

ABNORMAL REPETITIVE BEHAVIOR: EARLY EXPERIENCE AND STRESS
RESPONSIVENESS

By

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I dedicate this dissertation to my mother, who taught me to set my goals high, and my father, who taught me to be humbled by that which I did not know.

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ABNORMAL REPETITIVE BEHAVIOR: EARLY EXPERIENCE AND STRESS
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Stereotyped behaviors occur in humans with developmental disabilities, neurological and psychiatric disease, as well as non-human animals under conditions of maternal deprivation, environmental restriction, and thwarting or conflict. As stereotyped behaviors have been associated with sub-optimal environmental conditions, accounts of stereotypy have proposed a homeostatic function, the behavior serving to modulate levels of stress. The current studies examined the relationship between stereotypy and stress using a novel mouse model, deer mice that develop high rates of spontaneous stereotypy when housed in standard laboratory cages.

Deer mice were exposed to brief and extended maternal separation which produces stress hypo- and hyperresponsiveness in adult rats, respectively. Differential responsiveness to stress was hypothesized to affect the development of stereotypy. Although deer mice did not show the hypo- and hyperresponsiveness to stress

hormonally, the maternally separated mice exhibited decreased stereotypy in their home cages and following a mild stressor.

Producing deer mice that were non-stereotypic as well as stereotypic allowed for the assessment of whether the mice were differentially responsive to stress as a function of stereotypy. As stereotypy in deer mice appears to be a response to a restricted environment, these studies examined whether raising deer mice in larger, more complex environments would reduce the occurrence of stereotypy. Two forms of environmental enrichment resulted in a decreased stereotypy in deer mice.

The hypothesis was that stereotypic mice would show increased HPA axis responses (e.g., corticosterone, adrenocorticotrophic hormone [ACTH]) to acute stress, and that following the stress, stereotypy would increase in stereotypic mice and potentially, be induced in non-stereotypic mice. There were no differences, however, between stereotypic and non-stereotypic mice in either basal or post startle ACTH or corticosterone levels. The amount of stereotyped behavior decreased following acoustic startle in both groups. We also examined the relationship between stereotypy and basal ACTH levels. A positive correlation was observed between stereotyped jumping and ACTH levels.

Thus, these studies established an important role for early experience (maternal separation, environmental enrichment) in the development of stereotypy. They also failed to support a significant relationship between stereotypy and stress responsiveness and questioned the coping function of stereotypy.

CHAPTER 1

INTRODUCTION

Frequent and often intense, repetitive motor behaviors such as body rocking, hand waving, and object twirling are highly salient features of the behavior of individuals with severe developmental disabilities such as autism and mental retardation. Repetitive patterns of behavior similar in several important dimensions to those observed in humans also occur in a wide variety of animal species (Mason, 1991). Typically these stereotyped behaviors occur in response to conditions of deprivation, involving either lack of social contact, environmental complexity, or both. Common to most definitions of stereotypy is the repetitive, topographically invariant, and rhythmical nature of the motor behavior. As these attributes define many of the motor behaviors in an organism's repertoire (e.g., locomotion), there is the need to consider temporal characteristics, frequency, context, and motivation in labeling a behavior as stereotypy. Stereotypes often dominate the behavioral repertoire of the organism and can be considered abnormal due to their excessive frequency, inappropriateness to the context, and lack of any apparent goal or purpose to the behavior (Berkson, 1967; Dantzer, 1986; Lewis, Baumeister, 1982). Stereotyped behavior occurs in multiple species in a wide variety of environmental contexts and thus has led ethologists, veterinarians, psychologists, psychiatrists, and neurologists to ask fundamental questions regarding the nature and function of these seemingly purposeless behaviors.

Stereotypy has been considered an important feature of some psychiatric and neurological disorders as well as developmental disorders. Stereotypies were described in schizophrenic patients dating back to the writings of Kraepelin and Bleuler in the early 1900's prior to the introduction of antipsychotic medication (Rogers, 1992; Jones, 1965). In his early writings, Kraepelin (1919) described stereotypies as a defining feature of *dementia praecox*. The pioneering neurologist, Hughlings Jackson (1884) described repetitive behavior associated with psychosis and suggested that these repetitive motor movements as well as other positive symptoms of schizophrenia (e.g., hallucinations, delusions) may be attributed to a disinhibition of subcortical areas due to decreased functioning of inhibitory cortical inputs. Interestingly, later writings on schizophrenia do not emphasize stereotypies as being a key feature of the disease. As Frith and Done (1990) suggested, this decreased emphasis on stereotypies as a defining feature of schizophrenia in the psychiatric community may be due in part to the difficulty in categorizing and defining stereotypy potentially due to neuroleptic treatment. Neurological disorders such as basal ganglia lesions, Tourette syndrome, and Rett syndrome are also associated with stereotypy (Shulman et al. 1996). Repetitive, restricted patterns of behavior have been a defining feature of autism since its initial description (Kanner, 1943).

Excessive, repetitive behavior was recognized as a problem in animal husbandry and by zoological societies in the late 19th century and early 20th century. Rich descriptions of abnormal behaviors performed by animals in zoos were provided by Meyer-Holzapfel (1968). As early as the 1870's, the neurobiological basis of these bizarre, repetitive behaviors in domesticated cattle was beginning to be questioned. The

German scientist Feser injected cows and sheep with the recently discovered compound apomorphine and observed behaviors similar to those displayed by cattle with “licking sickness” and wool biting in sheep (reported in Sharman, 1978). Unaware of the mechanism of action of apomorphine, Feser predicted that the same areas of the brain affected by apomorphine were involved in the spontaneous repetitive behaviors observed in these farm animals (Sharman, 1978).

Conditions Associated with Stereotypy

Various labels such as self-stimulatory behavior, displacement activities, or adjunctive behavior have been used for repetitive behaviors and each label is associated with a particular explanatory hypothesis as to the function and/or etiology of the specific behavior. This section provides a brief introduction to these behaviors, their associated environmental conditions, and explanatory hypotheses.

Clinical Pathology

In humans, stereotypies (e.g., body rocking, arm waving, bouncing) appear in typically developing children when they are progressing through transitional stages in motor development (Thelen, 1996). If the same types of repetitive motor behaviors persist or occur later in childhood and into adulthood, they are most often associated with psychiatric and neurological disorders and developmental disabilities such as mental retardation and autism (Berkson, 1983; Bodfish et al. 1995; Baumeister, Forehand, 1973; Jones, 1965). Stereotyped behaviors in individuals with developmental disabilities include the body rocking, head rolling, and hand waving mentioned previously but can also involve manipulation of objects (e.g., object twirling) and self-injury (e.g., self-hitting). Stereotyped behaviors tend to co-occur with other repetitive behaviors such as

compulsions (e.g., checking, arranging), interfere with adaptive training, and are often refractory to treatment (Bodfish et al. 1995). Although stereotyped movements, along with social and communicative deficits, constitute the triad of symptoms essential to the diagnosis of autism, repetitive behaviors have been surprisingly understudied in this population (Rutter, 1996).

In addition to motor stereotypies, repetitive behaviors in individuals with developmental disabilities also include more elaborate behaviors such as checking and hoarding. Such compulsive behaviors were estimated to occur in approximately 38% of individuals with mental retardation residing in a state residential facility (Bodfish et al. 1995) and occur in approximately 100% of individuals with autism (McDougle et al. 1996). Compulsive behaviors in this population are comorbid with stereotyped and self-injurious behaviors (Bodfish et al. 1995). In fact, King (1993) has advanced the idea that self-injury can be characterized as a form of compulsive behavior. Stereotyped behaviors seen in individuals with mental retardation and autism are topographically similar to repetitive patterns of behavior seen in psychiatric disorders such as trichotillomania (compulsive hair pulling) and onycophagia (compulsive nail biting) which are considered part of a continuum of obsessive compulsive spectrum disorders (Hollander, Wong, 1995; Rasmussen, 1994). Stereotypies and compulsions are similar not only in terms of phenomenology, but also pathophysiology. There is considerable evidence indicating basal ganglia pathology in OCD as indicated by its comorbidity with other disorders of the basal ganglia and more recent *in vivo* neuroimaging studies (Schwartz et al. 1996). Thus, empirical studies addressing the function of compulsions may have important relevance to the present topic.

Maternal Deprivation

In classic studies initiated by Harry Harlow in the 1950s, rhesus monkeys were taken from their mothers within an hour after birth and raised without tactile or social contact with their mothers or peers and only limited contact with the external environment (Harlow et al. 1965). Monkeys and chimpanzees raised in this manner developed topographies of stereotypy similar to those observed in individuals with mental retardation (e.g., huddling, body rocking, self-injury; Berkson, Mason, 1964). Stereotyped body rocking appears relatively early (ca. 29 days of age) in the behavioral repertoire of chimpanzees raised in isolation with a stationary surrogate mother, but is not observed in chimpanzees raised in isolation with a moving surrogate mother (Mason, Berkson, 1975). These data suggest that the lack of movement is particularly important in the etiology of stereotypes developing in response to early social isolation.

Environmental Restriction

Housing animals in other adverse environmental circumstances has also been associated with the appearance of stereotyped behaviors (Mason, 1991). For example, stereotypes such as pacing and head swinging often develop in bears and elephants when housed in zoos (Meyer-Holzapfel, 1968). Veal calves exhibit eye-rolling when confined in stalls (Fraser, Broom, 1990), and sows engage in chain-chewing when tethered (Cronin, Wiepkema, 1984). Considering the sub-optimal housing conditions in which zoo and farm animals have been confined historically, the appearance of stereotyped, abnormal behavior in these animals has been associated with organisms in “distress” (for review see Mason, 1991). The stereotypes arising from such environmental conditions have been termed “conflict-induced,” such as jumping in bank voles (Ödberg, 1986), and

"frustration-induced," such as chain chewing in pigs (Dantzer et al. 1987), and interpreted as a response to conditions of chronic stress (Wiepkema, Schouten, 1992). The expression of such behavior (e.g., route tracing, chain chewing, cribbing) in zoo and farm animals is considered a major animal welfare concern by veterinarians and animal caretakers as it is postulated to represent the animal's response to an inadequate environment (Lawrence, Rushen, 1993; Cronin, Wiepkema, 1984).

Enriching the environment of animals has resulted in the reduction of stereotypy in some species. For example, when sows were placed in a more complex environment consisting of additional loose straw to explore and chew, the amount of stereotypy previously exhibited was significantly reduced (Fraser, 1975). Additionally, heifers that exhibited stereotyped behaviors such as bar biting and inner tongue playing while tethered did not show stereotypies after being moved to a pasture (Redbo, 1990). When housed under enriched conditions (e.g., larger cage size, addition of nest materials, hiding places, twigs on which to climb), substantially fewer bank voles developed stereotypy compared to bank voles housed in standard cages (Sorensen, 1987). Ödberg (1987) has also observed a decrease in the number of bank voles developing stereotypy through the addition of enrichment objects to the cage. Empirical evidence from methodologically sound studies for the role of environmental restriction in zoo and farm animals is relatively sparse and weak, however.

Other investigations of the relationship between cage size and stereotypy have focused on changes in the amount of stereotypy performed in relation to changes in cage size in adult animals (Berkson et al. 1963; draper et al. 1963). As adult animals (4-5 years of age), chimpanzees that were separated from their mothers at birth and raised in

small cages during the first 2 1/2 years of life engaged in more stereotyped behavior when temporarily placed in small enclosures (Berkson et al. 1963). Similarly, wild-born and laboratory-reared rhesus monkeys (*Macaca mulatta*) display higher rates of stereotyped behavior when placed in small versus large enclosures (Draper et al. 1963; Paulk et al. 1977). Although several studies have examined the effect of enrichment on behavior, these studies have used adult subjects who have well-established stereotypes. Little research has examined the effects of enrichment on animals during early development.

Frustration / Conflict

Rich descriptions of repetitive, species-specific behavior patterns observed in animals in response to environmental circumstances associated with conflict exist in the ethology literature and have been termed displacement activities. Displacement activities (e.g., grooming, pecking, digging, sniffing) are often stereotyped and “occur when two strongly motivated behaviors are in conflict” (Rushen et al. 1993b). For example, in the wild, birds may display non-functional pecking behavior in response to conflict situations such as approach-avoidance. Displacement activities occur in situations which appear to lack the stimuli typically eliciting the behavior and are thus considered functionally irrelevant in that situation (Delius, 1967). The type of displacement activity occurring in a particular situation depends on the environmental stimuli present. For example, when a Zebra finch is threatened, it will feed if there is food in close proximity or mount a female if one is present (Falk, 1971).

In animal husbandry, displacement activities generally occur in response to various feeding schedules. For instance, hens display spot pecking in response to food

restriction (Savory et al. 1992). The approach-avoidance conflict in this situation would be the motivation to obtain the food (approach) and the motivation to escape the frustrating environment produced by the food restriction (avoidance). In humans repetitive grooming activities such as head scratching, tie adjusting, fingernail biting, lint picking, and non-grooming activities such as thumb-sucking have been considered displacement activities (Kinsbourne, 1980). By giving these behaviors such a label, however, we infer that we understand the motivational state of the organism, which is often not the case.

Similar to displacement activities, stereotyped behaviors appear in animals and humans exposed to fixed temporal schedules of reinforcement (Falk, 1971; Staddon, Simmelhaag, 1971). In this paradigm the subject develops highly stereotyped sequences of behavior termed adjunctive or schedule-induced behavior early in the inter-reinforcement interval (Falk, 1971). Displacement activities and adjunctive behaviors are similar in that they occur in animals under a high drive state (e.g., food deprivation) when a consummatory behavior is thwarted and the resultant behavior is facilitated by the environmental stimuli (Falk, 1971). Additionally, both types of behavior are excessive in frequency and the adaptive value of the behavior is not clear (Dantzer, Mittleman, 1993). The assumption that there is no adaptive significance of adjunctive behaviors has been questioned, though (Falk, 1971). Adjunctive behavior may be adaptive by allowing the animal to remain in a situation by making it less aversive. In the case of intermittent food delivery, the situation is ultimately beneficial since food is delivered but is also aversive at the same time because the animal's behavior is thwarted by the intermittent delivery of the food (Dantzer, Mittleman, 1993). Dantzer and Mittleman (1993) assert that drinking

in that context may develop out of the conflict between the appetitive component of the paradigm (feeding behavior) and the aversive component of the paradigm (escape behavior). Additionally, it has also been suggested that the arousal associated with the food pellet is maintained after its delivery and is therefore focused on another activity cued by environmental stimuli (e.g., drinking) (Kileen et al. 1978). It is from these hypotheses and interpretations that the notion of adjunctive behavior serving a coping response was generated.

Although often conceptualized as a *class* of behaviors, stereotypy is a *descriptive* term for motor behavior. Displacement activities, on the other hand, are more inferential in that the behavioral state of the organism (e.g., conflict) is inferred by the use of the term. Arguably, the motivation for the behavior is critical for the definition of displacement activities, whereas stereotyped behavior can be described in contexts in which no apparent conflict situation exists. For example, the state of conflict said to elicit body rocking in an institutionalized individual with mental retardation is not always readily observable.

Stereotypy, Stress, and Arousal

Although the definition of stereotypy includes the apparent purposelessness of the behaviors, the function of stereotyped behaviors has received considerable discussion in both the clinical and animal literature. Discussions of the function or maintenance of stereotypy in developmentally disabled populations have focused on positive reinforcement (e.g., attention), automatic reinforcement (e.g., sensory stimulation), and negative reinforcement, or the removal of aversive stimuli (e.g., escape from demand

situations, reduction of arousal). Thus, the repetitive behaviors are thought to be maintained by the functional consequences produced by the behavior.

Historically, the observation of stereotypies in clinical populations residing in public institutions suggested the association of stereotypy with unstimulating, sterile environments. Stereotypies were thus considered to be functional in providing sensory stimulation (e.g., proprioceptive, kinesthetic). Indeed, in many clinical contexts these behaviors are referred to as self-stimulatory behavior (Lovaas et al. 1987; Rincover et al. 1977). Other theorists hypothesized that the behaviors were a response to a frustrating, stressful environment (see Baumeister, 1978; Dantzer, 1986 for review). Frustration in this context resulted not only from demands being placed on the individual or from environmental restriction (e.g., lack of stimulating activity), but also from the restriction produced by the disability itself. Some have argued that an individual with a developmental disability is limited from interacting with his/her environment due to an inability to communicate or manipulate aspects of that environment. For example, Baumeister (1978) asserted that “[p]erhaps it is not unreasonable to assume that as the ability to control the environment in socially adaptive ways diminishes, either owing to limitations in the individual and/or the environment, one can expect to see the emergence of less adaptive behavior such as stereotypy” (p. 356). Additionally, this association between arousal and stereotypy also stems from the observation that situations such as loud noises or demands often exacerbate the expression of stereotyped behaviors (Forehand, Baumeister, 1970).

The function of abnormal, stereotyped behaviors remains an interesting and, as yet, unanswered question. Most of the literature on the arousal or stress-reducing

properties of stereotypy relies on inferences based on the apparent sub-optimal environments in which the behaviors are observed. Such reasoning is, to some degree, tautological. Although much has been written about the potential importance of stress in the expression and development of abnormal repetitive behavior, few empirical studies have rigorously tested this hypothesis.

One of the most widely debated functions of stereotyped behavior is that the behaviors serve to reduce stress (Mason, 1991, Mason, 1993; Dantzer, 1986) and thus protect the organism against the effects of stress. As reviewed above, this proposed function of stereotypy emerged from the observation of stereotypies in humans with disabilities in institutional settings and in animals under conditions of thwarting or conflict (Falk, 1971; Duncan, Wood, 1972), extreme social or maternal deprivation (Berkson, 1967; Harlow et al. 1965), and environmental restriction (Meyer-Holzapfel, 1968). Additionally, a stress reducing or coping function of stereotypy stems from evidence that blocking stereotyped behavior (e.g., SIP) results in increased levels of the stress hormone, corticosterone, a finding that will be addressed in subsequent sections. In this context stress is considered an intervening variable important in understanding the behavioral response to environmental stimuli. For example, environmental stimuli (e.g., intermittent reinforcement) would lead to a behavioral and/or physiological state (“stress”) and the behavioral output (stereotypy) would be considered an attempt to decrease stress. In this theoretical framework, it is assumed that stereotypy alters the stress response in a functionally adaptive way.

In order to explore this functional relationship it is important to understand the body’s physiological response to changes in the external environment; establish an

association between these physiological responses and stereotypy, and then ask functional questions regarding the role of stereotypy in modulating these variables.

CHAPTER 2

STRESS AND STEREOTYPY

The Stress Response

Claude Bernard pioneered the notion of physiological regulation of bodily function with his introduction of the concept of *internal milieu*. Bernard suggested that animals have evolved to become less dependent on the external environment and more capable of self, or internal, regulation (reviewed in Kopin, 1995). In "The Wisdom of the Body," Walter Cannon (1932) extended this notion of the body's need to monitor and maintain a favorable internal milieu and suggested that the stress response was a critical aspect to the maintenance of the state he termed, *homeostasis*. Physiological responses to external stressors, Cannon proposed, allowed the organism to engage in either "fight or flight" responses. Selye (1976) was one of the first to extend this notion and define stress in terms of a set of common changes it exerts on the body. His model became known as the "General Adaptation Syndrome" and revolutionized the way scientists were addressing the body's reaction to conditions threatening homeostasis. Borrowing the term from physics, the notion of stress is used to describe the strain or pressure of environmental events on either the psychological and/or physiological state of an individual. Selye (1976) contended that the "General Adaptation Syndrome" involved (1) an alerting response or alarm reaction which was the homeostatic physiological response suggested by Cannon, (2) a resistance response and (3) a stage of exhaustion (see Kopin, 1995 for review). In other words, different environmental stimuli (e.g.,

extreme cold, threat of a predator) can elicit the same physiological response, otherwise known as the “stress response.” Albeit tautological, the various external stimuli which elicit the stress response have been termed “stressful stimuli” or “stressors.” Although commonly interpreted as a response to negative or threatening situations, it must be kept in mind that the stress response is also activated in conditions that are associated with positive reward. A large bulk of the work on the stress response, however, is focused on aversively motivated behavior, in part because it is easier to study in animals.

The stress response in mammals is characterized by activation of the sympathetic nervous system (SNS) and the hypothalamic-pituitary-adrenal (HPA) axis (Stratakis, Chrousos, 1995). These systems are, in turn, modulated by neurons in extrahypothalamic nuclei (e.g., amygdala) which release corticotropin releasing factor (CRF) and neurons of the locus coeruleus which release catecholamines (Stratakis, Chrousos, 1995; Whitnall, 1993). In response to a stressful stimulus, the paraventricular nucleus (PVN) of the hypothalamus secretes CRF and arginine vasopressin (AVP) which are carried through the hypophyseal portal vessel to the pituitary gland (Whitnall, 1993 for review). At the pituitary gland these secretagogues (primarily CRF) stimulate the production of adrenocorticotropin hormone (ACTH) from corticotropes. ACTH is released into the general circulation and then stimulates the adrenal cortex’s production of glucocorticoids (e.g., corticosterone in rodents, cortisol in humans). Glucocorticoids mobilize the body’s energy sources, enabling the organism to mount a fight or flight response, while also suppressing immune function, inflammatory responses, and reproduction (Munck et al. 1984). Negative feedback on the HPA axis is regulated through hypothalamic glucocorticoid receptors and extra-hypothalamic glucocorticoid receptors located

primarily in the hippocampus and cortex (Jacobson, Sapolsky, 1991; Sapolsky et al. 1984; DeKloet et al. 1991). Activation of adrenal hormones is relevant for an organism's survival, as it provides an adaptive response to challenging environmental situations (Munck et al. 1984). Prolonged activation of the HPA axis and the sympathetic adrenal medullary system, however, has been associated with decreased immune function, problems with reproduction and growth, and loss of neurons in the hippocampus (Stratakis, Chrousos, 1995; McEwen, Sapolsky, 1995; Sapolsky, 1992).

The stress response also involves activation of the autonomic nervous system, particularly the sympathetic division, which is responsible for the organism's "fight or flight" response. The sympathetic nervous system consists of innervation of the vascular smooth muscle, the kidneys and gut, and the adrenal medulla (Stratakis, Chrousos, 1995). In general, the autonomic nervous system uses primary neurotransmitters (acetylcholine, norepinephrine, and epinephrine), neuropeptide transmitters (somatostatin, neuropeptide Y, enkephalin, galanin, and neuropeptides), and other signaling molecules (nitric oxide and ATP; Stratakis, Chrousos, 1995). These neural messengers respond rapidly to an environmental event and are thus responsible for an acute response to the stressor.

