

NEUROPLASTICITY IN AN ANIMAL MODEL OF ABNORMAL STEREOTYPED MOTOR
BEHAVIOR: EFFECTS OF ENVIRONMENTAL ENRICHMENT

By

LINDA WEN-HUA LEE

A DISSERTATION PRESENTED TO THE GRADUATE SCHOOL
OF THE UNIVERSITY OF FLORIDA IN PARTIAL FULFILLMENT
OF THE REQUIREMENTS FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY

UNIVERSITY OF FLORIDA

2008

© 2008 Linda Wen-Hua Lee

To my parents, Jau-Ming and Chu-Chen Lee.

ACKNOWLEDGMENTS

I am grateful to many people for their time and help throughout the course of my graduate studies. First and foremost, I would like to thank Drs. Mark H. Lewis, Michael A. King, Kevin K.W. Wang, Darragh P. Devine, and Edwin M. Meyer for serving on my supervisory committee. I would also like to thank my fellow labmates, both past and present. Finally, I would like to thank the faculty, graduate students, and administrative staff of the Department of Neuroscience and the Interdisciplinary Program in Biomedical Sciences.

TABLE OF CONTENTS

	<u>page</u>
ACKNOWLEDGMENTS	4
LIST OF TABLES	8
LIST OF FIGURES	9
ABSTRACT.....	11
CHAPTER	
1 ABNORMAL STEREOTYPED MOTOR BEHAVIOR	13
Introduction.....	13
Prevalence and Significance	14
Human Stereotypies.....	14
Animal Stereotypies	16
Neurobiological Basis of Stereotypy	17
Clinical Studies.....	17
Animal Studies	19
Animal Models of Stereotypy.....	21
Genetic Models of Stereotypy	21
Models of Stereotypy Associated with Insults to the Central Nervous System	23
Models of Pharmacologically Induced Stereotypy.....	25
Models of Stereotypy Associated with Adverse Environmental Circumstances	25
Deer Mice (<i>Peromyscus maniculatus</i>).....	27
2 ENVIRONMENTAL ENRICHMENT	30
Historical Perspective	30
Behavioral Consequences.....	30
Structural and Morphological Modifications.....	32
Regulation of Gene Expression	35
Effects on Neuronal Signaling.....	37
3 SPECIFIC AIMS	40
4 EFFECTS OF ENVIRONMENTAL ENRICHMENT ON THE REGULATION OF DARPP-32 IN AN ANIMAL MODEL OF ABNORMAL STEREOTYPED MOTOR BEHAVIOR.....	42
Introduction.....	42
Materials and Methods	45
Animals.....	45
Behavioral Testing.....	45

Western Blotting.....	46
Statistical Procedures.....	48
Results.....	48
Behavioral Data.....	48
Levels of total DARPP-32, phospho-Thr34-DARPP-32, and phospho-Thr75-DARPP-32.....	48
Levels of phospho-Thr34-DARPP-32/total DARPP-32 and phospho-Thr75-DARPP-32/total DARPP-32.....	49
Discussion.....	49
5 EFFECTS OF ENVIRONMENTAL ENRICHMENT ON PATTERNS OF Δ FOSB EXPRESSION IN AN ANIMAL MODEL OF ABNORMAL STEREOTYPED MOTOR BEHAVIOR.....	59
Introduction.....	59
Methods and Materials.....	61
Animals.....	61
Behavioral Testing.....	62
Western Blotting.....	63
Double-Label Immunohistochemistry.....	64
Image Analysis.....	65
Statistical Procedures.....	65
Results.....	66
Behavioral Data.....	66
Δ FosB Expression in Selected Brain Regions.....	66
Regional Distribution of Striatal FosB/ Δ FosB Expression.....	67
Compartmental Distribution of Striatal FosB/ Δ FosB Expression.....	68
Discussion.....	69
6 EFFECTS OF ENVIRONMENTAL ENRICHMENT AND ABNORMAL STEREOTYPED MOTOR BEHAVIOR ON SYNAPTIC PROTEIN EXPRESSION.....	84
Introduction.....	84
Materials and Methods.....	86
Animals.....	86
Behavioral Testing.....	87
Western Blotting.....	88
Statistical Analysis.....	89
Results.....	89
Behavioral Data.....	89
Effects of Housing Condition and Stereotypy on Synaptic Protein Expression.....	90
Discussion.....	91

7	EFFECTS OF ENVIRONMENTAL ENRICHMENT ON SPATIAL AND STIMULUS-RESPONSE LEARNING DEFICITS ASSOCIATED WITH ABNORMAL STEREOTYPED MOTOR BEHAVIOR	98
	Introduction.....	98
	Materials and Methods	100
	Animals.....	100
	Quantification of Stereotypic Behavior	101
	Morris Water Maze (MWM) Testing	102
	Spatial MWM Task	103
	Cued MWM Task.....	103
	Western Blotting.....	103
	Data Analysis.....	105
	Results.....	105
	Subjects.....	105
	Morris Water Maze Testing.....	106
	Spatial MWM Task	106
	Cued MWM Task.....	108
	Synaptic Proteins	109
	Learning-induced changes in synaptic protein expression.....	109
	Relationships between MWM performance and synaptic protein levels	110
	Discussion.....	110
8	CONCLUSIONS	127
	Summary of Findings	127
	Future Directions	129
	LIST OF REFERENCES	132
	BIOGRAPHICAL SKETCH	160

LIST OF TABLES

<u>Table</u>	<u>page</u>
5-1 Stereotypy scores for experimental groups.....	74
5-2 Levels of Δ FosB expression in selected brain regions.	75
5-3 Correlations between FosB/ Δ FosB in striatal quadrants and stereotypy scores.	76
7-1 Stereotypy scores of experimental groups.	117
7-2 Immunoblotting analysis of synaptic marker proteins.....	118

LIST OF FIGURES

<u>Figure</u>	<u>page</u>
2-1	An example of an enriched environment shown in comparison to a standard laboratory cage.....39
4-1	Stereotypy scores for environmentally enriched, low stereotypy; environmentally enriched, high stereotypy; standard caged, low stereotypy; and standard caged, high stereotypy mice.55
4-2	Western blot analysis of total DARPP-32, phospho-Thr34-DARPP-32, and phospho-Thr75-DARPP-32 levels in the dorsolateral striatum.56
4-3	Concentrations of phospho-Thr34-DARPP-32 and phospho-Thr75-DARPP-32 normalized relative to total DARPP-32 levels.57
4-4	Relationship between the proportion of total DARPP-32 phosphorylated at Thr75 and stereotypy.58
5-1	Representative immunoblot for the detection of FosB isoforms in the striatum.77
5-2	Schematic illustrating striatal subregions.78
5-3	Photomicrographs of sections double stained for FosB/ Δ FosB nuclei and MOR-1-rich striosomes.79
5-4	Subregional distribution of FosB/ Δ FosB-immunoreactive nuclei.80
5-5	Correlations between the estimated number of FosB/ Δ FosB-immunoreactive nuclei and stereotypy scores.81
5-6	Distribution of FosB/ Δ FosB induction in the striosome and matrix compartments of the striatum.....82
5-7	Changes in ISMP value more closely parallel changes in stereotypy.83
6-1	Diagrammatic representation of the dissection procedure.95
6-2	Effects of environmental enrichment and motor stereotypy on synaptophysin protein expression in the dorsolateral striatum.96
6-3	Effects of environmental enrichment and motor stereotypy on NCAM-140 protein expression in the cerebral cortex.....97
7-1	Illustration depicting the timeline of experimental events.119
7-2	Performance during spatial MWM task acquisition.120

7-3	Performance during spatial MWM probe trials.	121
7-4	Performance during cued MWM task acquisition.	122
7-5	Performance during cued MWM probe trials.	123
7-6	Representative immunoblots of the synaptic proteins synaptophysin and neural cell adhesion molecule.	124
7-7	Relationship between synaptophysin levels in the dorsolateral striatum and cued MWM performance.	125
7-8	Relationship between NCAM-120 levels in the hippocampus and cued MWM performance.	126

Abstract of Dissertation Presented to the Graduate School
of the University of Florida in Partial Fulfillment of the
Requirements for the Degree of Doctor of Philosophy

NEUROPLASTICITY IN AN ANIMAL MODEL OF ABNORMAL STEREOTYPED MOTOR
BEHAVIOR: EFFECTS OF ENVIRONMENTAL ENRICHMENT

By

Linda Wen-Hua Lee

August 2008

Chair: Mark H. Lewis

Major: Medical Sciences--Neuroscience

Although abnormal, repetitive body movements (i.e., stereotypies) manifest in many neuropsychiatric illnesses, the neurobiological basis of these behaviors remains poorly characterized. Deer mice (*Peromyscus maniculatus*) provide an animal model of stereotypy in which the expression of stereotyped behaviors varies inversely as a function of environmental complexity. The objective of the present studies was to identify the molecular and cellular mechanisms that contribute to enrichment-related behavioral plasticity.

We first examined the impact of environmental manipulations on the functional status of DARPP-32, a key mediator of dopamine signaling. The data revealed an inverse relationship between stereotypy and the proportion of DARPP-32 phosphorylated at Thr75. Furthermore, enrichment-related attenuation of stereotypy was also associated with a decrease in total DARPP-32 expression.

Using Δ FosB as an index of neuronal activation, our second study examined the effects of environmental enrichment on long-term changes in gene expression. The results showed that stereotypy was associated with regionally and compartmentally specific patterns of Δ FosB induction with the effects of enrichment localized primarily to the dorsolateral striatum.

Our third study examined the effects of enrichment on synaptic protein expression. Stereotypy was associated with decreased levels of synaptophysin in the dorsolateral striatum and neural cell adhesion molecule-140 (NCAM-140) in the cortex, whereas enrichment was associated with increased expression of synaptophysin and NCAM-140 in the dorsolateral striatum and cortex, respectively.

Our final study examined whether stereotypy would be associated with other behavioral deficits and, coincidentally, whether the beneficial effects of environmental enrichment would also extend to these deficits. The results showed that stereotypy was associated with deficits in both spatial and stimulus-response learning and memory ability. In addition, we found that enrichment was able to counteract deficits in spatial but not stimulus-response learning.

Collectively, these findings suggest that decreases in stereotypy and associated deficits due to environmental enrichment are linked to regionally specific changes in neuronal communication, gene expression, and synaptic function. Hopefully, the novel mechanistic insights derived from this research will eventually reveal new therapeutic avenues for the treatment of human repetitive behavior disorders.

CHAPTER 1

ABNORMAL STEREOTYPED MOTOR BEHAVIOR

Introduction

Stereotypy refers to sequences of motor behavior which are characterized by excessive repetition, topographical invariance, and purposelessness (Lewis and Baumeister, 1982; Berkson, 1983; Mason, 1991; Ridley, 1994). These behaviors manifest in a wide variety of animal species, including man, and are striking in their frequency and intensity as well as in the rhythmical quality of their expression. Furthermore, because they occur absent a functional context and have, on occasion, been associated with maladaptive consequences, stereotypies are also generally regarded as being abnormal behaviors.

Motor stereotypies represent one specific form of abnormal repetitive behavior. Multiple categories of clinically relevant repetitive behaviors exist. These can include purely motoric behaviors, such as stereotyped body movements, self-injurious behaviors, repetitive manipulation of objects, tics, dyskinesias, and akathisia, as well as more complex behaviors, such as repetitive verbal behaviors (e.g., echolalia), compulsions, rituals and routines, insistence on sameness, and preoccupation with narrow, circumscribed interests (Lewis and Bodfish, 1998). The studies comprising this dissertation employed an animal model of abnormal stereotyped motor behavior. However, animal behaviors can be interpreted in many different ways by many different researchers. Therefore, the following literature review will focus primarily on stereotypy, however, with the understanding that these behaviors may share considerable phenomenological and conceptual overlap with a broad spectrum of abnormal repetitive behaviors.

