandled animals (Meaney & Aitken, 1985; Meaney, et al., 1988; Meaney, et al., 1985a; Meaney, et al., 1989; Meaney, et al., 1991a). Inasmuch as handling increases type II glucocorticoid receptors in the frontal cortex and hippocampus, the differences in stress-induced corticosterone and ACTH secretion are thought to represent a handling-induced enhancement in the sensitivity of glucocorticoid negative-feedback mechanisms (Meaney, Viau, Bhatnagar, Betito, Iny, O'Donnell, et al., 1991c). This increase in negative-feedback sensitivity may be mediated by serotonin-induced increases in cellular cyclic adenosine monophosphate (cAMP) levels (Meaney, et al., 1991a; Meaney, et al., 1991c).

The BALB/cByJ strain of mouse, it will be recollected, exhibits hypersecretion of corticosterone in response to stressors, and also fails to acquire the escape response in the Morris water-maze task. Thus, the possibility exists that this strain is exhibiting a performance disturbance relatively early in life, which is comparable to that seen in aged animals of other strains. Specifically, owing to the corticosterone hypersecretion in response to stressors (Shanks, et al.,

1990), BALB/cByJ mice may be vulnerable to early degeneration of hippocampal cells and type II glucocorticoid receptors, hence promoting the impaired Morris water-maze performance.

The focus of the present investigation will be to determine whether a) the impaired performance of BALB/cByJ mice is influenced by early-life stimulation, just as this manipulation influences the behavioral disturbances associated with aging, b) such an effect is related to maternal factors, and c) the effects of early-life handling are accompanied by changes in stressor-induced central amine and corticosterone release.

HPA Consequences of Early Life Stimulation

Early environmental stimulation exerts powerful effects on neuroendocrine functioning and behavior. (Meaney, et al., 1989; Meaney, et al., 1991a). These effects appear to be operative throughout the aging process, since they are still apparent in animals at 24-26 months of age. (Meaney, et al., 1988; Meaney, Aitken, Bhatnager, & Sapolsky, 1991).

Differences in stress-induced corticosterone secretion in handled versus nonhandled animals are not a function of differences in free corticosterone or corticosterone-binding globulin (CBG) levels (Meaney, Aitken, Sharma, & Viau, 1992; Meaney, et al., 1989; Meaney, et al., 1991c). Corticosterone-binding globulin is the principle binder for corticosterone within plasma, and has been proposed to enhance delivery of corticosterone to target tissues, assist in transporting corticosterone through cell membranes, and to compete in intracellular corticosterone sequestering in cells (Kloet, Voorhuis, Leunissen, & Koch, 1984; Koenig, Gudelsky, & Meltzer, 1987; Pardridge, 1987). Likewise, the differences cannot be attributed to cellular clearance of corticosterone or CBG (Grota, 1975; Meaney, et al., 1989). Moreover, the differences in corticosterone secretion are stress-related as no differences are observed during basal conditions

(Meaney, et al., 1992; Meaney, et al., 1989; Meaney, et al., 1991c). Inasmuch as the effects of handling on CRF and ACTH secretion in response to stressors parallel the aforementioned differences in stress-induced corticosterone, it was suggested that the effects of handling on HPA activity arise from mechanisms upstream from the pituitary (Meaney, et al., 1991c).

Postnatal handling has been demonstrated to affect stress-induced glucocorticoid negative feedback involving the hippocampus and pituitary gland. In particular, animals which experience 21 days of postnatal handling with brief periods of maternal separation have higher sensitivity to the negative-feedback effects of circulating glucocorticoids on HPA activity (Meaney, et al., 1985a). It has been shown that handled animals which received either corticosterone or dexamethasone prior to restraint stress exhibited corticosterone hypersecretion for 2 hours post-stress relative to nonhandled animals. Additionally, handled animals that received exogenous glucocorticoids showed down-regulation of hippocampal type II receptor binding to levels indistinguishable from that of nonhandled animals. Moreover, the dosage of exogenous corticosterone or dexamethasone required for suppression of stress-induced HPA activity was reported to be between 5 and 10 times lower in the handled animals (Meaney, et al., 1989). On the basis of these findings, it was suggested that handling differences in glucocorticoid negative-feedback efficacy is related to differences in hippocampal type II binding capacity (Meaney, et al., 1989; Meaney, et al., 1991a; Meaney, et al., 1991c)

The handling-induced aberrations in negative-feedback sensitivity to glucocorticoids is attributed to the increased density of type II glucocorticoid receptors within the hippocampus but not in the septum, amygdala, hypothalamus or the pituitary (Meaney, et al., 1988; Meaney, et al., 1985a; Meaney, et al., 1985b). Moreover, it seems that the increased number of receptor sites, rather

than changes in receptor affinity, was critical for the handling effects on HPA functioning (Meaney, et al., 1991c). In particular, it was demonstrated that chronic postnatal corticosterone administration antagonized the handling-induced increase in hippocampal glucocorticoid binding capacity by reducing receptor concentrations. As a result, the handling differences in post-stressor corticosterone concentrations were eliminated (Meaney, et al., 1991c). It was suggested that the increases in hippocampal type II receptor density enhances the sensitivity to circulating glucocorticoids. This enhanced sensitivity allows the hippocampus to inhibit HPA activity through corticosterone negative feedback much more effectively. Subsequently, a reduction in post-stress secretion of ACTH and corticosterone is observed in handled animals (Meaney, et al., 1991a; Meaney, et al., 1991c).

The effect of handling on HPA functioning are not limited to variations of glucocorticoid negative-feedback mechanisms. Handling may influence transcortin receptors thereby affecting inhibition of stress-induced HPA activity. The transcortin receptor is a soluble receptor physically similar to plasma CBG. Like CBG, the transcortin receptor has a high affinity to corticosterone and acts as a buffer by reducing corticosterone binding to glucocorticoid receptors. The neonatal rat has very low levels of transcortin which exposes the neonate to increased corticosterone uptake in cell nuclei, consequently resulting in an enhancement of glucocorticoid feedback on the pituitary release of ACTH (Sapolsky, Meaney, & McEwen, 1985; Walker, Sapolsky, Meaney, Vale, & Rivier, 1986). It has been suggested that this neonatal enhancement in glucocorticoid feedback on pituitary ACTH may account for the reduced ability of the neonatal animal to secrete ACTH in response to stress (Meaney, et al., 1991c; Sapolsky & Meaney, 1986; Sapolsky, et al., 1985). As animals age, transcortin receptor levels

increase, which reduces the negative-feedback effect on ACTH release (Meaney, et al., 1991c).

It has been proposed that changes in pituitary transcortin receptor levels may be related to handling differences in HPA activity during stress. However, such an effect may be dependent upon the gender of the animal. Handling in male and female rats increases hippocampal type II glucocorticoid receptor binding and reduces post-stress corticosterone levels in comparison to nonhandled animals (Meaney, et al., 1991). However, it has been repeatedly shown that among females, the effects of handling on stress-induced corticosterone levels are not evident (Meaney, et al., 1985a; Meaney, et al., 1985b; Meaney, et al., 1992). It has been suggested that handled male rats, owing to reduced pituitary transcortin levels, have increased pituitary corticosterone uptake during the neonatal period. Likely, these animals are more sensitive to corticosterone elevations and have more efficient glucocorticoid feedback mechanisms operating on pituitary ACTH release, culminating in reduced pituitary secretion of ACTH during stress (Meaney, et al., 1991c). In accordance with this hypothesis, handled female rats do not have reduced pituitary transcortin receptor levels during the neonatal period and do not differ in HPA activity in response to stress during adulthood (Meaney, et al., 1991; Meaney, et al., 1992; Meaney, et al., 1991c).

An alternative mechanism to account for differences in stress-induced HPA activity as a function of handling has recently been proposed. It was suggested that handling differences in post-stress HPA activity may occur independently of circulating levels of corticosterone. For example, it has been shown that stress induces the release of CRF and arginine vasopressin (AVP) from the median eminence of the hypothalamus (Kloet, 1991). Additionally, postnatal handling can affect CRF and AVP levels in the median eminence (Plotsky & Meaney, 1993; Viau, Plotsky, & Meaney, 1993). In comparison to animals exposed to 3 hours of

daily maternal separation or nonhandling, handled animals have significantly lower concentrations of CRF mRNA in the median eminence (Plotsky & Meaney, 1993). It was argued that handling-induced changes in median eminence levels of CRF and arginine vasopressin (AVP) may be responsible for the effects on stress-induced HPA activity (Viau, et al., 1993). For instance, it has been known for some time that if adrenalectomized (ADX) animals are provided with low levels of corticosterone in their drinking water, the termination of their subsequent ACTH stress responses are as efficient as in intact animals (Jacobson, Akana, Cascio, Shinsako, & Dallman, 1988). Viau et al. (1993) demonstrated that although handled and nonhandled ADX animals do not differ in stress-induced ACTH responses, very low levels of corticosterone replacement are sufficient to restore handling differences in stress ACTH responses. Inasmuch as, nonhandled animals were shown to have significantly higher basal levels of CRF and AVP in the median eminence, the differential effects of stressors in handled and nonhandled rats may have involved release of hypothalamic CRF and/or AVP (Viau, et al., 1993).

It is clear that handling differences in stress-induced HPA activity are related to differences in circulating corticosteroids and enhanced development of hippocampal type II glucocorticoid receptors. The type II receptor has been implicated in mediating hippocampal inhibition of HPA activity during stress conditions. In particular, under basal conditions only 10 to 15 percent of the type II receptor sites are occupied, while 80 to 90 percent of the type I receptor sites are occupied. It seems that the increased release of corticosterone under stressor conditions results in hippocampal type II receptor binding which acts to provoke glucocorticoid negative feedback effects on HPA activity (Reul, Bosch, & Kloet, 1987; Reul & Kloet, 1985). Moreover, the increased efficiency of negative-feedback effects and subsequent inhibition of stress-induced ACTH secretion in

handled animals is attributed to increased type II corticosteroid receptor density in the hippocampus and the frontal cortex (Meaney, et al., 1985a; Meaney, et al., 1985b). With greater numbers of type II receptors in these brain regions, handled animals are able to respond more quickly and efficiently to stress-induced corticosterone elevations and ACTH release (Meaney, et al., 1989).

The mechanisms(s) responsible for alterations in hippocampal type II receptor development associated with handling have not been elucidated fully. It has been proposed that hormonal changes coinciding with the handling procedure initiates serotonin (5-HT) mediated cellular events (Meaney, Mitchell, Betito, Bhatnager, Iny, Viau, et al., 1991b; Meaney, et al., 1991c; Mitchell, Betito, Rowe, Boksa, & Meaney, 1992; Mitchell, Iny, & Meaney, 1990a). The hippocampus receives serotonergic innervation from the median raphe nucleus (Azmitia & Segal, 1978), which interacts with corticosterone-sensitive hippocampal cells (Azmitia & Segal, 1984). Serotonin may have direct effects on hippocampal cells and affect type II receptor concentrations (Meaney, et al., 1991a; Meaney, et al., 1991b; Mitchell, et al., 1990a). Indeed, it has been demonstrated *in vitro*, that type II receptor binding in rat hippocampal cells is increased in response to chronic exposure to 5-HT and this effect is specific to neurons, not glial cells (Mitchell, Rowe, Bokfa, & Meaney, 1990b).

Hippocampal 5-HT concentrations in neonatal rat pups peak on day 14 (Mitchell, et al., 1990a), corresponding to the rise hippocampal type II receptor binding capacity during the first two weeks of life (Meaney, et al., 1991a; Rosenfield, Sutanto, Levine, & Kloet, 1988). Early life administration of 5,7-DHT, a 5-HT neurotoxin, decreases the binding capacity of hippocampal type II receptors (Mitchell, et al., 1990a). Likewise, administration of the 5-HT₂ antagonist, ketanserin, antagonizes the enhanced binding capacity of type II receptors induced by handling (Mitchell, et al., 1990a). Moreover, *in vitro* studies

have shown the 5-HT increases type II receptor binding in hippocampal cells, and this effect is reversed by ketanserin, and mimicked by a number of 5-HT₂ receptor agonists (Mitchell, et al., 1990b).

Serotonergic mediation of postnatal handling effects on type II receptor affinity has been suggested to result from hypothalamic-pituitary-thyroid axis activation during the handling procedure. Mild, transient drops in handled pups' body temperature likely involves increases in circulating thyroxine (T₄) and intracellular levels in triiodothyronine (T₃). Postnatal handling increases in plasma T₃ and T₄ hormones is hypothesized to induce alterations in type II receptor density and binding (Meaney, Aitken, & Sapolsky, 1987). It has been demonstrated that either T₃ or T₄ treatment during the first week of life results in dramatic increases in glucocorticoid type II receptor binding during adulthood (Meaney, et al., 1987; Meaney, et al., 1991b; Meaney, et al., 1991c). Moreover, postnatal administration for two weeks of the thyroid hormone synthesis inhibitor, propylthiouracil (PTU), blocks the handling effect on type II glucocorticoid receptor binding (Meaney, et al., 1987). It has been suggested that because thyroid hormones do not affect embryonic hippocampal type II receptor density *in vitro*, thyroid effects on type II receptor density likely occurs at a site distal to hippocampal cells (Meaney, et al., 1991c).

It is likely that both 5-HT and thyroid hormone activity contribute to the handling effects on type II receptor development. Thyroid hormones are known to regulate serotonergic neurons and increase 5-HT turnover in the hippocampal cells of neonatal rats (Mitchell, et al., 1990a). As previously discussed, both handling and thyroid hormones affect serotonergic hippocampal neuron stimulation (Mitchell, et al., 1992; Mitchell, et al., 1990a). Moreover, *in vitro* studies have demonstrated a 5-HT effect on hippocampal type II receptor binding which appears to be mediated by 5-HT₂ receptors (Mitchell, et al., 1990b). Thus, it

seems that the handling effect on hippocampal type II receptor binding, and the subsequent physiological responses to stress, may be mediated by a number of factors, including 5-HT as well as thyroid hormones.

It was recently suggested that the handling-induced serotonergic effect on type II binding capacity may be mediated by a number of secondary messenger systems, including the cAMP system. Serotonin increases cAMP levels in cultured hippocampal cells and, this effect is blocked by ketanserin. Similarly, cultured hippocampal cells treated with the stable cAMP analog, 8-bromo-cAMP, show increased type II receptor density. Interestingly, both 5-HT and 8-bromo-cAMP were comparable in their maximal stimulatory effects on hippocampal type II receptor binding, and, these effects were not apparent until 4 days after treatment (Mitchell, et al., 1992). Furthermore, it was demonstrated that the cyclic nucleotide-dependent protein kinase inhibitor, H8, but not a protein kinase C inhibitor, blocks 5-HT enhancement of type II receptor binding in cultured hippocampus cells, suggesting a possible involvement of protein kinase A on serotonergic regulation of type II receptor binding (Meaney, et al., 1991c; Mitchell, et al., 1992). In summary, alterations 5-HT activity can enhance type II receptor binding capacity in the hippocampus, and it seems that such an effect may be mediated by several secondary messenger systems, including the cAMP system (Meaney, et al., 1991b; Mitchell, et al., 1992; Mitchell, et al., 1990a).