Although often discussed separately, these neural responses interact extensively to form a complex and integrative system finely tuned to regulate physiological and behavioral responses to changes in the external environment. The hypothalamus plays a particularly important role in orchestrating the neuroendocrine and autonomic responses to external events (Dodd, Role, 1991). The hypothalamus sends direct projections to nuclei of the brain stem and spinal cord that activate preganglionic autonomic neurons controlling body temperature, heart rate, blood pressure and respiration (Dodd, Role,

1991). Indirectly, the hypothalamus stimulates the release of hormones which influence autonomic function. Often called the “head ganglion” of the autonomic nervous system, the hypothalamus integrates information from other structures such as the cerebral cortex, hippocampus, entorhinal cortex, thalamus, basal ganglia, cerebellum, and reticular formation (Dodd, Role, 1991; Whitnall, 1993). Ascending catecholaminergic projections from brain stem innervate the paraventricular nucleus of the hypothalamus (Swanson et al. 1981; Swanson et al. 1981) and when stimulated increase release of CRF in the infundibular stalk (Plotsky, 1987; Plotsky et al. 1989). Opioid peptides released from the arcuate nucleus innervate and are reciprocally innervated by CRF and AVP neurons of the PVN and inhibit CRF and AVP-producing neurons (Whitnall, 1993). There are also reciprocal connections between CRF and catecholaminergic neurons, and, through short negative feedback loops, each system regulates itself (Stratakis, Chrousos, 1995).

Catecholamines are released both peripherally and centrally in response to an environmental challenge (Stanford, 1993). Adrenaline (or epinephrine), which is released from the adrenal medulla in response to an acute stressor, was one of the first hormones identified as being important in an animal’s fight or flight response. It was subsequently discovered that noradrenaline was also released peripherally from the adrenal medulla as well as postganglionic sympathetic neurons (Stanford, 1993 for review). Central activation of noradrenaline involves release from brain stem nuclei including the locus coeruleus, which innervates cortex, amygdala, and the hypothalamus as mentioned above. It is now accepted that central dopamine systems are also activated in response to stress (LeMoal, Simon, 1991). Dopamine cell bodies in the ventral tegmental area project to the prefrontal cortex and nucleus accumbens to form the

mesocortical and mesolimbic dopamine pathways, respectively. In response to stress (e.g., novelty, restraint), there is an increase in dopamine release in the mesocortical and to a lesser extent, mesolimbic dopamine systems (Roth et al. 1988). Increases in dopamine utilization in the prefrontal cortex and nucleus accumbens occur in response to stressful stimuli such as moderate footshock (Thierry et al. 1976; Herman et al. 1982). In response to milder electric footshock, however, the mesoprefrontal dopaminergic neurons are more sensitive than are dopaminergic neurons projecting to the nucleus accumbens (Horger, Roth, 1996). Recent evidence suggests that dopamine release in the amygdala, however, was more responsive to mild handling stimulation than was the prefrontal cortex (Inglis, Moghaddam, 1999).

As the importance of stress in psychiatric diseases such as schizophrenia and drug addiction has become more highly recognized, the interaction of the HPA axis and dopamine systems has received a considerable amount of attention in the clinical and basic neurosciences (Piazza et al. 1991; Piazza, LeMoal, 1998). The dopaminergic system interacts with the HPA axis at several levels. First, there are glucocorticoid receptors localized on dopamine cell bodies in the substantia nigra and ventral tegmental area (DeKloet et al. 1991). Second, glucocorticoids potentiate dopamine release in mesolimbic dopamine neurons (DeKloet et al. 1991). This effect appears to be mediated by glucocorticoid receptors (GR) and not mineralcorticoid receptors (MR) as RU39305 a selective GR antagonist decreases K⁺ stimulated dopamine release in cultured mesencephalic neurons, but spironolactone, an MR antagonist, fails to attenuate K⁺ stimulated dopamine release (Rouge-Pont et al. 1999). Corticosterone also alters the response of dopamine neurons to glutamate agonists by potentiating responses of

dopamine neurons to NMDA as well as AMPA and kainic acid. This potentiation can be blocked by RU38486, a GR antagonist (Cho, Little, 1999). Since CRF neurons project to dopamine-containing neurons of the substantia nigra and ventral tegmental area (Gray, Bingaman, 1996) and dopamine release in the amygdala is preferentially sensitive to stress (Inglis, Moghaddam, 1999), CRF-dopamine interactions may be particularly important in mediating an animal's response to stress.

The amygdala is an important brain region for the coordination of sensory input and relay of incoming sensory information to other stress responsive brain areas (Gray, Bingaman, 1996). In addition to its actions on the stimulation of ACTH at the pituitary, extrahypothalamic CRF is also involved in orchestration of the stress response. When administered centrally, CRF elicits behavioral sequelae similar to behaviors seen during an acute stress response such as decreased exploratory behavior, increased freezing and potentiation of the startle response (Gray, Bingaman, 1996). CRF neurons of the amygdala project to the bed nucleus of the stria terminalis, midbrain central gray, parabrachial nucleus, mesencephalic nucleus of the trigeminal nerve, locus coeruleus, dorsal vagal nucleus, and the nucleus of the solitary tract (Gray, Bingaman, 1996). CRF expressing neurons in the central nucleus of the amygdala receive projections from the bed nucleus of stria terminalis, thalamus, lateral hypothalamus, substantia nigra and VTA, central gray, raphe nucleus, locus coeruleus, parabrachial nucleus, ventrolateral medulla, and nucleus of solitary tract (Gray, Bingaman, 1996). CRF neurons in the amygdala contain glucocorticoid receptors, and it has been shown that glucocorticoids act directly on CRF neurons and regulate CRF expression. For example, corticosterone

administered to adrenalectomized rats results in a substantial increase in CRF mRNA in the central nucleus of the amygdala (Makino et al. 1994).

Thus, noxious or pleasurable stimuli trigger autonomic and endocrine responses which are coordinated by the hypothalamus to alter the *internal milieu* and prepare the organism for fight or flight, sexual activity, or other adaptive behaviors. Upon interaction with the environment, structures such as the cerebral cortex which are involved in higher cognitive function then become important in modulating the organism's behavior.

As reviewed above, stress can be defined, physiologically, as a condition that threatens homeostasis. Its behavioral concomitants, however, are less well defined. The concept of "threatening homeostasis" does appear in the psychology literature, but it focuses on traumatic life events or sustained anxiety over everyday life occurrences, which have negative mental consequences for humans. This focus reveals one of the several common pitfalls in discussions of stress. Labeling environmental events, which induce these negative mental consequences and/or physiological sequelae, as "stressors" has led to equating the stimulus with "stress." A more useful view of stress is to consider it an intervening variable and attempt to define it in terms of the common physiological and behavioral response properties associated with presentation of or changes in the stimulus. To determine what constitutes a stress response, the goal would be to obtain control over the stimulus (e.g., manipulate the intensity and duration of the stimulus) and to characterize the behavioral and physiological responses. Another pitfall in discussions of the stress response is the assumption that when presented with the same stimuli, animals will respond in the same manner, as suggested by the general adaptation syndrome. The general adaptation syndrome has been challenged over the years as

theories involving the role of cognitive factors in humans and individual differences have been explored. Although many animals do respond to various threats in the same manner, subsequent work has demonstrated the diversity of reactions to environmental stressors evolving in different species or organisms. In fact, a variety of external factors can elicit differing physiological responses depending on the individual organism's genetic predisposition and prior experiential history (Veith-Flanigan, Sandman, 1985).

Yet a third pitfall in the study of stress is the difficulty in measuring the intensity of a stressful stimulus, a stress response, or even individual differences in susceptibility to stress. Environmental events which cause strain on the organism's psychological state, such as thwarting of species specific, motivated behaviors or withholding reinforcement of a previously reinforced response are not well understood in terms of either the physiological or behavioral responses of the organism. In these examples, the direct effects of the stimulus on the physiological response of the organism are even more difficult to assess than the effects of a physical stimulus such as physical restraint or cold temperatures. Neither the environmental events nor their impact on behavior is well understood in terms of either the magnitude of stimulation necessary to produce a response or the intensity of the response required to define it as a stress response. Moreover, the inferred psychological state of the organism is difficult to determine for "psychological stressors" such as these. Furthermore, the effects of "physical stress" on the physiological and behavioral response of the organism may depend on the animal's prior experience and genetic predisposition. Thus, when assessing the effects of environmental events on the "stress response" of an organism, one must keep in mind these important caveats.

In discussions of psychiatric conditions or animal welfare, it is nevertheless difficult to avoid terms such as “stress”. There has been a debate in the field as to whether or not it is beneficial to use such terms when addressing complex behaviors and whether or not they help to clarify the pertinent issues or hinder our understanding of behavior. We would argue that concepts such as stress provide a useful tool in assessing physiological and behavioral responses to stimuli as long as the behavioral and physiological endpoints are well defined and correlated. It is difficult, if not impossible, to assess accurately the subjective experience of an organism and thus, stress as it is conceptualized as a subjective experience is difficult to interpret in empirical studies, particularly if these studies employ animals or individuals with restricted communicative skills.

Stereotypy & HPA Axis

Similar to the hypothesis that stereotypy in individuals with mental retardation may be a response to some form of environmental restriction, the appearance of stereotyped, abnormal behaviors in zoo and farm animals, has been interpreted as reflecting distress or frustration (for review see Mason, 1991). As reviewed earlier, the hypothalamic pituitary adrenal axis responds to external environmental conditions which threaten homeostasis, and thus, the association of stereotypy with HPA axis activity has been examined in animals under conditions of environmental restriction as well as thwarting or conflict. Functional relationships between stereotypy and HPA axis activity have also been investigated in order to answer questions regarding stereotypy serving a coping response.

Association of Stereotypy with HPA Axis Activity

Investigations into the role of the HPA axis in stereotypy have focused mainly on measuring corticosteroid levels or adrenal function. Stereotyped behaviors in several species have been associated with decreased levels of circulating hormones or adrenal function. Pacing, which develops in laying hens housed in small cages, has been associated with a decrease in corticosteroid levels (Duncan, 1970). Repetitive head flicking, another stereotypy that emerges in domesticated fowl, has been negatively correlated with adrenal weight (Bareham, 1972; Dawkins, 1980). Young tethered sows, which do not typically exhibit stereotypies, often show increased corticosteroid levels relative to the older sows that perform stereotypies (Cronin, 1985). Stereotyped tongue playing in veal calves has also been negatively associated with the severity of gastric ulceration associated with restricted housing (Wiepkema et al. 1987). Dairy cows with high levels of stereotypy had lower plasma ACTH levels than did cows with low levels of stereotypy (Redbo, 1998).

Stereotypy, Stress, and Coping

Several physiological parameters involved in the stress response have been studied to test the coping hypothesis of stereotypy. One of the first studies providing empirical support for the coping hypothesis was conducted by Brett and Levine (1979) who reported that placing rats on fixed schedules of reinforcement resulted in elevations of corticosterone and that stereotyped drinking during the inter-reinforcement interval lowered this increase. These investigators also observed that the removal of the opportunity to engage in scheduled induced polydipsia (by removing the water bottle) was associated with increased corticosterone levels, which returned to basal values upon

reinstatement of the water bottle. The observation of reductions in corticosterone associated with SIP and the increase in corticosterone when the bottle was removed has now been replicated (Tazi et al. 1986) and extended to other species (e.g., voles). When housed in standard laboratory cages, bank voles develop stereotyped jumping (Ödberg, 1986). Preventing the jumping by lowering the cage ceiling results in a rise in corticosteroid levels which fall if the animals develop another form of stereotypy (Ödberg, 1989). Additionally, rats that are placed on fixed reinforcement schedule with exposure to water that do not develop SIP also display increases in corticosterone (Dantzer et al. 1988). When rats were adrenalectomized, they consumed less water in the SIP paradigm than did sham operated rats, suggesting that the increase in corticosterone is necessary to observe an increase in drinking (Levine, Levine, 1989). Mittleman et al. (1988) also showed that the amount of water consumed during a 30-minute test session was inversely correlated with plasma corticosterone levels. In other words, rats that did not develop SIP had higher levels of corticosterone. The authors interpret this finding as suggesting that instead of SIP modulating corticosterone, corticosterone levels may modulate SIP (Mittleman et al. 1988).

The coping hypothesis of stereotypy has been questioned as subsequent studies of HPA axis function and SIP have generated inconsistent results (Dantzer, Mittleman, 1993). Rats developing SIP can show increased corticosterone levels compared to baseline levels (Wallace et al. 1983) as well as to rats exposed to food schedules that were not given the opportunity to drink (Mittleman et al. 1988). In the Tazi et al. (1986) study mentioned above, the rats exposed to the food schedule that were not allowed to drink did not show increased corticosterone levels over baseline measures.

In addition to SIP, rats placed on fixed reinforcement schedules also develop other adjunctive behaviors such as wheel running, pica eating, air licking and increased locomotor activity (Dantzer, Mittleman, 1993). Wheel running has been associated with increases in corticosterone levels (Tazi et al. 1986), further questioning the validity of the coping hypothesis. Pigs develop stereotyped chain pulling when placed on intermittent schedules of food reinforcement and when presented a massed quantity of food (Dantzer, Mormede, 1981; Dantzer et al. 1987). Chain pulling is generally observed immediately after food delivery and decreases when the animal has access to food prior to the session but increases when the animal is food deprived (Dantzer, Mormede, 1981). When placed on fixed time schedules of food delivery in which the pigs are allowed access to a chain, there is a decrease in cortisol levels at the end of the session and an increase in cortisol in response to extinction (removal of food reinforcement) (Dantzer, Mormede, 1981). This decrease in cortisol with access to a chain has been replicated in pigs on intermittent schedules of food delivery, whereas, an increase in cortisol was observed in pigs receiving massed presentation of food (Dantzer et al. 1987). Since chain pulling developed in both massed and intermittent food presentation, Dantzer et al. (1987) suggest that chain pulling may be schedule-modulated rather than schedule-induced. Additionally, the different patterns of cortisol in response to the two types of food presentation also suggest that the repetitive behaviors may serve different functions in the two situations. If chain pulling in the intermittent reinforcement schedule serves a coping response as suggested by the decrease in cortisol levels, an increase in cortisol would be expected when the chain was not available. No differences

in cortisol levels, however, were reported between the beginning and end of the intermittent reinforcement sessions when no chain was available (Dantzer et al. 1987).

Further evidence questioning the assumption that SIP serves a coping function comes from studies with the neuropeptide, corticotropin releasing factor (CRF). When administered centrally, CRF elicits behavioral sequelae similar to behaviors seen during an acute stress response such as decreased exploratory behavior, increased freezing, and potentiation of the startle response (Gray, Bingaman, 1996; Cole, Koob, 1994).

Intracerebroventricular (icv) administration of CRF blocks the performance of SIP in a dose dependent manner and appears to be selective for SIP as the temporal pattern of the behavior was maintained (Cole, Koob, 1994). Interestingly, the CRF antagonist, α -helical CRF (icv), had no effect on SIP, suggesting that endogenous CRF does not play a role in the maintenance of the behavior (Cole, Koob, 1994). As outlined by Cole & Koob (1994), α -helical CRF does block many stress-induced physiological and behavior responses such as stress-induced increases in plasma epinephrine and decreases in exploratory behavior. The level at which CRF exerts its effects is unclear.

Administration of CRF stimulates the production of ACTH and subsequently corticosterone (Vale et al. 1981) as well as central dopamine and noradrenergic systems (Dunn, Berridge, 1987). These data call into question the role of SIP as a coping response. Considering that both intermittent schedules of reinforcement and administration of CRF increase plasma corticosterone levels, it seems contradictory that CRF blocked SIP in these studies. Cole & Koob (1994) suggest that SIP may respond to an inverted U shaped function of plasma corticosterone such that an optimal level of corticosterone is required to induce SIP and levels either too low or too high serve to

reduce the behavior. Evidence for this hypothesis comes from the observation that the acquisition of SIP can be blocked by adrenalectomy, inhibition of corticosterone synthesis, and administration of corticosterone (Mittleman et al. 1992).

Behavioral interpretations of schedule induced polydipsia have also challenged the coping hypothesis. Falk and Kupfer (1998) questioned the usefulness of assessing the function of a behavior and assuming that behavior is performed for a given purpose. With SIP, the putative conflict of being in a highly motivated state (food deprivation) in a situation in which a valued reinforcer is present but only available on a restricted schedule has suggested to some researchers that the animal is frustrated. In this situation the adjunctive behavior (e.g., polydipsia) has been interpreted as a coping response to this frustration. Falk and Kupfer (1998) argued that the coping theory fails to account for the bitonic function of SIP in which excessive water consumption only occurs in the middle range of inter-reinforcement intervals (2 minutes), but decreases as the inter-pellet interval reaches 5 to 10 seconds. Presumably, the animal would still be frustrated as the inter-pellet interval increases. Falk and Kupfer (1998) suggested that it is more useful to parametrically vary commodity of the relevant variable and assess the performance of the adjunctive behavior. For instance, with SIP, increasing the pellet size and thus changing "commodity acquisition" shifts the bitonic function to the right. Falk and Kupfer (1998) asserted that conflict is not an internal construct, but rather, two opposing response tendencies – the probability of consummatory behavior versus the probability of escape from the reinforcement schedule. As consummatory rate increases (shorter inter pellet interval), consummatory behavior increases and escape behavior decreases. Conversely, as consummatory rate decreases (longer inter pellet interval), consummatory behavior

decreases and escape behavior increases. It is in the middle range of reinforcement interval that adjunctive behavior is the most probable, because at the two extreme ends, either consummatory or escape behavior will predominate (Falk, Kupfer, 1998). This approach allows the experimenter to systematically vary the timing and amount of reinforcer and draw conclusions regarding the parameters that govern SIP without inferring the subjective experience of the animal.

Clinical Conditions and HPA Axis Activity

Schizophrenics and amphetamine addicts report that engaging in stereotypy reduces anxiety and often provides pleasure (Fox, 1971; Rylander, 1971; Robbins, Sahakian, 1981). Anecdotal reports from patients with Obsessive Compulsive Disorder (OCD) suggest a similar relationship between obsessive thoughts and the performance of compulsive behaviors. OCD patients report that the performance of compulsions decreases levels of anxiety associated with obsessive thoughts (Foa, et, 1984). In a study by Kasvikis et al. (1988), patients with obsessive compulsive disorder exposed to prolonged aversive stimuli evoking an urge to ritualize reported subjective increases in anxiety accompanied by increases in urinary cortisol levels when compared to baseline sessions. To our knowledge, there are no data on whether performance of the compulsion decreases measures of HPA axis activity (e.g., corticosterone or ACTH levels).

Castellanos et al. (1996) reported that DSM-IV Stereotypic Movement Disorder can be diagnosed in adolescents and adults functioning within the normal IQ range. These individuals typically display thumb sucking and body rocking and tend to have comorbid affective or anxiety problems. Interestingly, out of the 12 who met criteria for DSM-IV SMD, 11 had a lifetime history of an affective or a non-OCD anxiety disorder.

In case reports some of these individuals describe the social stigma, the interference with daily functioning, and the relaxing sensation produced by engaging in the repetitive behavior. Whereas studies in humans provide evidence of an association between stereotypy and arousal or anxiety, the physiological responses associated with the behavior are still unclear. Better measures of HPA axis function in response to the performance or blockade of the behavior (e.g., cortisol response to blocking of stereotypy) may prove useful when addressing the functional consequences of stereotyped behavior. There are multiple ways of manipulating the HPA axis (e.g., glucocorticoid antagonist, ACTH challenge) and assessing repetitive behaviors in humans, and if these systems prove to be important in the induction and/or maintenance of repetitive behaviors, potential pharmacological treatment targeting these systems may be beneficial (e.g., non-peptidergic CRF antagonists).

It is also important to ask whether there is any evidence for a role of the HPA axis in the *development* of stereotyped behaviors. Most of the studies reviewed here suggest a role for changes in HPA function affecting the *expression* of stereotyped behavior. Perhaps fluctuations in corticosterone or ACTH levels can modulate SIP, but may not be important in the acquisition of the behavior. Indeed, adrenalectomy decreases the acquisition as well as the performance of SIP, whereas, corticosterone administration decreased the acquisition but not the performance of SIP (Mittleman et al. 1992). Finally, few studies incorporate multiple physiological endpoints relevant to the stress response and very little consideration is given to the importance of individual differences.

Stereotypy and Dopamine Function

It is now well established that stereotyped patterns of behavior can be induced in a number of mammalian species following administration of drugs that alter nigrostriatal dopamine function (Cooper, Dourish, 1990; Lewis et al. 1996). When administered high doses of psychostimulants such as amphetamine, rats will display stereotyped behaviors including repetitive sniffing and head movements (Robbins, Sahakian, 1981). The stereotypy-inducing effects of psychostimulants have been attributed primarily to activation of the nigrostriatal dopamine system. For example, dopamine or dopamine agonists injected directly into the striatum induce stereotyped behaviors in rats (e.g., Ernst, Smelik, 1966). In fact, specific regions of the striatum have been implicated in specific topographies of stimulant-induced stereotypy (e.g., ventrolateral striatum and orofacial stereotypy; Dickson et al. 1994). Although the nigrostriatal dopamine system has been established as an important site for the induction of stereotypy, other dopamine pathways have been implicated as well. In particular, the mesolimbic dopamine pathway may be particularly important in the expression of locomotor stereotypies (LeMoal, Simon, 1991). For example, intra-accumbens injections of amphetamine have been reported to induce stereotypy (Annett et al. 1983) and work on schedule-induced polydipsia has suggested the importance of the nucleus accumbens in the acquisition of these behaviors (Robbins, Koob, 1980).

Amphetamine-induced stereotypy is thought to be dependent on increases in dopamine concentration following uptake inhibition, particularly in the nigrostriatal dopamine pathway. As mentioned previously, the role of the mesolimbic dopamine system, especially stress-induced dopamine activation, in stereotyped behavior has been

addressed. In particular, the importance of individual differences either due to genetic predisposition or prior exposure history has become a significant focus of work in the field of stress, sensitization, stereotypy, and drug self-administration (Piazza et al. 1991; Piazza et al. 1993). In these paradigms it is important to consider the prior behavioral history of the animal when assessing its response to drug challenge. For example, prior exposure to foot shock (Brett et al. 1982) or social isolation (Jones et al. 1989b) reduces acquisition of SIP (Jones et al. 1994). In this respect, delineating individual differences is perhaps one of the best ways to understand the adaptability of physiological and behavioral responses to stressors.