Prevalence and Significance

Human Stereotypies

Stereotypies are common in typically developing infants (Thelen, 1979) and often occur at subclinical levels within normal adult populations (Berkson et al., 1999; Rafaeli-Mor et al., 1999). However, stereotypies can also become behaviorally dominant in many clinical conditions. Indeed, the behavioral phenotypes of many neurodevelopmental (e.g., autism, mental retardation, Rett syndrome), psychiatric (e.g., schizophrenia, obsessive-compulsive disorder, chronic drug abuse), neurological (e.g., Tourette's syndrome, congenital blindness or deafness, brain injury, Parkinson's disease, Sydenham's chorea), and genetic (e.g., Lesch-Nyhan syndrome, Prader-Willi syndrome, Cri du Chat syndrome) disorders often include specific forms of stereotyped behaviors.

For example, body-rocking, head-rolling, and hand-flapping are among the most common topographies of stereotypic behavior observed in autistic and mentally retarded individuals. Repetitive hand movements (e.g., wringing, washing, clapping, and twirling) are characteristic of Rett syndrome (Temudo et al., 2007), while eye-pressing, eye-rubbing, and eye-poking are among the most prevalent stereotypies reported in congenitally blind children (Troster et al., 1991; Fazzi et al., 1999). Originally described in chronic abusers of amphetamine (Ellinwood, 1967; Rylander, 1972; Ellinwood et al., 1973), "punding," a complex and peculiar form of stereotypy characterized by an intense fascination with the repetitive manipulation of objects (e.g., continual handling, assembling and disassembling, collecting, sorting, etc.) has also been documented more recently in Parkinson's disease patients following dopamine replacement therapy (Evans et al., 2004; Lawrence et al., 2007). Finally, repetitive self-injurious behaviors, such as self-biting and skin-picking, feature prominently in the behavioral profiles of Lesch-Nyhan (Robey et al., 2003) and Prader-Willi patients (Symons et al., 1999), respectively.

Although considered relatively innocuous by some clinicians, stereotypes can, nevertheless, have profound and multitudinous consequences on adaptive functioning (Matson et al., 1997). First, because they are often perceived as being inappropriate to context, stereotypes can be stigmatizing (Jones et al., 1990). As a result, this may limit participation in social activities. In fact, it has been shown that mentally retarded individuals with stereotypes display more negative nonverbal social skills and fewer general positive social skills (Matson et al., 2006) and have greater difficulties in forming interpersonal relationships, communicating, and socializing than non-stereotypic controls (Matson et al., 1997).

Stereotypes are also considered problem behaviors because of their disruptive effects on learning. Stereotypes have been shown to impair the acquisition of new skills (Koegel and Covert, 1972; Dunlap et al., 1983) as well as the performance of established skills (Morrison and Rosales-Ruiz, 1997), therefore, controlling stereotypes may be a necessary precondition for effective learning. Indeed, correlational studies have shown that developmentally disabled individuals who exhibit more stereotypic behaviors also have lower IQs (Turner, 1999; Bishop et al., 2006).

Because clinical stereotypes occupy a large proportion of a patient's behavioral repertoire, they can hinder sustained interaction with the environment. In extreme cases, severe and persistent stereotypes can even become crippling in their interference with intentional activities. This is the case in Rett syndrome where the gradual loss of purposeful hand movements is a tragic consequence seen with disease progression (Temudo et al., 2007).

Perhaps the most clinically significant aspect of stereotype is its relationship to self-injury. Stereotypes are frequently comorbid with and have been proposed as a potential precursor of self-injurious behaviors (Kennedy et al., 2002; Richman and Lindauer, 2005; Richman, 2008).

Well-established self-injurious behaviors are more difficult to treat than emerging self-injurious behaviors. Therefore, it may be advantageous for clinicians to target stereotypies for therapeutic intervention antecedent to the development of more serious, self-injurious behaviors.

Animal Stereotypies

Although mostly absent in wild populations, stereotypies are commonly observed in animals kept in captivity, such as those housed in zoo, farm, and laboratory environments. Similar to the distinct topographies observed in clinical stereotypies, animal stereotypies are species-specific with widely varying morphologies. To illustrate, some examples of animal stereotypies include swaying and trunk-swinging in elephants (Elzanowski and Sergiel, 2006), pacing in bears (Vickery and Mason, 2004) and big cats (Jenny and Schmid, 2002; Bashaw et al., 2007), licking in giraffe and okapi (Bashaw et al., 2001), repetitive pawing in wombats (Hogan and Tribe, 2007), body-rocking, self-clasping, and self-biting in nonhuman primates (Lutz et al., 2003; Mallapur and Choudhury, 2003; Tarou et al., 2005), sham-chewing and bar-mouthing in tethered sows (Cronin and Wiepkema, 1984), crib-biting, box-walking, and wind-sucking in horses (Nicol, 1999), head-twirling and nodding in farmed mink (Mason, 1993), route-tracing in songbirds (Garner et al., 2003b), feather-picking and perch-dancing in parrots (Garner et al., 2003a; Meehan et al., 2004; Garner et al., 2006), digging in gerbils (Wiedenmayer and Brunner, 1993), bar-mouthing in bank voles (Garner and Mason, 2002), and wire-gnawing and jumping in ICR mice (Würbel et al., 1996).

Indeed, stereotypies are the most common category of abnormal behavior reported in captive animals (Würbel, 2001) with a recent estimate suggesting that stereotypies are displayed by over 85 million zoo, farm, and laboratory animals worldwide (Mason and Latham, 2004). The high occurrence of stereotypic behaviors in captive animals is a source of intense concern for animal caretakers. Among the deleterious consequences that have been attributed to stereotypy

are impaired growth, self-injury, decreased reproductive success, and increased mortality (Mason, 1991; Mason et al., 1995; Lutz et al., 2003). Because of their frequent association with adverse environmental circumstances and suboptimal health outcomes, stereotypies in captive populations have been construed as an indicator of poor animal welfare and a warning sign of potential suffering (Mason, 1991; Mason and Latham, 2004). Furthermore, with regard to the use of experimental animals, stereotypies have also been interpreted as symptomatic of abnormal brain functioning, thus, raising concerns about the validity, reliability, and replicability of scientific research conducted using stereotypic laboratory animals (Garner, 2005).

Neurobiological Basis of Stereotypy

Despite its widespread prevalence and significance, the critical factors contributing to the emergence and persistence of stereotypic behaviors remain poorly defined. However, while the specific mechanisms are unknown, substantial evidence from both clinical and animal studies indicates that behavioral stereotypy may originate as a consequence of basal ganglia dysfunction.

Clinical Studies

Many experimental approaches have been used to study stereotypic behaviors in clinical populations. For example, analysis of spontaneous eye-blink rates can be employed as a non-invasive probe of central dopamine activity (Karson, 1983). Several studies have shown that mentally retarded patients who exhibit frequent stereotyped behaviors have significantly lower blink rates than matched controls who do not engage in stereotypy, suggesting that hypodopaminergic function might be responsible for stereotypy in these subjects (MacLean et al., 1985; Bodfish et al., 1995; Roebel and MacLean, 2007). Dopamine functioning in mentally retarded individuals has also been evaluated by measuring plasma concentrations of homovanillic acid (HVA), a dopamine metabolite (Lewis et al., 1996). In this study, individuals

with high levels of stereotypy were found to have lower levels of HVA, providing further support for a hypothesis of dopamine deficiency in relation to stereotypy.

In addition to biobehavioral and biochemical markers of dopamine function, neuropsychological tasks designed to detect basal ganglia pathology have also been used to study stereotypic subjects. As an example of one task, patients are presented with two stimuli over a series of trials and instructed to find the rule governing reward presentation. In actuality, the chosen stimulus is rewarded at random for each trial. Whereas normal controls will produce a random sequence of responses over time, subjects with damage to the basal ganglia will demonstrate a tendency to repeat a specific response, a state called recurrent perseveration. The degree of recurrent perseveration, as measured by this task, has been shown to be significantly correlated with the severity of spontaneously occurring stereotypies in both autistic (Turner and Russell, 1997) and schizophrenic (Frith and Done, 1983) patients. This relationship has also been demonstrated using other tasks to measure perseveration. For example, in a study of high-functioning autistic individuals, a significant correlation was found between repetitive behavior scores and the number of perseverative responses in the Wisconsin Card Sorting Task (South et al., 2007). In another study of autistic adults (Lopez et al., 2005), subjects were given a battery of neuropsychological tests designed to measure executive functions (i.e., cognitive flexibility, working memory, response inhibition, planning, and fluency). Of the processes examined, cognitive rigidity was the only executive deficit which predicted the restricted, repetitive symptoms of the autistic subjects, suggesting that the cognitive impairments associated with the repetitive behaviors were selective for the basal ganglia rather than being global in nature.

The presence of neuroanatomical abnormalities associated with stereotyped behaviors have also been localized to the basal ganglia using neuroimaging techniques. Reductions in the

absolute volume of the caudate nucleus have been reported in autistic children with simple motor stereotypies involving repetitive movements of the arms and hands (Kates et al., 2005).

Similarly, abnormalities in caudate volume have also been found in association with both lower-order and higher-order repetitive behaviors in autism (Sears et al., 1999). Interestingly, a positive correlation was found between caudate volume and stereotyped motor mannerisms, a lower-order behavior, whereas a negative correlation was found between caudate volume and higher-order behaviors, such as compulsions, rituals, and resistance to change. This is in contrast to another study of repetitive behaviors in autism wherein increases in right caudate and total putamen volumes were found to be driven primarily by the presence of higher-order repetitive behaviors (Hollander et al., 2005). Although the direction of change is different in these studies, these results collectively demonstrate that a relationship exists between structural abnormalities of the basal ganglia and repetitive behaviors in autism.

On occasion, individual case reports within the clinical literature have also documented the appearance of motor stereotypies following damage to basal ganglia structures. In one instance, a 17-year-old boy began demonstrating circling behaviors and complex hand stereotypies after experiencing a right putaminal infarct (Maraganore et al., 1991). In another case, a previously healthy 27-year-old woman with face, neck, and limb stereotypies was later found to have a lesion of the right caudate nucleus (Edwards et al., 2004). For the most part, these observations are congruent with hypothesis-based experimental findings and also serve to provide further support for a role of compromised basal ganglia function in the pathogenesis of stereotyped behaviors.

Animal Studies

Experimental evidence from animal studies also lend credence to the theory that disruptions in the cortico-basal ganglia neural circuits which control motor behavior culminate in

stereotypy. In particular, pharmacological data have implicated the involvement of the nigrostriatal dopamine system in the production of stereotypies. It has been known since the late 1960s that motor stereotypies can be reliably induced in a wide variety of animal species with dopaminergic stimulation of the striatum (Ernst and Smelik, 1966; Randrup and Munkvad, 1967; Fog, 1969). Furthermore, upon repeated stimulation, the behavioral response becomes progressively augmented such that stereotypies are elicited more rapidly as well as demonstrate increased duration and intensity (Segal et al., 1980). In contrast, intrastriatal antagonism of dopamine receptors has been shown to have an attenuating effect on stereotypic behaviors (Arnt, 1985; Presti et al., 2003). Obstruction of dopaminergic neurotransmission accomplished by interfering with the biosynthetic production of dopamine (Odberg et al., 1987) or the destruction of dopamine neurons (Creese and Iversen, 1972; Fibiger et al., 1973) also produces a similar effect.

In line with a critical role of dopamine in the generation of stereotypies, genetic manipulations which increase extracellular levels of dopamine have also been found to evoke stereotyped patterns of behavior (Berridge et al., 2005). Furthermore, alterations in the density (Helmeste et al., 1981; Sharman et al., 1982; Fields et al., 1991; McBride and Hemmings, 2005) or sensitivity (Lewis et al., 1990) of dopamine receptors are also commonly associated with stereotypies. Similarly, changes in the concentration of the dopamine metabolites, HVA and DOPAC, have been observed coincident with the development of stereotypic behaviors (Fry et al., 1981).