Behavioral Consequences of Early Life Stimulation

Environmental enrichment and postnatal handling have both been shown to have profound effects on various basal and post-stressor behaviors. Both manipulations appear to "protect" the organism from age-related deficits in several learning tasks, and, also influence several physiological processes. For instance, it has been shown that rats exposed to postnatal environmental enrichment show increased thickness in forebrain and hippocampal tissue, largely attributable to

higher numbers of granule cells in the hippocampus (Rosenzweig, 1984). Additionally, enriched animals have higher levels of acetylcholinesterase (AChE) activity in subcortical brain regions (Renner & Rosenzweig, 1987; Rosenzweig, Bennett, & Diamond, 1972). Paralleling these findings, animals exposed to environmental enrichment show superior performance in complex spatial tasks. Enriched animals show enhanced use of extramaze cues and an enhanced ability to perform spatial task reversals (Renner & Rosenzweig, 1987). Similarly, enriched-reared rats learn more quickly and make less errors in a 17-arm radial maze task than rats reared in isolation (Juraska, Henderson, & Muller, 1984). The effects of environmental enrichment on subsequent behavior are not limited to younger animals. The effects of enrichment on incidental learning and on performance in a food seeking task have been reported in animals exposed to 150 days environmental enrichment beginning at 600 days of age (Warren, Castro, Rudy, & Maier, 1991).

The behavioral effects of postnatal handling on subsequent behavior in adulthood are particularly marked under stressor conditions. Rats handled during infancy have been shown to explore more and defecate less when placed in novel environments (Levine, Halmeyer, Karas, & Deneberg, 1967; Sherrod, Meier, & Conner, 1977). Animals so treated showed increased willingness to explore illuminated areas and displayed less emotional reactivity to handling in comparison to nonhandled animals (Fernandez-Teruel, Escorihuela, Driscoll, Tobena, & Battig, 1991; Fernandez-Teruel, Escorihuela, Driscoll, Tobena, & Battig, 1992). Additionally, rats exposed to either daily handling, cold stress, or a series of different mild stressors between postnatal days 3 to 15 show stimulated weight gain as young adults and fewer stressor-induced alterations in forced swim behavior during late adulthood (Gonzalez, Rodriguez, Cabrera, Foscolo, & Fracchia, 1990). Finally, postnatal handling has also been demonstrated to reverse

heightened emotionality in rats prenatally subjected to random bursts of light and noise (Wakshlak & Weinstock, 1990), reduce alcohol consumption and immobility in the Porsolt's swim test in comparison to nonhandled animals (Hilakivi-Clarke, Turkka, Lister, & Linnoila, 1991), and result in hyposensitivity to thermal stimulation in hot-plate and tail flick tests (D'Amore, Pieretti, Chiarotti, & Loizzo, 1991; Pieretti, D'Amore, & Loizzo, 1991).

Given that handled animals show less pronounced stressor-induced behavioral alterations in a number of behavioral domains, it has been suggested that handled animals are more prepared to withstand and adapt to stressor exposure. This enhanced coping ability, it will be recalled, is not only manifested behaviorally, but is also seen with respect to several endocrine changes ordinarily associated with stressors (Hilakivi-Clarke, et al., 1991; Meaney, et al., 1991a), including lowered plasma corticosterone, ACTH, and CRF release following stress (Ader, 1970; Meaney & Aitken, 1985; Meaney, et al., 1985a; Meaney, et al., 1989; Meaney, et al., 1991a) and faster returns to basal plasma corticosterone levels following stressor termination (Meaney, et al., 1988). It is likely that handling-induced alterations of various physiological responses to stress allow for a larger adaptive behavioral repertoire in response to stressors.

The aforementioned findings indicate that handled animals appear to be "protected" from age-related spatial learning deficits (Meaney, et al., 1988). It has been suggested that such an outcome can be related to improved abilities to cope effectively with stressors (Meaney, et al., 1991a; Sapolsky, 1992).

As discussed earlier, latencies and the distances swum in a Morris water-maze task increase dramatically with age and a substantial number of animals over 12 months of age fail to learn in this paradigm (Aitken & Meaney, 1989; Meaney, et al., 1988). These age-related spatial learning deficits are paralleled by loss of hippocampal CA1, CA3 and/or dentate gyrus cells (Conrad & Roy, 1993;

Jacobson & Sapolsky, 1991; Meaney, et al., 1988; Sapolsky, 1987; Sapolsky, et al., 1986). These age-related deficits in Morris water-maze performance are not observed in handled animals (Meaney, et al., 1988). Likewise, early-life handling has been shown to influence hippocampal long-term potentiation (LTP), which has been proposed to be effective on learning and memory (Wilson, Willner, Kurz, & Nadel, 1986). Inasmuch as early-life handling results in the moderation of glucocorticoid release engendered by stressors, it is conceivable that early handling abrogates hippocampal cell loss and prevents the age-related deterioration on Morris water-maze performance.

The effects of postnatal handling on subsequent adult behavior may interact with the strain of the animal. The Roman high and low avoidance rats strains, RHA/Verh and RLA/Verh, respectively, are bred for rapid versus slow learning of two-way shuttle avoidance. Postnatal handling has been shown to increase exploration and reduce emotionality, as expressed by increased entry into a brightly area of the hexagon maze, decreased defecation, and decreased vocalization during weighing of animals. This effect, however, was more pronounced in the highly emotional RLA/Verh line than in the less emotional the RHA/Verh strain (Fernandez-Teruel, et al., 1991; Fernandez-Teruel, et al., 1992).

As indicated earlier, work in this laboratory revealed that, in comparison to other mouse strains, the BALB/cByJ mouse shows greater emotionality and behavioral disturbances in response to stressors, and these are paralleled by marked alterations in neurochemistry and corticosterone (Anisman & Zacharko, 1990; Shanks & Anisman, 1993; Shanks, et al., 1990; Shanks, Zalzman, Zacharko, & Anisman, 1991). The observed behavioral disruptions in this particular strain have been observed in several paradigms, including a shuttle escape task (Shanks & Anisman, 1988; Shanks & Anisman, 1993), responding for electrical brain stimulation (Zacharko, Lalonde, Kasian, & Anisman, 1987) and consumption of a

highly palatable diet (Griffiths, Shanks, & Anisman, 1992). Of particular interest was the finding that a substantial proportion of BALB/cByJ mice seemed incapable of acquiring the Morris water-maze response, and if acquired, could be readily disrupted by stressor exposure (Francis, et al., 1994).

The finding that the BALB/cByJ mice did not learn the Morris water-maze response is surprising, given that this response is an easily learned one. Yet, given the particular stress reactivity of this strain, coupled with the glucocorticoid hypersecretion elicited by stressors in BALB/cByJ mice, the possibility exists that this deficit may be related to a progressive degeneration associated with stress-responsivity. Using the highly reactive BALB/cByJ mouse, and the less reactive C57BL/6ByJ strain, we will determine whether the profile of behavioral performance in the Morris water-maze is similar to that seen in animals with hippocampal damage and whether early-life handling will differentially influence Morris water-maze performance in these strains. Additionally, we will examine whether early-life handling influences central neurochemical and glucocorticoid secretion otherwise elicited by stressor exposure.

Experiment 1a: Cued and Noncued Acquisition

Consistent with previous findings (Upchurch & Wehner, 1988), it was observed in this laboratory that the BALB/cByJ mouse strain demonstrated impaired place learning and reversal performance in the Morris water-maze in comparison to other inbred strains (Francis, et al., 1991; Francis, et al., 1994). As discussed earlier, such a profile of performance has been demonstrated in animals that have sustained hippocampal damage. However, when hippocampal damaged animals are tested in a cued water-escape response, they exhibit proficient performance (Morris, Garrud, Rawlins, & O'Keefe, 1982; Schenk & Morris, 1985; Sutherland, Kolb, & Whishaw, 1982). Experiment 1 was conducted to determine whether the BALB/cByJ strain would fail to learn the escape response in the

Morris water-maze but learn an escape response in this paradigm when salient visual intramaze cues were present. Additionally, we determined the effect of footshock stress on the retention of cued and noncued escape responses in the Morris water-maze using both the BALB/cByJ and C57BL/6ByJ mouse strains.

Method

Subjects:

A total of 40 BALB/cByJ and 32 C57BL/6ByJ experimentally-naive male mice (Jackson Laboratory, Bar Harbor, Maine) aged approximately 120 days were used. Mice were acclimatized to laboratory conditions for at least 3 weeks prior to the beginning the experiment. During this period, mice were housed in groups of four in standard polypropylene cages. When the experiment began, each mouse was individually housed in standard polypropylene cages and maintained on a 12 hour light/12 hour dark cycle (lights on between 0700h and 1900h) All mice had access to standard Purina Laboratory Chow ad libitum. Testing procedures were conducted between 1000h - 1400h.

Apparatus

Mice were tested in a circular, white, polypropylene pool measuring 1.2 m in diameter and 28 cm in height. The pool was filled with water (20° C) to a depth of 22.5 cm and was made opaque using powdered milk. A platform, fashioned from a white plastic column (dimensions: 22 cm in height, 15 cm in width), was covered with a clear plastic mesh to allow the mice to raise themselves onto the platform. The platform was centred in the Northwest (NW) quadrant of the pool. In the Hidden condition, the platform was submerged approximately 0.5 cm below water level. The pool was situated in a laboratory room that contained several extramaze cues (e.g. white cupboard with handles, laboratory bench, wall corners etc.). A Panasonic PV-320-K video camera was suspended directly above the pool to record swimming behavior of mice.

In the Cued condition, visual cues included a three-dimensional black cardboard cross suspended 15 cm above the platform surface. Additionally, the platform had a 1.5 cm width border of black electrical tape around the platform to provide another salient maze cue. The water level was lowered such that the top of the platform was visible 2.5 cm above the water surface.

Procedure

All animals received 5 trials per day in the Morris water-maze for 5 days during which, response latencies were recorded, as was the time mice spent in each quadrant. On the sixth day, all animals received a spatial probe trial, which required each mouse to swim in the pool for 60 seconds without the platform present. The number of entries, the time spent in each quadrant and the percentage of the total time spent in each quadrant was recorded. Additionally, we determined the average number of times the mouse swam over the exact site of the original location of the platform ("hits") versus the average number of times the mouse swam over a similar area in another quadrant ("misses"). Half of the mice of each strain were randomly assigned to either the Cued condition or the Hidden condition ($n=20$ per group for BALB/cByJ mice and $n=16$ per group for C57BL/6ByJ mice).

Each acquisition trial consisted of an individual mouse being placed into the water, facing the outer edge of the pool, at one of four possible starting points: North (N), East (E), South (S) or West (W). The starting point for each trial was randomly determined with the provision that two successive trials never began from the same position and that all start locations were used in a given day. A trial was terminated when the mouse reached the platform and remained on it for 5 seconds. If a successful response was not observed within 60 seconds, the trial was terminated and recorded as an escape failure. During the 60 second intertrial intervals, animals were maintained in a dry holding cage. Escape latencies were

manually timed and were later confirmed through examination of the videotape record. The location of the platform remained constant throughout testing. Additionally, the order of the strains tested was reversed each testing day. The investigator was stationed in the same position throughout all testing trials.

Statistical Analysis

Mean latencies to escape were expressed in seconds per group. For the five acquisition days, a 2 (Strain) x 2 (Cue) x 5 (Days) analysis of variance with repeated measures (Days) was used. Where appropriate, Newman-Keuls comparisons were used to determine simple effects and group differences on testing days. Data for the Day 6 Probe trial were analyzed separately, using a two-way 2 (Strain) x 2 (Cue) analysis of variance for each of the following dependent variables: number of quadrant entries, time spent in each quadrant, percentage of total time spent in each quadrant, the number of "hits" made in the goal quadrant and the number "misses" made in the non-goal quadrants.

Results and Discussion

The mean latencies to escape as a function of strain and cue across the 5 acquisition days are presented in Figure 1a. An analysis of variance of escape latencies revealed a Strain x Cue x Days interaction, $F(4, 272)=4.03$, $p<.01$. Newman-Keuls multiple comparisons of the simple effects comprising this interaction ($\alpha=.05$) revealed that exposure to platform cues during the 5 acquisition days enhanced escape learning in both mouse strains. However, the alterations in escape behavior over days varied between the strains. Among BALB/cByJ mice trained in the noncued condition, escape performance did not vary over the first 3 days of training and, a significant decline in escape latencies was only observed between Day 3 and Day 4. In the cued condition, the escape performance of BALB/cByJ mice was superior to that observed in the noncued condition, and in addition the between-days improvement was apparent earlier

Specifically, a significant decline in escape latencies was observed as early as the third day of testing. Indeed, performance at this time approached that seen on the fifth training day. As observed previously (Francis, et al., 1994), C57BL/6ByJ mice tested in the noncued condition displayed superior escape performance relative to BALB/cByJ mice through the last 4 training days, and significant reductions of escape latencies were observed by the second day of training. Exposure to the platform cues enhanced escape acquisition in C57BL/6ByJ mice during the first 2 days of training. The differences in performance between strains observed in the noncued condition were still present when the position of the platform was cued. However, the magnitude of the strain differences was less pronounced, and absent by the fifth training day.

The mean number of escape failures over the 5 acquisition days is shown in Figure 1b. Paralleling the escape latencies, the number of escape failures varied as a function of the Strain x Cue interaction, $F(1, 68)=15.23$, $p<.01$. Newman-Keuls multiple comparisons ($\alpha=.05$) confirmed that BALB/cByJ mice exposed to the cues exhibited fewer escape failures than mice in the noncued condition. The C57BL/6ByJ mice also benefited from cue exposure, but this effect was not as robust as in the BALB/cByJ strain. In the cued condition a significant decline in escape failures was observed in both strains between Day 1 and Day 2, after which the occurrence of escape failures was infrequent. In contrast, in the noncued condition BALB/cByJ mice demonstrated a significant decline in escape failures between Day 1 and Day 2, but continued to make escape failures throughout the training period. The C57BL/6ByJ mice trained in the noncued condition likewise showed significant reductions in escape failures until the third training day, but escape failures were rare thereafter.

Insert Figure 1 about here

In an attempt to qualify what cued animals had learned over the 5 days of training, the average goal quadrant entries, the percentage of time spent in each quadrant, the time required to reach the goal quadrant, the number of annulus crossings ("hits") made in the goal quadrant, the number of crossings made in non-goal quadrants, the total number of crossings made in all areas of the swim apparatus and the ratio of annulus crossings ("hits") to total crossings were determined from the Probe trial (see Figures 2-4).

The mean number and percentage of goal quadrant entries for each group are illustrated in Figures 2a and 2b, respectively. The number and percentage of goal quadrant entries varied as a function of the Strain x Cue interactions, $F(1, 68)=5.36; 4.10, p's<.05$. Contrary to expectation, Newman-Keuls multiple comparisons of the simple effects comprising these interactions revealed that the BALB/cByJ mice did not make more goal quadrant entries or a higher percentage of goal quadrant entries as a function of previous cue exposure. Interestingly, C57BL/6ByJ mice in the cued condition made significantly fewer goal quadrant entries than mice in the noncued condition. It is conceivable that C57BL/6ByJ mice in the cued condition relied on these cues to determine the platform location, at the expense of learning about the position in space. As a result, when the cues were eliminated during the probe trial, the propensity to approach the appropriate spatial location (i.e., where the platform had previously been located) was diminished.

Insert Figure 2 about here

- Figure 1a: Mean ($\pm$ SEM) escape latencies (seconds) across blocks of 5 trials for 5 acquisition days in cued and noncued BALB/cByJ and C57BL/6ByJ mice (where CUE=cued; PLACE=noncued)
- Figure 1b: Mean ($\pm$ SEM) escape failures for blocks of 5 trials for 5 acquisition days in cued and noncued BALB/cByJ and C57BL/6ByJ mice (where CUE=cued; PLACE=noncued)

The mean latencies to reach the goal quadrant, the mean percentage of time spent in the goal quadrant, and the number of annulus crossings ("hits") for each group are shown in Figures 3a, 3b, and 3c, respectively. Analyses of variance revealed that these measures varied only as a function of Strain, $F's(1, 68)=22.23; 15.50; 20.08, p's<.0001$. Overall, BALB/cByJ mice were much slower to reach the goal quadrant, spent less time in this quadrant, and made fewer "hits" than C57BL/6ByJ mice. There was a slight trend for the cued BALB/cByJ mice making more annulus crossings relative to noncued animals of the same strain, but this trend was not significant ($p = .10$).