Research Questions

The focus of the current studies is to elucidate the role of stress responsiveness (e.g., HPA axis activity and dopamine function) in the development and moment to moment expression of stereotypy. The first arm of the studies focuses on altering the stress responsiveness of deer mice through an early maternal separation procedure and assessing the subsequent development of stereotypy in standard laboratory cages and in response to a mild stressor. The second arm of the studies focuses on: (1) establishing that environmental restriction is indeed an important part of the genesis of stereotyped behavior in this species, and (2) using various forms of environmental enrichment to produce stereotypic and nonstereotypic deer mice in order to compare indices of stress responsiveness.

CHAPTER 3
EFFECTS OF EARLY MATERNAL SEPARATION ON THE DEVELOPMENT OF
STEREOTYPY IN DEER MICE

Introduction

Several rodent species have been reported to exhibit spontaneous stereotypies (i.e., absent a specific environmental or pharmacological challenge) in the laboratory whether raised in captivity or caught in the wild (Mason, 1991; Powell et al. 1999). For example, trapped bank voles (*Clethrionomys glareolus*) develop stereotyped jumping, backwards somersaulting, and patterned running when housed in standard rodent cages (Ödberg, 1986). We have reported similar topographies of stereotyped behavior in laboratory housed deer mice (*Peromyscus maniculatus*) (Powell et al. 1999).

Stereotypies arising from adverse environmental conditions have been interpreted as a response to chronic stress (Wiepkema, Schouten, 1992). Thus, one hypothesized function of stereotypy is that it serves as a coping response, protecting the organism against the effects of stress (Dantzer, 1986; Mason, 1991; Ladewig et al. 1993). For example, schedule-induced polydipsia has been associated with decreased corticosterone (Tazi et al. 1986), whereas the loss of the opportunity to engage in this behavior resulted in increased corticosterone levels (Brett, Levine, 1979; Tazi et al. 1986; Dantzer et al. 1987). In addition, attenuating amphetamine-induced stereotypy in rats by 6-OHDA lesions of the caudate-putamen (Jones et al. 1989a) and preventing stereotyped jumping in bank voles by lowering the cage ceiling (Ödberg, 1989) resulted in increased plasma corticosterone levels. Some reports, however, have suggested that stereotypies are

associated with an increased response to stress, as stereotypic sows showed increased cortisol levels in response to ACTH stimulation compared to non-stereotypic sows (von Borell, Hurnik, 1991).

Although these studies suggest that stress is important in the expression of stereotyped behavior, they do not provide evidence for the role of stress in the pathogenesis or development of the behavior. Rushen (1993a) points out that a considerable amount of data suggest that stereotypy may not act as a coping response. Rather, differences in stress responses (e.g., sympathetic activity, HPA axis activity) may be explained by individual differences which predispose an animal to stereotypy, rather than a direct effect of engaging in stereotypic behavior (Schouten, Wiepkema, 1991; Schouten et al. 1991). There is evidence that animals and humans that are more sensitive to stress are more likely to develop stereotypy. For example, reactive breeds of horses are predisposed to develop stereotypy (Kiley-Worthington, 1983), and individuals that show elevated reactions to stress are more susceptible to the psychomimetic effects of amphetamine (reviewed by MacLennan, Maier, 1983). Thus, Rushen (1993a) suggested that it may be useful to manipulate experimentally the development of stereotypy in an attempt to establish causal relationships rather than relying on correlative studies. In the current study we sought to manipulate the stress responsiveness of deer mice through an early maternal separation procedure and assess the effects of such differential stress responsiveness on the development of stereotypy.

Previous studies have demonstrated that the hypothalamic-pituitary-adrenal axis response to environmental challenge can be altered through early experience (e.g. infantile handling) (Levine, 1957). Rats that have been separated from their mother for

short periods of time (3-15 min.) during the first few weeks of postnatal development exhibit decreased ACTH and corticosterone secretions both during and after a stressful experience (e.g. restraint, open field) when tested in adulthood (Meaney et al. 1991, Meaney et al. 1993a, Meaney et al. 1993b; Nunez et al. 1996). Handling rats early in development has been associated with decreased CRH content in the hypothalamus and increased glucocorticoid receptor sites in the hippocampus and frontal cortex, increasing the sensitivity of glucocorticoid negative-feedback which results in reduced post-stress secretion of ACTH and corticosterone (Meaney et al. 1991; Meaney et al. 1993a, Meaney et al. 1993b). Conversely, when pups are separated from their mother for more extended periods of time (e.g., 180 minutes), they show an exaggerated corticosterone and ACTH response to acute stress, increased CRH content in the hypothalamus, and decreased glucocorticoid receptors in the hippocampus (Plotsky, Meaney, 1993). After his initial observations of reduced stress responsiveness associated with neonatal handling, Levine (1957) interpreted the phenomenon as an animal benefiting from an early experience with stress by strengthening its ability to adapt to physiological and psychological stress as an adult. More recent evidence has suggested that the behaviors displayed by the dam (e.g., increased licking, grooming, and arched back nursing) upon reunion with her pups are the critical features mediating this effect (Liu et al. 1997).

The current study used the maternal separation procedure described by Meaney et al. (1991, 1993 a,b; Plotsky, Meaney, 1993) to manipulate stress responsiveness experimentally during ontogeny and assess the effects on the development of stereotypy in deer mice. We hypothesized that separating mice from the dam for brief periods of time (15 minutes/day) during the first two weeks of life would make deer mice

hyporesponsive to stress and be associated with reduced levels of stereotypy.

Conversely, we hypothesized that separating mice from the dam for more extended periods of time (180 minutes/day) would make deer mice hyperresponsive to stress and be associated with increased levels of stereotypy.

Methods

Subjects

Deer mice (*Peromyscus maniculatus bairdii*) were housed in a standard colony room kept at 24° C and maintained on a 16/8 hour light/dark cycle, with lights off at 0930h. The animals used in the present study came from a breeding colony maintained at the University of Florida. The time course of experimentation is displayed in Figure 2-1.

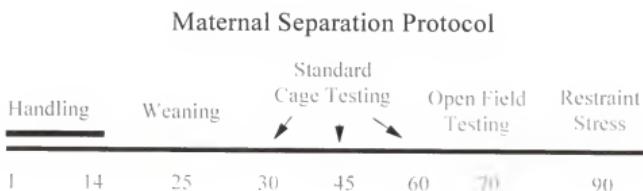


Figure 2-1. Timeline for maternal separation experiment. Numbers at bottom of figure represent the respective age of the animal.

Handling Procedure

A total of 30 litters were used in the study. Twenty litters were exposed to the maternal separation procedure and the remaining 10 litters were left undisturbed in their maternal cages until weaning at postnatal day (PND) 25. Cages were checked daily for the birth of pups, and at time of parturition, the father was removed from the cage. The

day of birth was designated postnatal day 0 (PND0). From PND1 until PND14, the experimental offspring underwent a handling procedure at approximately the same time (1200 +/- 1 hour) each day. The handling procedure consisted of gently removing the pups manually from their parental cage (45 x 24 x 14 cm) and transferring the pups to a smaller, plastic container with fresh bedding. The container was then placed in a pre-warmed incubator (36.3°C - 38.2°C) for either 15 (MS-15) or 180 (MS-180) minutes. The first two weeks of life were chosen because previous experiments in rats indicated that handling exerted the most profound effects when done during the first week of life and had minimal effects when done after the second week of life (Meaney, Aitken, 1985). Although cages are normally changed weekly, the cages in all three groups were not changed until after weaning in order to avoid disturbing the non-handled, control litters and excess, uncontrolled manipulation of the handled, experimental litters. At least once during the preweaning period, some of the dirty bedding was removed from the cage and replaced with some fresh bedding. Latex gloves were worn for handling and the bedding in the small cages used during separation was changed after each use.

On PND 25, the MS-15, MS-180 and non-handled (NH) pups were weaned from their mother and housed in groups of 2-3 in same sex standard laboratory cages (45 x 24 x 14 cm) according to the experimental condition. For identification purposes, each pup received an ear punch or sham punch at the time of weaning to indicate individual identity. Since the number of pups in a litter ranged from 1-6 in this species, when possible, two mice from each litter were used in the study. This yielded 21 mice in the MS-180 condition, 25 mice in the MS-15 condition, and 20 mice in the non-handled, control condition at the start of behavioral testing. Since there was not an equal number

of male and female mice in a given litter, the number of MS-180, MS-15, and NH males and females were matched as closely as possible. The number of mice at each time point varied due to mortality, escape from cages, loss of data (e.g., faulty videotape), and inability to obtain blood samples, but the same set of mice were tested in each procedure. Thus, the number of mice represented in each set of behavioral and neuroendocrine data are indicated appropriately.

Observational Methods

The development of stereotypy in the three groups was assessed for two consecutive days on PND 30, 45, and 60. Mice were singly housed in standard laboratory cages (22 x 15 x 28 cm), and videotaped for 3 hours (every 30 minutes) for a 6

Table 3-1. Stereotyped Behaviors in Deer Mice in Standard Caging

Jump	Rearing in one of the four corners of the cage and repeatedly jumping on his/her hind paws
Backward Somersault	A backward somersault with or without the animal pushing off from the cage top or side
Patterned Running	Repetitive circling, or route tracing, on the floor of the cage in a clear, continuous pattern
Cagetop Circling	Repetitive circling, or route tracing, on the cage top in a clear, continuous pattern.

hour period under dim red light between 1000 and 1700h. The cages were placed in a room in such a manner to record 4-6 cages of mice in one video frame, depending on the number of mice tested on a given day. The remaining siblings of the mice tested behaviorally were maintained in standard caging and left undisturbed. Due to the range in the date of birth of the litters used in the study, animals were observed in cohorts born within 3 days of each other. Videotape sessions were conducted under dim red light with

care taken by the observer not to make any unnecessary noise. No observation sessions were conducted during the light phase of the cycle since it has been documented in this same colony of animals that this species shows very little activity during the light phase (Baumgardner et al, 1980). Following the 6-hour testing session, mice were returned to their home cages until subsequent testing. At a later date the behavior of individual mice, including jumping, backward somersaulting, patterned running, cagetop circling, and non-stereotyped behavior were coded and analyzed using the Observer software (Noldus, 3.0). Behaviors were coded as states and coded from the time the mouse initiated the behavior until the time the mouse ended the bout. A bout was terminated when the mouse stopped engaging in the behavior for a two-second period. Ten minutes of video were analyzed in the middle of each 30 minute block, starting at 15 minutes into the 30 minute block. This analysis of the data produced a one-hour segment of behavior coded for each mouse. The topographies of stereotyped behaviors observed in deer mice raised in these cages are listed in Table 3-1. Sixty days of age was chosen for the upper age limit because previous studies have shown that most deer mice that develop stereotypy should do so by that age (Powell et al. 1999). In this paradigm, standard cages are the challenge for the mice and should, therefore, distinguish the groups based on stress responsiveness if indeed stereotypy is related to increases in HPA axis activity. This hypothesis stems from the idea that standard caging, in this species, is a stressful environment.

Open Field Testing

On PND 70 the same mice were tested in an open field for 10 minutes beginning around 1300 hours. The open field consisted of a brightly lit, circular plexiglass

enclosure (diameter = 18 inches; height = 12 inches) divided into two concentric circles. The inner circle (diameter = 4 inches) was divided into four equal quadrants, and the outer circle was divided into six equal divisions. Mice were placed in the center circle at the beginning of the test and their behavior in the open field was video taped for 10 minutes. At a later date, an observer blind to experimental group status coded the location of the mouse and the behavior of the mouse (rearing, grooming, locomotion, flipping, jumping) from the videotape using the Observer software (Noldus 3.0). Following the 10-minute open field test, the mice were returned to a standard cage (22 x 15 x 28 cm) at which time they were videotaped for an additional 10 minutes. The mice were then anesthetized with a cocktail of ketamine and acepromazine (4:1), and after 3 minutes, blood was taken from the retro-orbital sinus cavity using a Natelson blood collecting tube for corticosterone determinations. The samples were centrifuged at 8,500 G for seven minutes, and plasma was fractionated and stored at -80° C until time of assay. After recovery from the retro-orbital sinus bleed, mice were returned to their home cages.

Restraint Stress

On day 90 mice in the three experimental groups were restrained in 50 mL centrifuge tubes under bright white light (75 watt). After one hour in the restraint chambers, mice were anesthetized with a ketamine:acepromazine cocktail (4:1) and blood was then taken from the retro-orbital sinus cavity as described above. Mice were then returned to their home cages and left undisturbed until day 120 (+/- 4 days), at which point they were sacrificed by cervical dislocation followed by decapitation and brains removed, snap frozen in isopentane, and stored at -80° C.

In order to obtain corticosterone responses at varying points following restraint stress, untested siblings of the maternally separated (MS15 and MS180) and nonhandled mice were restrained for one hour and blood was sampled at three different timepoints (15, 30, 60 minutes) after removal from the restraint chamber. Mice were restrained in 50 mL centrifuge tubes under bright white light (75 watt) for 60 minutes and blood samples were obtained from the retro-orbital sinus cavity without the use of anesthesia. On average, 10 mice from each experimental condition were tested at the three timepoints. Each mouse, therefore, was exposed to two of the three timepoints following an hour of restraint stress. The restraint stress and blood draws on individual mice were separated by one week. Previous data from our lab suggest that repeated retro-orbital sinus eye sampling does not affect corticosterone levels in deer mice.

Corticosterone Determinations

Corticosterone concentrations were determined (in duplicate) from plasma samples using a [³H]-corticosterone radioimmunoassay kit (ICN Biomedicals, Inc., Costa Mesa, CA).

Results

Behavioral Analyses

A Kruskal-Wallis one way analysis of variance on ranks was conducted on the percent of time engaged in stereotypy in the home cage at day 45 for all experimental conditions. Although there was a trend for the MS15 (n=20) and MS180 (n=23) groups to be lower than the non handled (n=21) group, there was not a statistically significant difference in the amount of stereotypy displayed by the three groups on day 45 [$H(2) =$

4.002, P = 0.135; Figure 3-1]. These results should be interpreted with caution as there was insufficient power to ensure that a Type II error was not committed.

The activity of deer mice in the open field is shown in Figure 3-2. One way ANOVAs were performed where appropriate. When assumptions of normality and homogeneity of variance were violated, the Kruskal-Wallis one way analysis of variance on ranks was used as a non-parametric alternative. Behavioral data in the open field were obtained on 20 NH mice, 24 MS15 mice, and 22 MS180 mice. There were no differences among the groups in the total number of crossings (entries into one of the 10 quadrants; $F(2,63) = 0.466$, $p = 0.63$) or the number of crossings in the inner [$H(2) = 0.947$, $p = 0.623$; Kruskal-Wallis] or outer circles [$H(2) = 0.918$, $p = 0.632$]. There were also no differences between the groups in the amount of time spent in the inner circle [$H(2) = 1.854$, $p = 0.396$] or the outer circle [$H(2) = 1.925$, $p = 0.382$] of the open field. Mice in the three experimental conditions did not differ in the percent of time engaged in jumping, flipping, rearing, or grooming during the 10-minute open field test ($p > 0.05$; data not shown). The levels of stereotyped behavior during the 10-minute open field session were minimal in all of the groups.

During the 10-minute post open field session, the three experimental groups differed in the percent of time spent engaged in stereotypy (jumping, flipping, patterned running) [$H(2) = 15.982$, $p < 0.001$; Figure 3-3]. Mice from the non handled condition engaged in significantly more stereotypy than mice in both the MS15 ($p < 0.05$) and MS180 groups ($p < 0.05$). Post open field behavioral data was obtained on 11 NH mice, 20 MS15 mice, and 20 MS180 mice. The small number of mice represented in the NH group was due to a faulty videotape, which contained a greater number of NH mice that

MS15 and MS180 mice. Stereotyped behavior of the first 11 mice coded in the MS15 and MS180 groups was compared with the 11 NH mice and the reduction in stereotypy in the maternally separated groups was maintained (data not shown). Since more than one mouse per litter was used in the study, the data were analyzed using litter as the unit of analysis. This reduced the sample size of each group (NH= 7, MS15= 9, MS180= 10). The main effect of condition [$F(2,23)= 18.59$, $p< 0.001$] and the reduction in stereotypy in the MS15 and MS180 groups ($p< 0.05$) were maintained.

Table 3-2. Mean (S.E.M) percent of time engaged in each behavior during the 10-minute post open field session.

	NH Mean (S.E.M.)	MS15 Mean (S.E.M.)	MS180 Mean (S.E.M.)
Jumping	30.36 (9.32)	14.52 (4.70)	7.59 (3.64)
Flipping	14.73 (7.76)	1.44 (0.82)	2.12 (1.60)
Patterned Running	4.18 (3.91)	0.02 (0.02)	0.82 (0.70)
Rearing	10.32 (2.00)	12.86 (2.08)	11.48 (1.86)
Locomotion	15.50 (2.21)	22.97 (3.19)	15.93 (2.35)
Grooming	6.01 (1.25)	10.44 (1.87)	11.58 (2.07)
Burrowing	1.15 (0.85)	0.41 (0.17)	0.31 (0.13)
Inactive	17.77 (5.11)	36.8 (7.27)	50.18 (6.48)*

* significantly different from non handled group, $p< 0.05$.

The specific behavioral topographies coded following the open field are shown in Table 3-2. Exploratory analyses of the specific behaviors displayed during the post open field session were conducted. Although the groups did not differ in non-stereotyped locomotion [$F(2,49)= 2.305$, $p = 0.110$] or rearing [$H(2)= 0.556$, $p = 0.757$], they did

differ in the percent of time spent inactive [$H(2)= 7.589, p= 0.022$]. MS180 mice spent a greater percent of time inactive than did non handled mice ($p< 0.05$), but MS15 mice did not differ from non handled mice or from MS180 mice ($p> 0.05$). There were also no differences among the groups in burrowing [$H(2)= 0.095, p= 0.95$] or grooming [$H(2)= 3.53, p= 0.17$]. When specific topographies of stereotypy were analyzed, there were no differences among the groups in individual topographies of stereotypy ($p> 0.05$).

Corticosterone Response to Acute Stress

As shown in Figure 3-4, there were no differences in corticosterone response to open field testing [$H(2)=2.115, p= 0.347$] among the three experimental groups (MS15, n=20; MS180, n=19; NH, n=20). There were also no differences in corticosterone following one hour restraint [$F(2,50)= 1.932, p= 0.156$] among the three experimental groups (MS-15, n=14; MS-180, n=13; NH, n=17). In order to examine whether the three experimental groups differed in the return of corticosterone to baseline levels, siblings of those mice tested in the previous experiments were restrained for one hour and blood was taken at 15, 30, and 60 minutes following restraint. A one-way ANOVA was run on corticosterone levels at each of the three timepoints. Corticosterone levels did not differ between the non-handled and MS15 or MS180 groups at any of the three timepoints ($p > 0.05$; Figure 3-5).

Discussion

Deer mice in the brief and extended maternal separation groups showed a trend to display less stereotyped behavior than the non-handled controls when tested in standard cages at day 45, although this decrease was not statistically significant. More robust, statistically significant differences between the groups were observed following a mild

environmental challenge (open field). During the 10-minute post-open field test, both MS-15 and MS-180 mice showed decreased levels of stereotypy compared to nonhandled mice. Thus, early experience in the form of brief and extended maternal separation was associated with a reduction in stereotypy following a mild stressor. This observation suggests an important role for early experience, specifically, early mother-pup interactions in the development of stereotypy. It is interesting that both the brief (MS15) and extended (MS180) maternal separation groups showed the same reduction in stereotypy. Although this maternal separation procedure usually differentiates rats on neuroendocrine and behavioral parameters, the failure to separate brief from extended maternal separation groups has also been reported when rats were tested in the elevated plus maze and in an appetitive response task. Handled (15 minute separation) and MS (180 minute separation) female rats both showed increased time in the open arms of the elevated plus maze, which is often interpreted as a decreased fear response, and an increased consumption of a palatable "snack" (McIntosh et al. 1999).

Unlike studies of rats exposed to brief and extended maternal separation, we did not observe the expected decrease and increase in corticosterone response to an acute stressor, respectively. Generally, when compared to non-handled controls, rats exposed to brief maternal separation display decreased corticosterone and ACTH responses to an acute stress, whereas, rats exposed to extended maternal separation exhibit increased corticosterone and ACTH levels (Plotsky, Meaney, 1993; Meaney et al., 1991, 1993). In rats the decreased stress response in the brief maternal separation group is associated with increased glucocorticoid receptors in the hippocampus and frontal cortex and decreased hypothalamic CRH levels (Meaney et al., 1991, 1993). Conversely, the increased stress

response in the extended maternal separation group has been associated with decreased glucocorticoid receptors in the hippocampus and frontal cortex and increased hypothalamic CRH levels (Plotsky, Meaney, 1993).

The failure to observe the expected differences in corticosterone following mild (open field) and more severe (restraint) stress may be due to several possibilities. First, the differences in corticosterone observed in maternally separated rats can be accounted for by differences in the time taken for corticosterone to return to basal values (Meaney et al. 1991). Due to the increased number of glucocorticoid receptors in the hippocampus, handled rats have a more efficient negative feedback system. Thus, the magnitude of the difference between the handling conditions is not as great at peak corticosterone values. Rather, the group differences appear more strongly in the time taken for corticosterone to return to baseline values (Meaney et al. 1991). Due to methodological limitations, we were not able to obtain repeated blood samples from the deer mice because blood was taken from the retro-orbital sinus cavity. Obtaining multiple blood samples would require an indwelling catheter in the jugular vein, which would be not only technically difficult in deer mice but also interfere with behavioral studies. To address the question of whether mice from the different handling groups differed in the return of corticosterone to baseline levels, untested siblings of the maternally separated (MS15 and MS180) and nonhandled mice were restrained for one hour and blood was sampled at three different timepoints (15, 30, 60 minutes) after removal from the restraint chamber. There were no differences among the groups in corticosterone levels at any of the three timepoints. Interestingly, corticosterone levels did not show a return to baseline when plasma levels were compared across the three timepoints. As each mouse was tested at two timepoints

and not at all three, a repeated measures ANOVA was not performed in the data analysis. A one-way ANOVA was selected to independently compare the groups at the three timepoints, which did not allow us the directly test the effects of time on corticosterone levels. The data, however, do not suggest that corticosterone levels were decreasing systematically as function of time up to one hour following restraint. One hour following such an extreme manipulation such as 60 minute restraint may not be enough time to observe a return to basal corticosterone levels in this species. In order to estimate proper sampling times, a parametric study using multiple animals at each timepoint would need to be conducted since corticosterone time course data following stress are not readily available in deer mice.