Patterns of early gene activation in the neurochemically distinct, striosome and matrix, compartments of the striatum are highly accurate predictors of psychostimulant-induced stereotypies (Canales and Graybiel, 2000; Capper-Loup et al., 2002; Saka et al., 2004).

Interestingly, stereotypies in rodent (Garner and Mason, 2002) and avian (Garner et al., 2003b; Garner et al., 2003a) species are also correlated with impaired performance on neuropsychological tasks analogous to those used to detect basal ganglia pathology in clinical populations.

Overall, a synthesis of the extant research tends to implicate the basal ganglia as the neural substrate of stereotypy. Nevertheless, it is clear that much still remains to be learned about the neurobiological basis of stereotypy. For this reason, animal models of stereotypy may prove to be invaluable experimental tools.

Animal Models of Stereotypy

Animal models of stereotypy can generally be classified into four broad categories: (i) genetic models of stereotypy, (ii) models of stereotypy associated with insults to the central nervous system, (iii) models of pharmacologically induced stereotypy, and (iv) models of stereotypy associated with adverse environmental circumstances.

Genetic Models of Stereotypy

Motor stereotypies have been observed in conjunction with transgenic mouse models of various clinical disorders. Many neurodevelopmental disorders are characterized by patterns of behavior which may be described as restricted, repetitive, and stereotyped. Likewise, the behavioral aberrations characteristic of their genetic models also include specific forms of stereotyped behavior. For example, mutations in the methyl-CpG binding protein 2 (*MECP2*) gene are responsible for the majority of Rett syndrome cases. Mice expressing truncated MeCP2 protein exhibit repetitive forelimb movements resembling the distinctive hand stereotypies (e.g., hand-wringing, waving, and clapping) observed in Rett syndrome patients (Shahbazian et al., 2002; Moretti et al., 2005). The human genetic disorder Angelman syndrome (AS) results from a deletion or mutation in the 15q11-13 region of maternally-derived chromosome 15. The

GABRB3 gene, which codes for the $\beta 3$ subunit of the GABA_A receptor, lies within this region and is thought to contribute to the clinical manifestations of the disease. Paralleling the repetitive behaviors associated with AS, *gabbr3* homozygous knockout mice show stereotyped behaviors such as intense circling or “tail-chasing” which may continue for hours (Homanics et al., 1997; DeLorey et al., 1998). As another example, Ts65Dn mice are segmentally trisomic for the distal portion of mouse chromosome 16, the region containing murine orthologs to human chromosome 21, and provide a model for Down syndrome. The behavioral repertoire of Ts65Dn mice includes behaviors, such as repetitive jumping and cage-top twirling, which recall the abnormal repetitive behaviors often seen in individuals with mental retardation (Turner et al., 2001).

Because repetitive behaviors can be conceptualized as a neurobehavioral dimension which cuts across many neuropsychiatric disorders, mutant mouse models of other human repetitive behavior disorders, such as obsessive-compulsive disorder (OCD) and Tourette’s syndrome, also hold relevance to stereotypy. Hyperdopaminergic mice with a knockdown mutation of the dopamine transporter (*DAT*) gene have been advanced as a model of OCD and Tourette’s (Berridge et al., 2005). Gene-based knockdown of *DAT* reduces adult *DAT* expression to 10% of wild-type levels and elevates extracellular dopamine concentrations in the striatum to 170% of wild-type controls. Behaviorally, *DAT*-knockdown mice perform syntactic grooming chains which become increasingly more fixed and less resistant to disruption. Similarly, compulsive grooming behaviors are also evident in mice with genetic deletion of SAP90/PSD95-associated protein 3 (*Sapap3*), which codes for a postsynaptic scaffolding protein at excitatory synapses (Welch et al., 2007). SAPAP3 shows high levels of expression in the striatum; correspondingly, the cortico-striatal synapses of *Sapap3*-null mice demonstrate multiple defects, including

reduced glutamatergic transmission, altered NMDA receptor subunit composition, and changes in the structure of the postsynaptic complex.

In addition to transgenic mouse models, other genetic models of stereotypy have also been developed based on the observed heritability of the behavior. Selective breeding studies have shown that the offspring of stereotypic bank voles are six to seven times more likely to develop stereotypies than the offspring of non-stereotypic animals (Schoenecker and Heller, 2000). This pattern of inheritance holds true when only one of the parents is stereotypic. Moreover, the specific topography of stereotypy which develops in the offspring appears to be strongly related to the maternal form of stereotypy. Similarly, a genetic basis for stereotypy has also been demonstrated in other species. Stereotypies are four times more common in the offspring of stereotypic female African striped mice (*Rhabdomys pumilio*) than in the offspring of non-stereotypic females (Schwaibold and Pillay, 2001). Furthermore, cross-fostering experiments indicate that this tendency to develop stereotypy is independent of non-genetic factors (e.g., learned social behaviors) as the offspring of stereotypic mothers are still more likely to develop stereotypies than the offspring of non-stereotypic mothers regardless of whether they are raised by their own biological mothers or a foster mother. Finally, genetic transmission of stereotypy has also been demonstrated in farm mink (*Mustela vison*), and the development of divergent lines of low-stereotyping and high-stereotyping mink has been used to conduct biological- and welfare-related investigations into the significance of stereotypies (Jeppesen et al., 2004; Svendsen et al., 2007).

Models of Stereotypy Associated with Insults to the Central Nervous System

The behavioral sequelae of targeted insults to the central nervous system often include the appearance of stereotypic behaviors. As mentioned previously, stereotypies are a defining feature of autism. Rather than targeting the major candidate genes or loci thought to be associated with

autism, some animal models have examined the role of perinatal risk factors in the etiology of autism. These models have been generated based on the observation that prenatal exposure to teratogenic agents increases the risk of autism. For example, exposure to valproic acid (VPA), an anti-epileptic drug, on embryonic day 12.5 in rats not only reproduces neuroanatomical abnormalities similar to those reported in autistic patients but also long-term disturbances in postnatal behavior including increased time spent engaged in stereotypic activity (Ingram et al., 2000; Schneider and Przewlocki, 2005). Intriguingly, these behaviors appear to be sensitive to environmental manipulations as rearing VPA-treated pups in an enriched environment ameliorates many of these behavioral deficits (Schneider et al., 2006).

The pathogenesis of autism has also been linked to viral infection during early development. In support of this putative association, intracerebral inoculation of newborn rats with Borna disease virus induces neuroanatomical and neurochemical deficits correlated with those seen in autism and recapitulates many of the behavioral impairments, including stereotypies, in common with clinical aspects of the human disease (Hornig et al., 1999).

To demonstrate an autoimmune etiology for autism, studies have also been performed wherein rhesus monkeys were exposed prenatally to human IgG collected from the mothers of autistic children (Martin et al., 2008). Phenotypical analysis of these monkeys confirms that their behaviors are characterized by increases in whole body stereotypies. An autoimmune etiology has also been proposed for Tourette's syndrome. When sera from Tourette's patients with high levels of autoantibodies are infused into the ventrolateral striatum of rats, they begin to display high rates of oral stereotypies, such as wood chip eating, self-gnawing, biting, licking, and taffy-pulling (Taylor et al., 2002). As a final example of insult-based stereotypy, administration of the

dopaminergic neurotoxicant, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), also results in acute increases in stereotyped behaviors, such as grooming or gnawing (Linder et al., 1987).

Models of Pharmacologically Induced Stereotypy

Insights into the molecular mechanisms which underlie stereotypy have been primarily derived from neuropharmacological investigations. Early experiments established the importance of central dopamine transmission in the generation of stereotypies by demonstrating that stereotypies can be induced in numerous mammalian species with intrastriatal or systemic administration of dopamine agonists (e.g., amphetamine, methamphetamine, apomorphine, cocaine, pemoline, methylphenidate, phenmetrazine) (Ernst and Smelik, 1966; Randrup and Munkvad, 1967; Fog, 1969). Indeed, dopamine has received much of the focus of studies designed to elucidate the neurobiology of stereotypy; however, pharmacological agents which interact with other neurotransmitter systems have also been shown to consistently induce stereotypies in laboratory animals. These compounds include NMDA antagonists (e.g., MK-801, AP-5, phencyclidine [PCP], dextromethorphan, dextrorphan, ketamine) (Schmidt, 1986; Tiedtke et al., 1990; Ishmael et al., 1998; Vandebroek et al., 1998), opiate agonists (e.g., morphine, methadone, β -endorphin, levorphanol, pentazocine, nalorphine) (Iwamoto and Way, 1977; Nistico et al., 1981), serotonin agonists (e.g., lysergic acid diethylamide [LSD] and 8-hydroxy-2-(di-*n*-propylamino) tetralin [8-OH-DPAT]) (Fog, 1969; Dourish et al., 1985), and GABA agonists (Scheel-Kruger et al., 1978).

Models of Stereotypy Associated with Adverse Environmental Circumstances

Stereotypies often develop in response to abnormal rearing conditions. Higher mammals, such as nonhuman primates, seem to be especially vulnerable to the effects of partial or total social isolation during early development. In addition to profound deficits in social behavior, infant rhesus monkeys deprived of early social contact with their mother or peers exhibit more

stereotypic behaviors (e.g., body-rocking, self-clasping, thumb-sucking, and self-wounding) than their group-housed counterparts even after attempts are made to rehabilitate them by housing them with their normally reared peers (Cross and Harlow, 1965) (Harlow et al., 1965). Furthermore, when examined as adults, socially deprived monkeys show increased sensitivity to the effects of dopamine (Lewis et al., 1990) as well as substantial alterations in the neurochemical composition of the striatum (Martin et al., 1991).

Stereotypies also arise in frustrative situations such as when animals are chronically deprived of the opportunity to perform highly motivated behaviors. For example, in the wild, Mongolian gerbils (*Meriones unguiculatus*) build elaborate networks of underground tunnels with numerous entrances to different chambers that serve as nest, food storage, and waste areas. Gerbils raised in standard laboratory cages are prevented from engaging in this natural behavioral pattern. As a result, they develop stereotypic digging behaviors, which consist of long-lasting digging bouts directed at the corners of their cages (Wiedenmayer and Brunner, 1993). Neither variations in cage size (Wiedenmayer, 1996) nor digging substrate (Wiedenmayer, 1997b) alone are able to reduce these behaviors. In contrast, when raised in laboratory cages connected to artificial burrow systems, young gerbils do not develop stereotypic digging behaviors (Wiedenmayer, 1997a). However, these burrows can only prevent the development of stereotypy when the entrances appear tunnel-like in shape (Wiedenmayer, 1997b; Waiblinger and nig, 2004), suggesting that the development of these behaviors is tied to the lack of highly specific environmental stimuli.

Environmental restriction, in the form of standard laboratory caging, has also been shown to induce stereotypic behaviors in bank voles (*Clethrionomys glareolus*). The most common form of captivity-induced stereotypy in bank voles is repetitive up-and-down jumping (Odberg,

1986). Consistent with an etiological role for environmental factors, the augmentation of environmental complexity (e.g., the addition of shelter, nesting materials, climbing structures, and dietary enrichments) significantly reduces the development of these behaviors in young voles (Ödberg, 1987; Sorensen, 1987). Of note, stereotypies can also be pharmacologically induced in bank voles. Systemic injections of apomorphine elicit stereotyped licking (Vandebroek and Ödberg, 1997), whereas MK-801 administration induces intensive sniffing and unidirectional circling behaviors (Vandebroek et al., 1998). In both cases, not only are the topographies of drug-induced stereotypy distinct from the stereotyped jumping normally seen in caged voles but the pharmacological manipulations also have no effect on levels of captivity-induced jumping stereotypy. These data suggest that restriction-induced and drug-induced stereotypies may not share the same underlying neural mechanisms and speak to the importance of identifying valid animal models of clinical stereotypies.