 Insert Figure 3 about here

Probe trial videotapes were also analyzed for the number of "misses" (i.e. crossing over an area similar to the annulus area, but in the incorrect quadrant) in each quadrant, the total number of misses, the number of total crossings and the ratio of "hits" to total crossings for each animal. Additionally, the number of entries and the time spent in each incorrect quadrant was analyzed. The most notable variations of swim behaviors observed were the number of entries, the number of misses and the time spent in the south-east quadrant, the quadrant opposite to the goal quadrant (see Figures 4a, 4b, and 4c, respectively). The number of entries, misses, and the time spent in this quadrant varied as a function of Strain x Cue interactions, $F's(1, 68)=4.07; 4.85; 4.93, p's<.05$. Newman-Keuls multiple comparisons of the simple effects comprising these interactions revealed that in the cued condition the BALB/cByJ mice displayed fewer misses in the south-east quadrant than noncued mice of the same strain, but did not differ from C57BL/6ByJ cued animals. In contrast, noncued C57BL/6ByJ mice made significantly fewer south-east misses than noncued BALB/cByJ mice and cued

- Figure 2a: Mean ($\pm$ -SEM) number goal quadrant entries made by cued and noncued BALB/cByJ and C57BL/6ByJ mice during the Probe trial
- Figure 2b: Mean ($\pm$ -SEM) percentage of quadrant entries that were goal quadrant entries in cued and noncued BALB/cByJ and C57BL/6ByJ mice during the Probe trial

Figure 3a: Mean ($\pm$ SEM) latencies (seconds) to reach the goal quadrant during the Probe trial in cued and noncued BALB/cByJ and C57BL/6ByJ mice

Figure 3b: Mean ($\pm$ SEM) proportion of time spent in the goal quadrant during the Probe trial in cued and noncued BALB/cByJ and C57BL/6ByJ mice

Figure 3c: Mean ($\pm$ SEM) number of annulus crossings ("hits") made by cued and noncued BALB/cByJ and C57BL/6ByJ mice during the Probe trial

C57BL/6ByJ mice. These observations were supported by the findings that cued animals of both strains exhibited an equivalent number of entries, and spent similar amounts of time in this quadrant. Additionally, noncued C57BL/6ByJ mice made fewer entries, fewer misses, and spent less time in this quadrant than both cued animals of the same strain and noncued BALB/cByJ mice. Further analyses revealed that while C57BL/6ByJ mice spent the greatest amount of time in the goal quadrant relative to the remaining quadrants, among BALB/cByJ mice the time spent in the four quadrants was more equally distributed. Moreover, in the latter strain the number of entries between the various quadrants was relatively equally distributed. In effect, the BALB/cByJ mice seemed to have learned less about the correct position of the platform, and hence displayed an increase in the entries into incorrect quadrants. Accordingly, the frequency of "misses" within these quadrants was increased. It is of particular interest, that the BALB/cByJ mice also showed the greatest propensity to wander away from the goal quadrant (showing the greatest entries into the opposite quadrant), further attesting to their inability to learn the spatial response.

 Insert Figure 4 about here

Experiment 1b: Retention Following Footshock

It will be recalled that marked interstrain variability exists in behavioral responses to stressors. Specifically, the BALB/cByJ strain demonstrates longer latencies to escape in a shuttle-box escape task following uncontrollable stress in comparison to the C57BL/6ByJ strain (Shanks & Anisman, 1993). Likewise, the BALB/cByJ strain has been shown to be incapable of learning a reversal escape response in the Morris water-maze following uncontrollable footshock (Francis, et al., 1994) In Experiment 1b, we determined the effect of acute uncontrollable

- Figure 4a: Mean ($\pm$ -SEM) number of southeast (SE) quadrant entries (quadrant opposite to the goal quadrant) made by cued and noncued BALB/cByJ and C57BL/6ByJ mice during the Probe trial
- Figure 4b: Mean ($\pm$ -SEM) number of "misses" made in the southeast (SE) quadrant (quadrant opposite to the goal quadrant) by cued and noncued BALB/cByJ and C57BL/6ByJ mice during the Probe trial
- Figure 4c: Mean ($\pm$ -SEM) time spent (seconds) in the southeast (SE) quadrant (quadrant opposite to the goal quadrant) in cued and noncued BALB/cByJ and C57BL/6ByJ mice during the Probe trial

footshock stress on previously acquired Morris water-maze escape responses, and whether stress differentially affected responses acquired during cued versus noncued trials. Subjects from Experiment 1a were exposed to either uncontrollable footshock or no treatment and tested for retention of their respective Morris water escape responses (cued versus noncued responses) acquired during the acquisition trials.

Method

Subjects:

All mice from Experiment 1a were used 14 days following the termination of the Probe trial from the previous experiment. Mice remained individually housed during this 14 day period.

Apparatus

Morris water-maze escape responses were evaluated in the same water-maze apparatus and videotaped, as previously described in Experiment 1a. For Shock groups, inescapable shock was administered in five black Plexiglas chambers that measured 30.0 x 14.0 x 15.0 cm. The chamber floors consisted of twenty-eight 0.32 cm. stainless steel rods spaced 1.0 cm apart (centre to centre) connected in series by neon bulbs. Stainless steel plates, connected in series with the grid floor, lined the insides of each chamber. The cover of each chamber were made from red translucent Plexiglas to prevent illumination. Shocks (150 mA, 60 Hz, ac) were delivered to the chamber floors through a 3000 - V source.

Procedure

Following the 14 day "waiting period", mice of each strain and cue condition were given 2 baseline days of Morris water-maze escape training, using procedures identical to those outlined previously. On the third day, animals were matched according to baseline escape latencies and then assigned to the Shock or No Treatment conditions. Animals in the Shock groups were exposed to 66

minutes of uncontrollable footshock (360 shocks, 150 mA, 2 seconds duration, 9 seconds intertrial time), while nonshocked animals were placed in the shock boxes for an equivalent period but shock was withheld. Immediately thereafter, animals had 5 retention trials, identical to their previous respective acquisition trials (i.e. cued versus noncued). Escape latencies and escape failures were recorded.

Results and Discussion

During the baseline testing days, there were no strain differences in escape performance among cued mice. In contrast, in the noncued condition the performance of the C57BL/6ByJ mice was superior to that of BALB/cByJ mice just as it had been during the initial acquisition days.

The mean latencies to reach the platform and the number of escape failures made by both strains and in both paradigms as a function of the shock condition are shown in Figures 5a, and 5b, respectively. Analysis of variance of the response latencies and the number of escape failures revealed significant Strain x Cue interactions $F's(1, 64)=12.78; 9.95, p's<.01$. As observed during the 5 acquisition days, Newman-Keuls multiple comparisons ($\alpha = .05$) indicated that the noncued BALB/cByJ mice exhibited poorer escape performance and more escape failures than both the cued BALB/cByJ and noncued C57BL/6ByJ mice.

Insert Figure 5 about here

It was expected that the shock treatment would differentially affect mice depending upon the training paradigm used. Although the Strain x Cue x Shock interaction of the response latencies was not significant, $F(1, 64)=2.33, p=.13$, Newman-Keuls multiple comparisons of the simple main effects were conducted since an a priori prediction had been made regarding this interaction. These

- Figure 5a:** Mean ($\pm$ -SEM) escape latencies (seconds) across one block of 5 trials in cued and noncued BALB/cByJ and C57BL/6ByJ mice immediately following inescapable footshock (150 mA) or no shock
- Figure 5b:** Mean ($\pm$ -SEM) number of escape failures across one block of 5 trials in cued and noncued BALB/cByJ and C57BL/6ByJ mice immediately following exposure to inescapable footshock (150 mA) or no shock

comparisons indicated that the shock treatment did not influence escape performance in C57BL/6ByJ mice, regardless of the cue condition, or among BALB/cByJ mice tested in the cued learning condition. However, BALB/cByJ mice tested in the noncued paradigm exhibited significantly longer escape latencies than nonshocked mice of this strain. It is apparent that stressor treatment may disrupt Morris water-maze performance, but this effect is dependent upon the strain of mouse and the nature of the learning paradigm employed. It is noteworthy that the lack of shock effect in C57BL/6ByJ mice was not likely a result of the response being well entrenched in this strain. In separate experiments we observed that footshock did not affect acquisition of the response under any conditions in C57BL/6ByJ mice (Francis, et al., 1994).

Experiment 2: Determination of Possible Age-Related Performance Deficits

As animals age, circulating glucocorticoid levels rise, possibly resulting from down-regulation of type II glucocorticoid receptors (Sapolsky, et al., 1983b) and the loss of hippocampal cells (Landfield, 1987; Landfield, et al., 1981; Landfield, et al., 1978; Sapolsky, et al., 1984b). Additionally, the behavioral deficits associated with hippocampal dysfunction, such as deficits of spatial learning and memory (e.g. in the Morris water-maze task) are most evident in aged rats that exhibit the greatest hippocampal cell loss (Landfield, 1987; Landfield, et al., 1981; Landfield, et al., 1978; Sapolsky, et al., 1985).

It has been reported that the impaired acquisition in Morris water-maze escape performance (Francis, et al., 1991; Francis, et al., 1994; Upchurch & Wehner, 1988) and in radial-maze performance in BALB/cByJ mice was correlated with size of intra- and infrapyramidal mossy fiber terminal field (Crusio, Schwegler, & Lipp, 1987; Schwegler, Crusio, Lipp, & Heimrich, 1988). It is possible that the observed Morris water-maze performance deficits in the BALB/cByJ mouse may be a manifestation of hippocampal dysfunction due to

early degeneration of hippocampal neurons and/or type II glucocorticoid receptors. Our laboratory has demonstrated that Morris water-maze disruptions in the BALB/cByJ strain occurs in animals as early as 90 days of age. It is possible, however, that relative to other mouse strains, the BALB/cByJ strain may be particularly vulnerable to hippocampal damage earlier in life, and this damage manifests itself as impaired Morris water-maze performance.

Experiment 2 was conducted to determine whether the inability of the BALB/cByJ mouse to acquire the Morris water-maze escape response was symptomatic of early aging. Therefore, we evaluated Morris water-maze escape performance in animals at 30, 60 and 90 days of age.

Method

Subjects

A total of 30 BALB/cByJ and 30 C57BL/6ByJ male mice were used. Mice were bred at this laboratory from parent stock obtained from Jackson Laboratories (Bar Harbor, Maine). Pups were kept with their dam until they were 21 days of age.

During postnatal day 1 (PN1) to postnatal day 21 (PN21), each mouse litter and dam were housed in standard polypropylene cages, which were changed twice weekly. Mice were weaned from the dam at 22 days of age. When evaluation of swim performance commenced, each mouse was individually housed in standard polypropylene cages and were be maintained on a 12 hour light/12 our dark cycle (lights on between 0700h and 1900h). All mice had access to standard Purina Laboratory Chow ad libitum. Testing procedures were conducted between 1000h - 1400h.

Apparatus & Procedure

Morris water-maze performance was evaluated in the same maze as previously described in Experiment 1a. All animals were tested using procedures

described in Experiment 1a. Independent groups of mice of each strain were tested when they were either 30, 60 or 90 days of age. Animals were tested for escape response acquisition for 5 days, 5 trials per day with a 60 second interval between trials. As in Experiment 1a, the order of strains tested was reversed each day. Additionally, counterbalancing procedures for age groups were used to minimize testing order effects. As in Experiment 1a, latencies to escape were averaged over 5 trials, and escape failures were recorded. Likewise, mice were exposed to a probe trial on the sixth day of testing. The number of entries, the time spent, and the percentage of total time spent in each quadrant was recorded. Furthermore, we determined the average number of "hits" versus the average number of "misses" per group.

Results and Discussion

Mean latencies to escape for each mouse were averaged over 5 trials and expressed in seconds. A 2 (Strain) x 3 (Age) x 5 (Days) analysis of variance with repeated measures (Days) was used. As in Experiment 1a, data for the Day 6 probe trial were analyzed separately, as a 2 (Strain) x 3 (Age) analysis of variance for each of the following measures; the number of quadrant entries, time spent in each quadrant, percentage of total time spent in each quadrant, number of "hits" and number of "misses". Where appropriate, Newman-Keuls comparisons were used to determine, simple effects and group differences on days.

The mean latencies to escape for each acquisition day for the 30, 60 and 90 day old mice of each strain are presented in Figure 6a. Analysis of variance of these latencies indicated that the performance of BALB/cByJ mice, as in the preceding experiments, was inferior to that of C57BL/6ByJ mice, $F(1, 54)=71.91$, $p<.01$. Additionally, the analysis indicated that the Strain x Age interaction approached, but did not reach statistical significance, $F(2, 54)=2.54$, $p=.09$. Nevertheless, Newman-Keuls multiple comparisons were conducted since the

specific prediction of this particular experiment was that performance would deteriorate with age in the BALB/cByJ but not in the C57BL/6ByJ mice. These comparisons revealed that the C57BL/6ByJ mice did not differ in their escape performance as a function of their age. Contrary to prediction, performance was not found to deteriorate with age in the BALB/cByJ mice. While the escape latencies of 30 and 60 day old BALB/cByJ mice did not differ from one another, the latencies of the 90 day group were actually shorter than in the 30 day animals. Regardless of age, C57BL/6ByJ mice displayed superior escape performance relative to BALB/cByJ mice of the same age. These observations suggest that the poor performance of the BALB/cByJ strain is not due to early aging (at least not that which could be detected by 30 days of age), and indeed, the impaired performance in this strain is apparent within several days of weaning.

The mean number of escape failures for all training days in 30, 60 and 90 day old mice of both strains are shown in Figure 6b. Analysis of variance revealed that the number of escape failures across training days varied as a function of the Strain x Age interaction, $F(2, 54)=3.92$, $p<.05$. There were few escape failures in C57BL/6ByJ mice, regardless of the age of the animals. Consistent with the escape latency data, Newman-Keuls multiple comparisons of the simple effects revealed that the frequency of escape failures in this strain did not vary over days. In contrast, the 90 day old BALB/cByJ mice exhibited significantly fewer escape failures than either the 30 or 60 day old mice of this strain.

 Insert Figure 6 about here

The mean number of goal quadrant entries during the probe trial are illustrated in Figure 7. Since there was a problem with video equipment during

Figure 6a: Mean ($\pm$ -SEM) escape latencies (seconds) across blocks of 5 trials for 5 acquisition days in 30, 60 and 90 day old BALB/cByJ (i.e., B30, B60, B90-left panel) and C57BL/6ByJ (C30, C60, C90-right panel) mice

Figure 6b: Mean ($\pm$ -SEM) number of escape failures across blocks of 5 trials for 5 acquisition days in 30, 60 and 90 day old BALB/cByJ (B30, B60, B90-right panel) and C57BL/6ByJ (C30, C60, C90-left panel) mice

videotaping of mice, probe data from one animal from the C57BL/6ByJ 30 day old group was missing. The number of goal quadrant entries was dependent upon the Strain x Age interaction, $F(2, 53)=3.30$, $p<.05$. The multiple comparisons revealed that C57BL/6ByJ mice displayed significantly more goal quadrant entries than did BALB/cByJ mice across all ages. In the former strain, the number of such entries in the 60 day old mice was somewhat higher than at the other ages, thus accounting for the Strain x Age interaction.