The failure to observe corticosterone differences in the experimental groups may also be due to differences in this species' response to the maternal separation procedure. The important component of the handling effect in rats appears to be the mother's behavior in response to the pups when they are reintroduced to the cage (Liu et al. 1997). For example, rates of licking, grooming and arched back nursing in Long Evans hooded rats were positively correlated with hippocampal glucocorticoid receptor density, negatively correlated with hypothalamic CRF content and message, and negatively correlated with ACTH and corticosterone levels following acute stress (Liu et al. 1997). The same maternal behaviors were increased in rats exposed to brief maternal separation and decreased in rats exposed to extended maternal separation (Liu et al. 1997). Since maternal behavior may be the critical component mediating the handling effect, important strain and species differences in maternal behavior may exist that could result in a different pattern of neurobiological changes in the offspring.

Indeed, the neonatal handling / maternal separation procedure produces differing effects on HPA axis function and behavior in rats and mice depending on the strain tested. Most of the neonatal handling studies have focused on Long Evans and Sprague Dawley rat strains (Levine, 1957; Meaney et al. 1991; Plotsky, Meaney, 1993). Durand et al. (1998), however, reported different patterns of HPA axis changes in Lewis rats exposed to neonatal handling. High emotionality and a hyporesponsive HPA axis characterize the Lewis rat strain. Handled Lewis rats did not show a decreased behavioral response to an elevated plus maze although plasma corticosterone was decreased (Durand et al. 1998). In these same experiments, handled Lewis and Long Evans rats did not show an expected decrease in hippocampal glucocorticoid receptors (Durand et al. 1998). Whether or not the different effects of neonatal handling between rat strains is due to differences in maternal behavior cannot be determined from these studies as data on maternal behavior were not obtained on Lewis rats in the Durand et al. (1998) study.

The studies on neonatal handling in mice are relatively sparse and mostly focus on the effects of handling on nociceptive responses in which both increases (Pieretti et al. 1991) and decreases (Clausing et al. 1997) in pain threshold have been reported. In an effort to disentangle potentially important genetic factors associated with neonatal handling, investigators have begun to examine the effects of early maternal separation in various strains of mice. BALB/cByJ mice exposed to brief handling during the first three weeks of life showed improved performance on the Morris water maze and reduced corticosterone response to acute stress compared to non handled BALB/cByJ mice. Early handling, however, was not associated with any improvement in the Morris water maze

or reduction in corticosterone in C57BL/6ByJ mice (Zaharia et al. 1996) Anisman et al. 1998. The importance of maternal behavior in the effects on spatial memory was suggested by the improved performance in the Morris water maze in BALB/cByJ mice when they were cross fostered with C57BL/6ByJ dams (Zaharia et al. 1996). Data on rats and mice suggest an important contribution of genetic and environmental factors to the neuroendocrine and behavioral effects of maternal separation. Potentially important differences in maternal behavior may exist between mice and rats that could contribute to varying outcomes of the maternal separation paradigm. Whether similar maternal behaviors in mice are important mediators of HPA axis development should be tested.

In our experiments the maternally separated groups did not differ from the non-handled group in total activity or the pattern of activity (e.g., time spent in the inner and outer circles) during open field testing. Although the open field in these experiments was intended to provide a mild stressor for the mice, it may be interesting to compare the behavior of deer mice in the open field with that of rats and different strains of mice exposed to similar handling procedures. In general, the data on open field activity in neonatally handled animals have not produced consistent results. In rats some investigators have reported increases in activity in the open field in briefly handled rats (Meaney, Aitken, 1985); whereas, others have reported no difference (Costela et al. 1995; Vallee et al. 1997) or decreased activity (Ogawa et al. 1994) in handled compared to nonhandled rats. Extended (3 hour) maternal separation, on the other hand, resulted in an increased neuroendocrine response to stress (Plotsky & Meaney, 1993) and an attenuation of locomotor responses in a novel environment, as well as anhedonia (reduced responsiveness to alterations in the magnitude of sucrose reinforcement)

(Matthews et al. 1996). When tested in an open field DBA/1 mice exposed to early brief maternal separation showed decreased crossings, decreased center time, decreased rearing, increased grooming, and decreased number of fecal boli (Clausing et al. 1997). Differing effects of open field testing in neonatally handled animals may be due to difference in the open field test itself as well as different early handling procedures. Although increased activity in the open field is often interpreted as a decreased fear response (Ader, 1969), behavior in the open field does not always correlate with the expected changes in HPA activity. For example, in some studies rats that show increased locomotion in the open field also show elevated corticosterone and an increased propensity to engage in schedule-induced polydipsia and amphetamine self-administration (Piazza et al. 1991, Piazza et al. 1993).

Although maternal separation in deer mice did not result in the expected changes in corticosterone, the decreases in stereotypy associated with maternal separation is interesting independent of any changes in HPA axis activity. Several other behavioral differences associated with early handling have been reported which are not necessarily dependent on HPA axis alterations. Brief maternal separation in Sprague Dawley rats was associated with a reduction in amphetamine-induced conditioned place preference, but no change in locomotor response to novelty or amphetamine challenge or stress-induced sensitization of locomotor activity (Campbell, Spear, 1999). Outbred albino mice that were exposed to brief maternal separation showed increased apomorphine-induced climbing (Cabib et al. 1985). Prenatal stress has also been reported to affect HPA axis activity as well as dopaminergic function. Specifically, prenatal stress has been associated with increased locomotor responses to novelty and amphetamine

challenge (Deminiere et al. 1992), facilitation of amphetamine induced sensitization and an increase in D2 receptors in nucleus accumbens (Henry et al. 1995), as well as increases in self-administration of amphetamine (Deminiere et al. 1992). Considering the effects of maternal separation and prenatal stress on the dopamine system and dopamine-related behaviors, the differences in stereotypy between handled and nonhandled deer mice in the current study may potentially be explained through alterations in dopaminergic systems. Potentially important glucocorticoid / dopamine interactions should be examined in the maternally separated deer mice since there are glucocorticoid receptors on dopamine containing neurons of the VTA that project to the nucleus accumbens (Harfstrand et al. 1986) and are capable of modulating dopamine release (Rothschild et al. 1985).

Our data suggest that behavioral differences in maternally separated mice may be due to some mechanism other than differences in HPA axis function or some alteration in HPA activity other than changes in corticosterone response to an acute stressor. The data also suggest that important species differences exist between rats and deer mice (e.g., in maternal behavior) that may be crucial to the early handling effect. These are some of the first studies showing that an early environmental manipulation affects the later development of stereotypy in adulthood. The mechanism of this effect, however, is still not well understood through these studies, and they do not allow us to make conclusions regarding the role of HPA axis activity in the development of stereotypy.

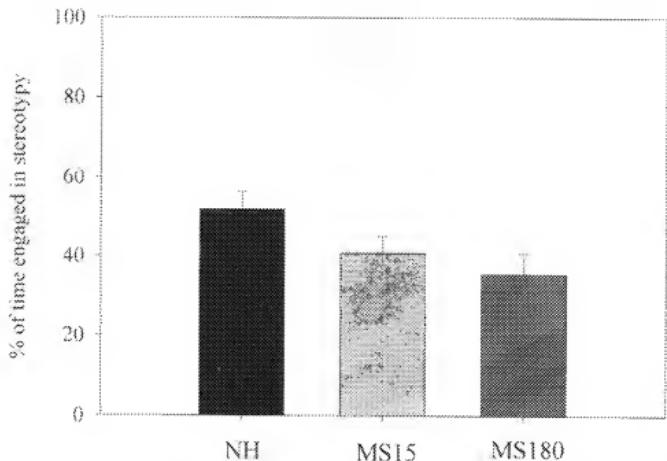


Figure 3-1. Home cage stereotypy (Mean, S.E.M.) in maternally separated mice at Day 45. No significant difference among groups was found.

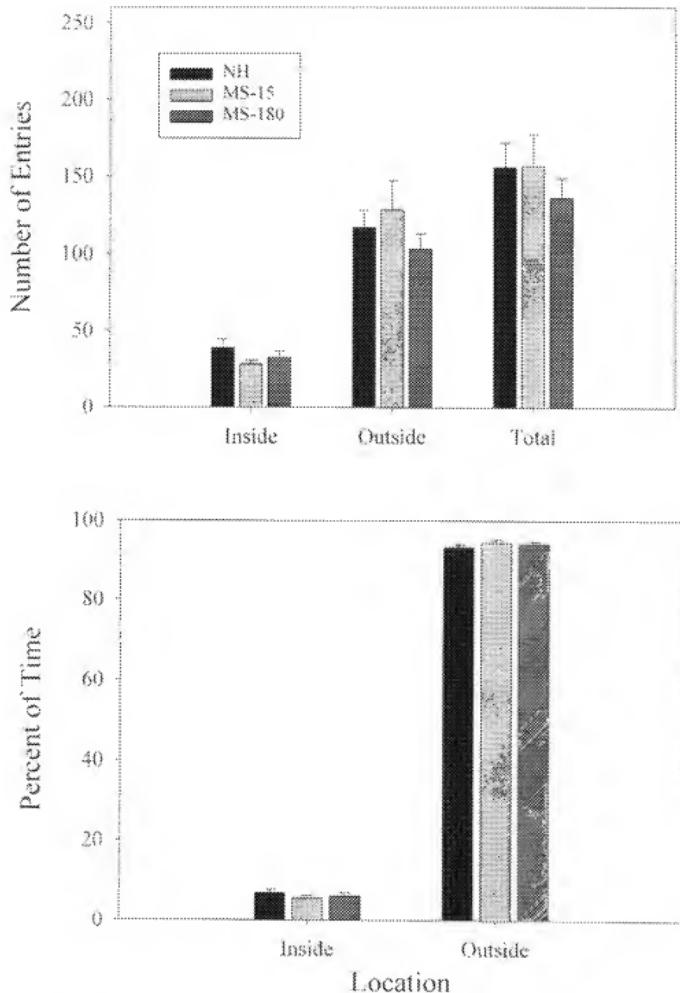


Figure 3-2. Activity of deer mice in the open field. A. Number of entries (Mean, S.E.M) into each circle of the open field. B. Percent of time spent in the inner and outer circles (Mean, S.E.M). There were no significant differences among the groups on any measure.

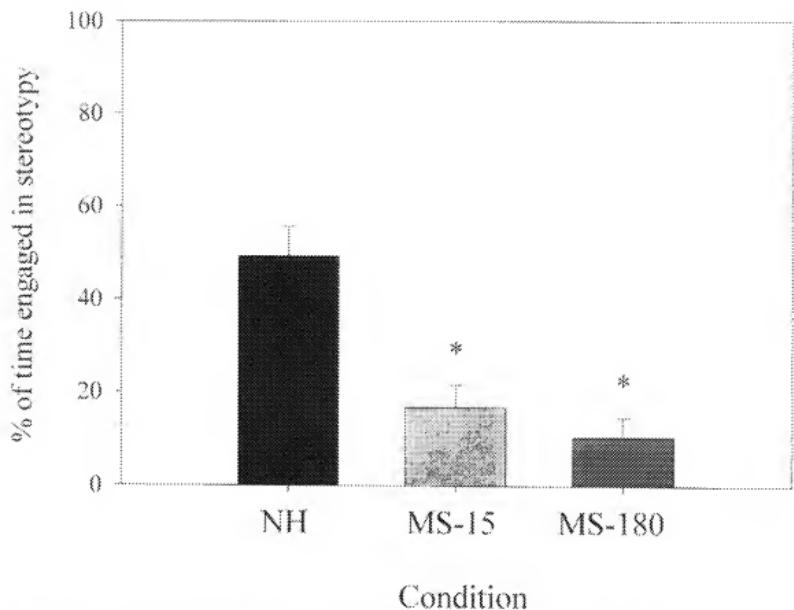


Figure 3-3. Percent time engaged in stereotypic behavior (Mean, S.E.M) following the 10-minute open field test; * significantly different from non-handled mice $p < 0.05$.

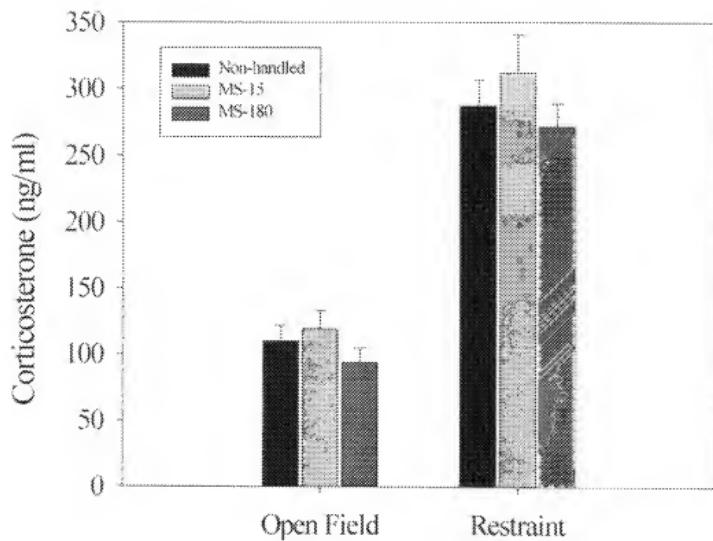


Figure 3-4. Corticosterone levels (ng/ml) in deer mice following a 10-minute open field test and 60-minute restraint stress (Mean, S.E.M.). There were no differences among the groups in corticosterone levels after either open field or restraint.

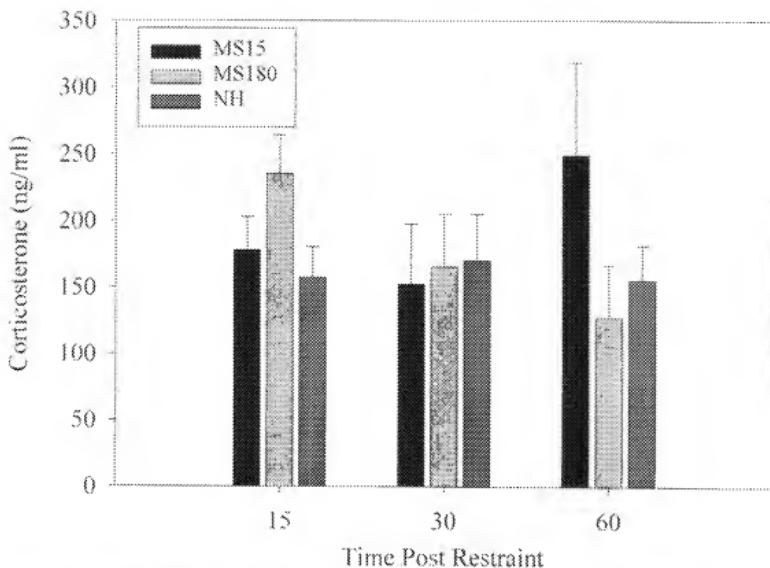


Figure 3-5. Corticosterone (Mean, S.E.M) response to 60 minute restraint stress in untested siblings of maternally separated mice tested behaviorally. No differences were observed among groups at any time point ($p > 0.05$).

CHAPTER 4
DEVELOPMENT OF SPONTANEOUS STEREOTYPED BEHAVIOR IN DEER
MICE: EFFECTS OF EXPOSURE TO A MORE COMPLEX ENVIRONMENT

Introduction

Stereotypies often develop in animals that experience environmental restriction including confinement and movement restraint (Levy, 1944; Meyer-Holzapfel, 1968). As environmental impoverishment is considered a major factor in the development of stereotypies, the effects of environmental enrichment on these behaviors have been examined in various species. In these studies, a decrease in the amount of stereotypy has been associated with an increase in the complexity and size of the animal's environment (Fraser, 1975; Redbo, 1990). In general these studies have used adult subjects which had well-established stereotypies. Little research has examined the effects of enrichment during early development on the prevention or amelioration of stereotypy. Notable exceptions are the work of Ödberg (1987) and Sorensen (1987) who have shown that exposure to enriched cages after weaning markedly reduced the number of bank voles exhibiting stereotyped behavior. Whether such enrichment protected these animals from the stereotypy-inducing effect of subsequent standard laboratory housing was not tested.

The question of whether exposure to an enriched environment at different points in development has differential effects on stereotypy was addressed in two studies. The first study (Cooper et al. 1996) found that when young bank voles were placed in an enriched environment, stereotypies ceased, whereas older animals placed in the same condition continued to engage in stereotyped behaviors. One problem with this study,

however, was the lack of a control group against which to compare the effectiveness of enrichment at different ages. On the other hand, Ödberg (1987) reported that enriching the environment after day 60 of life appeared to be as effective as early enrichment. These discrepant results in the same species leave open the question of a sensitive period associated with environmental enrichment effects.

In the current study, deer mice (*Peromyscus maniculatus bairdii*) were used as a model of spontaneous stereotypies which develop under standard laboratory conditions. In such an environment, these behaviors occur absent pharmacological challenge or challenge with a specific environmental stimulus, and in many cases, dominate the repertoire of the animal (Powell et al. 1999). Preliminary observations have suggested that increasing the level of environmental complexity was associated with decreased stereotypy (Powell et al. 1999). Thus, one goal of the present study was to examine the effect of a significantly larger, more complex environment on the development of stereotypies. In addition, it was hypothesized that stereotypies could be prevented by providing less restrictive environments early in development, that early enrichment would have a protective effect on the later development of stereotypy, and that stereotypies firmly established in the repertoire of the animal will be difficult to reverse by environmental manipulation.

Study 1: Effects of Early and Late Enrichment Using Barn Facility

Study 1 was designed to test whether environmental enrichment at two different points during development would result in a decrease in stereotyped behavior.

Methods

Subjects: A sample of 44 deer mice (*Peromyscus maniculatus bairdii*), approximately half male and half female, from a breeding colony maintained at the University of Florida was used in this study. Animals for this study represented 14 litters and were born to parents maintained under standard cage conditions in a colony room and were kept in these conditions until weaning (24 days of age). Although no systematic data were taken on the parents of these animals, their behavior was representative of the high levels of stereotyped behaviors typically observed in this colony.

Housing Conditions: Two housing conditions were employed in the study. At the time of weaning, each mouse was randomly assigned to either a standard cage or a larger, more complex enclosure (i.e., environmental enrichment). Environmental enrichment involved housing same sex mice in one of four large enclosures (609 cm x 480 cm X 100 cm each) that subdivided a renovated cow barn (Figure 4-1). The enclosures were equipped with hardware cloth dividers, bedding, six nesting squares, large wire mesh cylinders, PVC pipe fittings used as places to hide, and rodent chow and water available *ad lib*. The chow was placed in containers on the floor of the enclosures and the water bottles were hung from the wire mesh. Standard caging involved housing two to three same sex mice in a standard laboratory mouse cage (29 x 18 x 13 cm) that included bedding, two squares of nesting material and food and water available *ad lib*. Standard cages were placed on the ground in one of three smaller areas (363 cm x 242 cm). Animals were left undisturbed in their respective housing conditions except for routine cage maintenance. All mice whether in large enclosures or standard cages received varied

visual, auditory, olfactory stimulation as well as light and temperature changes as a function of time of day and season.

Experimental Design: At the time of weaning, mice were separated by sex and assigned to one of three experimental conditions: early enrichment (EE) ($n = 15$), late enrichment (LE) ($n = 16$) or a control group (C) ($n = 13$). Whenever possible, littermates were assigned to different housing conditions. Early enrichment animals were housed in the large enclosures for approximately 60 days after weaning (until 84 ± 1 days of age) and then transferred to standard cages for an additional 60 day period (until 145 ± 1 days of age). Conversely, late enrichment animals were first housed in standard cages for 60 days after weaning at which time they were transferred to large enclosures for an additional 60 days. Control animals remained in standard cages from weaning until the conclusion of the experiment. The duration of Phase 1 was selected based on previous data from this lab suggesting that most deer mice develop stereotypies by three months of age (Powell et al., 1999).

Since animals were transferred from Phase 1 to Phase 2 at different times depending on age, it was occasionally necessary to add non-experimental mice to maintain at least two animals per standard cage. Therefore, the number of animals in standard cages and enclosures varied throughout the study for LE and EE conditions. As control animals remained in standard cages for the duration of the experiment, the number of animals per cage remained constant.

Observational Procedures: Mice were observed for the last five days of each experimental phase. On the day prior to the initiation of observations, each animal to be observed was marked on the rump using a commercially available blond hair dye for

identification purposes. Observation sessions were conducted under dim red light at two standard times during the animal's dark cycle, between 7 and 9 PM and between 12 and 2 AM every night. Each session involved three 10-minute periods divided into five second scoring intervals. Thus, each animal was observed for a total of five hours. The percentage of animals being observed that exhibited stereotypy was recorded during each five second scoring interval. Due to the large number of animals in the enclosures and our inability to discriminate reliably up to five distinct markings at a time, data on individual animals were not taken. Nonetheless, stereotypic behavior in the enclosures was highly detectable with good inter-observer reliability. The primary stereotyped behaviors observed in standard housing included repetitive jumping, repetitive backward somersaulting and patterned running, consistent with the topographies of stereotypies observed in the colony room. Stereotypies observed in enriched housing included repetitive jumping and patterned running. Inter-rater reliability was obtained prior to the initiation of the study and judged to be acceptable ($k>0.70$). Observers were not blind to housing condition.

Data Analysis: The primary dependent measure was the proportion of animals being observed which exhibited stereotypy per five second interval. For example, the number of mice displaying stereotypy during the 10-minute period was divided by the number of intervals (120) multiplied by the number of animals being observed. Three such 10-minute observation periods were used and averaged to generate daily scores. Since collection of data on individual animals was not possible, the cage became the unit of analysis for graphical description and statistical analysis of the data. In the results section this dependent measure is referred to as the "percentage of stereotypic animals

per scoring interval." For each phase, a one way analysis of variance (ANOVA) was used to test the effect of experimental condition on the dependent variable of mean percentage of stereotypic animals per scoring interval. Post hoc pair-wise comparisons were made using Fisher's least significant difference test.