Deer Mice (*Peromyscus maniculatus*)

Peromyscus maniculatus bairdii or prairie deer mice are wild-type, muroid rodents among the most abundant of North American mammals. Extremely active animals, deer mice require a relatively large home range in the wild of 242 to 3,000 m² (Wolff, 1989). When confined to standard laboratory cages, these animals develop remarkably high rates of motor stereotypies. For this reason, our lab has adopted deer mice as an animal model of stereotypy.

Despite their species-specific nature, the stereotypic behaviors (i.e., patterned running, repetitive jumping, and backward somersaulting) exhibited by deer mice mimic the fundamental characteristics observed in clinical populations; i.e., these invariant and apparently functionless behaviors emerge early in development and persist throughout the lifespan of the animal. Moreover, our lab has established that, while these stereotypies occur spontaneously under

standard laboratory housing, their development may be delayed and their frequency reduced by rearing the mice in an enriched environment (Powell et al., 1999).

The spontaneous nature of these behaviors is an important aspect of this model. By far, much of what is currently known about the neurobiology of stereotypy comes from studies of drug-induced stereotypies. However, studies in our own (Presti et al., 2002; Presti et al., 2004a) and other labs (Vandebroek and Ödberg, 1997; Vandebroek et al., 1998) have demonstrated that the topographies of spontaneously-emitted and pharmacologically-induced stereotypies are often qualitatively different in the same species. Therefore, it is likely that they are subserved by different mechanisms. Clinically relevant stereotypies most often emerge spontaneously. As such, an animal model of spontaneous stereotypy would provide a more accurate representation of the pathophysiological mechanisms underlying clinical stereotypies.

The fact that these behaviors are subject to environmental regulation is also of particular scientific utility. Environmental enrichment reduces stereotypy in deer mice. Environmental enrichment also recruits mechanisms similar to those active during neuroplasticity. By comparing the brains of environmentally enriched, low stereotypy mice to environmentally enriched, high stereotypy and standard caged mice, we can identify the specific mechanisms which underlie the ameliorative effects of environmental enrichment on stereotypy. This information is useful not only because it can provide clues into the biological basis of stereotypy but also because it can provide potential targets for therapeutic applications.

Finally and most importantly, this model also appears to demonstrate construct validity. As reviewed previously, clinical studies implicate basal ganglia dysfunction in the etiology of stereotypy. Similarly, our work with this model has linked the expression of stereotypic behaviors to alterations in the cortico-basal ganglia loop circuits which control the selection and

initiation of motor behavior (Presti et al., 2002; Turner et al., 2002; Presti et al., 2003; Turner and Lewis, 2003; Turner et al., 2003; Presti et al., 2004a; Presti et al., 2004b; Presti and Lewis, 2005).

CHAPTER 2 ENVIRONMENTAL ENRICHMENT

Historical Perspective

The concept of experience-dependent plasticity was first proposed by Donald Hebb in the late 1940s (Hebb, 1949). In his revolutionary monograph *The Organization of Behavior*, Hebb reported that rats he initially brought home as pets for his children later demonstrated superior problem-solving ability when compared to their laboratory-reared littermates. He concluded that “the richer experience of the pet group during development made them better able to profit by new experiences at maturity.” Although this may have been a somewhat uncontrolled experiment, subsequent replications of Hebb’s work, under more controlled conditions, also yielded similar findings (Forgays and Forgays, 1952; Hymovitch, 1952). In the 1960s, Rosenzweig and colleagues at the University of California-Berkeley were able to expand on this early research by providing evidence linking improved learning ability in “enriched” rats to specific neurochemical and neuroanatomical alterations in the brain (Rosenzweig and Bennett, 1996). More systematic investigations into the effects of environmental enrichment were to follow in later decades, most notably with the contributions of Greenough and co-workers (Markham and Greenough, 2004), giving birth to what is now considered a classical line of research wherein the brains and behavior of animals reared in an enriched environment are compared to those reared in a standard laboratory environment (Figure 2-1).

Behavioral Consequences

Environmental stimulation modifies a myriad of behavioral characteristics in normal, wild-type animals. The beneficial effects of environmental enrichment on learning and memory ability are among the most well-studied of these behavioral changes. Environmental enrichment has been shown to enhance performance in a wide variety of tasks designed to test spatial and non-

spatial learning (Krech et al., 1962; Nilsson et al., 1999; Duffy et al., 2001; Williams et al., 2001; Bruel-Jungerman et al., 2005; Leggio et al., 2005; Barbelivien et al., 2006) as well as protect against memory decline due to normal aging (Winocur, 1998; Soffié et al., 1999; Kobayashi et al., 2002; Frick and Fernandez, 2003; Frick et al., 2003; Bennett et al., 2006; Harburger et al., 2007a; Harburger et al., 2007b).

Environmental enrichment also confers behavioral advantages with respect to emotionality and stress responsiveness. For example, enriched animals show decreased fear and anxiety not only when tested on artificial constructs, like the elevated plus-maze (Chapillon et al., 1999; Benaroya-Milshtein et al., 2004; Friske and Gammie, 2005; Pena et al., 2006), but also when confronted with natural stressors, like predators (Klein et al., 1994). Enriched animals also tend to be less neophobic and, as a result, exhibit greater novelty-seeking and exploratory behaviors (Widman and Rosellini, 1990). Decreased reactivity to the deleterious effects of stress is also thought to contribute to the fewer depressive-like behaviors observed in enriched animals (Brenes and Fornaguera, 2008; Brenes et al., 2008).

The effects of environmental enrichment on social behaviors appear to be gender-specific. When housed in an enriched environment, female mice generally display sociopositive behaviors (e.g., grooming cagemates), whereas male mice show increased aggression (Haemisch et al., 1994). Yet, in spite of the possible negative effects of aggressive behavior (e.g., increased stress, risks of injury, etc.), enriched male mice also show more play behaviors, an indicator of good welfare, implying that they are able to cope with the increased aggression brought about by enrichment (Marashi et al., 2003).

Of direct relevance to the present studies, environmental enrichment also has a major impact on the development of stereotypic behaviors. As reviewed previously (Chapter 1),

environmental impoverishment is known to induce stereotypic behaviors in captive animals often with detrimental effects on the wellbeing of the animal. Consequently, environmental enrichment has emerged as a means to eradicate these behaviors. In this context, enrichment is used as an animal husbandry tool; however, researchers have also utilized environmental stimulation as an experimental tool to improve functional outcome in animal models of human disease.

Similar to the positive effects observed in studies of intact animals, these experiments have demonstrated that enrichment can ameliorate the cognitive deficits associated with fetal alcohol syndrome (Hannigan et al., 1993; Hannigan and Berman, 2000), developmental lead exposure (Schneider et al., 2001; Guilarte et al., 2003), fragile X syndrome (Restivo et al., 2005), Down syndrome (Martinez-Cue et al., 2002), traumatic brain injury (Hamm et al., 1996; Hoffman et al., 2007; Kline et al., 2007), ischemic stroke (Dahlqvist et al., 2004), Huntington's disease (Nithianantharajah et al., 2007), and Alzheimer's disease (Arendash et al., 2004; Jankowsky et al., 2005; Berardi et al., 2007; Costa et al., 2007). In addition to its cognitive effects, exposure to an enriched environment can also accelerate the functional recovery of motor skills following traumatic brain injury (Hoffman et al., 2007; Kline et al., 2007) and stroke (Ohlsson and Johansson, 1995; Johansson, 1996), improve motor function in Parkinson's disease (Jadavji et al., 2006; Steiner et al., 2006), and delay the onset and progression of motor symptoms in Huntington's disease (van Dellen et al., 2000; Hockly et al., 2002; Schilling et al., 2004; Spires et al., 2004).

Structural and Morphological Modifications

The behavioral effects of environmental enrichment are mediated through a host of structural alterations in the brain. First evidence for experience-induced morphological plasticity was suggested by the observation that animals reared in an enriched environment possess heavier (Bennett et al., 1969) and thicker (Diamond et al., 1976) cerebral cortices than their standard-

caged counterparts. Subsequent studies were later able to pinpoint the underlying changes in neuronal, dendritic, and synaptic properties which contribute to these gross anatomical differences.

Although there is a decrease in neuronal density following enrichment (Diamond et al., 1966), the neurons of enriched animals demonstrate larger cell bodies and nuclei (Diamond et al., 1967). Enrichment-related neuronal remodeling also involves changes in dendritic field dimensions: not only are there increases in the number and length of dendritic branches following enrichment (Wallace et al., 1992), but the dendritic arbor is also typified by the presence of more higher-order branches (Volkmar and Greenough, 1972; Greenough and Volkmar, 1973; Greenough et al., 1973). In addition to synaptogenesis, as measured by increased dendritic spine density (Turner and Greenough, 1985) or greater number of synapses per neuron (Sirevaag and Greenough, 1987; Jones et al., 1997), changes in existing synaptic morphology also occur in response to environmental stimulation. These can include increases in the size of pre- and post-synaptic components (Diamond et al., 1975; Sirevaag and Greenough, 1985), alterations in the shape of dendritic spine (Comery et al., 1996), the appearance of perforations in the post-synaptic density (Greenough et al., 1978), or the formation of multiple synaptic boutons (Jones et al., 1997).

Many of these initial morphological studies focused on cortical regions; however, experience-induced modifications in brain structure have since been demonstrated in several other regions, including the cerebellum (Floeter and Greenough, 1979; Greenough et al., 1986), superior colliculus (Fuchs et al., 1990), basal ganglia (Comery et al., 1995; Comery et al., 1996), and hippocampus (Faherty et al., 2003). The hippocampus, in particular, appears to be especially sensitive to the effects of environmental enrichment.

Once regarded with a certain amount of scientific skepticism, adult neurogenesis in the mammalian brain is now a well-accepted phenomenon. Exposure to an enriched environment, both during early adulthood (Kempermann et al., 1997) and old age (Kempermann et al., 2002), induces hippocampal neurogenesis. Not only is this effect regionally specific to the hippocampus (Brown et al., 2003) but enrichment has also been shown to reduce spontaneous apoptosis in the hippocampus in effect promoting the survival of the new neurons (Young et al., 1999). The newly generated cells develop into mature neurons with morphological characteristics and electrophysiological response properties similar to those found in dentate granule cells (van Praag et al., 2002). Furthermore, when neurogenesis is blocked by treatment with an anti-mitotic agent during the enrichment period, enrichment-related behavioral improvements in hippocampus-dependent learning tasks are abolished (Bruehl-Jungerman et al., 2005). These data suggest that the new neurons are not only functionally integrated into the existing neural circuitry but are also critical for the behavioral improvements seen following enrichment.

Although traditionally less well-studied, environmental enrichment also impacts non-neuronal elements in the brain. Astrocytic hypertrophy (Sirevaag and Greenough, 1987) and hyperplasia (Szeligo and Leblond, 1977; Sirevaag and Greenough, 1991) as well as increased ensheathment of synapses by astrocytic processes (Jones and Greenough, 1996) have been reported following enrichment. Similarly, increases in the volume fraction (Sirevaag and Greenough, 1987) and number (Szeligo and Leblond, 1977) of oligodendrocytes as well as increases in axon myelination (Juraska and Kopcik, 1988) have also been observed. In addition to glial proliferation, the cerebrovasculature of enriched animals are also characterized by larger, more elaborately branched capillaries (Black et al., 1987; Sirevaag and Greenough, 1987; Sirevaag et al., 1988; Black et al., 1991). Because the spatiotemporal pattern of gliogenesis and

angiogenesis often parallels the induction of experience-dependent neuronal plasticity, it is hypothesized that these changes serve to support and optimize the changes in neuronal connectivity which occur as a result of enrichment.