 Insert Figure 7 about here

The latencies to reach the goal quadrant were shorter in C57BL/6ByJ mice than in BALB/cByJ animals, the time spent in that quadrant was longer in C57BL/6ByJ mice, and the ratio of hits to total crossings was also greater in this strain $F_s(1, 53)=33.71$; 66.55 ; 58.06 , $p's<.01$ (see Figures 8a, 8b, 8c, respectively). The analysis of variance of the number of annulus crossings revealed that hits were made more frequently in the C57BL/6ByJ mice, $F(1, 53)=61.17$, $p<.01$ and that accuracy of performance improved with Age, $F(1, 53)=3.79$, $p<.05$. None of the interactions proved to be statistically significant (see Figure 8d).

 Insert Figure 8 about here

The number of entries and the time spent in each of the non-goal quadrants was analyzed to determine if the strain and age of the mice affected specific spatial learning errors. Analyses of variance revealed that the number of entries into the south-west quadrant (adjacent to the goal quadrant) and the number of misses in this quadrant varied as a function of the Strain x Age interactions, $F_s(2, 53)=3.21$; 3.37 , $p's<.05$. (see Figures 9a and 9b). Paralleling the data from

Figure 7: Mean ($\pm$ -SEM) number goal quadrant entries made during the Probe trial by 30, 60 and 90 day old BALB/cByJ (B30, B60, B90-left panel) and C57BL/6ByJ (C30, C60, C90-right panel) mice

- Figure 8a: Mean ($\pm$ -SEM) latencies to the goal quadrant during the Probe trial in 30, 60 and 90 day old BALB/cByJ (B30, B60, B90-left panel) and C57BL/6ByJ (C30, C60, C90-right panel) mice
- Figure 8b: Mean ($\pm$ -SEM) time spent goal quadrant (sec.) during the Probe trial by 30, 60 and 90 day old BALB/cByJ (B30, B60, B90, respectively) and C57BL/6ByJ (C30, C60, C90, respectively) mice
- Figure 8c: Mean ($\pm$ -SEM) ratio of the number of annulus crossings ("hits") to the total number of crossings made during the Probe trial by 30, 60 and 90 day old BALB/cByJ (B30, B60, B90) and C57BL/6ByJ (C30, C60, C90) mice
- Figure 8d: Mean ($\pm$ -SEM) number of annulus crossings ("hits") made during the Probe trial by 30, 60 and 90 day old BALB/cByJ (B30, B60, B90) and C57BL/6ByJ (C30, C60, C90) mice

Experiment 1a, the number of misses made in the south-east quadrant (opposite to the goal quadrant) was dependent upon the Strain x Age interaction, $F(2, 53)=5.17, p<.05$ (see Figure 10).

 Insert Figures 9 and 10 about here

Newman-Keuls multiple comparison of the simple effects comprising these interactions revealed that the 30 day and 60 day old BALB/cByJ mice made fewer entries into the south-west quadrant in comparison to the C57BL/6ByJ mice. The number of entries made by 90 day old BALB/cByJ mice increased relative to that seen at the younger ages, such that performance between the two strains at 90 days of age did not differ from one another. Furthermore the number of misses in this quadrant increased significantly among 90 day old BALB/cByJ mice in comparison to 30 day old mice. It was also noted that the 60 day and 90 day old BALB/cByJ mice made more misses in the south-east quadrant (that opposite to the goal quadrant) than C57BL/6ByJ mice. With age, the number of south-east misses declined in C57BL/6ByJ mice, whereas this decrease was not observed in the BALB/cByJ strain. Moreover, an effect of Strain was observed with respect to both the number of entries and the time spent in the south-east quadrant, $F's(2, 53)=5.31; 26.08, p's<.05$. As in Experiment 1A, Newman-Keuls multiple comparisons indicated that BALB/cByJ mice made more entries and spent significantly more time in this quadrant suggesting that they paid more attention to irrelevant extramaze cues (see Figure 10).

Experiment 3a: Postnatal Handling Effects on Morris Water-Maze Acquisition

It has been previously demonstrated that rats exposed to postnatal handling for 21 days do not show age-related hippocampal cell loss or impairments in

Figure 9a: Mean ($\pm$ -SEM) number of southwest (SW) goal quadrant entries (quadrant adjacent to the goal quadrant) made during the Probe trial by 30, 60 and 90 day old BALB/cByJ (B30, B60, B90) and C57BL/6ByJ (C30, C60, C90) mice

Figure 9b: Mean ($\pm$ -SEM) number of "misses" made in the southwest (SW) quadrant (quadrant adjacent to the goal quadrant) during the Probe trial by 30, 60 and 90 day old BALB/cByJ (B30, B60, B90) and C57BL/6ByJ (C30, C60, C90) mice

- Figure 10a: Mean ($\pm$ -SEM) number of "misses" made in the southeast (SE) quadrant (quadrant opposite to the goal quadrant) during the Probe trial by 30, 60 and 90 day old BALB/cByJ (B30, B60, B90) and C57BL/6ByJ (C30, C60, C90) mice
- Figure 10b: Mean ($\pm$ -SEM) number of entries made in the southeast (SE) quadrant (quadrant opposite to the goal quadrant) during the Probe trial by 30, 60 and 90 day old BALB/cByJ (B30, B60, B90) and C57BL/6ByJ (C30, C60, C90) mice
- Figure 10c: Mean ($\pm$ -SEM) proportion of time spent in the southeast (SE) quadrant (quadrant opposite to the goal quadrant) during the Probe trial in 30, 60 and 90 day old BALB/cByJ (B30, B60, B90) and C57BL/6ByJ (C30, C60, C90) mice

hippocampal-related behaviors, such as performance in the Morris water-maze task (Meaney, et al., 1988; Meaney, et al., 1991a; Sapolsky, 1992). It has been suggested that deficits in spatial learning (e.g. in Morris water-maze performance) are a manifestation of hippocampal cell loss resulting from age-related changes in glucocorticoid secretion and HPA negative feedback mechanisms (Jacobson & Sapolsky, 1991; Sapolsky, 1987; Sapolsky, et al., 1983b; Sapolsky, Krey, & McEwen, 1984c; Sapolsky, et al., 1986). It seems that postnatal handling may "protect" the organism from hippocampal damage by increasing type II glucocorticoid receptors numbers within the hippocampus and providing the organism with more effective regulation of stress-induced HPA activity (Jacobson & Sapolsky, 1991; Meaney, et al., 1985a; Meaney, et al., 1985b; Meaney, et al., 1989; Meaney, et al., 1991a).

We suggested earlier that the BALB/cByJ mouse may be more susceptible to early hippocampal cell loss, since this strain demonstrates corticosterone hypersecretion in response to stressors (Shanks, et al., 1990). Experiment 3a evaluated whether early-life handling would facilitate Morris water-maze learning during later adulthood, and whether handling effects interacted with the strain of the animal. It was hypothesized that the Morris water-maze performance in the BALB/cByJ mouse would be enhanced by early-life handling.

Method

Subjects:

Ten BALB/cByJ and 10 C57BL/6ByJ female mice and 5 male mice of both strains (Jackson Laboratory, Bar Harbor, Maine) aged approximately 7 weeks were obtained. Mice were group housed (2 females; 1 male per cage) with food and water ad libitum, on a 12 hour light/12 hour dark cycle. Dams were removed from breeding cages when visibly pregnant and individually housed for

approximately 5 days before giving birth. Dams were placed into cages with extra bedding 1-2 days prior to giving birth.

All mouse pups were weaned at 22 days of age. A total of 36 BALB/cByJ and 32 C57BL/6ByJ male mice aged between 120-140 days were used. They were housed with their respective siblings in cages of 3 to 5 animals until experimentation began.

Apparatus & Procedure

All mice were tested in the same Morris water-maze apparatus with the same extramaze cues and the experimenter was in same position as in previous experiments. At birth, all litters were culled to 8 pups. They were then randomly assigned to either the Handled or the Nonhandled condition. For the Handled group, handling procedures were carried out on postnatal days (PN) 1-21. On PN1, the dam was removed from the home cage and placed into a holding cage. Pups were then individually handled for a few seconds and placed into a new cage with standard Betachip bedding and remained there 15 to 20 minutes. Following this time period, pups were individually placed back into the nest of the home cage, after which, the dam was reintroduced. This procedure was conducted daily from PN1 to PN21 at approximately the same time each day (+/- 2 hours) until pups were weaned. The handling regimen was conducted only by a single investigator, who wore standard latex gloves for all handling procedures. For the Nonhandled group, cages were left undisturbed until weaning at PN22. Additionally, cages of each group were not changed until PN14, when half of the bedding material was manually removed and fresh nesting material and bedding were added. After mouse weanlings reached 120-140 days of age, experimentation began. Thirty-six male BALB/cByJ and 32 male C57BL/6ByJ mice represented the respective Handled and Nonhandled conditions. Prior to testing, mice were individually housed in standard polypropylene cages, had

access to standard Purina Laboratory Chow and water ad libitum. All mice were maintained on a 12 hour light/12 hour dark cycle (lights on between 0700h and 1900h).

All animals were tested for Morris water-maze performance for 5 days, 5 trials per day with procedures used in Experiment 1a. For this experiment, the platform remained hidden for all trials. On Day 6, the platform was removed, and, a single 60 second Probe trial was administered to determine group differences in the number of quadrant entries, the time spent in each quadrant, the percentage of total time spent in each quadrant, the number of "hits" and the number of "misses". All testing procedures were conducted between 1000h - 1400h.

Results and Discussion

Figures 11a and 11b show the mean escape latencies and the mean number of escape failures for each strain on each training day as a function of the handling treatment. The analysis of variance of the escape latencies revealed a significant Strain x Handling interaction, $F(1, 63)=8.75$, $p<.01$. Newman-Keuls multiple comparisons ($\alpha=.05$) of the simple effects comprising this interaction indicated the escape latencies in nonhandled BALB/cByJ mice were longer than in similarly treated C57BL/6ByJ mice. Early-life handling reduced latencies significantly in the BALB/cByJ relative to the nonhandled animals, but had no such effect in the C57BL/6ByJ strain. Nevertheless the between-strains differences were still maintained in the handled condition.

It was expected that if handling were to have any effects on the swim performance of BALB/cByJ mice relative to their nonhandled counterparts, then such effects would become apparent as training progressed. Thus, although a Strain x Handling x Day interaction was not significant in the present investigation, Newman-Keuls comparisons were conducted between means comprising the simple main effects of this interaction. These comparisons

confirmed that in the absence of handling performance in C57BL/6ByJ mice improved between days, with a significant decline of response latencies being evident as early as the second day of testing. In contrast, among the BALB/cByJ mice performance improved only between the second and third testing day and remained unchanged thereafter. Postnatal handling significantly improved escape performance in the BALB/cByJ strain on the fourth and fifth days of testing relative to nonhandled mice of this strain, whereas handling did not affect the performance of C57BL/6ByJ mice at any time. However, on these test days, the performance of the C57BL/6ByJ mice was still superior to that of the handled BALB/cByJ mice.

Analysis of the mean number of escape failures revealed a Strain x Handling interaction, $F(1, 63)=9.78$, $p<.01$, and a Day x Strain interaction, $F(4, 252)=21.00$, $p<.001$. Paralleling the escape latency data, Newman-Keuls multiple comparisons ($\alpha =.05$) of simple effects confirmed that in nonhandled BALB/cByJ mice the frequency of escape failures exceeded that seen in nonhandled C57BL/6ByJ mice. Handling was found to reduce escape failures in BALB/cByJ mice, without affecting the performance in the C57BL/6ByJ strain. Over days, the frequency of escape failures in C57BL/6ByJ mice declined appreciably. Indeed, by the second day of training the occurrence of escape failures was rare. In contrast, nonhandled BALB/cByJ mice did not show a decline in the number of escape failures across acquisition days.

 Insert Figure 11 about here

Consistent with Experiment 1A, in the Probe trial, C57BL/6ByJ mice made more annulus crossings, more goal quadrant entries and a greater proportion of goal quadrant entries than did BALB/cByJ mice, $F_s(1, 63)=19.37; 22.46; 8.74;$

- Figure 11a: Mean ($\pm$ -SEM) escape latencies (seconds) across blocks of 5 trials for 5 acquisition days in handled and nonhandled BALB/cByJ and C57BL/6ByJ mice (where BH=BALB Handled; BNH=BALB Nonhandled; CH=C57BL Handled; CNH=C57BL Nonhandled)
- Figure 11b: Mean ($\pm$ -SEM) number of escape failures across blocks of 5 trials for 5 acquisition days in handled and nonhandled BALB/cByJ and C57BL/6ByJ mice (where BH=BALB Handled; BNH=BALB Nonhandled; CH=C57BL Handled; CNH=C57BL Nonhandled)

16.89; 19.52, p 's<.01 (see Figures 12a, 12b, and 12c, respectively). Additionally, the C57BL/6ByJ strain exhibited significantly shorter latencies to the goal quadrant and spent much more time in the goal quadrant, indicating that in this strain the escape response was more entrenched than in the BALB/cByJ mice, F 's (1, 63)=16.89; 19.52, p 's<.01 (see Figures 13a and 13b).

 Insert Figures 12 and 13 about here

As might have been expected, the total number of misses (in the incorrect quadrants) varied as a function of the Strain x Handling interaction, F (1, 63)=5.27, p <.05 (see Figure 14).. The BALB/cByJ nonhandled group made fewer misses than the BALB/cByJ handled group, whereas the C57BL/6ByJ nonhandled made more total misses than the C57BL/6ByJ handled group and the BALB/cByJ handled group.

 Insert Figure 14 about here

This finding may have been a reflection of interstrain differences in the level of swim activity. Indeed, C57BL/6ByJ mice made significantly more quadrant entries and total crossings than BALB/cByJ mice, F 's(1, 63)=17.34; 16.52, p 's<.01. As well, the ratio of hits to total crossings were also much higher in C57BL/6ByJ mice, F (1, 63)=16.52, p <.01 (see Figures 15a, 15b and 15c, respectively).

The analyses of the percentage of non-goal quadrant entries revealed that both strain made proportionately equal entries in the quadrants adjacent to the goal quadrant. Commensurate with the preceding studies, BALB/cByJ mice tended to enter the southeast quadrant (opposite to the goal quadrant) much more frequently than C57BL/6ByJ mice, F 's(1, 63)=8.54, p <.01, although percentage of

Figure 12a: Mean ($\pm$ -SEM) number of annulus crossings ("hits") made by handled and nonhandled BALB/cByJ and C57BL/6ByJ mice during the Probe trial

Figure 12b: Mean ($\pm$ -SEM) number of goal quadrant entries made by handled and nonhandled BALB/cByJ and C57BL/6ByJ mice during the Probe trial

Figure 12c: Mean ($\pm$ -SEM) proportion of quadrant entries that were goal quadrant entries made by handled and nonhandled BALB/cByJ and C57BL/6ByJ mice during the Probe trial

Figure 13a: Mean ($\pm$ -SEM) latencies (seconds) to reach the goal quadrant in handled and nonhandled BALB/cByJ and C57BL/6ByJ mice during the Probe trial

Figure 13b: Mean ($\pm$ -SEM) proportion of time spent in the goal quadrant in handled and nonhandled BALB/cByJ and C57BL/6ByJ mice during the Probe trial

Figure 14: Mean ($\pm$ -SEM) total number of "misses" made by handled and nonhandled BALB/cByJ and C57BL/6ByJ mice during the Probe trial

entries in other non-goal quadrants were equivalent between strains (see Figure 15d).