We also examined the number of animals in each housing condition that developed stereotypies. In order to determine this number, at the end of the observation period, observers endorsed the number of individual animals exhibiting stereotyped behavior in each condition. In every case, there was full agreement across observers. These data were analyzed using a chi square analysis. All analyses were conducted using an alpha level of 0.05.

Results

No overall significant effect of housing condition was found [$F(2,30)=2.49$, $p=.10$] on the percentage of stereotypic animals per scoring interval for Phase 1. Pairwise comparison tests, however, revealed that the early enrichment group exhibited significantly less stereotyped behavior than control animals ($p= 0.04$; Figure 4-2, left panel). No significant difference was found between the amount of stereotyped behavior in early enrichment and late enrichment animals ($p> 0.05$), or between late enrichment and control animals ($p> 0.05$).

Since animals in both late enrichment and control conditions were housed in standard cages during Phase 1, the averages for animals in the late enrichment and control groups were collapsed in order to compare the overall effect of standard caging versus enriched caging. A significant difference in the amount of stereotypy was found

between the standard cage animals and the animals housed in enrichment enclosures, [t = 2.07, df=31, p = 0.02].

The data from Phase 2 are also depicted in Figure 4-2. A significant effect of housing condition was found, [$F(2,30) = 4.17, p = 0.03$] with early enrichment animals exhibiting significantly less stereotyped behavior than control animals ($p= 0.01$). Late enrichment animals also displayed significantly lower levels of stereotyped behaviors than control animals ($p= 0.05$). No significant difference was found, however, in the frequency of stereotyped behavior between late enrichment and early enrichment animals in Phase 2 ($p = 0.52$).

There was a significant association between the number of animals exhibiting stereotypies and housing condition in Phase 1 [$\chi^2(2) = 6.44, p = 0.04$] (see Figure 4-3). As expected, the number of late enrichment animals (standard cages) exhibiting stereotypies (11/16 or 69%) was similar to the number of stereotypic animals in the control condition (10/13 or 77%). The number of animals exhibiting stereotypy in the early enrichment condition (5/15 or 33%), however, was less than the number of stereotypic animals in the two standard cage conditions (late enrichment and control).

There was also significant association between the number of mice exhibiting stereotypies and the three experimental conditions during Phase 2 [$\chi^2(2) = 7.5, p = 0.02$] (Figure 4-3). Fewer late enrichment animals (4/14 or 28%) exhibited stereotyped behavior compared to control animals (6/9 or 67%) in the same phase. The lowest percentage of animals exhibiting stereotypy (2/15 or 13%) was observed in the early enrichment condition.

Phase 1-Phase 2 Comparisons: In order to compare stereotyped behavior across phases, a change in the percentage of stereotypy in each treatment condition was determined. There was an overall difference in the change scores between the three treatment conditions [$F(2,23) = 8.12$, $p = 0.002$]. Both late enrichment ($p = .001$) and early enrichment ($p = 0.002$) animals exhibited significantly lower difference scores (-2.0 and +0.7% respectively) than did controls (+18%).

As seen in Figure 4-3, the percentage of animals exhibiting stereotyped behavior in the control condition is similar from Phase 1 (77%) to Phase 2 (69%). The percentage of animals in the late enrichment condition decreased from Phase 1 (69%) to Phase 2 (35%). The number of animals in the early enrichment condition also decreased from Phase 1 (35%) to Phase 2 (18%).

Summary of Study 1

Between group analyses in both phases as well as phase differences support a strong effect of environmental enrichment on the development of stereotyped behavior in this species. This effect was evident in both the number of animals developing stereotypy as well as quantitative measures of stereotypic behavior. The relatively low rate of stereotyped behavior in late enrichment mice in Phase 1 made the assessment of late enrichment effects ambiguous and prevented conclusions regarding sensitive period effects. In addition, the outcomes of differential housing were assessed in the animals' respective home environments. Thus, it may be unclear whether some of the effects observed were due to long-term exposure to a complex or standard environment or due to the test environment. Finally, the number of mice in the early and late enrichment conditions changed over time due to age differences whereas cagemates stayed constant

for the standard cage group. This housing condition difference may have been a confound. In order to address these issues, two additional experiments were conducted. Study 2 employed individual animal analyses of early enrichment using automated apparatus and a standardized test chamber. Study 3 assessed late enrichment effects also employing automated measurement of stereotypy in a standardized test environment.

Study 2: Effects of Early Enrichment Using Barn Facility and Standard Testing Methods

Subjects: Deer mice from the same breeding colony as described in Study 1 were used in this experiment.

Experimental Design and Housing Conditions: Litters were kept with their mothers in the colony room until weaning (PND25). At this time, mice were transferred to the barn and placed in either standard cages (n=70) or large enclosures (n=64), assigning litters from a given mating pair to both conditions. Each litter was ear-punched for identification purposes. Mice were housed 3/cage in same sex standard cages (45 x 24 x 14 cm) and 16/enclosure in the larger, more complex enclosures. Animals were maintained in their respective housing conditions for approximately 70 days. Enriched and standard housing conditions were identical to those described in Study 1.

Automated Testing: Starting at 95 days of age (\pm 5 days) groups of 12 mice (6 enriched, 6 standard cage) were singly housed in standard test cages (22 x 15 x 28 cm) approximately 30 hours prior to testing in the automated apparatus. The apparatus (Columbus Instruments, Columbus, OH) measured vertical activity with photocell detectors arranged to register only jumping and flipping but not rearing. Test cages were also videotaped in order to assess patterned locomotion. Mice were tested during the dark

phase of the cycle (between 9 PM and 4 AM) under dim red light for two hours per night over two nights.

Results

Figure 4-4 depicts the hourly average stereotypic activity of deer mice in each housing condition in the barn. The effects of housing condition and litter on stereotypy were assessed using a 2-factor factorial design with both housing condition and litter treated as fixed effects. There was a significant effect of housing condition on stereotypy [$F(1,47)= 30.05, p = 0.0001$] with mice housed in larger enclosures exhibiting substantially less stereotypy than standard cage mice ($p= 0.0001$). There was also a significant effect of litter on the expression of stereotypy [$F(17,47)= 3.27, p= 0.0007$]. No significant housing condition by litter interaction was found ($p> 0.05$).

Study 3: Effects of Enrichment Using Renovated Kennels

As we were precluded from using the barn facility, designing a different form of enrichment was necessary to generate stereotypic and non-stereotypic mice for subsequent neurobiological and hormonal assessments. In designing new forms of enrichment, we sought to obtain more control over exposure to external environmental stimuli, thus reducing the effects of partial enrichment in standard caged mice. Thus, this study was conducted to determine whether modifying the form of enrichment would also result in a reduction in stereotyped behavior in deer mice.

Methods

Housing Condition: Mice were kept with their mothers in the colony room until weaning (PND25). At this time, mice were transferred to an animal housing facility and placed in either standard cages ($n=16$) or large, renovated dog kennels ($n=16$), assigning

litters from a given mating pair to both conditions. Each mouse in a given cage or kennel was ear-punched for identification purposes. Mice were housed 3-4/cage in same sex standard cages (45 x 24 x 14 cm) and 8/kennel in the larger, more complex enclosures (27 W x 40 L x 30 H inches; Figure 4-5). The mice were kept on a 16/8 hour reverse light/dark cycle with lights off at 10:30 am. In these experiments, the larger, complex enclosures consisted of dog kennels renovated with wire mesh to house mice. The kennels had three levels connected with ladders so the animal could traverse them freely. They were equipped with a running wheel and various objects and toys on which the mice could climb and hide. The objects included toy houses, Habitrails®, wire mesh structures, plastic dolls, blocks, and kitchenware. The objects were rotated three times a week on Monday, Wednesday, and Friday in order to provide novelty for the animals. All objects except the ones the animals were inhabiting at that time were removed from the kennel, washed with dilute chlorine bleach, and replaced with new objects. Mice were placed in clean, new kennels every other week. Throughout all of these procedures care was taken by the animal care provider to conduct all procedures with minimal disturbance to the mice.

Behavioral Testing: Mice were singly caged and tested in automated apparatuses starting the following day. Behavioral testing procedures were identical to those described in Experiment 1 except mice were tested for two, one hour sessions a day (between 11:30 am – 2:00 pm and 3:30 pm – 6:00 pm) for two days.

Results

The effect of housing condition and litter on stereotypy were assessed using a general linear model, 2-factor factorial design with both housing condition and litter

treated as fixed effects. There was a significant effect of housing condition [$F(1,13)=7.716, p=0.016$]. As shown in Figure 4-6, deer mice raised in enriched environments (kennels) displayed significantly less stereotypy than did deer mice raised in standard cages. In this experiment there was no effect of litter on the expression of stereotypy [$F(9,13)=0.318, p=0.954$].

Discussion

In Study 1, early enrichment animals displayed significantly less stereotyped behavior than animals housed in standard cages at the end of Phase 1. Environmental enrichment effects were also evident in the number of animals exhibiting stereotypies. The criterion for this analysis was liberal, as the amount of stereotyped behavior was not considered in judging an animal as stereotypic. Far fewer animals developed stereotypies in large enclosures versus those in standard cages and as expected, similar percentages of animals exhibited stereotypies in both control and late enrichment conditions during Phase 1. The level of stereotypy in late enrichment mice, however, was relatively low compared to control mice which were also housed in standard cages. This made it difficult to assess late enrichment effects. In subsequent studies in which baseline rates of stereotypy were used to match mice assigned to standard and enriched cages, late enrichment resulted in decreased amounts of stereotypy (unpublished observations).

A significant effect of enrichment across housing conditions was also found during Phase 2. These differences reflect a persistent effect of early enrichment as animals in this condition displayed significantly less stereotypy than controls, despite both groups being housed in standard cages. This group difference was also apparent in the frequency of animals exhibiting stereotypy. As alterations in neuronal

chemoarchitecture have been reported to accompany other forms of environmental enrichment (Comery et al. 1996; Rosenzweig et al. 1978), this persistent effect may be considered as neuroprotective. Neuroprotective effects of enrichment have been demonstrated for both the response to CNS insult as well as to recovery from such injury (Young et al. 1999).

Arguably, the differences observed in housing conditions reflect the animal's response to its home environment and would not generalize to other test contexts even though control and early enrichment mice were housed in the same way in Phase 2. Study 2 addressed this potential confound of housing and test context by adopting an automated evaluation of the frequency of stereotyped behavior in a standard test chamber. The results from this experiment provide additional evidence for enrichment and suggest that results obtained in Study 1 were not an artifact of evaluating the animal in its home environment. These results along with results from Study 3 also suggest that Study 1 differences were not due to differences in the social composition or number of animals in the early and late enrichment conditions.

The low levels of stereotypy exhibited by animals housed in large enclosures during Phase 2 of Study 1 suggested an effect of late enrichment. The relatively low levels of stereotypy observed in these animals when in standard cages in Phase 1 precluded any conclusions being drawn, however, despite substantially fewer animals in this condition displaying stereotyped behavior compared to controls.

These studies support both increased spatial area as well as increased novelty as important factors contributing to the reduction in stereotypy. Enrichment in the barn consisted of a large enclosure with wire mesh on which to climb, providing a large area

for the mice to traverse. The kennels, on the other hand, were much smaller compared to the enclosures in the barn, but consisted of a higher degree of novelty associated with changing enrichment objects. Although the two forms of enrichment cannot be directly compared as there were many other variables (e.g., light/dark cycle, exposure to ambient light, outside noises and odors) which were not held constant between the two studies, it can be noted that raising mice in the kennels did not have as dramatic an effect on reducing stereotypy as did the barn facility.

The comparison of animals housed in large enclosures versus standard cages is a conservative test of environmental enrichment. This is because mice housed in standard cages were exposed to many and varied stimuli associated with the barn including changes in ambient light and temperature and numerous auditory and olfactory stimuli. Thus, the standard cage condition could be construed as partial enrichment. These variables most likely contributed to the relatively low levels of stereotypy observed in standard cages housed in the barn in Study 2. The standard cages used for comparison with the mice raised in kennels in Study 3, on the other hand, were exposed to the same conditions as those in the colony room (16/8 hour light dark cycle, constant temperature (24°C), constant humidity, no exposure to external environment) which most likely contributed to the overall increase in stereotypy in standard and enriched conditions.

Other research has supported a decrease in levels of stereotypy as a result of enriching the animal's environment. For example, moving adult heifers with stereotyped behaviors to an open pasture after being tied up in stalls eliminated stereotypies which were reinstated when the animals were returned to stalls (Redbo, 1990). Although these results suggest a late enrichment effect, the specific time course of the development and

amelioration of these behaviors was not known. This was because age of the animal was not held constant (range = 14 to 23 months) with exposure to condition. Additionally, the lack of a control group makes it difficult to attribute the effects to the experimental condition. Finally, the reinstatement of the stereotypy when the animals were returned to stalls suggests the differences observed were due to the immediate environment and no standardized testing context was employed.

Fraser (1975) found that tethered sows housed in stalls with bare concrete floors exhibited stereotyped behaviors such as bar biting. When straw bedding was placed in the stall, however, the amount of stereotyped behaviors was reduced. The design of Fraser's experiment was similar to the present study (Study 1) in that animals were held in either an "impoverished" environment or a more "enriched" one and then switched after a certain period. Similar to the Redbo (1990) study, Fraser's experiment used sows that were sexually mature. Therefore, the effects of early enrichment or prevention of stereotypy were not addressed. The lack of a control group in this study also makes interpretation of the results difficult. Finally of two studies conducted in bank voles, one (Sorensen, 1987) suggested that stereotypies were harder to disrupt through environmental enrichment in older voles (14 months of age) than in young voles (2 months of age). Using younger animals, Ödberg (1987) was able to demonstrate enrichment effects after 60 days of age but also a late enrichment effect on stereotypies using bank voles exposed to enrichment conditions after day 60 of life. Neither of these studies tested the persistence of enrichment effects when animals were returned to standard environments. Additionally, neither of these studies used an independent and standardized test condition.

In summary, the present findings suggest that the spontaneous stereotyped behavior observed in deer mice under standard laboratory conditions is associated with environmental restriction. Additionally, it appears that early exposure to an enriched environment may prevent the development of stereotypy when animals are housed in restrictive environments later in life. These effects can be observed in the housing condition to which the animal has been assigned as well as in a standardized test context. These studies not only provide support for the importance of environmental restriction in the development of stereotypy, but they also allow us to generate stereotypic and non stereotypic mice for comparisons of stress responsiveness.

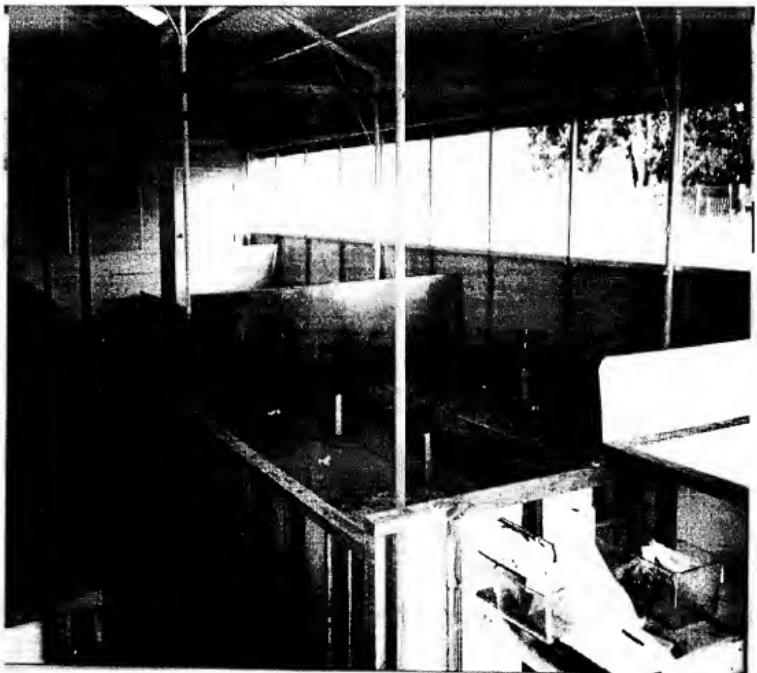


Figure 4-1. Photograph of the barn facility used to house mice in Studies 1 and 2.

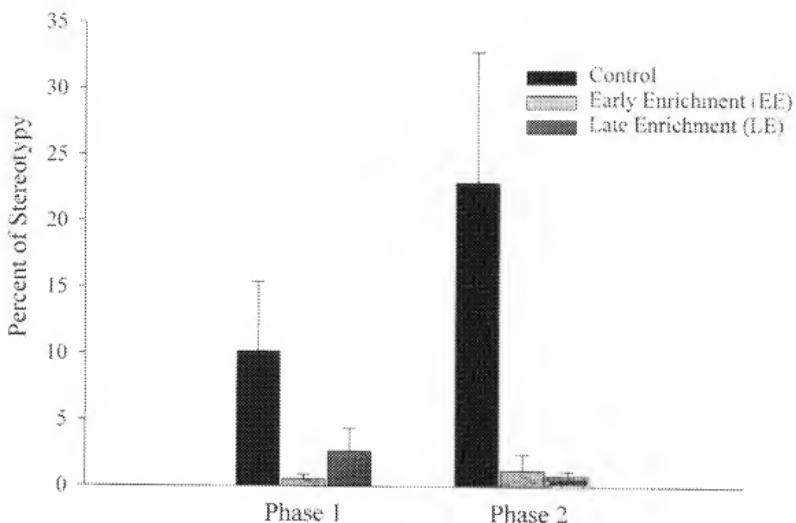


Figure 4-2. Mean (S.E.M) percent of stereotypy in Phase 1 and Phase 2 for control, early enrichment, and late enrichment conditions.

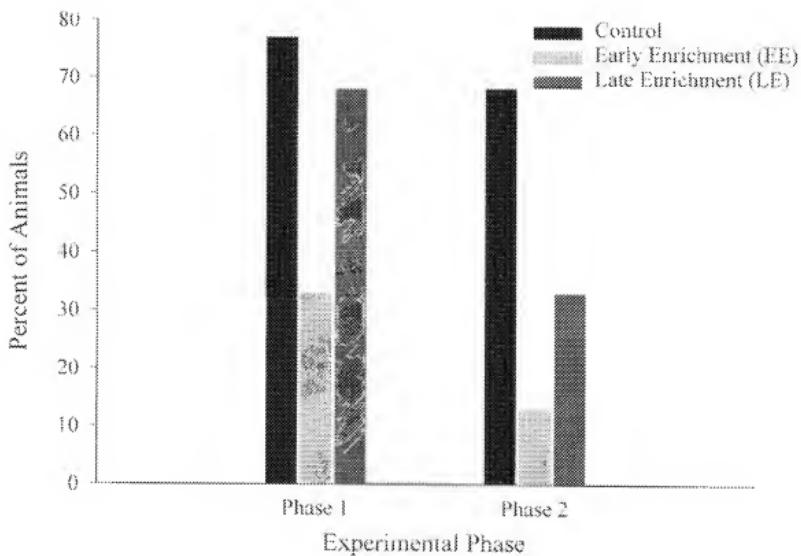


Figure 4-3. Percent of animals exhibiting stereotyped behavior in control, late enrichment and early enrichment conditions for Phases 1 and 2.

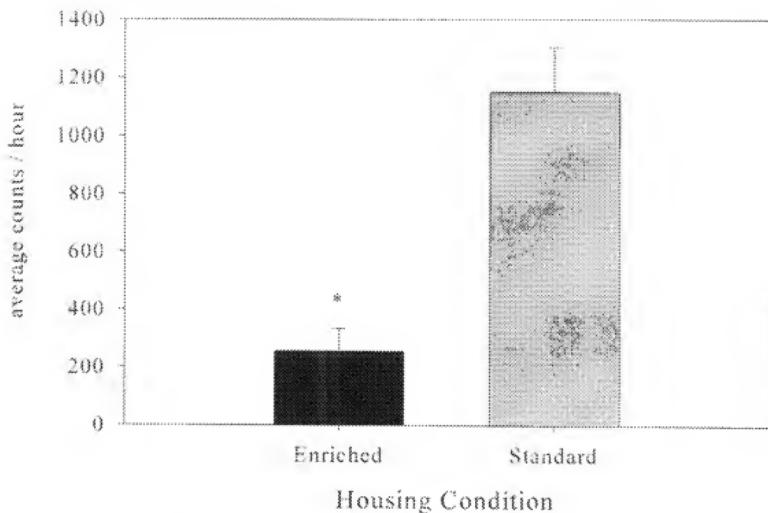


Figure 4-4. Hourly average stereotypic activity (Mean, S.E.M.) of deer mice in early enrichment (barn) and standard housing conditions when tested in a standardized automated test chamber. Mice raised in environmental enrichment exhibited significantly less stereotypy than mice raised in standard cages; $p < 0.001$.

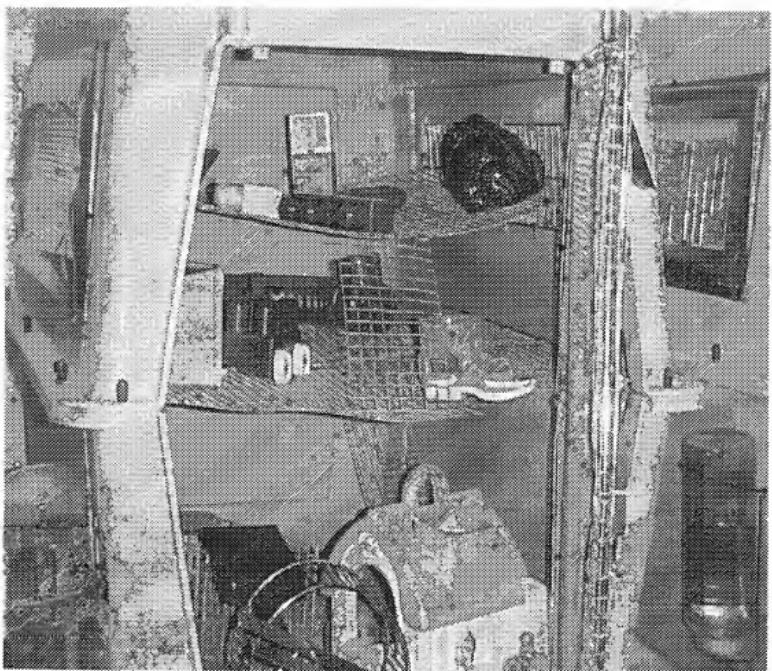


Figure 4-5. Photograph of kennels used in Study 3.