Regulation of Gene Expression

Long-lasting morphological plasticity in the brain requires changes in gene expression. Exposure to complex environmental stimuli enhances the activity of immediate early genes and transcription factors which regulate the expression of structural proteins involved in neuronal reorganization. Site-specific induction of *egr-1* (also known as *zif-268*, *NGFI-A*, *krox-24*, and *zenk*) (Wallace et al., 1995; Pinaud et al., 2002; Toscano et al., 2006), *arc* (Pinaud et al., 2001), *egr-2* (aka *krox-20*) (Ronnback et al., 2005), *homer-1a*, *junB* (Koh et al., 2005), and *CREB* (Young et al., 1999; Williams et al., 2001) have all been reported following enrichment. These increases are not elicited in response to other stimuli, such as handling, and only occur in regions known to experience enrichment-related structural change. For the most part, levels of gene induction also correlate with improvements on learning tasks known to be affected by enrichment.

In addition to experiments designed to elucidate the effects of environmental enrichment on individual genes, the advent of microarray technology has allowed researchers to characterize how the genome changes as a whole in response to environmental stimulation. To this end, gene expression profiling has shown that enrichment upregulates genes involved in the synthesis or processing of DNA, RNA, and protein molecules, genes involved in the formation of new synapses or the reorganization and strengthening of existing synapses, and genes involved in the regulation of neuronal excitability (Rampon et al., 2000). In contrast, genes encoding proteolytic and pro-apoptotic proteins are downregulated following enrichment. Many of these differences are apparent after only 3 hours of enrichment; however, with prolonged exposure to an enriched

environment, a shift is observed towards the selective upregulation of genes involved in neuronal transmission and structural change.

Microarray analysis has also been used to gain a better understanding of the molecular mechanisms which underlie the beneficial effects of environmental enrichment in animal models of disease. For instance, environmental enrichment produces a pronounced reduction in cerebral β -amyloid deposition in the APP^{swe} \times PS1 Δ E9 transgenic mouse model of Alzheimer's disease (Lazarov et al., 2005). It also promotes the expression of immediate early genes as well as genes involved in β -amyloid sequestration, endothelial function, and phospholipid metabolism. As many of these differentially expressed genes are known to regulate vasculogenesis, one theory has been that the influence of enrichment on β -amyloid clearance is related to modifications in the properties of the blood-brain barrier.

Despite the large-scale analytical power afforded by microarrays, one potential limitation of this approach is that it may only detect experience-dependent changes in protein expression due to *de novo* protein synthesis and may not be able to identify changes in functional protein levels which arise due to posttranslational modifications, trafficking, or degradation. To circumvent this problem, a recent study used mass spectrometric methods to evaluate the global changes which occur in the hippocampal proteome following enrichment (McNair et al., 2007). In this experiment, the co-alignment of principal cells, a feature unique to the anatomical organization of the hippocampus, was exploited to allow for independent analysis of the soma and dendritic fields. Functional classification of differentially expressed proteins revealed that structural and metabolic proteins were the most highly upregulated in response to enrichment. Signal transduction proteins also demonstrated differential expression; however, in contrast to the structural and metabolic proteins, which were upregulated in both the somatic and dendritic

fractions, increased expression of signal transduction proteins were significantly more pronounced in the synaptosomal sample, suggesting a major contribution of neurotransmission-related processes to the effects of environmental enrichment.

Effects on Neuronal Signaling

Intracellular signaling pathways lie at the nexus between environmental stimulation and experience-dependent changes in gene expression. Environmental enrichment induces alterations in neuronal signaling via several mechanisms. For example, the functional properties of NMDA and AMPA receptors depend on their subunit composition. Enrichment has been shown to modify the expression of NMDA (i.e., NR1, NR2A, NR2B) and AMPA (i.e., GluR1-4) receptor subunits (Naka et al., 2005; Andin et al., 2007; Li et al., 2007). Furthermore, in many of these studies, these changes were observed concomitant with behavioral improvements known to involve glutamate-mediated synaptic plasticity (Tang et al., 2001; Guilarte et al., 2003; Bredy et al., 2004). The effects of environmental enrichment on receptor expression also extend to other neurotransmitter systems as well: changes in dopamine (Keyvani et al., 2004; Del Arco et al., 2007), serotonin (Rasmuson et al., 1998), GABA (Keyvani et al., 2004), and adrenergic (Keyvani et al., 2004) receptor expression have all been reported following enrichment.

In addition to neurotransmitter receptors, environmental enrichment also regulates the expression of other proteins involved in neurotransmission. These can include neurotransmitter transporters (Zhu et al., 2005) (Andin et al., 2007) and ion channels (Keyvani et al., 2004; Li et al., 2007) as well as various kinase/phosphatase proteins (Rampon et al., 2000; Keyvani et al., 2004; McNair et al., 2007).

Augmentation of long-term potentiation (LTP) is another mechanism by which enrichment can enhance synaptic transmission. One way enrichment accomplishes this is by altering the protein kinase A-dependence of LTP (Duffy et al., 2001). Additionally, environmental

manipulations also appear to be able to gate the efficacy of specific signaling pathways involved in the induction of LTP (Li et al., 2006). In young enriched mice, LTP induction can occur via cAMP-dependent, NMDA receptor-mediated activation of p38, a MAP kinase. Remarkably, this signaling cascade is completely non-functional in standard caged mice. Furthermore, this pathway is also inactive in mature mice even if they are housed in an enriched environment later in life, implying a developmental time window. Overall, these studies show that environmental enrichment can exert multiple levels of control on neuronal signaling processes.



Figure 2-1. An example of (a) an enriched environment is shown here in comparison to (b) a standard laboratory cage. Enriched environments provide “a combination of complex inanimate and social stimulation” beyond that which is normally found in standard laboratory housing (Rosenzweig et al., 1978). In practice, experimental paradigms can vary widely from laboratory to laboratory although some common elements of enrichment include the addition of objects designed to provide cognitive or sensory stimulation, greater numbers of conspecific cagemates, increased spatial density, a running wheel for exercise, and novelty (i.e., routine replacement or relocation of enrichment items). Occasionally, foraging opportunities and dietary enrichments may also be provided.

CHAPTER 3 SPECIFIC AIMS

The overarching goal of this research was to identify the molecular and cellular mechanisms which contribute to environmentally mediated induction of functionally beneficial behavioral plasticity in an animal model of abnormal stereotyped motor behavior.

We have observed frequent and intense stereotypies dominating the behavioral repertoire of deer mice (*Peromyscus maniculatus*). These motor stereotypies are extremely context-dependent: phenotypically distinct groups of mice, i.e., high stereotypy and low stereotypy, can be generated by differentially housing deer mice in either environmentally impoverished or environmentally enriched conditions, respectively. Previous work in our lab has demonstrated that enrichment-related reduction of motor stereotypies in these animals correlates with region-specific increases in measures of neuroplasticity. The objective of the present studies was to further explore and extend these findings.

Neuroplasticity refers to the brain's ability to adapt and change over time in response to experience. Long-term, experience-dependent alterations in neural function can result from changes on multiple, interacting levels. The intracellular signaling pathways of the brain form the initial link between external stimuli and the translational machinery of the cell. In response to environmental stimulation and cell signaling, complex programs of gene expression are activated which modify the synaptic connections between neurons. Synaptic reorganization occurs as the brain remodels itself to absorb new information until, eventually, this neural plasticity gives rise to behavioral plasticity as an organism's behavior, too, adjusts to changing experience.

If neuroplasticity allows an organism to adapt to its environment, then the performance of stereotypy, a behavior marked by rigidity and invariance, implies an underlying impairment in neuroplasticity. Hence, the specific aims of this research were to determine the mechanisms by

which environmental impoverishment restricts and environmental enrichment restores neural and behavioral plasticity.

1. Converging lines of research link motor stereotypies to perturbations in dopaminergic neurotransmission. Dopamine- and cAMP-regulated phosphoprotein of 32kDa (DARPP-32) is a key player in dopamine signal transduction processes. Thus, the intertwining goals of our first specific aim were (i) to determine whether DARPP-32 would contribute functionally to stereotypy and (ii) to determine the impact of environmental manipulations on DARPP-32 regulation.
2. Enduring changes in gene expression often occur following exposure to various forms of chronic stimuli. The transcription factor, Δ FosB, is hypothesized to mediate these long-term, experience-dependent neuroadaptations. Therefore, the goal of our second specific aim was to determine patterns of Δ FosB induction following exposure to persistent stereotypy and prolonged environmental enrichment.
3. On a molecular level, experience-dependent neural plasticity can also manifest as lasting alterations in synaptic structure and function. For this reason, the goal of our third specific aim was to determine the sites of enrichment- and stereotypy-related changes in synaptic plasticity by analyzing levels of the synaptic proteins, synaptophysin and neural cell adhesion molecule (NCAM), in multiple brain regions.
4. The striatum does not mediate motor functions exclusively: Striatal neuropathology interferes with both striatal-based motor activity and learning ability. As such, the goal of our final specific aim was to determine whether the striatal dysfunction associated with stereotypy would also affect the learning and memory functions of the striatum. As a corollary goal, we also sought to determine if the beneficial effects of environmental enrichment on stereotypy would also extend to improved behavioral performance on striatum-dependent learning tasks.

CHAPTER 4
EFFECTS OF ENVIRONMENTAL ENRICHMENT ON THE REGULATION OF DARPP-32
IN AN ANIMAL MODEL OF ABNORMAL STEREOTYPED MOTOR BEHAVIOR

Introduction

Stereotypy refers to patterns of movements characterized by frequent and invariant repetition (Berkson, 1983; Mason, 1991). These behaviors occur absent a functional context and are a prominent feature of many psychiatric and neurological disorders, including autism, schizophrenia, and Rett syndrome. Persistent stereotypies are a source of distress for patients and concern for clinicians not only because they can be stigmatizing in social settings but also because they can severely interfere with more adaptive activities, essentially disabling some individuals. Under certain circumstances, stereotypies can also devolve into repetitive self-injurious behaviors (e.g., head-banging in autistic children, eye-poking in the congenitally blind, and skin-picking in Prader-Willi patients), thereby assigning them a high priority for clinical intervention (Kennedy et al., 2002).

Although relatively little is known about the critical factors contributing to the emergence and persistence of stereotypic behaviors, substantial evidence from both human and animal studies points to a relationship between stereotypy and disturbances of the nigrostriatal dopamine system. Human stereotypies appearing in diverse clinical populations often share a common connection to dopamine. Several studies have linked alterations in dopamine functioning to the amount of stereotypy observed in subjects with mental retardation (Bodfish et al., 1995; Lewis et al., 1996; Roebel and MacLean, 2007). In chronic abusers of amphetamine, behavioral stereotypy is a well-documented side effect (Ellinwood et al., 1973). Similarly, stereotyped behaviors have also been reported following dopamine replacement therapy in Parkinson's disease patients (Evans et al., 2004; Lawrence et al., 2007). In studies using experimental animal models, genetic (Berridge et al., 2005) and pharmacological (Randrup and Munkvad, 1967;

Segal et al., 1980) manipulations which result in increased levels of extracellular dopamine reliably induce stereotypies, while, conversely, stereotypies can be suppressed by blocking dopaminergic neurotransmission (Fog, 1972; Presti et al., 2003). Alterations in the density (Helmeste et al., 1981; Sharman et al., 1982; Fields et al., 1991; McBride and Hemmings, 2005) or sensitivity (Lewis et al., 1990) of dopamine receptors are also commonly associated with stereotypy, lending further support for a role of dopamine in the expression of stereotyped behaviors.