Insert Figure 15 about here

Experiment 3b: Early-Life Handling Effects on Retention Following Footshock

It has been demonstrated in this laboratory that uncontrollable footshock stress does not affect Morris water-maze escape response retention in BALB/cByJ mice, but did affect the ability of this strain to learn an escape reversal response, in comparison to other strains. However, in BALB/cByJ mice that learned the response, inescapable footshock retarded the acquisition of an escape-reversal response.

We hypothesized that the Morris water-maze performance of BALB/cByJ mice would be enhanced by postnatal handling. It was of interest to determine whether inescapable footshock would affect the performance of BALB/cByJ mice, and whether such an effect was dependent upon the strain of the animal as well as whether early-life handling was received.

Method

Subjects

All animals from Experiment 3a were be used in Experiment 3b. During this time they were individually housed in standard polypropylene cages with food and water provided ad libitum.

Apparatus & Procedure

The Morris water-maze apparatus was the same as that described earlier. Likewise, the shock apparatus and parameters were the same as that described in Experiment 1b. Fourteen days after the Probe trial, all animals were given 2 days

- Figure 15a: Mean ($\pm$ -SEM) number of goal quadrant entries made by handled and nonhandled BALB/cByJ and C57BL/6ByJ mice during the Probe trial
- Figure 15b: Mean ($\pm$ -SEM) number of total crossings made by handled and nonhandled BALB/cByJ and C57BL/6ByJ mice during the Probe trial
- Figure 15c: Mean ($\pm$ -SEM) ratio of "hits" to total crossings in handled and nonhandled BALB/cByJ and C57BL/6ByJ mice during the Probe trial
- Figure 15d: Mean ($\pm$ -SEM) proportion of southeast (SE) quadrant entries (quadrant opposite to goal quadrant) made by handled and nonhandled BALB/cByJ and C57BL/6ByJ mice during the Probe trial

of baseline escape testing (5 trials per day). Animals were ranked and matched according to escape latencies, and assigned to either the No Treatment (NT) condition or the Shock (SH) condition. Animals in the Shock condition received 360 shocks (150 mA), of 2 sec. duration, with a 9 sec. intertrial interval. Animals were given 5 retention trials immediately following treatments.

Results and Discussion

During the two days of baseline testing prior to shock treatment, the response latencies and the number of escape failures observed varied as a function of the Strain x Handling interactions $F(1, 63)=9.21; 6.29, p's<.05$. Newman-Keuls multiple comparisons ($\alpha = .05$) of the simple effects comprising these interactions indicated that handled BALB/cByJ mice had significantly shorter response latencies and fewer escape failures than nonhandled animals of the same strain. However, the interstrain differences were still apparent; both handled and nonhandled C57BL/6ByJ mice rarely made escape failures and exhibited shorter latencies than their respective BALB/cByJ counterparts. Furthermore, handled and nonhandled C57BL/6ByJ mice did not differ from one another on either of these measures.

Mean latencies and the number of escape failures for each of the shocked and non-shocked groups are presented in Figures 16a and 16b, respectively. Analysis of variance revealed that exposure to postnatal handling resulted in shorter response latencies, irrespective of strain and stressor condition, $F(1, 59)=9.89, p<.01$. Additionally, response latencies and the number of escape failures varied as a function of the Strain x Shock interactions, $F(1, 59)=5.93; 4.43, p's<.05$. Response latencies were significantly longer and more escape failures were observed among shocked BALB/cByJ mice in comparison to mice which had only been exposed to the apparatus. Commensurate with the data

from Experiment 1B, exposure to shock in C57BL/6ByJ mice did not affect either response latencies or the number of escape failures made.

 Insert Figure 16 about here

Experiment 4: Maternal Factors in Handling-Induced Behavioral Alterations

It has been shown that postnatal handling enhances Morris water-maze performance in 24 month old Long Evan rats (Meaney, et al., 1988). As discussed earlier, several mechanisms have been suggested to account for this observation, such as early-life activation of serotonergic and cAMP mechanisms, which have been shown to affect hippocampal type II receptor development (Meaney, et al., 1991c; Mitchell, et al., 1992; Mitchell, et al., 1990a; Mitchell, et al., 1990b). Another factor which may contribute to such handling effects concerns the dam's behavior towards pups following the daily handling procedure. It has been shown that maternal behavior, such as feeding and anogenital licking, differentially affects HPA activity. In particular, maternal feeding suppresses pups' corticosterone secretion, whereas stroking inhibits ACTH secretion (Suchecki, Rosenfeld, & Levine, 1993). The separation from the dam and the handling of pups may affect maternal behavior, which, in turn, affects the regulation of the pups' HPA activity.

Maternal behavior following the separation and the handling of pups may vary across mouse strains. The BALB/cByJ mouse has been demonstrated to be a highly emotional animal which shows stress-induced behavioral disruptions in a number of different paradigms (Shanks & Anisman, 1988; Shanks, et al., 1990). Conceivably, the alterations in maternal behavior following separation of pups in this strain may interact with possible postnatal handling to influence subsequent

Figure 16a: Mean ($\pm$ SEM) escape latencies (seconds) across one block of 5 trials in handled and nonhandled BALB/cByJ and C57BL/6ByJ mice immediately following inescapable footshock (150 mA) or no shock

Figure 16b: Mean ($\pm$ SEM) number of escape failures made during one block of 5 trials in handled and nonhandled BALB/cByJ and C57BL/6ByJ mice immediately following inescapable footshock (150 mA) or no shock

adult behavior. Experiment 4 examined whether strain differences in maternal behavior (using a variant of a cross-fostering paradigm) would influence handling-induced alterations in Morris water-maze performance.

Method

Subjects

As in Experiment 3a, 10 BALB/cByJ and 10 C57BL/6ByJ female mice and 5 male mice of each strain (Jackson Laboratory, Bar Harbor, Maine) aged approximately 7 weeks served as a breeding colony. Dams were removed from breeding cages when visibly pregnant and individually housed approximately 5 days before giving birth. Dams were placed into cages with extra bedding and nesting material 1-2 days prior to giving birth. As in Experiment 3a, all litters were culled to 8 pups per litter on PN0. Mouse pups were weaned at 22 days of age (PN22). A total of 36 BALB/cByJ and 36 C57BL/6ByJ male mice aged between 120 to 140 days were used. These mice were housed with their respective siblings (3-5 animals per cage) until experimentation began.

Apparatus & Procedure

Morris water-maze escape performance was assessed in the apparatus described earlier. At birth (PN0), half of the total number of mouse litters were randomly assigned to the Cross-Fostering condition where a C57BL/6ByJ mouse dam was removed from its birthing cage and placed into a BALB/cByJ birthing cage, and vice versa. Thus, cross-fostering was specific to the dam of the alternate strain. The rest of the mouse litters were not exposed to cross-fostering and remained with their respective dam until PN22, when pups were weaned. As in Experiment 3a, all litters were culled to 8 pups per litter. On PN1, litters also were randomly assigned to either the Handled condition or the Nonhandled condition. Handling protocol was performed as outlined in Experiment 3a.

After mouse weanlings reached 120-140 days of age, experimentation commenced. Thirty-six male BALB/cByJ and 36 male C57BL/6ByJ mice represented all cross-fostering and handling conditions ($n=9$ per group). Prior to testing, mice were individually housed in standard polypropylene cages, had access to standard Purina Laboratory Chow and water ad libitum and were maintained on a 12 hour light/12 hour dark cycle (lights on between 0700h and 1900h).

All animals were tested for Morris water-maze escape performance for 5 days, 5 trials per day following the procedures used in Experiment 1a. As in Experiment 3a, the platform remained hidden for all trials.

Results and Discussion

Mean latencies to escape and the number of escape failures as a function of the handling and cross-fostering manipulations for the BALB/cByJ and C57BL/6ByJ mice are shown in Figures 17a and 17b, respectively. Since we did not expose some animals to the Probe trial during our pilot study, there were a lesser number of degrees of freedom for the Probe trial analyses. Analyses of variance of both response latencies and escape failures revealed Strain $\times$ Handling interactions, $F's(1, 70)=5.38; 7.66, p's<.05$ and Strain $\times$ Cross-Fostering interactions, $F's(1, 70)=4.76; 10.25, p's<.05$. Commensurate with the preceding studies, Newman-Keuls multiple comparisons ($\alpha=.05$) indicated that in nonhandled BALB/cByJ mice, escape latencies were longer and more escape failures were made than in similarly treated C57BL/6ByJ mice. Early-life handling of BALB/cByJ mice significantly reduced both the latencies and the number of escape failures relative to the nonhandled animals of this strain, whereas no such effect was apparent in the C57BL/6ByJ mice. Regardless of handling condition, the between-strains differences were still maintained. The multiple comparisons also revealed that cross-fostering had strain-specific effects on escape latencies

and frequency of escape failures. In particular, the increased latencies and escape failures ordinarily evident in BALB/cByJ mice was significantly reduced when pups of this strain were reared with C57BL/6ByJ mice. Interestingly, among C57BL/6ByJ mice raised by the BALB/cByJ parent increased latencies or escape failures were not evident.

 Insert Figure 17 about here

The Strain x Handling x Cross-Fostering x Day interactions with respect to response latencies and escape failures did not reach statistical significance, $F's(4, 280)=1.82; 2.14, p's=.12; .07$. However, it is important to emphasize that the effects of the two manipulations (i.e., the cross-fostering and handling) were, in fact, not additive. In particular, Newman-Keuls multiple comparisons of the simple main effects for the 4-way interaction confirmed that performance in C57BL/6ByJ mice improved over days and was unaffected by any of the manipulations. In contrast, among BALB/cByJ mice that were not handled and were raised with a BALB/cByJ mother the frequency of escape failures did not vary over days. Moreover, while declining somewhat between the first and third test days, escape latencies actually deteriorated thereafter. If mice were either handled or were raised with a C57BL/6ByJ mother, escape failures declined markedly and were rare by the third test day, and escape latencies generally were shorter on the last two days of testing than in animals that did not receive either of the two treatments. There were no differences between BALB/cByJ mice that received either or both the handling and/or cross-fostering treatments.

Taken together, the data indicate that the disturbances of Morris water-maze performance characteristic of BALB/cByJ mice are modifiable by either early-life stimulation or by being raised by a C57BL/6ByJ dam. Inasmuch as the two

Figure 17a: Mean ($\pm$ -SEM) escape latencies (seconds) across blocks of 5 trials for 5 acquisition days in handled and nonhandled BALB/cByJ (left panel) and C57BL/6ByJ mice (right panel) which were either cross-fostered (CF) or not cross-fostered (NCF) for 21 days following birth

Figure 17b: Mean ($\pm$ -SEM) escape latencies (seconds) across blocks of 5 trials for 5 acquisition days in handled and nonhandled BALB/cByJ (left panel) and C57BL/6ByJ mice (right panel) which were either cross-fostered (CF) or not cross-fostered (NCF) for 21 days following birth

treatments did not have additive effects, it is conceivable that early-life stimulation and cross-fostering influenced performance through similar mechanisms (e.g., having a C57BL/6ByJ mother may have resulted in endocrine/neurochemical changes comparable to that elicited by early-life stimulation). It is unlikely, however, that simply having a BALB/cByJ rearing mother was responsible for the induction of the behavioral deficits, since the performance of C57BL/6ByJ mice was not compromised in those animals that had been cross-fostered.

Consistent with the preceding studies, relative to C57BL/6ByJ mice, the BALB/cByJ mice made fewer goal quadrant entries and hits, took longer to reach the goal quadrant and spent significantly less time in this quadrant, $F(1, 54)=20.63; 19.72; 12.71; 9.39, p's<.01$. These particular differences were observed irrespective of the handling or cross-fostering conditions (see Figures 18a, 18b, 18c and 18d, respectively). Analyses of the types of errors made in each group, revealed that significant Strain x Cross-Fostering interactions were observed with respect to the number of misses in the northeast (adjacent to the goal quadrant) and the southeast quadrants (opposite to the goal quadrant), respectively, $F(1, 54)=9.15; 11.11, p's<.01$ (see Figures 19a and 19b). The cross-fostered BALB/cByJ mice made significantly more misses in both of these quadrants than noncross-fostered animals of the same strain or cross-fostered C57BL/6ByJ mice. This may have been due to differences in the number of quadrant changes among cross-fostered groups. Indeed, a significant Strain x Cross-Fostering interaction was observed with respect to this measure, $F(1, 54)=5.32, p<.05$ (see Figure 20). Newman-Keuls multiple comparisons revealed that cross-fostered BALB/cByJ mice made significantly more quadrant changes than noncross-fostered mice of the same strain and cross-fostered C57BL/6ByJ mice.

Insert Figures 18, 19 and 20 about here

Experiment 5: Early-Life Handling Effects on Stressor-Induced Neurochemical and Corticosterone Alterations

It has been shown that animals handled from birth to 21 days of age have altered adrenocortical and HPA responses to stress which persist throughout aging (Meaney, et al., 1988; Meaney, et al., 1991a). Relative to nonhandled animals, handled rats secrete less CRF, ACTH and corticosterone following a wide variety of stressors (Meaney, et al., 1988; Meaney, et al., 1985a; Meaney, et al., 1985b). It has been suggested that such effects are attributable to increased efficiency of HPA negative-feedback processes owing to increased type II corticosteroid receptor density in the hippocampus and the frontal cortex (Meaney, et al., 1985a; Meaney, et al., 1985b). With greater numbers of type II receptors in these brain regions, handled animals are able to respond more quickly and efficiently to stress-induced corticosterone elevations and ACTH release (Meaney, et al., 1989).

As discussed earlier, relative to other mouse strains, the BALB/cByJ is more vulnerable to stress-induced neurochemical and corticosterone alterations. Specifically, the BALB/cByJ mouse is more likely to show stress-induced norepinephrine (NE) changes in the hypothalamus, the locus coeruleus and the mesocortex, and dopamine (DA) alterations in the mesocortex and the VTA. (Herve, Tassin, Barthelemy, Blanc, Laviell, & Glowinski, 1979; Shanks, et al., 1991). Moreover, this strain also displays hypersecretion of corticosterone in response to stressors (Shanks, et al., 1990). Since handling influences Morris water-maze performance, it was of interest to determine whether this manipulation also affects physiological responses to stress. Experiment 5 examined the effects

- Figure 18a: Mean ($\pm$ -SEM) number of goal quadrant entries made during the Probe trial by handled and nonhandled BALB/cByJ and C57BL/6ByJ mice which were either cross-fostered (CF) or noncross-fostered (NCF) for 21 days following birth
- Figure 18b: Mean ($\pm$ -SEM) number of "hits" made during the Probe trial by handled and nonhandled BALB/cByJ and C57BL/6ByJ mice which were either cross-fostered (CF) or not cross-fostered (NCF) for 21 days following birth
- Figure 18c: Mean ($\pm$ -SEM) latencies (seconds) to reach the goal quadrant in the Probe trial in handled and nonhandled BALB/cByJ and C57BL/6ByJ mice which were either cross-fostered (CF) or not cross-fostered (NCF) for 21 days following birth
- Figure 18d: Mean ($\pm$ -SEM) proportion of time spent in the goal quadrant in the Probe trial in handled and nonhandled BALB/cByJ and C57BL/6ByJ mice which were either cross-fostered (CF) or not cross-fostered (NCF) for 21 days following birth

- Figure 19a: Mean ($\pm$ SEM) number of "misses" made in the northeast (NE) quadrant (the quadrant adjacent to the goal quadrant) in the Probe trial by handled and nonhandled BALB/cByJ and C57BL/6ByJ mice which were either cross-fostered (CF) or not cross-fostered (NCF) for 21 days following birth
- Figure 19b: Mean ($\pm$ SEM) number of "misses" made in the southeast (SE) quadrant (the quadrant opposite to the goal quadrant) in the Probe trial by handled and nonhandled BALB/cByJ and C57BL/6ByJ mice which were either cross-fostered (CF) or not cross-fostered (NCF) for 21 days following birth

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Figure 20: Mean ($\pm$ SEM) total number of quadrant entries made during the Probe trial by handled and nonhandled BALB/cByJ and C57BL/6ByJ mice which were either cross-fostered (CF) or not cross-fostered (NCF) for 21 days following birth

of postnatal handling on stress-induced alterations of plasma corticosterone and central catecholamines.