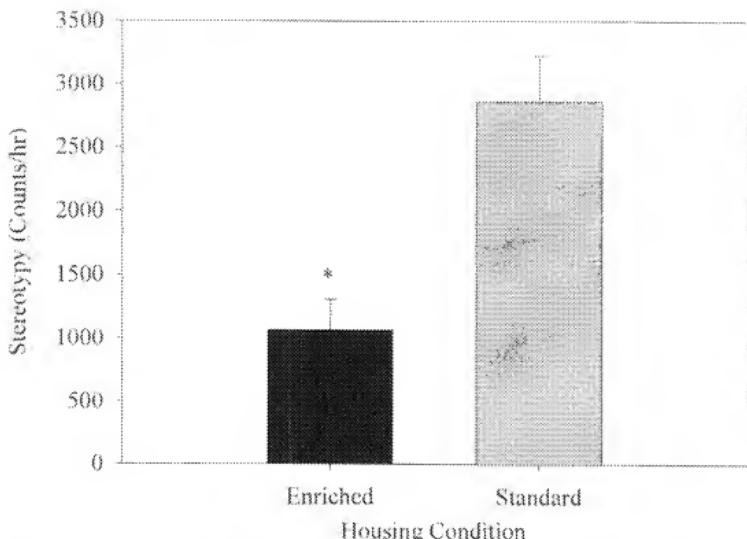


Figure 4-6. Hourly average stereotypic activity (Mean, S.E.M.) of deer mice in early enrichment (kennels) and standard housing conditions when tested in a standardized automated test chamber. Mice raised in environmental enrichment exhibited significantly less stereotypy than mice raised in standard cages; $p < 0.001$.

CHAPTER 5
STRESS RESPONSIVENESS IN STEREOTYPIC AND NON STEREOTYPIC DEER
MICE: ANALYSIS OF HPA AXIS AND DOPAMINE FUNCTION

Introduction

Data from the previous chapter suggest an important relationship between environmental restriction and the development of stereotypy in deer mice. Many investigators have hypothesized that stereotypies developing under conditions of environmental restriction do so as a result of the stress associated with the lack of the opportunity to perform species typical behaviors (Wiepkema, Schouten, 1992). There is evidence that stereotyped behaviors are associated with both decreased (Duncan, 1970; Bareham, 1972; Dawkins, 1980; Cronin, 1985) and increased (von Borell, Hurnik, 1991) indices of HPA activity.

Most of these studies rely on correlative data obtained from farm animals of varied age and duration of restrictive caging and somewhat indirect correlates of HPA function (e.g., adrenal weights). Additionally, distinctions between basal differences in HPA responses (e.g., corticosterone, ACTH) and stress induced elevations of HPA responses in relation to stereotypy have not been adequately addressed in previous work. The potentially important role of dopamine systems which are responsive to stress (particularly mesolimbic and mesocortical dopamine) has been discussed in several models of stereotyped behavior (e.g., apomorphine induced climbing; Cabib, 1993; Cabib et al. 1985), but has not been adequately addressed in relation to spontaneous stereotypy.

The strategy in the second arm of these studies is to (1) probe systems that are responsive to stress by assessing basal measures of HPA axis activity and dopamine function in stereotypic and non stereotypic deer mice, and (2) compare the neuroendocrine and behavioral responses to an acute stress in stereotypic and non stereotypic deer mice. Because stress may play an important role in the genesis of stereotyped behavior, we hypothesized that the level of stereotypy in deer mice would be associated with basal ACTH levels and alterations in dopamine transporter density. We also hypothesized that stereotypic deer mice would show increased corticosterone and ACTH responses compared to non-stereotypic mice when challenged with an acute stressor. Additionally, we proposed that stereotyped behavior would increase following an acute stress that increases ACTH and corticosterone.

There are two important caveats to consider in the following studies. First, these studies were not designed to test directly the coping hypothesis of stereotypy. Second, some of the studies used ACTH levels while others used corticosterone or both as indices of HPA function. The discrepancies in whether ACTH and/or corticosterone were used as endpoints of HPA activity in certain studies stems primarily from our increasing technical capabilities as we progressed through these studies and our increased awareness (through consultations with our collaborator Paul Plotsky) of the relative sensitivity of the two hormones to acute stress. For instance, in studies with deer mice in which multiple samples cannot be obtained and peak responses are of interest, ACTH may be a better endpoint than corticosterone since the adrenal glands saturate more quickly.

Study 1: Stereotypy and Basal ACTH

If indeed frustration or stress is associated with conditions of environmental restriction, such stress may be an important factor in the development of stereotyped behavior. It is now well established that the hypothalamic-pituitary-adrenal (HPA) axis is involved in an animal's response to an environmental stressor (Selye, 1976; Stratakis, Chrousos, 1995). Investigations into the role of the HPA axis in stereotypy have focused mainly on measuring corticosteroid levels or adrenal function. Many stereotyped behaviors have been associated with decreased levels of circulating hormones or adrenal function and this outcome has been interpreted as evidence of a coping function. Pacing, which develops in laying hens housed in small cages, has been associated with a decrease in corticosteroid levels (Duncan, 1970). Repetitive head-flicking, another stereotypy that emerges in domesticated fowl, has been negatively correlated with adrenal weight (Bareham, 1972; Dawkins, 1980). Young tethered sows, which do not typically exhibit stereotypies, often show increased corticosteroid levels relative to the older sows that perform stereotypies (Cronin, 1985). Stereotyped tongue-playing in veal calves has also been reported to be negatively associated with the severity of gastric ulceration associated with restricted housing (Wiepkema, van Hellemond, Roessingh & Romberg, Wiepkema et al. 1987). Additionally, dairy cows with high levels of stereotypy had lower plasma ACTH levels than did cows with low levels of stereotypy (Redbo, 1998).

Some reports, however, have suggested that stereotypies are associated with an increased HPA response to stress. Stereotypic sows have been reported to spend more time active than non-stereotypic sows and show higher locomotion scores in the open field test (von Borell, Hurnik, 1991). Most of the studies testing the coping hypothesis of

stereotypy have focused on schedule-induced polydipsia (SIP). Whereas there are several studies in support of the coping hypothesis for SIP (Tazi et al. 1986; Mittleman et al. 1988), the coping hypothesis of stereotypy has been questioned as subsequent studies of HPA axis function and SIP have generated inconsistent results (Dantzer, Mittleman, 1993). Rats developing SIP can show increased corticosterone levels compared to both baseline levels (Wallace et al. 1983) and to rats exposed to food schedules but not given the opportunity to drink (Mittleman et al. 1988). In the Tazi et al. (1986) study just cited, the rats exposed to the food schedule that were not allowed to drink did not show increased corticosterone levels over baseline measures. Additionally, adjunctive wheel running has been associated with increases in corticosterone levels (Tazi et al. 1986).

Rushen (1993a) has argued that differences in stress physiology (e.g., sympathetic activity, HPA axis activity) in these studies may be explained by individual differences which predispose an animal to stereotypy, rather than a direct effect of engaging in stereotypic behavior (Schouten, Wiepkema, 1991; Schouten et al. 1991). Thus, animals sensitive to stress may be particularly vulnerable to the development of stereotypies. There is evidence that animals and humans that are more sensitive to stress are more likely to develop stereotypy. For example, reactive breeds of horses, *Equus caballus*, are predisposed to develop stereotypy (Kiley-Worthington, 1983), and individuals that show elevated reactions to stress are more susceptible to the psychotic-inducing effects of amphetamine (reviewed by MacLennan, Maier, 1983). Interestingly, rats that were more susceptible to develop amphetamine self-administration were also more likely to develop schedule-induced polydipsia and show increased locomotor responses to novelty (Piazza et al. 1993).

Arguably, the notion of individual differences predisposing an organism to stereotypy may be more appropriate in the deer mouse model than attempting to test the coping hypothesis of stereotypy. Testing the coping hypothesis adequately would require a close temporal register between the performance of the behavior and the resultant changes in corticosterone and/or ACTH levels. In studies of HPA axis activity and stereotypy occurring under conditions of environmental restriction, it is difficult to distinguish between individual differences due to genetic predisposition and early experience and effects of performing the behavior over long periods of time. Thus, the current study was designed as an initial assessment of basal ACTH function in deer mice with varied levels of stereotyped behavior. We examined the degree of correlation between plasma ACTH levels and the behavior and we hypothesized that stereotypy would be associated with increased levels of basal plasma ACTH.

Methods

Subjects. Deer mice (*Peromyscus maniculatus bairdi*) were housed in a standard colony room kept at 24° C and maintained on a 16/8 hour light/dark cycle, with lights off at 0930h. Mice used in this study were approximately 6-15 months of age.

Behavioral Testing. Deer mice were screened in their home cages during the dark phase of the cycle to estimate levels of stereotyped behavior. From the initial screening procedure, 44 mice that ranged from low to high levels of stereotypy were selected for behavioral evaluation. Mice were individually housed in standard hamster cages (22 x 15 x 28 cm) approximately 18 hours prior to testing in automated apparatus. The automated apparatus (Columbus Instruments, Columbus, OH) measured the number of photocell interruptions through detectors, which were arranged to register only jumping and

flipping and not rearing. Behavioral assessment involved placing cages between two frames of photocell beams and videotaping the animals for a one hour period. Cage tops were removed and a wire mesh frame was placed on top of the cage so the cage top itself would not break the beams. Testing took place during the animals' dark phase (between 1100 and 1730) under dim red light on two days. Thus, two hours of behavioral data were obtained on each mouse. Videotapes were screened for accuracy of the automated counters and for topographies of stereotypy such as patterned running and cage top circling that did not register as photocell interruptions. In cases in which the automated apparatus was not accurate (90% accuracy) or the mice displayed patterned running or cage top circling, the entire one hour session was coded from the videotape by an experimenter. Patterned running and cage top circling were counted as an occurrence when the animal completed one full rotation through the pattern (e.g., completion of a circle). A certain amount of non systemic error was due to the small number of counts in the automated apparatus attributed to mice jumping to the cagetop for exploration.

Basal ACTH Determinations. On the day after the two days of behavioral testing, mice were quickly removed from their cages and blood (approximately 250 µL) was taken from the retro-orbital sinus cavity into non-heparinized Natelson tubes and dispensed into microcentrifuge tubes containing 25 µL EDTA (20mg/mL). ACTH determinations were made using an immuno radiometric assay (IRMA) kit (Nichols Institute Diagnostics; San Juan Capistrano, CA) in which the anti-ACTH antibody was labeled with ¹²⁵I.

Results

Stereotyped behavior in the deer mice varied sufficiently to permit a correlational analysis. The topographies of flipping and jumping were analyzed separately since these behaviors differ in the time taken to perform the behavior and thus, the number of beam breaks in a given one hour testing session. For example, the completion of a jump for some animals may take only 0.33 seconds; whereas, a flip may take 1.0 second to complete. Only mice engaging in significant rates of stereotypy ($> 1,000$ counts) were used in the analyses since it was difficult to assign a specific topography to lower rate behaviors which varied more and were often the result of leaping to the cage top.

Stereotyped jumping was positively associated with basal ACTH levels ($r= 0.634$, $p < 0.001$; Figure 5-1). No consistent relationship was observed, however, for flipping and ACTH ($r= 0.124$, $p > 0.05$). As there was some variation in the time it took the experimenters to obtain the blood sample as well as different litters represented in the analysis, bleed time (the latency to obtain blood sample) and litter were added to the model. A multiple linear regression including stereotyped jumping, bleed time, and litter as independent variables was performed to determine the contribution of each parameter to the variance associated with ACTH levels. Adding these variables increased the correlation modestly ($R= 0.72$, $F(3,21)= 7.48$, $p= 0.001$). Jumping ($p= 0.014$) and bleed time ($p= 0.038$) significantly contributed to the ability to predict ACTH, but litter ($p= 0.41$) did not.

Discussion

The results of Study 1 indicate that levels of stereotypic jumping are positively associated with basal ACTH levels, supporting our hypothesis that stereotypy in deer

mice is associated with elevations of basal HPA axis function. These data contradict many other studies suggesting an inverse relationship between stereotypy and HPA axis activity.

As mentioned previously, Rushen (1993a) pointed out the importance of assessing basal differences in stress responsiveness as they relate to stereotypy and not solely attributing HPA axis function to modulations by the behavior as is done in studies testing the coping hypothesis. In other words, animals with increased HPA axis activity may be particularly vulnerable to the development of stereotypies. The observation of a positive association of stereotypy and ACTH is supported by other evidence that animals and humans that are more sensitive to stress are more likely to develop stereotypy (Kiley-Worthington, 1983; MacLennan, Maier, 1983; Piazza et al. 1993). It is unclear from this study, however, whether the mice differ in ACTH levels due to predisposing environmental or genetic factors or whether engaging in the stereotyped behavior itself modulates levels of ACTH. Experiment 2 in Study 3 will address the effect of increased ACTH and corticosterone on the immediate expression of stereotypy.

Study 2: Dopamine Transporter Density in Stereotypic and Non-Stereotypic Deer

Mice

In addition to activation of the HPA axis, dopamine systems are also activated in response to stress (LeMoal, Simon, 1991). Dopamine cell bodies in the ventral tegmental area project to the prefrontal cortex and nucleus accumbens to form the mesocortical and mesolimbic dopamine pathways, respectively. In response to stress (e.g., novelty, restraint), there is an increase in dopamine release in the mesocortical and to a lesser extent, mesolimbic dopamine systems (Roth et al. 1988). Increases in dopamine

utilization in the prefrontal cortex and nucleus accumbens occur in response to stressful stimuli such as moderate footshock (Thierry et al. 1976; Herman et al. 1982). In response to mild electric footshock, however, the mesoprefrontal dopaminergic neurons are more sensitive than are dopaminergic neurons projecting to the nucleus accumbens (Horger, Roth, 1996).

It is now well established that stereotyped patterns of behavior can be induced in a number of mammalian species following administration of drugs that alter nigrostriatal dopamine function (Cooper, Dourish, 1990; Lewis et al. 1996). When administered high doses of psychostimulants such as amphetamine, rats will display stereotyped behaviors including repetitive sniffing and head movements (Robbins, Sahakian, 1981). The stereotypy-inducing effects of psychostimulants have been attributed primarily to activation of the nigrostriatal dopamine system. For example, dopamine or dopamine agonists injected directly into the striatum induce stereotyped behaviors in rats (e.g., Ernst, Smelik, 1966). In fact, specific regions of the striatum have been implicated in specific topographies of stimulant-induced stereotypy (e.g., ventrolateral striatum and orofacial stereotypy; Dickson et al. 1994). Although the nigrostriatal dopamine system has been established as an important site for the induction of stereotypy, other dopamine pathways have been implicated as well. The mesolimbic dopamine pathway may be particularly important in the expression of locomotor stereotypies (LeMoal, Simon, 1991). For example, intra-accumbens injections of amphetamine have been reported to induce stereotypy (Annett et al. 1983) and work on schedule-induced polydipsia has suggested the importance of the nucleus accumbens in the acquisition of these behaviors (Robbins, Koob, 1980).

Dopamine concentrations at the synapse are regulated primarily by high affinity uptake into the presynaptic terminal via the dopamine transporter (Kilty et al. 1991). Perhaps the most compelling evidence for the critical role of the dopamine transporter (DAT) in regulating dopamine comes from studies using dopamine transporter knockout mice. These animals, despite dramatic compensatory responses (e.g., downregulation of D₁ and D₂ receptors, decreased dopamine release), exhibit profound increases in extracellular dopamine and locomotor behavior (Giros et al. 1996)

Differences in DAT density can be interpreted as either a marker of dopamine nerve terminals or a marker of regulatory changes in the transporter in response to alterations in presynaptic dopamine function at the nerve terminal. The dopamine transporter has been shown to be regulated (Reith et al. 1997). For example, the psychostimulant methamphetamine has been shown to decrease dopamine uptake (Fleckenstein et al. 1997), as well as the affinity of the transporter and the Vmax (Bennett et al. 1998). Chronic amphetamine treatment increased DAT mRNA in the substantia nigra (SN) and ventral tegmental area (VTA) (Shilling et al. 1997). In contrast, cocaine has been reported to downregulate DAT mRNA and protein (Letchworth et al. 1997). Other studies have reported alterations in DAT following antidepressant treatment (Petrie et al., 1998) and by second messengers such as tyrosine kinase (Simon et al., 1997) and protein kinase A (Batchelor, Schenk, 1998).

Important to the current study was the observation that DAT binding in the shell of the nucleus accumbens is also regulated by corticosterone (Sarnyai et al. 1998). In these studies adrenalectomy (ADX) resulted in a decrease in DAT in the shell of the nucleus accumbens, but had no effect in the core of the accumbens or in any regions of

the dorsal striatum, olfactory tubercle, VTA, or substantia nigra (Sarnyai et al. 1998). The effects of ADX on dopamine transporter levels appears to be directly related to the effects of ADX on corticosterone since high doses of corticosterone reversed the effect in ADX rats. Additionally, plasma CORT levels were positively correlated with DAT binding in the shell of the accumbens, selectively (Sarnyai et al. 1998).

We used quantitative receptor autoradiography to measure DAT binding in the striatum, nucleus accumbens, and prefrontal cortex, the major terminal fields of dopamine pathways. Increased levels of CORT may lead to an increase in DAT in shell of nucleus accumbens. This observation suggests that we would observe an increase in DAT in stereotypic deer mice if stress is associated with the expression of the behavior. According to the findings in DAT knockout mice (Giros et al. 1996), however, we might observe a decrease in the dopamine transporter since such pronounced increases in motor behavior were observed in DAT knockout mice.

Methods

Subjects. Mice used in this experiment were housed in the barn as described in Chapter 4 (Experiment 2). Ten mice from standard cages with high levels of stereotypy and 10 mice from large enclosures with low or no stereotypy were used. Stereotypic mice were selected from the standard cage condition if their rate of stereotypy (as measured in the automated apparatus) was greater than 1500 counts/hour (Mean= 2397.85, SD= 773.3. Nonstereotypic mice were selected from the enriched housing condition if (1) they did not engage in stereotypy in the enclosures and (2) their rate of stereotypy in the automated apparatus was less than 25 counts/hour (Mean= 2.7, SD= 2.84).

Dopamine Transporter Binding Assay and Quantitative Autoradiography. Brains were sectioned (20 μ m) and thaw mounted onto Superfrost^{+/+} slides for [³H]-mazindol binding in the prefrontal cortex, nucleus accumbens, and caudate putamen. Slides were then stored at -20°C for no longer than 3 weeks. At the time of assay, slides were warmed to room temperature and preincubated in assay buffer (50 mM Tris, 120 mM NaCl, 5 mM KCl, pH=7.4) for 30 minutes. Slides were then incubated in either total or non-specific buffers for one hour at 4°C. Five slides with sections from the three regions of interest were incubated with 15 nM [³H]-mazindol for total binding using 0.3 μ M desmethylimipramine (DMI) to block norepinephrine uptake sites. Adjacent sections were incubated in buffer with 15 nM [³H]-mazindol in the presence of 0.3 μ M DMI and 1 μ M GBR 12909 to define non-specific binding. Slides were then rinsed with 3, 1-minute washes of ice cold assay buffer and then 1, 30-second wash with ice cold deionized H₂O. Slides were dried under the hood overnight and then apposed to tritium sensitive film (Amersham, Arlington Heights, IL) in X-ray cassettes and stored at -20°C for three weeks. A set of tritium standards was also placed in the cassette with the slides. After three weeks, the film was developed with Kodak D-19 developer.

Images were analyzed using the MCID image analysis system (Imaging Research, St. Catherines, Ontario, Canada). Standard curves (fmol/mg) for each film were generated using known concentrations of tritium standards (ARC, St. Louis, MO). Two levels of the caudate putamen were analyzed, three sections at the anterior level and three sections at the central level. Each section of the caudate putamen was divided into four regions (dorsolateral, dorsomedial, ventrolateral, and ventromedial) and sampled on each

side of the brain. The nucleus accumbens was analyzed separately, but the core and the shell were not differentiated.

Results

The binding of [³H]-mazindol in caudate nucleus and nucleus accumbens are shown in Figure 5-2. There was no specific binding of [³H]-mazindol in the prefrontal cortex. A three way ANOVA with repeated measures on two factors (level of caudate, region) was performed on the data to determine the contribution of housing condition, level of caudate (anterior-central), and region (dorsolateral, dorsomedial, ventrolateral, ventromedial) on [³H]-mazindol binding. A Student's t test was used to compare the binding of [³H]-mazindol in the nucleus accumbens. There was a significant effect of level [$F(1,54) = 8.75, p = 0.008$], and region [$F(3,54) = 90.36, p < 0.001$] on [³H]-mazindol binding, but there was no effect of housing condition in the caudate putamen [$F(1,18) = 0.54, p = 0.40$]. There was also no difference between the housing conditions in [³H]-mazindol binding in the nucleus accumbens [$t(18) = -1.033, p = 0.32$]. Representative autoradiograms of total and nonspecific binding of [³H]-mazindol are shown in Figure 5-3.

Discussion

Binding of [³H]-mazindol to regions of the caudate nucleus was slightly increased in non-stereotypic, enriched mice when compared to stereotypic, standard caged mice, although these differences were not statistically significant. There were also no differences in [³H]-mazindol binding in the nucleus accumbens. Our hypothesis was that dopamine transporter may provide some index of dopamine function in brain areas relevant to stereotypy and stress. As we were unable to detect any specific dopamine

transporter binding in the prefrontal cortex, no conclusions can be drawn regarding the role of prefrontal dopamine in the expression of stereotypy. One methodological consideration was the low levels of specific binding of [³H]-mazindol in certain regions of the caudate (20-30%). Although the level of specific binding was low, regional differences in [³H]-mazindol binding in the caudate putamen were detected. Similar regional differences in [³H]-mazindol binding were reported by Marshall et al. (Marshall et al. 1990). Considering the trend for a reduction in [³H]-mazindol binding in enriched mice in all regions studied and the relatively low levels of specific binding, it may be useful to assess a different ligand (e.g., [³H]-WIN 35,428) for analysis of dopamine transporter density in these mice.