Dopamine- and cAMP-regulated phosphoprotein of 32kDa (DARPP-32) mediates many of the biological effects of dopamine (for a review see (Svenningsson et al., 2004)). Highly enriched in the projection neurons of the striatum, DARPP-32 is a bifunctional signal transduction molecule whose biochemical properties are determined by its state of phosphorylation. When phosphorylated at Thr34 by protein kinase A (PKA), DARPP-32 is a potent inhibitor of protein phosphatase-1 (PP-1) (Hemmings et al., 1984). On the other hand, when phosphorylated at Thr75 by cyclin-dependent kinase 5 (Cdk5), DARPP-32 becomes an inhibitor of PKA activity (Bibb et al., 1999). Therefore, depending on its site of phosphorylation, DARPP-32 has the ability to modulate the activity of striatal output neurons by either amplifying or dampening the intracellular response to kinase/phosphatase signaling cascades.

Because of the central role it plays in striatal signal transduction, DARPP-32 mediates many of the behavioral functions of dopamine, including the regulation of motor activity. Numerous studies have established that DARPP-32 participates in the motor response to various dopamine agonists, including cocaine (Fienberg et al., 1998; Valjent et al., 2005; Zachariou et al., 2006), amphetamine (Svenningsson et al., 2003), SKF-81297 (Scott et al., 2005), and apomorphine (Fienberg and Greengard, 2000). The motor side effects of pathological conditions

affecting brain dopamine systems have also been linked to abnormalities in DARPP-32 regulation. For example, dysregulation of DARPP-32 has been implicated in the pathogenesis of levodopa-induced dyskinesias in Parkinson's disease (Picconi et al., 2003; Hakansson et al., 2004; Aubert et al., 2005; Santini et al., 2007). Similarly, alterations in DARPP-32 expression have been found in transgenic mouse models of Huntington's disease and have been hypothesized to contribute to its characteristic motor symptoms (Bibb et al., 2000; Spires et al., 2004).

The goal of this study was to determine whether DARPP-32 would also contribute functionally to motor stereotypies. Previous studies in mutant DARPP-32 mice have indicated that drug-induced stereotypy requires DARPP-32 phosphorylation (Svenningsson et al., 2003). However, models of stereotypic behavior which rely on administration of pharmacological agents may not offer the most appropriate paradigm with which to explore the underlying pathophysiology of clinical stereotypies. Indeed, it has been shown that the topography of spontaneously emitted and drug-induced stereotypies are qualitatively different in the same species (Vandebroek et al., 1998; Presti et al., 2002; Presti et al., 2004a), suggesting that they also may not share the same neural substrates. The present study utilizes an animal model of spontaneous (i.e., non-drug-induced) stereotypy. When housed under standard laboratory conditions, deer mice (*Peromyscus maniculatus*) engage in high rates of motor stereotypies which occur absent any pharmacological challenge (Powell et al., 1999). Interestingly, when reared in an enriched environment, the intensity of the stereotypies produced by these mice are substantially reduced (Powell et al., 1999). Environmental stimulation has been shown to alter the expression of a number of genes involved in neuronal signaling (Rampon et al., 2000; Li et al., 2006; Li et al., 2007), including DARPP-32 (Spires et al., 2004). Therefore, a related goal of

this study was to determine whether changes in the regulation of DARPP-32 might also be responsible for the decreases seen in stereotypic behaviors following environmental enrichment.

Materials and Methods

Animals

Deer mice (*Peromyscus maniculatus bairdii*) were obtained from a breeding colony maintained at the University of Florida. The mice were housed in a room kept at 25°C, maintained on a 16:8 light:dark cycle (lights off at 2200), and provided with access to food and water *ad libitum*. At weaning (3 weeks of age), mice were randomly assigned to either standard cage (SC, n = 39) or environmentally enriched (EE, n = 40) housing conditions. Standard caged animals were housed in standard laboratory rodent cages (29 × 33 × 15 cm) with two other same-sex weanlings, while environmentally enriched animals were housed in groups of five or six in modified large dog kennels (122 × 81 × 89 cm). These enrichment kennels were fitted with galvanized wire mesh to create multiple interconnected levels and equipped with bedding, a running wheel, and assorted ceramic and plastic objects varying in color, shape, and size. Throughout the 2 month experimental period, enrichment items were removed and replaced with novel objects on a weekly basis. To encourage foraging behaviors, approximately 50 grams of birdseed were also randomly distributed throughout the kennels three times a week. All animal procedures were approved by the University of Florida Institutional Animal Care and Use Committee and were performed in accordance with the NIH *Guide for the Care and Use of Laboratory Animals*.

Behavioral Testing

Animals were tested for stereotypy after 2 months in their respective housing conditions. Mice were removed from their home cages and placed singly into Plexiglas testing cages (22 ×

15 × 28 cm) two hours prior to the beginning of their dark cycle. Food, water, and bedding were provided, and the mice were left undisturbed for 2 hours to allow for habituation to the new environment and recovery from the stress of handling. Stereotypic behaviors were quantified using an automated testing apparatus (Columbus Instruments, Columbus, OH) designed to register stereotypic motor activity (e.g., repetitive jumping or backward somersaulting) as photobeam breaks. All testing sessions were recorded using a digital video surveillance system (GeoVision Inc., Taipei, Taiwan) and checked in order to verify the accuracy of the automated counts. Data were collected for two dark cycles, and the stereotypy score for each animal was calculated as the average number of photobeam interruptions per hour of testing. Following behavioral testing, the stereotypy scores for all animals tested were rank ordered, and animals belonging to the highest and lowest tercile of stereotypy scores were designated as high stereotypy (HS, greater than 600 photobeam breaks/hr) and low stereotypy (LS, less than 400 photobeam breaks/hr) animals, respectively. Animals with intermediate stereotypy scores between 400 and 600 photobeam breaks/hr were not used for analysis of DARPP-32 protein levels.

Western Blotting

Levels of total (i.e., non-phosphorylation-specific) DARPP-32, phospho-Thr34-DARPP-32, and phospho-Thr75-DARPP-32 in the dorsolateral striatum were determined using immunoblotting analysis. Animals (n = 10-18 per group) were sacrificed by cervical dislocation. The brains were rapidly removed, snap-frozen in isopentane, and stored at -80°C until processed for protein extraction. Briefly, 300 µm thick coronal sections were collected through the striatum (AP: +1.42 mm to +0.14 mm from bregma) (Franklin and Paxinos, 1997) in a cryostat held at -20°C. Next, the dorsolateral striata were microdissected by punch method and sonicated in a

homogenization buffer containing 20 mM Tris, 150 mM NaCl, 5 mM EDTA, 5 mM EGTA, 1% Triton X-100, 10 μ M DTT, and a protease inhibitor tablet (Roche). Samples were then placed on ice and vortexed every 15 min for 1 hr before clarification via centrifugation at 16,000g for 15 min. Subsequently, the supernatants were collected, and protein concentrations were determined using the DC Protein Assay kit (Bio-Rad Laboratories, Hercules, CA), following manufacturer's instructions. Equal amounts (20 μ g) of total protein from each sample were loaded onto 12% polyacrylamide gels and separated by SDS-PAGE before transfer to polyvinylidene difluoride membranes. Membranes were then blocked with 5% nonfat milk in Tris-buffered saline and 0.05% Tween (TBST) for 1 hr before incubation with the primary antibody (1:2,500 anti-DARPP-32; 1:1,000 anti-phospho-Thr34-DARPP-32; and 1:1,000 anti-phospho-Thr75-DARPP-32; all antibodies from Cell Signaling Technology, Beverly, MA) overnight at 4°C. The next day, blots were washed in TBST for 15 min before incubation with a horseradish peroxidase-conjugated anti-rabbit secondary antibody (1:5,000; Santa Cruz Biotechnology, Santa Cruz, CA) for 1 hr at room temperature. After washing with TBST, blots were developed using enhanced chemiluminescence and exposed to CL-XPosure film (Pierce Biotechnology, Rockford, IL) for visualization of protein bands. To control for variations in sample loading, blots were then incubated in stripping buffer (62.5 mM Tris-HCl, 100 mM β -mercaptoethanol, 2% SDS) for 30 min at 50°C before being reprobed for α -tubulin (1:1,500, BioDesign, Saco, ME). Quantification of immunoreactive protein bands was done by densitometry using ImageJ software (version 1.37, NIH, Bethesda, MD). Densitometric values for DARPP-32 bands were normalized relative to those of the loading control and are expressed as relative optical densities (ROD).

Statistical Procedures

All data are expressed as mean \pm standard error of the mean (SEM). An independent samples t-test (two-tailed) was used to determine the effect of housing condition on stereotypy levels (SPSS, Chicago, IL). One-way analyses of variance (ANOVAs) were used to determine group differences in stereotypy and protein expression with post hoc analysis of significant effects accomplished using Tukey's honestly significant differences (HSD) test. Correlations between protein concentrations and stereotypy were calculated according to the method of Pearson. The minimum level of significance was set at $p < 0.05$ for all analyses.

Results

Behavioral Data

Consistent with our previous findings (Powell et al., 1999; Powell et al., 2000), environmental enrichment resulted in a marked attenuation of stereotypic behavior ($t(77) = -2.36$, $p = 0.02$). The interaction of housing condition with stereotypy yielded our four experimental groups of mice: environmentally enriched, low stereotypy (EE-LS, $n = 13$), environmentally enriched, high stereotypy (EE-HS, $n = 13$), standard caged, low stereotypy (SC-LS, $n = 10$), and standard caged, high stereotypy (SC-HS, $n = 18$). As shown in Figure 4-1, a one-way ANOVA revealed significant differences ($F(3,50) = 39.1$, $p < 0.001$) in the frequency of stereotypic behaviors between the high stereotypy groups (EE-HS and SC-HS) and the low stereotypy groups (EE-LS and SC-LS). Stereotypy scores for the low stereotypy groups were not significantly different from each other ($p = 0.999$). Likewise, stereotypy scores also did not significantly differ between the high stereotypy groups ($p = 0.143$).

Levels of total DARPP-32, phospho-Thr34-DARPP-32, and phospho-Thr75-DARPP-32

We examined levels of total DARPP-32, phospho-Thr34-DARPP-32, and phospho-Thr75-DARPP-32 in the dorsolateral striatum based on previous studies in our lab (Turner et al., 2002;

Turner and Lewis, 2003; Turner et al., 2003) implicating this region as being the most sensitive to the effects of environmental enrichment and motor stereotypy. Our analysis revealed that levels of total DARPP-32 were significantly lower ($F(3,50) = 4.15, p = 0.011$) in EE-LS mice when compared to EE-HS, SC-LS, and SC-HS animals (Figure 4-2a). No group differences ($F(3,50) = 0.823, p = 0.488$) were found for phospho-Thr34-DARPP-32 levels (Figure 4-2b), while significantly higher levels of phospho-Thr75-DARPP-32 ($F(3,50) = 4.56, p = 0.007$) were observed in SC-LS mice when compared to SC-HS mice (Figure 4-2c).

Levels of phospho-Thr34-DARPP-32/total DARPP-32 and phospho-Thr75-DARPP-32/total DARPP-32

Because there were significant differences in total DARPP-32 concentrations between groups, we normalized levels of the two phosphoproteins to total DARPP-32 levels to determine if differences in relative rather than overall phosphoprotein concentrations would more effectively differentiate the experimental groups. No group differences ($F(3,50) = 1.21, p = 0.316$) were found in phospho-Thr34-DARPP-32/total DARPP-32 levels (Figure 4-3a). However, significantly higher ($F(3,50) = 4.99, p = 0.004$) levels of phospho-Thr75-DARPP-32/total DARPP-32 were found in both EE-LS and SC-LS animals when compared to SC-HS animals (Figure 4-3b). A significant main effect of stereotypy ($t(52) = 3.12, p = 0.003$) was also found for phospho-Thr75-DARPP-32/total DARPP-32 with LS animals showing significantly greater levels of phospho-Thr75-DARPP-32/total DARPP-32 than HS animals. Correlational analysis further confirmed the negative relationship ($r(52) = -0.292, p = 0.032$) between phospho-Thr75-DARPP-32/total DARPP-32 and stereotypy (Figure 4-4).