Method

Subjects

Ten BALB/cByJ and 10 C57BL/6ByJ female mice and 5 male mice of both strains (Jackson Laboratory, Bar Harbor, Maine) aged approximately 7 weeks served as a breeding colony. As in the preceding experiments, dams were removed from breeding cages when visibly pregnant and individually housed for approximately 5 days before giving birth. Dams were placed into cages with extra bedding 1-2 days prior to giving birth. Mouse pups were weaned at 22 days of age. A total of 72 BALB/cByJ and 66 C57BL/6ByJ male mice aged approximately 120-140 days were used. They were housed with their respective siblings in cages of 3 to 5 animals until experimentation began.

Apparatus & Procedure

Uncontrollable footshock was delivered in the Shock apparatus previously described. At birth, all litters were randomly assigned to either the Handled and Nonhandled conditions and exposed to the same handling procedures as previously outlined in Experiment 3a. After mouse weanlings reached 120-140 days of age, animals were randomly assigned to one of three treatment conditions: No Treatment, 60 Shock and the 360 Shock condition. Animals in the 60 Shock condition received 60 shocks over a period of 66 minutes whereas the 360 shock groups received 360 shocks over the same time period (150 mA, 2 seconds duration, 9 second intertrial time). The no treatment groups remained in their home cage until sacrifice.

Immediately after the stressor session, mice were sacrificed by decapitation. Brains were removed and sectioned on dry ice for neurochemical analysis using High Performance Liquid Chromatography (HPLC). Brain tissue was sectioned

using an aluminum dissecting block with 0.5 mm thick templates which were separated by brass plates of 0.25 mm in thickness. Each slot created by these brass plates served as a guide for singled edged razor blades. The brain slices were gently removed off of the blades onto precleaned glass microscope slides (Canlab Cat. #M6145) and immediately frozen on dry ice. Using a glass petri dish containing dry ice, brain slices were sectioned under a stereomicroscope illuminated by a fiber optic cool light source. Under low magnification, the prefrontal cortex, nucleus accumbens (Nas), caudate nucleus, the dorsal hippocampus, the substantia nigra (SN), the ventral tegmental area (VTA), and the locus coeruleus (LC) were punched out using hollow microdissection needles ranging from 22 to 16 gauge in diameter. The hypothalamus was removed in its entirety prior to the microdissection procedure. The hypothalamus and all of the microsections were placed and stored in round-welled titer trays (Falcon #3910, Baxter Corp.) at -70°C until HPLC analysis took place.

Brain tissue was analyzed for norepinephrine (NE) and its metabolite, 3-methoxy, 4-hydroxyphenylethylene (MHPG), serotonin (5-HT) and its metabolite, 5-hydroxyindoloacetic acid (5-HIAA), and dopamine (DA) and its respective metabolites, 3, 4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA) and 3-methoxytyramine (3-MT). High performance liquid chromatography (HPLC) with coulometric detection using a modified procedure of Seegal et al. (1986) was used to determine amine and metabolite concentrations in all microsectioned brain regions.

Frozen brain microsections were sonified in 0.3 M monochloroacetic acid containing 0.1 mM disodium ethylenediamine tetraacetate (EDTA - Fisher cat. #SS311-500). The protein content of each sample was measured colorometrically following the method of Lowry et al (1951) Brain homogenates were centrifuged at 1500 g (approximately 13,000 r.p.m), and the supernatants were filtered

through 0.2mm of regenerated cellulose fiber. Aliquots of each filtrate were injected onto a C18 reverse-phase column (Chromatography Sciences Company Inc.) in a high performance liquid chromatography (HPLC) system (Waters Co. #T12-WISP) equipped with a coulometric detector (ESA). The output signals from the detector were plotted and measured using a Hewlett-Packard HP-3390A plotting integrator. The mobile phase used for separation of all catecholamines and their metabolites was 1.3 g/L heptanesulfonic acid, HSA (Fisher #0-3013), 7.3 g/L triethylamine (Fisher #04884), 0.1 g/L disodium ethylene tetraacetate, EDTA (Fisher #S311) and 60 ml/L acetonitrile (Fisher #998B). The pH of the mobile phase was adjusted to 2.45 with phosphoric acid (Fisher #H260). Prior to use, the mobile phase was filtered with a 0.22 mm filter paper. The flow rate of the mobile phase was adjusted to 1.5 ml/minute. For NE, MHPG, DA, DOPAC, and 3-MT, working standards were used at a 50 ng/ml concentration, and for 5-HT, 5-HIAA and HVA, working standards of 100 ng/ml were used.

Trunk blood was collected and centrifuged at 700 g. Plasma samples were frozen and stored at -70°C. Plasma corticosterone levels were determined using rat H₃ corticosterone radioimmunoassay kits (ICN Chemicals, Montreal, Quebec, Cat. # 07-120002).

Results and Discussion

Each of the central amine and metabolite levels were analyzed independently. Since some brain samples were lost during the microdissection and HPLC procedures, the degrees of freedom varied across brain areas.

Mean concentrations of hypothalamic NE and MHPG in ng/ml of protein are shown in Figure 21. In accordance with earlier reports, exposure to stress reliably altered levels of hypothalamic NE and that of MHPG, $F(2, 119)=5.21$; 8.11 $p's<.01$. Multiple comparisons confirmed that both 60 and 360 shocks increased the accumulation of the metabolite and reduced the concentrations of NE. None

of the main effects or interactions involving the strain or handling variables approached statistical significance.

Commensurate with the hypothalamus, locus coeruleus NE and MHPG levels varied with the shock treatment, $F(2, 119)=13.41, 23.46$ $p's<.01$ (see Figure 21). Not unexpectedly, shock treatments reduced the concentrations of NE and increased the accumulation of MHPG, but the magnitude of these effect did not vary with the number of shocks applied.

 Insert Figure 21 about here

As in the case of the locus coeruleus, analysis of NE and MHPG in prefrontal cortex confirmed previous findings (Shanks, Griffiths & Anisman, 1994; Shanks et al, 1991) that shock treatment increased MHPG accumulation, $F(2, 126)=10.71$, $p<.01$. The NE and MHPG means for each group are presented in Figure 22. The multiple comparisons indeed revealed that both shock groups displayed elevated accumulation of the metabolite. In contrast, NE levels were not affected by the shock treatment, although levels of the amine were greater in C57BL/6ByJ than in BALB/cByJ mice, $F(1, 126)=9.26$, $p<.01$. It might be noted that as reported by Shanks et al (1994; 1991), there was a modest trend for stressor-induced NE reductions to be more pronounced in BALB/cByJ than in C57BL/6ByJ mice, $F(2, 126)= 1.86$, $p=.16$.

The stressor treatment did not influence concentration of hippocampal NE (see Figure 22). However, as observed in the other brain regions, the accumulation of MHPG was found to vary with the shock treatment, $F(2, 112)=9.21$, $p<.01$. Once again, it appeared that both the 60 and 360 shock treatments increased the metabolite concentrations to a similar extent. Early-life handling was found to have the effect of reducing hippocampal NE

Figure 21: Mean ($\pm$ SEM) concentrations (ng/mg protein) of NE (left panels) and MHPG (right panels) in the hypothalamus and the locus coeruleus in handled and nonhandled BALB/cByJ and C57BL/6ByJ mice immediately following exposure to either 60 footshocks, 360 footshocks or no treatment

concentrations, $F(1, 113)=3.30$, $p<.05$, while MHPG accumulation varied as a function of the Strain x Handling interaction, $F(1, 112)=5.02$, $p<.05$. Newman-Keuls multiple comparisons revealed that in nonhandled BALB/cByJ mice MHPG was higher than in C57BL/6ByJ mice. Handling produced small MHPG increase in the latter mice and a modest decrease in the former strain. As a result the between strain difference was abrogated.

 Insert Figure 22 about here

Mean concentrations of DA and DOPAC within the ventral tegmentum are presented in Figure 23. The concentrations of DA within the ventral tegmentum were found to vary as a function of the Strain x Shock interaction, $F(2, 114)=9.76$, $p<.01$. Newman-Keuls multiple comparisons indicated that both shock treatments reduced DA concentrations significantly in BALB/cByJ mice. Conversely, in C57BL/6ByJ mice the shock treatments increased the levels of DA, although only the effect of 360 shocks was statistically significant. The accumulation of DOPAC was increased by the shock treatment, $F(2, 115)=10.85$, $p<.01$, whereas HVA levels were not altered. Finally, the extraneuronal DA metabolite, 3-MT, did not vary significantly with the shock treatment or any of the interactions involving shock (see Figure 23).

 Insert Figure 23 about here

Within the nucleus accumbens the concentration of DA did not vary significantly with the shock treatment, although a reduction of approximately 10 percent was induced by the stressor (see Figure 24). This finding is consistent with that of earlier reports suggesting that although reductions in DA concentrations

Figure 22: Mean ($\pm$ -SEM) concentrations (ng/mg protein) of NE (left panels) and MHPG (right panels) in the prefrontal cortex and the hippocampus in handled and nonhandled BALB/cByJ and C57BL/6ByJ mice immediately following exposure to either 60 footshocks, 360 footshocks or no treatment

Figure 23: Mean ($\pm$ -SEM) concentrations (ng/mg protein) of DA, DOPAC, HVA and 3-MT in the ventral tegmental area (VTA) in handled and nonhandled BALB/cByJ and C57BL/6ByJ mice immediately following exposure to either 60 footshocks, 360 footshocks or no treatment

have been observed within the BALB/cByJ strain, DA levels within the accumbens were not readily altered by footshock in several other mouse strains (Shanks et al., 1994; Shanks et al, 1991). In contrast to the DA levels, the DOPAC accumulation was substantially increased by the shock treatment, $F(2, 119)=4.5$, $p < .05$, and this was the case in both stressor conditions. Likewise, DOPAC accumulation was greater in C57BL/6ByJ than in BALB/cByJ mice, $F(1, 119)=6.22$, $p < .05$. Interestingly, the accumulation of HVA, was lower in the C57BL/6ByJ strain, $F(1, 119)=10.95$, $p < .01$. The latter two findings might suggest the route of degradation of DA varied between strains. Indeed, Shanks et al (1991, 1994) had previously reported such findings, although these investigators also indicated that stressor exposure differentially influenced the two metabolites in the different strains (see Figure 24).

Concentrations of the extraneuronal DA metabolite, 3-MT, within the nucleus accumbens were found to vary as a function of the Strain x Shock x Handling interaction, $F(2, 119)=2.93$, $p=.05$. It appeared that the stressor treatments influenced the metabolite concentrations, but that such effects were strain- and handling-specific. In particular, Newman-Keuls multiple comparisons ($\alpha=.05$) indicated that in nonhandled BALB/cByJ mice the 360 shock treatment greatly increased 3-MT whereas in handled mice of this strain the increase was marginal. Interestingly, in nonhandled C57BL/6ByJ mice the shock treatment did not affect 3-MT, whereas in handled C57BL/6ByJ mice both the 60 and 360 shock treatments provoked marked increases in the metabolite concentrations.

 Insert Figure 24 about here

Typically, DA concentrations in the prefrontal cortex have been found to decline following stressor exposure (Dunn, 1988; Puglisi-Allegra, Imperato,

Figure 24: Mean ($\pm$ SEM) concentrations (ng/mg protein) of DA, DOPAC, HVA and 3-MT in the nucleus accumbens in handled and nonhandled BALB/cByJ and C57BL/6ByJ mice immediately following exposure to either 60 footshocks, 360 footshocks or no treatment

Angelucci, & Cabib, 1991; Shanks, et al., 1991). In the present investigation this was not observed to be the case. Among nonhandled mice of either strain DA levels were unaffected by footshock, whereas in handled BALB/cByJ mice DA levels actually increased markedly following 360 shocks, but were unaffected by the lesser shock treatment. Consistent with these strain- and handling-specific effects of shock, the analysis of variance indicated that DA levels varied as a function of the Strain x Shock x Handling interaction, $F(2, 125)=3.01$, $p=.05$.

It has been reported consistently that stressors increase the accumulation of DA metabolites within the prefrontal cortex. While most studies involving rats have revealed that stressors reliably increase DOPAC accumulation in this region (Cabib, Kempf, Schleef, Oliverio, & Puglisi-Allegra, 1988), studies in mice have indicated that the changes of HVA are more dramatic than those of DA (e.g., Shanks, Griffiths & Anisman, 1994). In the present investigation, the levels of DOPAC in the prefrontal cortex were not increased with footshock treatment. Quite the contrary, the stressor treatments reduced DOPAC significantly in nonhandled BALB/cByJ mice, while producing a small nonsignificant increase in handled BALB/cByJ mice. Conversely, in nonhandled C57BL/6ByJ mice DOPAC was not affected, while a significant decline was apparent in handled C57BL/6ByJ mice. Indeed, the analysis of variance revealed a significant Strain x Handling x Shock interaction, $F(2, 122)=3.25$, $p<.05$, and the specific differences outlined above were confirmed by Newman-Keuls multiple comparisons. In contrast to DOPAC, however, accumulation of HVA was increased by stressors in a strain-specific fashion, $F(2, 106)=3.68$, $p<.05$. The multiple comparisons confirmed that in BALB/cByJ mice the shock treatments increased HVA accumulation, whereas in C57BL/6ByJ mice, there was not a significant increase of the metabolite. Finally, 3-MT was found to be reduced by the shock treatments, $F(2, 111)=3.18$, $p<.05$. The Strain x Shock interaction did not reach

statistical significance; however, it did appear that the reductions of 3-MT were more pronounced in C57BL/6ByJ than in BALB/cByJ mice (see Figure 25).

 Insert Figure 25 about here

Mean concentrations of hypothalamic 5-HT and 5-HIAA as a function of shock treatment are presented in Figure 26. Analysis of variance revealed that concentrations of hypothalamic 5-HT were greater in C57BL/6ByJ than BALB/cByJ mice, $F(1, 120)=8.03$, $p<.05$. The accumulation of hypothalamic 5-HIAA was found to vary as a function of the Strain and Shock interaction, $F(2, 117)=4.58$, $p<.01$. Newman-Keuls comparisons revealed that among BALB/cByJ mice the two shock treatments significantly increased 5-HIAA accumulation, whereas in C57BL/6ByJ mice a small nonsignificant decline was evident.

Hippocampal 5-HT varied as a function of the Strain x Shock interaction, $F(2, 105)=3.64$, $p<.05$ (see Figure 26). The multiple comparisons revealed that the shock treatment did not affect 5-HT levels in BALB/cByJ mice. In contrast, in the C57BL/6ByJ strain, both the 60 and 360 shock treatments significantly reduced the amine concentrations. The accumulation of 5-HIAA was not found to vary across strains of mice, or as a function of the shock and/or handling treatments.

Finally, within the prefrontal cortex the shock treatment was found to reduce the concentrations of 5-HT, $F(2, 91)=4.19$, $p<.05$ (see Figure 26). This effect did not interact with either the strain or handling manipulation. The accumulation of 5-HIAA did not vary with the shock treatment or the strains. There was a modest decline of the metabolite in handled C57BL/6ByJ mice relative to nonhandled mice of this strain, whereas no such change was evident in BALB/cByJ mice, $F(1, 114)=3.14$, $p=.08$.