Although the dopamine transporter appears to be a key regulator of dopamine activity at the synapse (Giros et al. 1996), a more dynamic measure of dopamine function in the brains of stereotypic and non-stereotypic mice might have been useful (e.g., dopamine release, dopamine uptake). Dopamine is released during stress, preferentially in mesolimbic and mesocortical projections, and as Cabib (1993) has suggested, spontaneous stereotypy may be maintained because of the increased release of dopamine associated with both the environmental context eliciting the behavior and the behavior itself. If amphetamine-induced stereotypies are sensitized by prior exposure to stress and repeated administration of amphetamine, it may be possible that environmentally-induced stereotypies are associated with dopamine sensitization following chronic stress (Cabib, 1993).

Stereotypic mice were selected from standard cages and non-stereotypic mice were selected from enriched housing in order to analyze the brains of mice from the two

extreme ends of the distribution of stereotypy. This selection process, however, fully confounded stereotypy status with housing condition and is a major caveat to the current analysis of DAT density. However, this selection process also allowed us to evaluate differences in dopamine transporter density associated with environmental enrichment. Previous studies have reported that enriched caged (EC) rats showed decreased baseline locomotor activity, lower concentration of DA in striatum and nucleus accumbens, and decreased *in vivo* basal outflow of dopamine in nucleus accumbens and total levels of dopamine in nucleus accumbens and striatum when compared to isolation caged (IC) (Bowling et al. 1993). EC rats have been shown to be more sensitive to locomotor stimulating and rewarding effects of amphetamine (Bowling et al. 1993; Bardo et al. 1995), but less sensitive to sensitizing effects of repeated amphetamine administration. Even though EC and IC rats differed in sensitization to amphetamine, they did not differ in amphetamine stimulated release of DA or DOPAC in the nucleus accumbens or striatum. Thus, several studies have reported decreased dopamine function in rats housed in environmental enrichment.

The trend for a decrease in DAT density in enriched mice in our study may be the result of increased pruning or decreased neuronal density. Several early studies suggested that EC rats have lower neuronal density in cortex due to greater dendritic arborization (Diamond et al. 1964) and increased numbers of glia cells (Diamond et al. 1966) and capillary size (Diamond et al. 1964; Sirevaag, Greenough, 1988). The same changes in neuronal density may occur in sub cortical areas as well, which could potentially result in a decreased number of dopamine axon terminals in the striatum and nucleus accumbens.

Study 3: Response to Acute Stress in Stereotypic and Non-Stereotypic Mice

As reviewed previously, the association of stereotypy with HPA activity has been addressed in a number of species in response to environmental restriction. Most of these studies, however, have examined endpoints of adrenal function that do not allow distinctions between effects due to environmental restriction and effects due to the performance of the behavior. In other words, the association between the moment to moment expression of the behavior as it relates to changes in HPA activity have not been fully addressed in these studies. Additionally, very few studies have assessed differences in stress responsiveness in stereotypic and non-stereotypic animals. To our knowledge, no studies on environmental restriction-induced stereotypy have examined the effects of an acute environmental challenge on the expression of stereotypy and corresponding increases in stress hormones (ACTH, corticosterone).

The potential importance of stress systems in mediating neurobiological effects of environmental enrichment has been addressed through studies on alterations of HPA axis associated with increased environmental complexity. Increased glucocorticoid receptor mRNA in the hippocampus of rats housed in larger, more complex environments has been reported (Olsson et al. 1994). Similar increases in cortical thickness and increased dendritic arborization as that seen with environmental enrichment have been observed following adrenalectomy (ADX) (reviewed by Devenport et al. 1992). These two lines of evidence support the notion that stress (or changes in HPA activity) could be among the mediators of the effects of environmental enrichment on morphological and neurochemical changes in the brain, and potentially, the development of stereotypy.

The main impetus for the following studies was threefold: (1)The potential importance of adrenal function in the neurobiological effects of environmental enrichment, (2) the lack of empirical studies on the moment to moment expression of stereotypy and (3) the lack of empirical studies on neuroendocrine responses of stereotypic and non-stereotypic mice when challenged. We hypothesized that standard caged, stereotypic mice would show increased basal and stress-induced corticosterone and ACTH. Additionally, we hypothesized that an acute stress such as loud, unpredictable acoustic stimuli, which increases ACTH and corticosterone, would increase levels of stereotypy, particularly in standard caged mice.

Experiment 1: Basal and restraint induced corticosterone in enriched and standard caged mice housed in the barn.

Methods

Mice used in this experiment were housed in the barn in either large enclosures or standard cages from weaning until approximately day 110 (Range 101-128) as described in Chapter 4. Enriched (n=12) and standard (n=11) caged mice were singly caged (Day 1) and left undisturbed for 2 days (Day 2-3). On the following day (Day 4), blood was taken from the retro-orbital sinus cavity for baseline plasma corticosterone determinations. These baseline determinations were not used, however, due to problems processing the blood. Therefore, on Day 6 another estimation of baseline corticosterone was taken. On Day 8 mice were restrained for 60 minutes in 45 mL plastic centrifuge tubes containing multiple air holes and blood was taken immediately after removal from the restraint chambers. Mice were returned to their respective housing conditions and left undisturbed for 16 days (+/- 1 day) at which point they were behaviorally assessed in the

automated apparatus for two hour sessions over two nights as described previously in chapter 3.

Results

The data on basal and restraint-induced corticosterone levels are shown in Figure 5-4A. A two way repeated measures ANOVA was used to assess the effects of housing condition and time (pre, post restraint) on corticosterone. There was a significant increase in corticosterone levels following restraint [$F(1,21)= 109.02$, $p< 0.001$, for time] in both standard and enriched housed mice. Mice housed in enriched environments had slightly higher basal and restraint-induced corticosterone levels [$F(1,21)= 4.00$, $p= 0.058$, for condition]. Contrary to our previous findings, however, this set of mice did not differ in stereotypy as a function of housing condition ($T= 124.5$, $p= 0.55$, Mann Whitney Rank Sum; Figure 5-4B). There was also no correlation between amount of stereotypy and either basal ($r= -0.30$, $p= 0.19$) or restraint induced ($r= 0.041$, $p= 0.86$) corticosterone levels. We were not able to compare standard caged, stereotypic mice to enriched, nonstereotypic mice because there were not enough mice at the two extreme ends of the stereotypy distribution to establish large enough groups. As shown in Figure 5-4, the enriched group showed a much higher number of total counts (~850) than the group of mice tested previously in study 2 in chapter 4 (~250 counts; Figure 4-4). The animals tested in this study were individually housed and used to assess basal and restraint-induced corticosterone levels previously. Prior exposure to being singly housed and exposure to a stressor may explain the elevated levels of stereotypy in these mice.

Experiment 2: Behavioral and hormonal response to an acute stressor of enriched and standard caged mice housed in kennels***Methods***

Mice used in this study were the same group of mice housed in the kennels as described in Chapter 4. Following behavioral testing in the automated apparatus (Figure 4-5), mice were transferred to the UF Brain Institute and acclimated to the behavioral testing room in this facility. The following day at approximately 1500 - 1600 hours blood samples were obtained on all mice via retro-orbital sinus sampling for baseline corticosterone and ACTH determinations.

In order to determine both behavioral and hormonal responses to an acute stress in each mouse, a crossover design was used. Two days following baseline blood sampling, 16 mice (8 standard, 8 enriched) were assessed for their behavioral response to an acute stressor. Behavioral testing consisted of videotaping the mice in their home cage for 20 min. to determine baseline levels of stereotypy. The mice were then exposed to acoustic stimulus for 20 min. (120 dB + with an ITI of 10 seconds on average; San Diego Instruments) and then placed back in their home cages and videotaped for another 20 minutes. Two days later the mice were tested for their hormonal response to the same acoustic stimulus. Assessment of the hormonal response to the acoustic stimulus consisted of exposing the mice to the startle chamber for 20 minutes (120 dB; ITI 10 seconds on average) and immediately taking a blood sample from the retro-orbital sinus cavity for ACTH and corticosterone determinations. A second group of 16 mice received the same treatments in the reverse order, blood sampling first followed by behavioral

testing. Videos were scored for the duration of stereotypy (jumping, flipping, patterned running, cagetop circling), locomotion, grooming, rearing, and inactivity.

Blood (approximately 100 µL) was taken from the retro-orbital sinus cavity using non-heparanized Natelson tubes. The sample was quickly expelled into microcentrifuge tubes containing 25 µL of EDTA (20 mg/ml). Blood was centrifuged at 9,500 rpm for 7 minutes and plasma was removed and stored at -80°C until time of assay. Corticosterone determinations were made using a ³H-corticosterone radioimmunoassay kit (ICN Biochemicals Inc., Costa Mesa, CA). ACTH determinations were made as described in Experiment 1 using the ¹²⁵I antibody IRMA kit (Nichols Institute Diagnostics, San Juan Capistrano, CA).

Results

Behavioral Comparisons. Based on stereotypy data obtained from the automated apparatus, mice from standard cages (n=15) and mice from enriched cages (n=14) were tested to examine whether an acute stressor would increase stereotyped behavior. Baseline and post acoustic stimulus stereotypy data are shown in Figure 5-5. A repeated measures analysis of variance (ANOVA) was conducted to examine the main effects of condition (standard v. enriched) and time (repeated factor; pre v. post startle) and any interaction between the two on stereotyped behavior. There was not a significant effect of condition [$F(1,27) = 0.64, p = 0.43$] or time [$F(1,27) = 3.49, p = 0.073$] on stereotyped behavior and no interaction [$F(1,27)= 0.45, p= 0.51$]. Contrary to our hypothesis, there was a trend for stereotypy to decrease in both groups following acoustic startle. Although the primary objective of exposure to unpredictable acoustic stimuli was to increase ACTH and corticosterone levels, the startle data from the acoustic paradigm can

also be obtained. There were no systematic effects of housing condition on startle amplitude (data not shown).

The data on non-stereotyped behavior (locomotion, rearing, grooming, inactivity) as well as stereotypy by topography pre and post acoustic startle are shown in Figure 5-6. Exploratory two way (housing condition x time) repeated measures ANOVAs were conducted on specific topographies of stereotypy and non-stereotyped behavior with time as the repeated factor. The analyses from these ANOVAs are shown in Table 5-1. There was a significant effect of time on jumping and grooming. There was a significant effect of condition on rearing and a significant interaction between condition and time. Post-hoc comparisons using the Tukey test revealed that standard caged mice showed significantly less rearing than did enriched caged mice ($p < 0.05$).

Table 5-1. Two way repeated measures ANOVA results on stereotyped and non-stereotyped behaviors pre and post acoustic startle.

Behavior	Time (Pre v. Post Startle)		Condition (Std v. Enr)		Interaction	
	F Ratio*	P value	F Ratio*	P value	F Ratio*	P value
Jumping	4.52	0.043	0.30	0.59	0.71	0.41
Flipping	0.11	0.74	2.31	0.14	0.10	0.75
Cagetop Circling	0.72	0.41	1.07	0.31	1.42	0.24
Grooming	7.15	0.013	2.44	0.13	0.12	0.74
Rearing	2.01	0.17	5.92	0.022	4.64	0.04
Locomotion	0.16	0.69	2.77	0.11	0.18	0.67
Inactivity	0.082	0.78	0.36	0.55	1.10	0.31

* The degrees of freedom for each comparison were 1 and 27.

ACTH and Corticosterone Comparisons. A two way repeated measures ANOVA (housing condition x time) was also performed on corticosterone and ACTH levels to determine basal and post startle differences between the groups. Some mice had to be removed from the analysis because one of the timepoints for blood samples was missing due to problems with blood sampling or escape from the startle chamber. There was a main effect of time [$F(1,27)= 25.38$, $p < 0.001$], but no main effect of condition [$F(1,27)= 0.97$, $p= 0.33$] and no time by condition interaction [$F(1,27)= 0.19$; $p = 0.67$] on ACTH levels (Figure 5-7A). Similarly, there was a significant effect of time ($F(1,21)= 11.5$, $p= 0.003$) but not condition ($F(1,21)= 0.051$, $p = 0.82$) on corticosterone levels (Figure 5-7B) and no significant interaction ($F(1,21)= 0.047$, $p= 0.83$).

Discussion

The results from Experiment 1 indicate that standard caged, stereotypic mice have slightly lower basal and restraint-induced corticosterone levels than enriched mice. Although this relationship is contrary to what we predicted, there is some evidence that environmental enrichment in mice is associated with increased corticosterone levels. Mice (DBA/2J) raised in enriched cages had higher corticosterone levels than did standard caged mice (Haemisch et al. 1994). The increase in corticosterone levels in deer mice in enriched housing may also be due to a more immediate response to being singly caged for assessment purposes. The housing condition used for testing was similar to the home cage environment of the standard caged mice but dissimilar from the barn enclosures. Mice were singly housed three days prior to being bled for corticosterone determinations, which should have provided ample time for them to habituate to the new

environment. It should be considered, however, that the mice from the enclosures may be displaying a response to restricted housing.

In comparisons of mice housed in kennels and standard cages (Experiment 2), however, there were no differences in corticosterone or ACTH levels either prior to or following exposure to a loud, acoustic stimulus. These studies do not provide information regarding the role of stereotypy as a coping response since comparisons were made between mice housed in environmental restriction and a larger, more complex environment. The failure to observe a difference in corticosterone or ACTH in stereotypic and non-stereotypic mice has been supported by other studies of spontaneous stereotypy. Outbred ICR mice separated into high and low levels of wire gnawing did not differ in corticosterone levels (Wurbel, Stauffacher, 1996). Additionally, when wire gnawing was blocked, corticosterone levels initially increased in mice with high levels of stereotypy but did not persist when corticosterone was assessed 4 and 9 days following prevention of stereotypy. Considering that no other topographies of stereotypy developed (e.g., jumping, another common topography) the return to baseline corticosterone levels suggested that the initial increase was not due to prevention of stereotypy but prevention of a behavioral output (Wurbel, Stauffacher, 1996).

In Experiment 2 deer mice did not show the predicted increase in stereotypy with increased corticosterone and ACTH following loud acoustic stimuli. Although we predicted a more direct relationship between increased corticosterone and ACTH and stereotypy, other studies have also failed to show a relationship between fluctuations in corticosterone levels and the expression of stereotypy. For example, when breeder chickens are placed on fixed feeding schedules, they show increased pacing, drinking,

and pecking at non food objects before feeding (Savory, Mann, 1997). Corticosterone levels did not decline over the day, however, when birds were placed on the restricted ration eventhough there is a decline in the stereotyped behavior (Savory, Mann, 1997). Tethered heifers show stereotyped oral activities (mainly tongue rolling) after being taken from the pasture and tethered in stalls. Although levels of stereotypy increased over the first four weeks at the time when urinary cortisol levels were also high and then declined after four weeks of being tethered when urinary cortisol levels also dropped off, cortisol did not correlate with stereotypy level (Redbo, 1993). Additionally, the stereotypic and non stereotypic heifers did not differ in their cortisol response to ACTH challenge (Redbo, 1993).

In another set of experiments, Savory & Mann (Savory, Mann, 1997) showed that administration of corticosterone increased object pecking in restricted fed chickens, but blocking corticosterone with metyrapone had no effect. In our experiment, the increased corticosterone and ACTH following acoustic stimuli may have increased other neuroendocrine, neurochemical systems or induced behaviors competing with stereotypy. From the analysis of non-stereotyped activity, it appears that grooming increased in both groups following the challenge, but the overall levels of grooming were not extremely high (Figure 5-6). Considering the increased stereotypy in breeder chickens following corticosterone administration (Savory, Mann, 1997), it may be useful to challenge the deer mice with corticosterone to directly test the relationship between corticosterone and stereotypy in this model.

Our initial hypothesis was that increased corticosterone and ACTH would result in an induction of stereotypy in nonstereotypic mice and that the same hormonal

increases would exacerbate stereotypies already in the repertoire of stereotypic mice. We also predicted that stereotypic mice would be more sensitive to an environmental challenge resulting in increased HPA axis activation. Our data from both the barn studies as well as the kennel studies do not suggest increased HPA axis sensitivity of stereotypic mice compared to non stereotypic mice.

General Discussion

Although a positive relationship between basal ACTH and jumping was observed in mice reared in standard laboratory cages in the colony room (Study 1), in studies comparing stereotypic mice from standard caging with non-stereotypic mice from enriched caging there were no differences in basal or post startle corticosterone or ACTH levels. There may be several reasons for these discrepancies. First, the two studies were comparing mice with varied levels of stereotypy. All mice in Study 1 (ACTH - stereotypy correlation) had a fairly high level of stereotypy, whereas, some of the mice in Study 3 had low levels of stereotypy both in the standard and enriched cages. Perhaps stress hormones may play an important role in the degree of stereotypy if the mice have developed relatively high rates of the behavior, but may not be involved in the performance of low rate behaviors or the moment to moment expression of stereotypy. For example, a relationship was not found for flipping, a lower rate behavior, and ACTH. Second, mice in Study 1 (ACTH correlation) were significantly older than mice used in Study 3 (6-15 mo. versus 3-4 mo.). The performance of high rates of stereotypy over long periods of time in standard caged mice in the colony may have affected basal levels of circulating ACTH. There is evidence that chronic elevations in glucocorticoids result in decreased glucocorticoid receptors and potentially cell death in the hippocampus.

The design of the studies allowed for comparisons between enriched and standard caged mice as well. Considering the potential role of glucocorticoids in the mediation of the increased brain weight associated with enrichment, it is surprising that enriched mice did not differ more on endpoints of HPA activity (Devenport et al. 1992). Previous studies have shown that glucocorticoid receptors (GR) are increased in the hippocampus of rats exposed to environmental enrichment (Olsson et al. 1994). An increased number of hippocampal neurons (neurogenesis) in adult mice raised in an enriched environment (Kempermann et al. 1997) has also been reported. Additionally, 5-HT1A receptor mRNA and binding was in the hippocampus of environmentally enriched rats (Rasmuson et al. 1998). Considering the increased GR associated with enrichment, we predicted that when challenged, enriched mice would show a decreased ACTH and corticosterone response. Some of the same problems discussed in Chapter 3 with demonstrating changes in negative feedback while only sampling blood at one timepoint also apply to these studies. It may be useful to examine glucocorticoid or 5-HT1A receptors in enriched and standard caged deer mice and in stereotypic / non-stereotypic mice housed in the same environment.

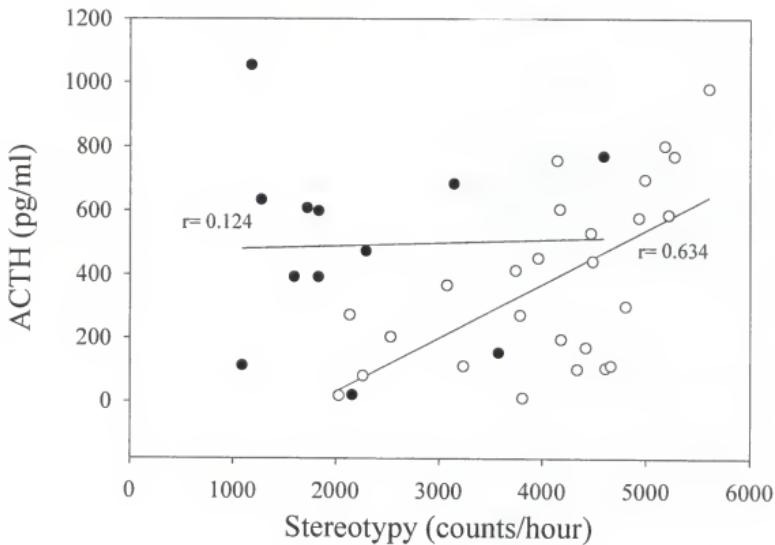


Figure 5-1. Relationship between stereotypy and ACTH (pg/ml). Open circles indicate jumping. Closed circles indicate flipping. There was a significant positive correlation between stereotyped jumping and ACTH levels ($r= 0.634$; $p < 0.001$). There was no significant relationship between stereotyped flipping and ACTH levels ($r= 0.124$; $p > 0.05$).

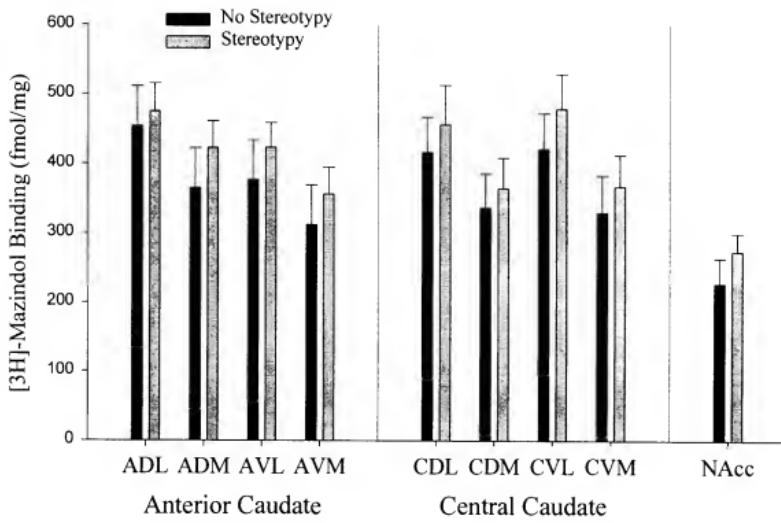


Figure 5-2. Mean (S.E.M.) [^3H]-mazindol binding (15nM) in caudate putamen and nucleus accumbens.

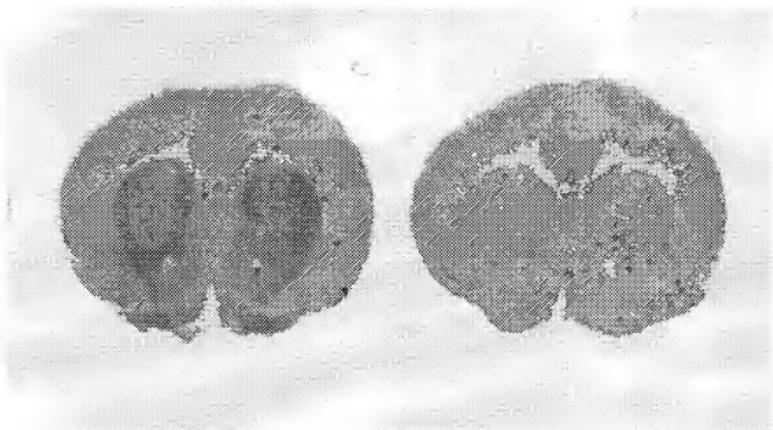


Figure 5.3. Representative autoradiograms of total (left) and nonspecific (right) binding of [^3H]-mazindol in the caudate putamen and nucleus accumbens of deer mouse brains.