Discussion

Previous studies from our lab have consistently demonstrated that early exposure to an enriched environment substantially reduces stereotypy in deer mice (Powell et al., 1999; Powell

et al., 2000; Turner et al., 2002; Turner and Lewis, 2003; Turner et al., 2003). Moreover, we have shown that these decreases in stereotypy are accompanied by neurobiological changes exclusive to environmentally enriched, low stereotypy mice. Specifically, these studies demonstrated that enrichment-related attenuation of stereotypy was associated with changes in various measures of synaptic plasticity, such as neuronal metabolic activity (Turner et al., 2002), dendritic morphology (Turner et al., 2003), and neurotrophin levels (Turner and Lewis, 2003). Here, we examined the effects of environmental enrichment on DARPP-32 regulation to determine if differences in the striatal signaling pathways which mediate dopamine function would also be associated with environmentally mediated decreases in stereotypy. The results show that environmentally enriched, low stereotypy mice have lower levels of total DARPP-32 protein when compared to either environmentally enriched, high stereotypy or standard caged animals. Neurobiological differences which distinguish this group of mice from the other groups are significant because they are specific for the beneficial effects of environmental enrichment. Therefore, this finding suggests that at least one additional mechanism by which enrichment functions to reduce stereotypy is by producing enduring changes in DARPP-32 protein expression.

Changes in total DARPP-32 expression have important functional consequences for motor behavior. Genetic deletion of DARPP-32 results in decreased sensitivity to the motor stimulatory effects of a wide variety of substances, including cocaine (Fienberg et al., 1998), apomorphine (Fienberg and Greengard, 2000), SKF-81297 (Scott et al., 2005), *para*-chloroamphetamine (Svenningsson et al., 2002), 5-HT (Svenningsson et al., 2002), and caffeine (Lindskog et al., 2002). In contrast, increases in total DARPP-32 levels have been associated with increases in abnormal motor behaviors, such as levodopa-induced dyskinesias (Aubert et al., 2005). These

observations are consistent with our finding of reduced stereotypy occurring concomitantly with decreases in total DARPP-32 expression in enriched, low stereotypy mice. However, given the disparate actions of the DARPP-32 phosphoproteins, any interpretations drawn from the analysis of total protein levels alone are incomplete because they omit consideration of DARPP-32 phosphorylation state.

Indeed, while this decrease in total DARPP-32 levels was an important aspect of its regulatory control in environmentally enriched, low stereotypy animals, the data demonstrate that the relative proportion of DARPP-32 phosphorylated at Thr75 (i.e., phospho-Thr75-DARPP-32/total DARPP-32) was the best overall predictor of stereotypy level. Regardless of housing condition, stereotypy was inversely related to phospho-Thr75-DARPP-32/total DARPP-32 levels; i.e., low stereotypy animals had higher levels of phospho-Thr75-DARPP-32/total DARPP-32, whereas high stereotypy animals had lower levels of phospho-Thr75-DARPP-32/total DARPP-32. Because phosphorylation at Thr75 converts DARPP-32 into an inhibitor of PKA, the physiological consequence of increased phospho-Thr75-DARPP-32/total DARPP-32 in low stereotypy animals would be a relative decrease in PKA activity. As a result, downstream targets of PKA, which include neurotransmitter receptors, ion channels, and transcription factors, would be maintained at a predominantly dephosphorylated state. Presumably, the converse would be true in high stereotypy animals, and decreased phospho-Thr75-DARPP-32/total DARPP-32 levels would result in a release of PKA inhibition and, therefore, an increase in neuronal excitability. Thus, the responsiveness to endogenous dopamine would differ in high and low stereotypy animals.

Previous findings in our lab support this hypothesis. Our research suggests that stereotypy might be expressed as a consequence of imbalanced activity along the direct and indirect

pathways of the basal ganglia (Presti et al., 2003; Presti and Lewis, 2005). Specifically, we have shown that hyperactivity of the indirect pathway is associated with low levels of stereotypy, whereas hypoactivity is associated with high levels of stereotypy (Presti and Lewis, 2005). The dopamine D₂ receptors of the indirect pathway are negatively coupled to adenylyl cyclase activation. Therefore, enhanced activity of the indirect pathway in low stereotypy mice would result in a decrease in cAMP production and decreased activation of PKA. In contrast, decreases in the activity of the indirect pathway in high stereotypy mice would result in a relative increase in cAMP and increased levels of catalytically active PKA. As a consequence, dopamine signaling would be differentially potentiated in high and low stereotypy mice.

No differences were found in either overall or normalized levels of phospho-Thr34-DARPP-32. This is in contrast to studies of drug-induced stereotypies which have indicated that this phosphorylation site may be crucial in the mediation of abnormal repetitive behaviors. The motor stereotypies typically induced by D-amphetamine, LSD, or PCP administration are all significantly reduced in DARPP-32 transgenic mice in which the Thr34 site has been replaced by a non-phosphorylatable alanine residue (Svenningsson et al., 2003). In other studies using Thr34Ala mutant mice, decreases in the motor stimulatory effect of acute cocaine challenge have also been observed (Valjent et al., 2005; Zachariou et al., 2006). The development of abnormal repetitive behaviors following L-DOPA treatment also appears to involve phosphorylation of DARPP-32 at Thr34. The striata of dyskinetic rats show abnormally high levels of phospho-Thr34-DARPP-32 when compared to non-dyskinetic rats (Picconi et al., 2003). Moreover, the severities of these repetitive movements are tightly correlated with levels of phospho-Thr34-DARPP-32 (Santini et al., 2007). Nevertheless, these results notwithstanding, the lack of an effect of Thr34 phosphorylation in our study may be explained, in part, by the differences

inherent between experimental paradigms of pharmacologically induced and spontaneously emitted stereotypies.

Drug-induced stereotypies are typically elicited following acute administration of a pharmacological agent, whereas our model involves a behavior which is both chronic and persistent. Therefore, the results of the present study likely reflect the adaptive changes which occur following prolonged stereotypy rather than the immediate changes. In support of this, it has been shown that the site-specific pattern of DARPP-32 phosphorylation differs following acute versus repeated psychostimulant administration. For example, acute administration of cocaine increases phosphorylation at Thr34 and decreases phosphorylation at Thr75 (Nishi et al., 2000), whereas repeated administration induces behavioral sensitization and is associated with a decrease in phosphorylation at Thr34 and an increase in phosphorylation at Thr75 (Scheggi et al., 2004). This increase in phospho-Thr75-DARPP-32 levels has been linked to enhanced Cdk5 activity (Bibb et al., 2001) and is thought to represent a homeostatic mechanism that develops in response to increased cAMP/PKA signaling during sensitization (Nestler, 2001). These data suggest that differences in the regulation of DARPP-32 can occur following short- and long-term exposure to the same stimulus.

Another reason we might not have detected a difference in phospho-Thr34-DARPP-32 concentrations may pertain to the contrasting actions of DARPP-32 in striatonigral and striatopallidal neurons. Activation of the cAMP/PKA pathway, and consequently phosphorylation of DARPP-32 at Thr34, in striatonigral neurons produces motor stimulation, whereas activation of the same pathway in striatopallidal neurons produces motor depression (Borgkvist and Fisone, 2007). Because the neuronal cell types of the striatum are anatomically intermixed, it was not possible to differentiate the biochemical regulation of DARPP-32 in

specific striatal cell subpopulations in this study using immunoblotting analysis. Therefore, additional studies may be necessary to more precisely localize the changes in phosphorylation of DARPP-32 at Thr34 and Thr75.

In summary, this study provides first evidence for a role of DARPP-32 in the mediation of spontaneously occurring repetitive behaviors. The data indicate that this is achieved primarily through the regulation of DARPP-32 phosphorylation at Thr75, although enrichment-related attenuation of stereotypy also involved transcriptional control of overall DARPP-32 protein expression. Because DARPP-32 is an important modulator of the intracellular signaling events in dopaminergic neurons, these results offer further empirical support for a link between perturbed dopaminergic function and the expression of stereotyped behaviors.

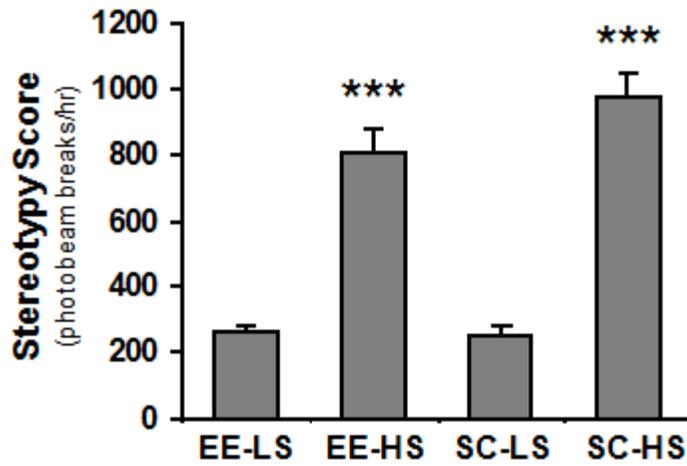


Figure 4-1. Stereotypy scores for environmentally enriched, low stereotypy (EE-LS, n = 13), environmentally enriched, high stereotypy (EE-HS, n = 13), standard caged, low stereotypy (SC-LS, n = 10), and standard caged, high stereotypy (SC-HS, n = 18) mice. *** indicates $p < 0.001$ versus EE-LS and SC-LS mice as determined by Tukey's HSD test.