Figure 25: Mean ($\pm$ -SEM) concentrations (ng/mg protein) of DA, DOPAC, HVA and 3-MT in the prefrontal cortex in handled and nonhandled BALB/cByJ and C57BL/6ByJ mice immediately following exposure to either 60 footshocks, 360 footshocks or no treatment

Insert Figure 26 about here

Mean plasma corticosterone concentrations as a function of shock treatment are illustrated in Figure 27. Several samples were lost owing to assay difficulties. Analysis of variance revealed that corticosterone concentrations were greatly increased by the Shock treatment, $F(2, 105)=86.81$, $p<.01$. Contrary to earlier work in the present laboratory (Shanks, et al., 1990), there were no differential effects observed with respect to the shock treatment in the two strains of mice. This was surprising given the marked strain-specific effects of stressors on corticosterone concentrations previously observed in this laboratory (Shanks, Griffiths, & Anisman, 1994; Shanks, et al., 1990). In the present study, mean corticosterone concentrations in the non treated groups of both strains were higher than previously reported, particularly in the C57BL/6ByJ mice. Moreover, the shock treatment in both the C57BL/6ByJ and BALB/cByJ strains induced corticosterone increases appreciably greater than those previously observed (Shanks, et al., 1990). Despite the differences in the results between the present investigation and those previously, the general patterns were similar. Accordingly, a separate analysis was conducted to assess the changes of corticosterone as a percentage of nonstressed animals. The results thus obtained were indeed reminiscent of those reported by Shanks et al (1990). These mean percent changes in corticosterone are illustrated in Figure 27.

The change of plasma corticosterone levels varied as a function of the Strain x Handling x Shock interaction, $F(2, 105)=9.77$, $p<.01$. Plasma corticosterone concentrations were appreciably increased by the shock treatment in both strains; however, the extent of the change was greater in BALB/cByJ than in C57BL/6ByJ mice. In particular, following stressor exposure the percent increase

Figure 26: Mean ($\pm$ SEM) concentrations (ng/mg protein) of 5-HT (left panels) and 5-HIAA (right panels) in the hypothalamus, hippocampus, and in the prefrontal cortex in handled and nonhandled BALB/cByJ and C57BL/6ByJ mice immediately following exposure to either 60 footshocks, 360 footshocks or no treatment

of corticosterone in the former mice significantly exceeded that seen in the latter. The handling procedure influenced the stress-induced corticosterone changes in a strain-dependent manner. Nonhandled BALB/cByJ mice exposed to either 60- or 360-shocks exhibited significantly higher changes in plasma corticosterone than did handled mice of the same strain or C57BL/6ByJ mice that were similarly treated. Contrary to the pattern observed in the BALB/cByJ strain, handling in C57BL/6ByJ mice resulted in a greater change in corticosterone values in comparison to nonhandled animals.

 Insert Figure 27 about here

It might be noted that the studies of Shanks et al were performed using a fluorometric analysis of corticosterone, whereas in the present investigation a radioimmunoassay (RIA) procedure was employed. Inasmuch as the antibody employed in the RIA, however, was one for rat corticosterone, it is tempting to speculate that nonspecificity of the assay may have limited detection of potential strain differences or effects of handling. Accordingly, in a separate experiment we assessed the effects of handling and shock treatment on corticosterone using a mouse antibody in the RIA. The results of this study are shown in Figure 28 (n=10-14/group). We observed that corticosterone levels in the absence of stressor exposure did not differ between strains. The Shock treatment (360 shocks only) increased plasma corticosterone in both strains, but this effect was significantly greater in BALB/cByJ mice, $F(1, 94)=26.63$, $p<.01$. Of particular interest, however, was the finding that if BALB/cByJ mice received early-life handling, the increased corticosterone accumulations ordinarily provoked by stress was limited, $F(1, 94)=3.29$, $p=.07$. In fact, among handled BALB/cByJ mice,

Figure 27a: Mean ($\pm$ -SEM) plasma corticosterone concentrations (mg/dl) in handled and nonhandled BALB/cByJ and C57BL/6ByJ mice immediately following exposure to either 60 footshocks, 360 footshocks or no treatment using a radioimmunoassay with a rat antibody for corticosterone

Figure 27b: Mean ($\pm$ -SEM) percent change (from control values) in plasma corticosterone concentrations in handled and nonhandled BALB/cByJ and C57BL/6ByJ mice immediately following exposure to either 60 footshocks, 360 footshocks or no treatment

stress levels of corticosterone were comparable to those observed in C57BL/6ByJ mice.

Like corticosterone, plasma levels of ACTH was increased by stressor application in both strains. As in the case of corticosterone, the stressor provoked a greater increase in ACTH in BALB/cByJ than in C57BL/6ByJ mice, $F(1, 89)=9.55$, $p<.01$. However, handling hardly affected ACTH in either strain, regardless of shock treatment (see Figure 28).

 Insert Figure 28 about here

General Discussion

Consistent with previous reports (Francis, et al., 1991; Francis, et al., 1994), it was observed in the present investigation that the performance of BALB/cByJ mice in a Morris water-maze task was vastly inferior to that of C57BL/6ByJ mice. A substantial number of BALB/cByJ mice tested in this paradigm exhibited little or no between-days improvement of performance. Moreover, as reported by Francis et al (1991), in some BALB/cByJ mice learning appeared to progress during the initial testing days, but was then followed by a deterioration of performance. Upon examination of swimming behavior during the Probe trial, C57BL/6ByJ mice demonstrated their superior response acquisition with increased entries, annulus crossings and time spent in the goal quadrant. In contrast, the BALB/cByJ mice made fewer annulus crossings, fewer goal quadrant entries and spent less time in the goal quadrant, attesting to the weakness in their spatial-learning during the acquisition period. Relative to the former strain, BALB/cByJ mice tended to display a random swimming pattern, such that both the number of entries and the time spent in the goal quadrant and in each of the non-goal quadrants were comparable. Interestingly, it seemed that

- Figure 28a:** Mean ($\pm$ -SEM) plasma corticosterone (mg/dl) concentrations (mg/dl) in handled and nonhandled BALB/cByJ and C57BL/6ByJ mice immediately following exposure to either 360 footshocks or no treatment using a radioimmunoassay with a mouse corticosterone antibody
- Figure 28b:** Mean ($\pm$ -SEM) plasma ACTH concentrations (pg/ml) in handled and nonhandled BALB/cByJ and C57BL/6ByJ mice immediately following exposure to either 360 footshocks or no treatment

the two strains could also be distinguished from one another with respect to the area of the pool (apart from the goal quadrant) in which time was spent. It appeared that when C57BL/6ByJ mice were not exploring the goal quadrant, they were more likely to spend time in the adjacent quadrants. The BALB/cByJ mice, in contrast, tended to venture further afield and spent more time in the area furthest from the goal quadrant.

It has been suggested that the apparent inability of BALB/cByJ mice to acquire the Morris water-maze response is a reflection of the visual disturbances associated with the albinism of this strain (Upchurch & Wehner, 1988). Both the present data, and previous data from this laboratory, do not support this position. In particular, in the present study approximately 20 percent of the BALB/cByJ mice tested became proficient in the Morris water-maze task. Likewise, in earlier reports 8-20 percent of BALB/cByJ mice acquired the escape response. Clearly, albinism per se in these animals was not sufficient to prevent learning. Besides, we have observed that the CD-1 mouse strain, which is also albino, readily acquired this response (Francis, et al., 1991; Francis, et al., 1994).

Recent studies in our laboratory have demonstrated that the impairment observed in BALB/cByJ strain is not related to their swimming speeds (Zaharia, Shanks, & Anisman, 1994). Specifically, strain differences were not observed with respect to the number of squares crossed per unit time. Moreover, it did not appear that the strain differences were related to the feedback procedures that were employed. In particular, in the present experiments when mice landed on the platform they were removed after a few seconds, and when an escape failure occurred the mouse was kept in a holding cage during the intertrial interval. Inasmuch as escape failures were more frequent in the BALB/cByJ mice, it could be supposed that they also received less feedback concerning the safety associated with the platform. In an additional experiment conducted in this

laboratory ($n=8/\text{group}$), however, we observed that placement on the platform following a failure trial did influence overall learning relative to mice that were not placed on the platform following escape failures.

It will be recalled that spatial learning and memory deficits are associated with hippocampal cell loss, particularly within the CA1, CA3 and dentate gyrus (Eichenbaum & Otto, 1992; Landfield, et al., 1978; McEwen, Watanabe, Gould, & Cameron, 1992; Meaney, et al., 1988; Sapolsky, et al., 1985). Commensurate with such findings, behavioral impairments have been reported to be particularly marked in aged rats that exhibit the greatest hippocampal cell loss (Landfield, 1987; Landfield, et al., 1981; Landfield, et al., 1978; Sapolsky, et al., 1985). When tested in a Morris water-maze, those aged rats which exhibited impaired Morris water-maze performance were also those shown to have higher basal corticosterone levels and greater hippocampal pyramidal cell loss relative to aged rats that did not exhibit behavioral impairments (Issa, Rowe, Gauthier, & Meaney, 1990). Furthermore, it was reported that age-related hippocampal cell/receptor loss was exacerbated by stress-induced glucocorticoid release, as well as by exogenous corticosterone administration (Bodnoff, Humphreys, Rose, & Meaney, 1992; Meaney, et al., 1988; Sapolsky, 1992; Sapolsky, et al., 1985). Inasmuch as BALB/cByJ mice display hypercorticoid secretion in response to stressors, we hypothesized that the poor performance of BALB/cByJ mice in the Morris water-maze may have been a manifestation of hippocampal dysfunction (recall that hippocampal cell loss or loss of corticoid receptors leads to excessive corticosterone release). Moreover, the possibility was considered that such an effect may have reflected early degeneration of hippocampal neurons and/or type II glucocorticoid receptors, rather than an innate characteristic of this strain. Given that performance of 90 day old BALB/cByJ mice was actually somewhat superior to that of younger mice of the same strain, the impairments of

performance could not be attributed to early aging. However, it is certainly possible that these deficits developed during the first 30 days postpartum. As mice cannot be tested prior to this age (mice younger than 30 days are not proficient swimmers) it could not be determined whether the behavioral disturbances are related to developmental degenerative processes.

Despite the fact that the performance deficits in BALB/cByJ mice do not appear to be progressive between 30 and 90 days of age, a role for hippocampal disturbances should not be excluded. It will be recalled that hippocampal damage is associated with spatial learning disturbances in the Morris water-maze, whereas cued learning is unaffected by damage to this region (Aggleton, Hunt, & Rawlins, 1986). In a like fashion, in the present investigation the deficits of performance of BALB/cByJ mice tested in the spatial task were largely eliminated when mice were tested with visual cues present. Yet, it should be noted that even after 5 days of training in the cued condition, the performance of BALB/cByJ mice was still somewhat inferior to that of C57BL/6ByJ mice. Whether this difference reflects nonassociative factors (e.g., motor effects, attentional disturbances), or factors related to learning ability remains to be determined. Nevertheless, it does appear that the deficits present in BALB/cByJ mice are of a spatial learning nature, and thus might reflect hippocampal involvement.

The deficits in Morris water-maze performance observed in BALB/cByJ mice were modifiable by the early postnatal handling procedure. Marked decreases in average latencies and escape failures were observed among handled mice of this strain. In contrast, handling did not affect performance among C57BL/6ByJ mice, where a high level of proficiency was demonstrated even in the absence of handling. Although the exposure to handling resulted in shorter response latencies and fewer escape failures among BALB/cByJ mice when the platform was present, handling did not influence the strength of association of extramaze

cues during the Probe trial (i.e., when the platform was not present). Indeed, handled and nonhandled BALB/cByJ mice did not differ from one another with respect to the number of entries, "hits" or amount of time spent in the acquisition quadrant. These findings essentially parallel the observation that handling prevented the age-related decline of Morris water-maze performance previously observed in Long-Evans rats. In these studies, aged animals with the most severe Morris water-maze response impairments were those that displayed the most pronounced hippocampal cell loss and the most prolonged elevations of corticosterone following stressor exposure (Issa, Rowe, Gauthier, & Meaney, 1990). Although, we did not observe age-related decrements in escape responding among BALB/cByJ mice, it is conceivable that the handling-induced improvements in swim performance were a reflection of mechanisms similar to those which "protected" against the age-related decrements of Morris water-maze performance.

The vulnerability to performance disturbances among BALB/cByJ mice exposed to a stressor has previously been reported (Francis, et al., 1994). Specifically, it was found that in BALB/cByJ mice reversal of a Morris water-maze response was severely retarded by stressor exposure. In contrast, in C57BL/6ByJ mice, reversal learning was not disrupted by such a manipulation. The results of the present investigation likewise revealed that footshock stress could hamper Morris water-maze performance in BALB/cByJ mice. Unlike the study of Francis et al. (1994), where reversal performance was evaluated in very well trained mice (8 trials/day over 7 days), in the present investigation it was demonstrated that acquisition performance could be disrupted in mice that had not previously received extensive training. This finding was contrary to that of other investigators who had reported that inescapable shock did not disrupt Morris water-maze performance among rats (Warren, et al., 1991) and suggested

that uncontrollable stressors did not influence a place learning response. The present results, like those of Francis et al., (1991; 1994) suggest that performance in this paradigm is, in fact, vulnerable to disruption, but that such an outcome is dependent on the strain of animal employed, and occurs primarily when the response is not yet well entrenched. In addition, the present findings suggest that although early-life handling may reduce the performance deficits that are characteristic of BALB/cByJ mice, such a manipulation does not protect against the behavioral disturbances engendered by a relatively strong stressor, such as footshock.

As alluded to earlier, several mechanisms have been suggested to account for the observed effects on HPA activity, hippocampal dysfunction and Morris water-maze performance among animals exposed to early-life handling. For instance, it has been argued that the effects of early-life handling may be related to activation of serotonergic and cAMP processes. It was suggested that temporary separation from the dam results in mild temperature drops in pups, which in turn, affects hippocampal type II receptor development (Meaney, et al., 1991c; Mitchell, et al., 1992; Mitchell, et al., 1990a; Mitchell, et al., 1990b). Other possible mechanisms that may have contributed to these observations involve alterations of maternal behaviors resulting from the daily handling procedure. As indicated earlier, maternal behaviors, such as feeding and anogenital licking, have been demonstrated to differentially affect HPA activity; maternal feeding has been reported to suppress pups' corticosterone secretion, whereas stroking inhibits ACTH secretion (Suchecki, et al., 1993). Since the BALB/cByJ mouse is a highly emotional animal (Shanks & Anisman, 1988; Shanks, et al., 1990), the possibility was considered that removal of pups from the dam may have influenced her subsequent interactions with these pups, hence leading to behavioral changes. Accordingly, it was of interest to ascertain whether (a) the

poor performance of BALB/cByJ mice was due to maternal factors, as opposed to either genetic or other environmental events, and (b) the effects of early handling were related to the stimulation per se, or was instead secondary to maternal factors.