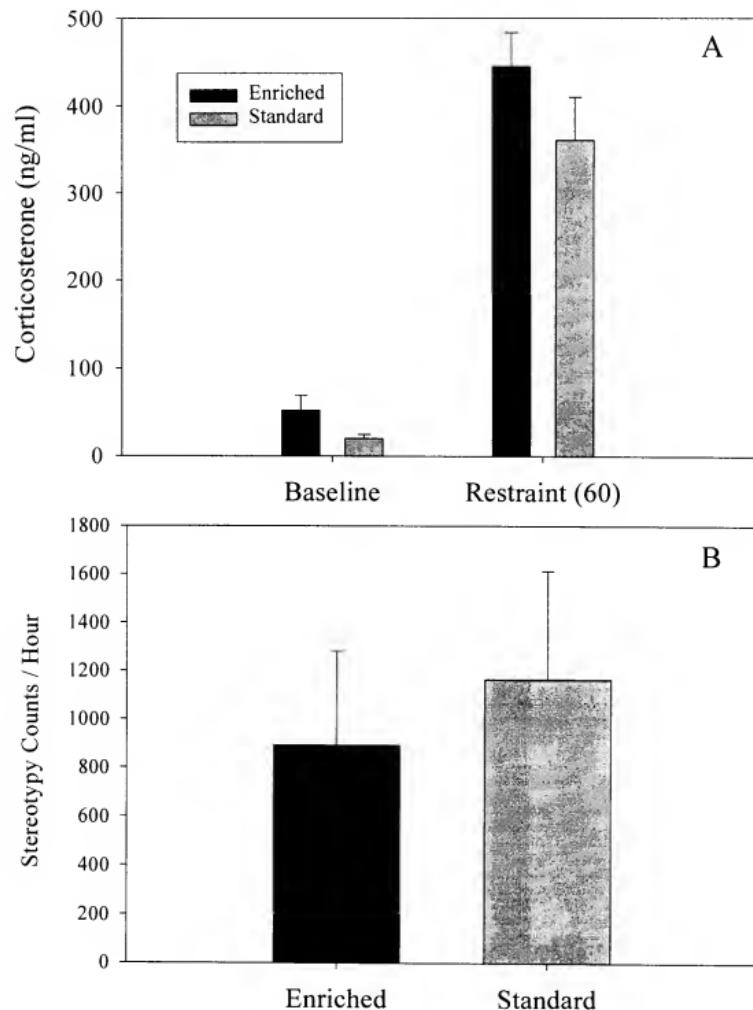


Figure 5-4. A. Corticosterone response (Mean, S.E.M.) in enriched ($n=12$) and standard caged ($n=11$) deer mice housed in the barn. B. Stereotyped behavior (Mean; S.E.M.) of the same deer mice used in the basal and stress induced corticosterone comparison in A.

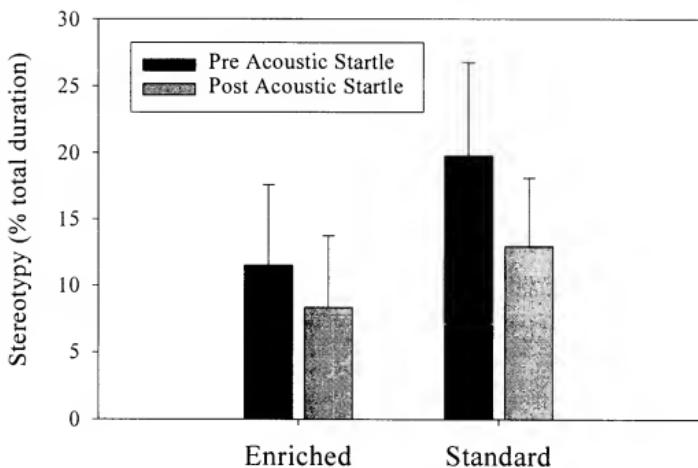


Figure 5-5. Mean (S.E.M.) stereotypy levels (% total duration) pre- and post-acoustic startle in standard ($n=15$) and enriched (kennels; $n=14$) housed mice. There were no significant differences between housing condition or as function of time.

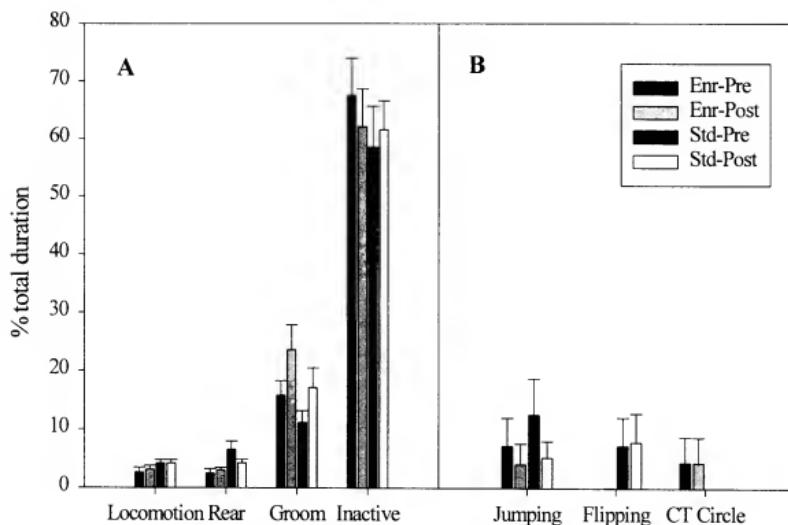


Figure 5-6. Mean (S.E.M.) percent total duration of non stereotyped behavior, A, and stereotyped behavior by topography, B, pre- and post-acoustic startle testing. CT= cage top. There was a significant effect of time for rearing and grooming ($p < 0.05$) and a significant time by housing condition interaction for rearing ($p < 0.05$). There was also a significant effect of time for the jumping topography ($p < 0.05$).

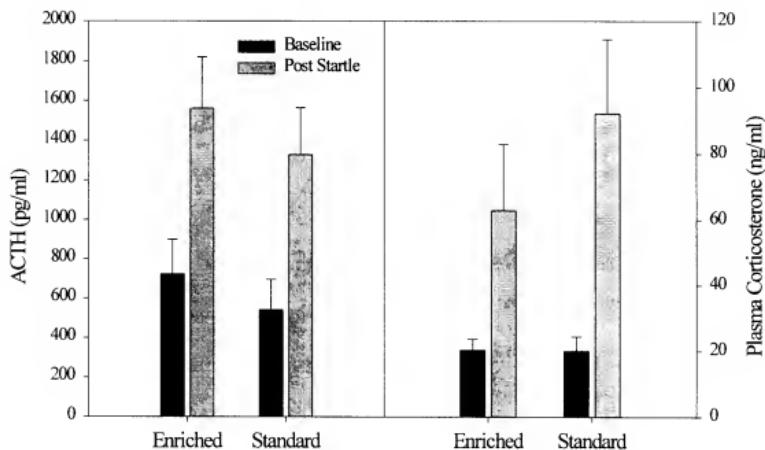


Figure 5-7. Corticosterone (ng/ml) and ACTH (pg/ml) levels pre- and post-acoustic startle in stereotypic and non-stereotypic mice.

CHAPTER 6 GENERAL DISCUSSION

Summary of Results / Future Studies

Stereotypy and HPA Axis Function

In order to determine associations between stress responsiveness and the expression of stereotypy in the deer mouse model, we chose a complementary two strategy approach. To circumvent some of the problems that have plagued the examination of stress and stereotypy, Rushen (1993a) suggested the need to manipulate stress levels and assess the effects on stereotypy. Thus, our first strategy was to induce experimentally differences in HPA axis response to stress and assess stereotyped behavior. We sought to do this by altering stress responsiveness during ontogeny through early maternal separation and assess the later development of stereotypy. Although deer mice exposed to brief and extended maternal separation did not show the predicted stress hypo- and hyperresponsiveness, respectively, maternal separation was associated with decreased levels of stereotypy following challenge in both maternal separation conditions. Although the data suggest an important role for early experience, particularly early mother-pup interactions, in the development of stereotypy, we cannot conclude from the current studies the neurobiological mechanism associated with this reduction in behavior.

The second strategy in these studies was to examine indices of HPA axis activity and dopamine function associated with stereotypy. Two approaches were employed in

carrying out this strategy, (1) assessment of individual differences in the amount of stereotypy and corresponding basal HPA activity; and (2) comparisons of HPA axis function in stereotypic and non-stereotypic mice. In order to begin to assess the importance of individual differences in HPA axis activity associated with stereotypy, we took advantage of the heterogeneity of expression of stereotypy in deer mice and assessed basal ACTH levels. Stereotyped jumping was significantly positively associated with basal plasma ACTH concentrations. Although data supporting the coping hypothesis would suggest the opposite relationship between basal ACTH and stereotypy, our data are supported by other studies reporting a positive relationship (von Borell, Hurnik, 1991). We would hypothesize that individual differences in HPA function may predict differences in the development of stereotypy, and specifically, that mice with higher basal ACTH levels would be more likely to exhibit stereotypy. Although we did not provide evidence for early differences in HPA function and their ability to predict the development of stereotypy, the results are suggestive of a positive relationship. Future studies should include an assessment of basal ACTH and corticosterone levels as well as an environmental challenge (e.g., open field motor activity, response to novelty) early during ontogeny and then assess the later development of stereotypy.

In order to compare HPA endpoints in stereotypic and non-stereotypic mice, the two groups were generated through differential housing (standard caging, environmental enrichment). When stereotypic and non-stereotypic mice were challenged with acoustic stimuli, both groups showed increases in ACTH and corticosterone. Our initial hypothesis was that stereotypic mice would show increased ACTH and corticosterone responses to the environmental challenge compared to non-stereotypic mice. The

magnitude of the increase in both groups was similar, however. We also predicted that the level of stereotypy would increase following the increase in ACTH and corticosterone. Contrary to our hypothesis, the level of stereotypy, particularly in the standard caged mice, decreased following acoustic stimuli. These studies were not designed to test directly the coping hypothesis since a precise temporal register between behavior and blood sample could not be obtained. The data suggest, however, that stereotypy does not occur in direct response to elevations in corticosterone and ACTH produced by an environmental challenge.

The coping hypothesis does not adequately address individual differences in HPA response to environmental restriction and/or intermittent reinforcement. Conversely, the individual differences perspective does not provide hypotheses regarding how the performance of the behavior modulates levels of stress hormones. In studies assessing differences in basal levels of stress hormones, interpretations of the ways in which the behavior is modulated by the hormones and the hormones modulated by the behavior are difficult and misleading, especially if the animals have been housed in restrictive environments for extended periods of time. The temporal relationship between the behavior and stress related variables cannot be inferred through corelative studies in which the hormonal endpoint is temporally removed from the initial development of the behavior. In the deer mouse model, there are several potential ways to address these issues. First, mice could be raised in enriched housing conditions and then subsequently exposed to environmental restriction and assessed for temporal changes in hormonal responses and the development of stereotypy. Second, in developmental studies early individual differences in HPA activity could be determined and then behavioral and

hormonal levels tracked through development. Another useful strategy would be to block the stereotypic behavior using pharmacological manipulations such as a non-peptide CRF antagonist (e.g., CP-154,526) or metyrapone which blocks corticosterone synthesis.

Environmental Enrichment and the Development of Stereotypy

One of the most consistent findings in the current set of studies is that stereotyped behavior can be decreased or prevented through exposure to early environmental enrichment. Although later enrichment was somewhat effective in reducing stereotypy in Study 1 (Chapter 4), conclusions regarding the effects of late enrichment cannot be made from the current set of studies. Subsequent studies in our laboratory using a more controlled design with standard test cages and adequate baseline levels of stereotypy have suggested that late enrichment is also effective in reducing stereotypies in deer mice (unpublished observations).

Two different types of enrichment, one consisting of a large spatial area (barn) and the other consisting of a somewhat smaller spatial area but increased novelty (kennels), were effective in reducing stereotypy. Future studies should address the relative importance of area, novelty, social density, and exposure to external stimuli in the effectiveness of enrichment. Our strategy for using environmental enrichment was (1) to establish the importance of environmental restriction in the genesis of stereotypy; and (2) to provide a method for generating non-stereotypic animals for comparison studies. One important consideration of the current model should be establishing similar effects of environmental enrichment in deer mice as those reported in rats (e.g., improved learning and memory, changes in brain morphology). In studies of environmental enrichment in rats, the importance of glutamate in mediating the increased synaptic

strength (Foster et al. 1996) could be of particular importance to the study of enrichment-related differences in stereotypy. There are important descending glutamatergic projections from cortex to striatum and nucleus accumbens (Vezina, Kim, 1999; Carlsson, 1995), which may contribute to differences in stereotypy. One explanatory hypothesis for the development of stereotypy is that the behavior results from a disinhibition of subcortical areas through decreased activity of descending cortical projections (Cabib, 1993). Release of cortical inhibition associated with stereotypy is not a novel hypothesis, as early writings in psychiatry and neurology describe stereotyped behavior and attribute them to a potential disinhibition of subcortical areas (Jackson, 1884). Thus, there is evidence to suggest an association between enrichment related differences in cortical structures (particularly glutamate projections) and the development of stereotypy in the deer mouse model.

Stereotypy and Dopamine Function

Although the data did not reach statistical significance, there was a trend for enriched mice to have lower levels of [³H]-mazindol binding in the caudate putamen. More dynamic indices of dopamine function in these brain areas should be assessed to more adequately determine the role of dopamine in repetitive behavior. Previous studies with this model have failed to establish a relationship between either D1 and D2 dopamine receptors and dopamine concentrations in the striatum (Powell et al. 1999). A more comprehensive assessment of dopamine function in this model is warranted, however, considering the well established role of dopamine in drug-induced stereotypy (Cooper, Dourish, 1990; Lewis et al. 1996).

Implications

Welfare Concerns

To animal behaviorists concerned with the welfare of animals housed in cages for either food production (e.g., tethered sows, caged veal calves) or human entertainment (e.g., zoos), stereotyped behaviors may indicate the need to increase cage size or complexity of the environment. The present results support this approach and contribute to the expanding body of evidence indicating the importance of increased environmental complexity for captive animals. Primarily concerned with the notion that stereotypy may be an indication of poor animal welfare, the veterinary field has been interested in assessing the validity of this assumption and potential treatments or amendments to the practice of animal husbandry. In this case, the primary question has been whether animals used in intensive husbandry housed in restricted environments are under chronic stress. The current studies suggest that stereotypy in animals housed in environmental restriction for a significant period of time may be related to increased basal ACTH levels. On the other hand, they also suggest that stereotypy does not appear to be an immediate response to increased levels of ACTH and corticosterone and that mice from enriched housing may not differ dramatically in basal stress hormone levels. From the current studies we can conclude that stereotypy results from environmental restriction but that using stereotypy as an indication of chronic stress may be misleading. As Mason (1993) suggested, other indices of chronic stress should be used to assess independently welfare issues and thus avoid the prevailing circularity in the field.

Clinical Significance

To clinicians faced with recurrent stereotyped behaviors in individuals with mental retardation or autism, the coping hypothesis may indicate that the repetitive behaviors should not be targets of behavioral management or pharmacological treatment, since they may serve to reduce arousal or stress. The current studies, however, add to previous work questioning the validity of the coping hypothesis of stereotypy for which there is very little empirical support, particularly in clinical studies. Additionally, effective reduction of stereotyped behaviors in individuals with developmental disabilities does not appear to cause increases in other problem behaviors. In fact, pharmacological and behavioral treatments effective in reducing repetitive behaviors in individuals with mental retardation have resulted in an overall improvement in adaptive training and other problem behaviors (Lewis et al. 1995).

Environmental Enrichment. Our data indicating a reduction or prevention in stereotypy following exposure to a more complex environment may be potentially important to early intervention strategies in developmentally disabled children. The impact that environmental enrichment studies may have on early intervention strategies for at risk children has become increasingly emphasized at the experimental as well as the policy level (Ramey, Ramey, 1999). Important factors contributing to the effects of environmental enrichment on improved learning performance and increased synaptic strength appear to be the extent to which the organism manipulates objects in its environment in a functionally relevant manner (Rosenzweig, Bennett, 1996). Early childhood intervention practices have also highlighted the importance of response contingencies in the effectiveness of the intervention. Combined with the observation

that early enrichment is associated with neuroprotective effects such as reduced apopototic cell death and an increased resistance to CNS insult (e.g., excitotoxicity, seizures) (Young et al. 1999), the current studies which indicate a protective effect of early enrichment on the development of stereotypy (Chapter 3, Study 1) have important implications for the field of mental retardation and developmental disabilities.

Individual Differences. In individuals with mental retardation, the distal causes for the development of stereotypy are much less clear due to the heterogeneity of the population at risk for the development of these abnormal behaviors (e.g., unknown etiology, varied degrees of brain damage). Proximal causes (e.g., frustration, demand), however, may exacerbate stereotypy in these individuals. Our data, however, do not indicate that stereotypy occurs as an immediate response to an acute increase in stress hormones in the deer mouse model. Similar studies on the cortisol response to an acute challenge (e.g., frustrating situation) in individuals with mental retardation could be conducted as well. Examining important individual differences in stress response may prove useful in addressing these issues. For example, stereotyped behavior may develop preferentially in individuals who are more at risk for disregulated HPA axis. In this case, instead of treatments targeting the stereotyped behaviors themselves, treatments may focus on treating the underlying pathology (e.g., anxiety disorder, motor disturbance) and minimizing environmental factors eliciting the target behavior. Our increased understanding of individual differences associated with stereotyped behavior may lead to potential pharmacological treatments for at risk individuals.

Notable exceptions to the dearth of studies on HPA axis activity and stereotypy in humans are the studies of Sandman and his colleagues examining neuroendocrine

responses associated with stereotypy and, primarily, self-injury in individuals with developmental disabilities. These are some of the only clinical studies that have attempted to obtain the close temporal register needed between the performance of the behavior (e.g., self-injury) and a physiological endpoint related to stress (e.g., ACTH). Patients with SIB and stereotypy did not differ in basal cortisol concentrations when compared to individuals with mental retardation without stereotyped or self-injurious behavior (Sandman et al. 1990). Sandman et al. (1997) also reported increased plasma β -endorphin and decreased ACTH after a bout of self-injury when compared to morning levels of control subjects but no difference in β -endorphin and ACTH when compared to a no-SIB condition at the same time of day. Thus, clinical evidence that performance of self-injury modulates levels of neuroendocrine responses is lacking.

Theoretical Concerns

Circularity

Arguably, one of the biggest obstacles in the discussion of the role of stress in stereotypy is the tendency to engage in circular reasoning. For instance, stereotypies have been interpreted as a response to stress since they occur in environments that appear to be sub-optimal. The appearance of stereotypy has been interpreted as an indication that an environment is stressful or sub-optimal for the organism. For example, when describing how stereotypic behavior (presumably repetitive behavior in response to environmental restriction) and schedule-induced behavior are similar, Ladewig et al. (1993) assert that “both include a routine type activity and both develop over time under conditions of apparent stress” (p. 106). In this example, the authors are using function to

provide an explanation of similarity between the behaviors without strong evidence of functionality.

Baumeister (1978) points to the same “conceptual circularity” of the self-stimulatory hypothesis of stereotypy. When assessing whether an environment is unstimulating, it is often difficult to determine what are the critical features to the amount of stimulation. This leads us to assume that if stereotyped behaviors occur, then an environment is essentially unstimulating. “That is, the environment that is supposed to produce such behavior is defined in terms of the very behaviors it is purported to produce - a clearly unsatisfactory definition” (Baumeister, 1978, p. 362). With this theoretical perspective it has also become common to assess the quality of care or quality of life which an institution maintains based on the occurrence of bizarre, repetitive, “self stimulatory” behaviors.

Interpretation of “Stress Responses”

The same circular reasoning regarding the function of stereotypy plagues the interpretations of neurochemical studies as well. Rather uncritically, ACTH and corticosterone have become the hallmarks of the stress response. Extrapolating from one neuroendocrine measure (e.g., plasma corticosterone) and relating it to stress has been one of the major problem in this area of research. Considering that no one study on the physiological correlates of stereotypic behavior will reveal the function of that behavior, the current studies employed several strategies to assess neuroendocrine function associated with stereotypy and did not make conclusions regarding the function of stereotypy based on the data. Experiments such as these are useful, however, in generating convergent information leading to clues about the nature and function of

abnormal repetitive behavior. Integrating our knowledge of neurochemical and endocrine responses to environmental challenges in association with stereotyped behavior across a variety of species may answer many of the questions posed by researchers in the field.

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BIOGRAPHICAL SKETCH

Susan B. Powell was born in Atlanta, Georgia, to Clabron and Rick Powell. She has two sisters, Beth and Laura, and two brother-in-laws, Robbie and Jim. She attended Dunwoody High School and then went on to college at the University of North Carolina at Chapel Hill. She graduated from UNC with a degree in psychology with honors. At UNC she conducted research with Drs. Mark Lewis and Louis Gariépy on postpartum aggression in mice from lines selectively bred for aggressive behavior. She then went to work at Western Carolina Center in Morganton, NC, where she began her study of stereotypic movement disorder in individuals with mental retardation with Dr. Lewis and Dr. Jim Bodfish. After traveling for several months both abroad and within the U.S., she packed her bags and headed to Gainesville to begin her studies in psychobiology at the University of Florida. In Gainesville she enjoys sitting on her porch weathering the summer heat, canoeing in the swamps, swimming in the springs, and taking an occasional bike ride.

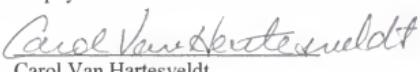
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Professor of Psychology

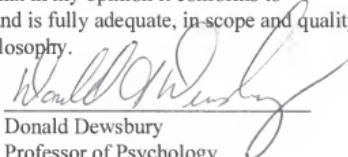
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Professor of Psychology

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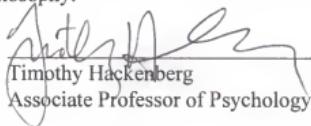
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