The Morris water-maze performance among C57BL/6ByJ mice was not affected by either handling or cross-fostering. In contrast, it was observed that nonhandled BALB/cByJ mice cross-fostered with a C57BL/6ByJ dam exhibited shorter response latencies and fewer escape failures than nonhandled, noncross-fostered mice of the same strain. This finding is consistent with the notion that the behavior of BALB/cByJ mice was related to maternal factors. It is important to note, however, that although both handling and cross-fostering influenced performance of BALB/cByJ mice, the two procedures did not have additive effects. Thus, it is conceivable that improvements in escape performance resulting from either the cross-fostering or handling procedures may have involved the same mechanism(s). Certainly the processes subserving the effects of early-life handling appear to be exceedingly complex, and likely involve numerous mechanisms (e.g. temperature-induced thyroid hormonal and serotonergic activation, secondary messenger systems such as the cAMP system and the possible involvement of protein kinase A. Nevertheless, the possibility cannot be dismissed that poor maternal behaviors would have effects comparable to that of repeated, transient separation from the dam. Regardless of the mechanisms involved in the cross-fostering effects, it was clear that having a BALB/cByJ mother did not induce spatial learning deficits per se. Rather, the influence of having a BALB/cByJ genetic background (which may have imparted increased vulnerability to stressor-related disturbance) coupled with a BALB/cByJ dam was more likely to culminate in these particular behavioral deficits. Conversely, having

a stronger, more stress-resistant genetic backdrop may "protect" the organism from possible deficits that might otherwise be shaped by maternal behavior.

The neurochemical profiles and plasma corticosterone levels of handled and nonhandled mice were examined in order to determine whether the exposure to postnatal handling would affect stress-induced neurochemical alterations. In the main, it was observed that stressors influenced neurochemical activity, but the effects of strain and handling were limited. Stress reliably reduced NE with corresponding increases of MHPG in the hypothalamus and the locus coeruleus, regardless of the strain and handling condition. In the prefrontal cortex, stress increased MHPG without an accompanying change of NE. Of particular interest, however, was the finding that in both mouse strains handling resulted in lower concentrations of hippocampal NE, while, MHPG accumulation was affected by the interaction between strain and handling exposure. Specifically, nonhandled BALB/cByJ mice had greater accumulation of hippocampal MHPG than did either handled mice of this strain or nonhandled C57BL/6ByJ mice. Given that the BALB/cByJ mouse strain is particularly vulnerable to stressors, manifested by marked behavioral, neurochemical and corticosterone alterations, it is conceivable that stressors similarly have a marked influence on hippocampal type II receptors in these mice, just as previously observed in rats. Moreover, it is not unreasonable to assume that these changes may involve a NE-mediated mechanism. A number of NE cells within the brainstem project to the hippocampus and, the effects of NE input on hippocampal cells is suppressed by corticosterone (Joels & DeKloet, 1989; Moore & Bloom, 1979). Moreover, it has been shown that 6-OHDA lesioning of NE pathways within the pendunculus cerebellaris superior (PCS) followed by corticosterone replacement administration decreases the affinity of hippocampal type II receptors (Maccari, Mormede, Piazza, Simon, Angelucci, & Moal, 1992). Since the BALB/c and BALB/cByJ strains exhibit both elevated

hippocampal MHPG levels following stress (Shanks, et al., 1991; Shanks et al., 1994) as well as increased hippocampal corticosterone binding capacity relative to the C57BL/6J strain, (Patacchioli, Puglisi-Allegra, Maccari, Casolini, Angelucci, Cabib, et al., 1990) it is possible that a NE mechanism may be operative in the alterations of hippocampal NE and MHPG in the BALB/cByJ strain. Indeed, we observed that nonhandled BALB/cByJ mice had elevated levels of hippocampal MHPG in comparison to nonhandled C57BL/6ByJ mice. More importantly, exposure to handling resulted in a slight increase of hippocampal MHPG in C57BL/6ByJ mice, while provoking an opposite effect among BALB/cByJ mice, such that the interstrain differences in this metabolite were eliminated.

The stress-induced alterations of DA in the VTA were more dependent upon the strain of mouse than previous handling exposure. For example, DA concentrations in the VTA were affected by shock. However, in the BALB/cByJ strain, DA was affected by both 60 and 360 shocks, whereas, among C57BL/6ByJ mice, only 360 shocks induced DA reductions in the VTA. As observed previously (Shanks, et al., 1991), exposure to shock reliably increased DOPAC concentrations in this brain region. In the nucleus accumbens, it appeared that BALB/cByJ and C57BL/6ByJ mice had distinctive patterns of DA degradation. Exposure to shock increased DOPAC in both strains, but such an effect was greater in C57BL/6ByJ mice. Additionally, HVA concentrations were lower in C57BL/6ByJ mice suggesting that the stressor may have influenced the route of DA degradation.

Postnatal handling affected DA utilization in the prefrontal cortex and the nucleus accumbens. Dopamine levels in the prefrontal cortex were elevated in handled BALB/cByJ mice following stressor exposure, whereas this was not the case in nonhandled mice. Additionally, DOPAC levels were not affected by shock with the exception of BALB/cByJ nonhandled mice, where prefrontal DOPAC

levels were reduced. These observations were contrary to expected since previous reports had shown stress-induced reductions in prefrontal cortex DA levels (Dunn, 1988; Puglisi-Allegra, Imperato, Angelucci, & Cabib, 1991; Shanks et al., 1991) coupled with increased DOPAC accumulation (Cabib, Kempf, Schlee, Mele, & Puglisi-Allegra, 1988). Interestingly, among nontreated, nonhandled BALB/cByJ mice, DOPAC levels were elevated in comparison to handled mice. It is conceivable that the basal DA utilization in BALB/cByJ mice (in the absence of handling) may have been relatively high, thereby limiting further increases of DA utilization elicited by stressor exposure. Of course, this in itself would not account for the fact that other investigators have reported increases of DA utilization following stressor exposure. However, since the purpose of the study was explicitly to assess the effects of early-life experiences, it is possible that relative to other studies, in the present investigation greater attention was devoted to assuring consistent early-life experiences across groups.

Paralleling the behavioral effects of stressors, analyses of 3-MT levels within the nucleus accumbens revealed strain-specific effects of the footshock treatment that were influenced by handling exposure. In particular, among BALB/cByJ mice that had not been handled, the 3-MT levels were increased, whereas this was not the case in handled mice. In contrast, in C57BL/6ByJ mice, increases in 3-MT levels following stress were only observed in the handled animals. It is not clear, of course whether the strain-specific alterations were responsible for the behavioral alterations, or were a reflection of the increased reactivity to the stressor. Specifically, it has been reported that accumbal DA alterations, including variations of 3-MT, are a sensitive index of the reactivity or arousal associated with stressor exposure (Fadda, Argiolas, Melis, Tisari, Onali, & Gessa, 1978; Puglisi-Allegra, et al., 1991; Roth, Tam, Ida, Yang, & Deutch, 1988). Thus, the

present findings might suggest that handling served to diminish reactivity to stressors in BALB/cByJ mice.

The nature of dopaminergic involvement in the effects of handling among the two mouse strains is not clear, since DA utilization patterns in the prefrontal cortex and the nucleus accumbens of the present investigation were not always consistent with previous reports (Shanks et al., 1994; Shanks et al., 1991). However, the possible role of early-life stimulation on subsequent behavior and central DA activity in adulthood should not be ignored. It was recently demonstrated that 6-OHDA lesions of the VTA resulted in reduced basal and stress-induced plasma corticosterone concentrations, as well as an increase in type II glucocorticoid receptors within the ventral striatum. Moreover, decreases in hippocampal type II receptor affinity were observed following the intraperitoneal administration of the D1 receptor agonist, SKF38393, suggesting that DA may influence type II corticoid receptor affinity (Casolini, Kabbaj, Piazza, Rouge-Pont, Angelucci, Simon, et al., 1993). Given that corticosterone levels or glucocorticoid receptor sensitivity may be affected by early-life experiences, and that early-life handling influences DA release ordinarily induced by stressors, it is tempting to speculate that the behavioral changes associated with early-life stimulation may involve the conjoint effects of central DA and adrenal glucocorticoids.

It has been maintained that the effects of handling on performance of aged rats may involve serotonergic mechanisms. As discussed earlier, developmental changes of serotonin, particularly within the hippocampus, paralleled that of glucocorticoid receptor binding (Mitchell, et al., 1990a; Rosenfield, et al., 1988). Early-life handling or administration of thyroid hormone had the effect of increasing both hippocampal 5-HT turnover and corticosterone receptor binding. Moreover, administration of a serotonergic neurotoxin resulted in diminished type

II corticosterone binding in this region, while treatment with the glucocorticoid receptor antagonist, ketansarin, antagonized the altered glucocorticoid binding ordinarily induced by handling (Mitchell, et al., 1990a). In effect, it seems that early-life manipulations may alter glucocorticoid receptor sensitivity in a permanent fashion, and that 5-HT facilitates this process.

In the present investigation stressors influenced 5-HT levels or activity in some brain regions. For instance, footshock reduced frontal cortex 5-HT, and in the C57BL/6ByJ mice hippocampal 5-HT was reduced, as well. In hypothalamus, 5-HIAA accumulation was increased in BALB/cByJ mice. However, neither 5-HT nor 5-HIAA varied as a function of Handling or the Handling x Stress interaction, with the exception of the prefrontal cortex, where handling exerted a modest decline in stress-induced 5-HIAA levels among C57BL/6ByJ mice. Thus, while it is possible that the performance disturbances in BALB/cByJ mice may involve a serotonergic component, there was no evidence in the present investigation that serotonergic mechanisms accounted for the enhanced performance induced by handling in BALB/cByJ mice. Furthermore, there was no reason to suppose that handling influenced stress-induced serotonergic alterations. Previous reports had suggested that environmental stimulation permanently alters hippocampal type II receptor number and binding, and that serotonergic processes may be involved in this respect (Meaney & Aitken, 1985; Meaney, et al., 1989). Inasmuch as the present investigation was limited to assessment of levels and utilization of 5-HT, as opposed to analyses of receptor changes, it premature to make conclusions regarding the contribution of serotonergic mechanisms in subserving the strain and handling effects on performance in a spatial learning paradigm.

It will be recalled that in aging rats corticosterone levels increase, presumably owing to degeneration of hippocampal receptors (Jacobson & Sapolsky, 1991; Meaney, et al., 1988; Sapolsky, et al., 1986). Moreover, in aged

animals that exhibit performance deficits in the Morris water-maze, hippocampal cell loss is more profound than in animals that exhibit proficient performance. Corticoid and adrenal manipulations have been demonstrated to alter exploratory patterns (Veldhuis, Kloet, Zoest, & Bohus, 1982), and subtle hippocampal alterations and disturbances of Morris water-maze behavior may result from increased circulating glucocorticoids (Dachir, Kadar, Robinson, & Levy, 1993; Sapolsky, 1992). Since corticoid manipulations influence hippocampal glucocorticoid receptors, and affect cell loss, it is thought that manipulations such as early-life handling may be acting through hypothalamic-pituitary-adrenal processes (Meaney, et al., 1988; Meaney, et al., 1991a; Meaney, et al., 1991c; Sapolsky, 1992). The results of the present study are certainly consistent with this proposition. The BALB/cByJ strain was previously shown to hypersecrete corticosterone in response to stressors, a finding that was confirmed in the present investigation. Moreover, as reported in rats, we observed that handling mice during the first weeks of life limited the behavioral disturbance otherwise seen in 90 day old animals (Meaney, et al., 1988). Paralleling these findings, we observed that the rise of corticosterone elicited by stressors in BALB/cByJ mice was limited in animals that had received the early-life handling. If handling precluded hippocampal cell loss, hence limiting the rise of glucocorticoids (owing to the preservation of an efficient negative feedback system), then it might have been expected that the longevity of the adrenocorticoid release would also be diminished. To date, however, we have not assessed the time course of the corticosterone changes associated with stressors in handled versus nonhandled mice.

As in the case of corticosterone, we observed that plasma ACTH levels in stressed BALB/cByJ mice exceeded that of C57BL/6ByJ mice. However, unlike the limited corticosterone release seen in BALB/cByJ mice that had received

early-life handling, plasma ACTH was unaffected by this manipulation. Thus, these data suggest that the effects of handling on plasma glucocorticoids were independent of effects on pituitary ACTH secretion. Of course, this does not imply that behavioral deficits often seen in BALB/cByJ mice, including the profound disturbances of shuttle escape performance and the marked anhedonia induced by stressors in this strain (Anisman & Zacharko, 1990; Shanks & Anisman, 1993), was independent of ACTH secretion.

One final note should be added at this juncture. The present investigation was limited to the analysis of catecholamines and serotonin in several brain regions, and the changes of corticosterone and ACTH in plasma. The BALB/cByJ mice, however, has been shown to be subject to other anomalies. A large proportion BALB/cByJ mice display gross genetic anatomical aberrations, such as absent or reduced corpus callosum (Wahlsten, 1989). It is conceivable that the disturbances in Morris water-maze performance reflect an interaction between glucocorticoid hypersecretion and the genetic anomalies characteristic of this strain (Crusio, et al., 1987; Schwegler, et al., 1988). Although the correlation between corpus callosum size and navigation in a swim maze was not strong (Lipp, Buechel, Scheffrahn, Wolfer, 1991), it is possible that the combination of callosal deficits, alterations in hippocampal mossy fibers, and glucocorticoid hypersecretion act in concert to limit navigational performance in this strain.

Summary

The BALB/cByJ mouse has been demonstrated to be hyperresponsive to stressors, displaying behavioral impairments in a number of paradigms including the Morris water-maze. The performance deficits we observed in the present investigation may be related to hippocampal dysfunction since these deficits were absent when salient cues were present, and were also modifiable by early-life handling. The deficits in the Morris water-maze were evident as early as 30 days

of age. However, it is not known whether these disturbances result from degeneration of physiological processes as opposed to an innate disturbance.

It was demonstrated previously that in aged rats performance impairments were evident in the Morris water-maze in about 50 percent of animals. These deficits, which coincided with hippocampal cell loss, could be prevented by early-life handling coupled with brief separation from the dam. In a similar fashion, it was observed in the present investigation that early-life handling limited the behavioral impairments in BALB/cByJ mice. Interestingly, the behavioral disturbances were also minimized among BALB/cByJ mice that were raised with a C57BL/6ByJ dam. The effects of handling and cross-fostering were not additive, however, raising the possibility that these treatments may have involved similar physiological processes during postnatal development. Parenthetically, poor performance in BALB/cByJ could not be attributed to maternal behavior *per se*, since having a BALB/cByJ dam did not affect performance among C57BL/6ByJ mice, irrespective of handling condition. Rather, it is more likely that having a particular genetic background coupled with having a BALB/cByJ mother culminates in poor water-maze acquisition during adulthood.

It is likely that the changes in performance in handled BALB/cByJ mice were related to strain-specific alterations in HPA activity. Again, as observed in studies with rats (Meaney et al., 1991; Sapolsky, 1992), exposure to early-life handling attenuated stress-induced corticosterone release. Since ACTH responses were not affected by such a manipulation it is likely that the handling-induced changes in HPA activity were independent of this endocrine. The neurochemical concomitants associated with handling, particularly within the hippocampus, and a lesser extent within the nucleus accumbens, paralleled both the strain-specific behavioral and corticoid alterations. It is possible that early-life handling alters stress-induced neurochemical activity in a strain-specific manner, particularly with

respect to accumbal DA and hippocampal NE utilization. Finally, although serotonergic mechanisms have been implicated in determining the effects of handling on behavioral deficits associated with aging in rats, the role of central serotonergic activity in mediating the results of the present investigation are unclear. While the data of the present investigation are suggestive of hippocampal mechanisms in subserving the behavioral deficits in BALB/cByJ mice, more detailed analyses of hippocampal morphology is required. Moreover, it is necessary to establish whether the behavioral impairments, and their reversal by early-life stimulation, are restricted to the Morris water-maze or are evident in other spatial and/or nonspatial paradigms

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