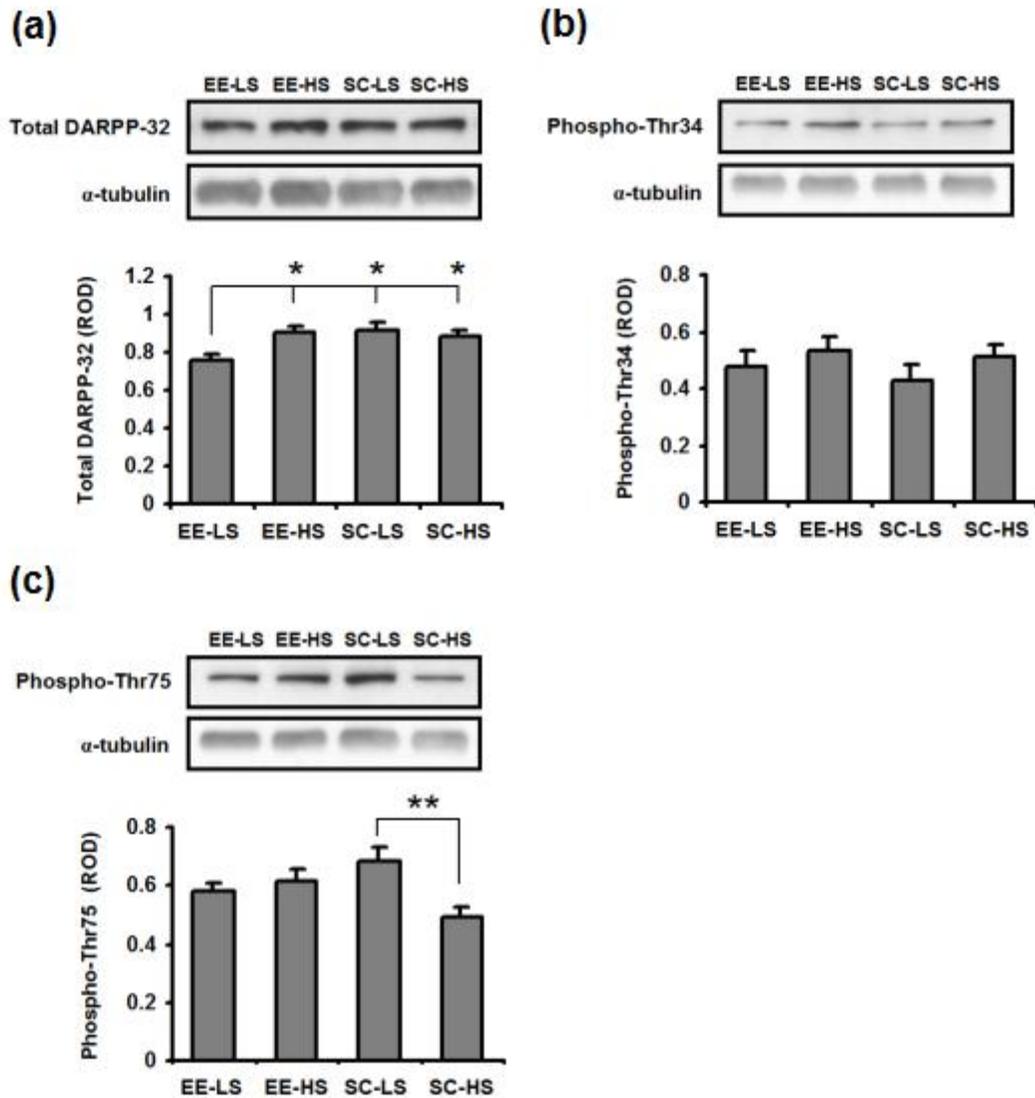


Figure 4-2. Western blot analysis of (a) total DARPP-32, (b) phospho-Thr34-DARPP-32, and (c) phospho-Thr75-DARPP-32 levels in the dorsolateral striatum. Representative immunoblots for the detection of total DARPP-32 and each phosphoprotein are shown above their respective graphs. Densitometric values for DARPP-32 bands are normalized relative to those of the loading control, α -tubulin, and are expressed as mean relative optical densities (ROD) \pm SEM. * indicates $p < 0.05$ versus EE-LS, and ** indicates $p < 0.01$ versus SC-HS as determined by Tukey's HSD test.

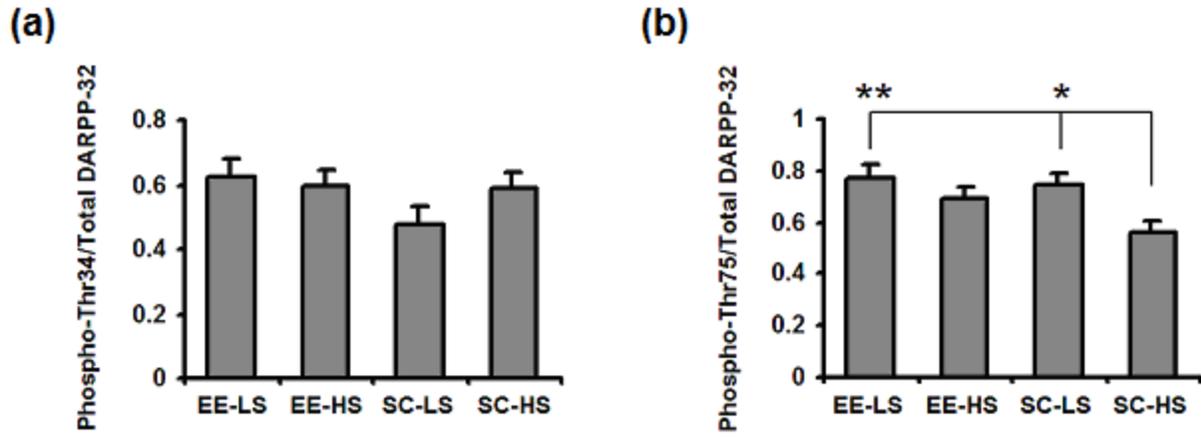


Figure 4-3. Concentrations of (a) phospho-Thr34-DARPP-32 and (b) phospho-Thr75-DARPP-32 normalized relative to total DARPP-32 levels. * indicates $p < 0.05$ and ** indicates $p < 0.01$ versus SC-HS as determined by Tukey's HSD test.

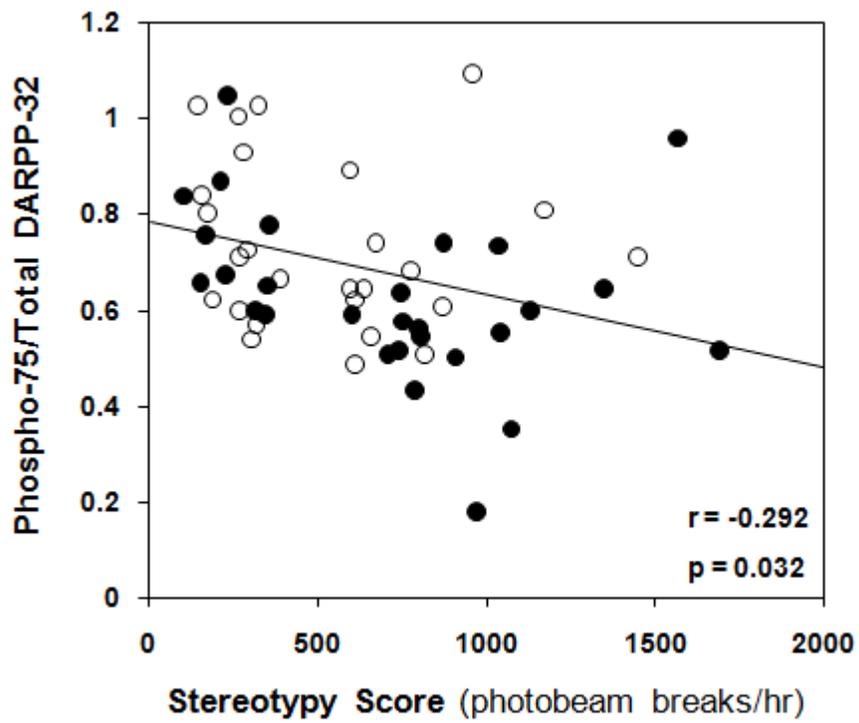


Figure 4-4. Relationship between the proportion of total DARPP-32 phosphorylated at Thr75 and stereotypy. Open symbols (○) indicate environmentally enriched animals. Closed symbols (●) indicate standard caged animals.

CHAPTER 5
EFFECTS OF ENVIRONMENTAL ENRICHMENT ON PATTERNS OF Δ FOSB
EXPRESSION IN AN ANIMAL MODEL OF ABNORMAL STEREOTYPED MOTOR
BEHAVIOR

Introduction

Abnormal repetitive behaviors have long been considered an important dimension of psychopathology. From its earliest description (Kanner, 1943) to current diagnostic criteria (American Psychiatric Association, 1994), patterns of behaviors and interests which may be described as being “restricted, repetitive, or stereotyped” have been a defining feature of autism. The phenomenological profile of these behaviors in autism is diverse and encompasses multiple categories of abnormal repetition, including stereotyped body movements, self-injurious behaviors, repetitive manipulation of objects, echolalia, compulsions, rituals and routines, insistence on sameness, and preoccupation with narrow, circumscribed interests. Although especially prominent in autistic symptomatology, repetitive behaviors are also characteristic of many other clinical disorders. Indeed, repetitive behaviors are associated with a wide variety of neurodevelopmental (e.g., mental retardation, Rett syndrome), psychiatric (e.g., schizophrenia, OCD, chronic drug abuse), and neurological (e.g., Tourette’s syndrome, Parkinson’s disease, Sydenham’s chorea) conditions.

Despite their clinical significance, the pathophysiology of abnormal repetitive behaviors remains poorly defined. Nevertheless, accumulating evidence from both human and animal studies suggest a link between repetitive behaviors and basal ganglia dysfunction. For example, stereotyped body movements in individuals with mental retardation are associated with alterations in both biochemical (Lewis et al., 1996) and biobehavioral (Bodfish et al., 1995; Bodfish et al., 2001; Roebel and MacLean, 2007) indices of dopamine functioning. Similarly, the frequency of repetitive motor behaviors in autistic (Turner and Russell, 1997) and schizophrenic

(Frith and Done, 1983) patients is correlated with profound impairments on neuropsychological tasks designed to detect signs of basal ganglia pathology. Neuroimaging research has also identified abnormalities in basal ganglia volume associated with “higher-order” cognitive-type repetitive behaviors in autism (Sears et al., 1999; Hollander et al., 2005).

In experimental animal models, motor stereotypies can be reliably induced with dopaminergic stimulation of the striatum (Randrup and Munkvad, 1967; Segal et al., 1980). With chronic administration of dopamine agonists, behavioral sensitization, a state in which the behavioral response to subsequent drug exposure is potentiated, develops (Kalivas and Stewart, 1991). This state is marked by behavioral changes, including enhanced locomotor activity and the production of stereotypies, often accompanied by changes in gene expression. In fact, it has been demonstrated that patterns of early gene activation within the neurochemically distinct, striosome and matrix, compartments of the striatum can accurately predict the severity of psychostimulant-induced stereotypies (Canales and Graybiel, 2000; Capper-Loup et al., 2002; Saka et al., 2004).

By far, much of what has been learned about the neurobiological basis of abnormal repetitive behaviors has been derived from studies of drug-induced stereotypies. However, models of stereotypic behavior which rely on pharmacological induction may not offer the most appropriate paradigm with which to explore the underlying pathophysiology of human repetitive behavior disorders. Indeed, it has been shown that the topography of spontaneously-emitted and drug-induced stereotypies are often qualitatively different in the same species (Vandebroek et al., 1998; Presti et al., 2002), suggesting that they also may not share the same neural substrates. Our lab has identified an animal model of spontaneously occurring (i.e., non-drug-induced) stereotypy. When housed under standard laboratory conditions, deer mice (*Peromyscus*

maniculatus) exhibit high rates of motor stereotypies which recapitulate key features of the abnormal repetitive behaviors observed in clinical phenomena; i.e., these stereotypies are spontaneous, emerge early in development, and persist throughout the lifespan of the animal. Remarkably, we have also discovered that the severity of these behaviors can be substantially reduced by rearing the mice in an enriched environment (Powell et al., 1999; Powell et al., 2000).

The goals of this study were to determine whether specific patterns of Δ FosB activation would serve to index the intensity of stereotypic behaviors in an animal model of spontaneous stereotypy and to determine whether the effects of environmental enrichment on stereotypy would correspond to changes in Δ FosB induction. We chose to evaluate Δ FosB because, unlike other Fos family proteins, Δ FosB levels accumulate rather than become desensitized with chronic stimulation (Chen et al., 1997); thus, it may provide a better marker for the long-term, experience-dependent neuroadaptations which occur following both persistent stereotypy and prolonged environmental enrichment.

Methods and Materials

Animals

Deer mice (*Peromyscus maniculatus bairdii*) were obtained from a breeding colony maintained at the University of Florida. The mice were housed in a room kept at 25°C, maintained on a 16:8 light:dark cycle (lights off at 2100), and provided with access to food and water *ad libitum*. At weaning (3 weeks of age), mice were randomly assigned to either standard cage (SC, n = 49) or enriched (EE, n = 50) housing conditions. Standard caged animals were housed in standard laboratory rodent cages (29 × 33 × 15 cm) with two other same-sex weanlings, whereas environmentally enriched animals were housed in groups of five or six in

modified large dog kennels (122 × 81 × 89 cm). These enrichment kennels were fitted with galvanized wire mesh to create multiple interconnected levels and equipped with bedding, a running wheel, and assorted ceramic and plastic objects varying in color, shape, and size. Throughout the 2 month experimental period, enrichment items were removed and replaced with novel objects on a weekly basis. To encourage foraging behaviors, approximately 50 grams of birdseed were also randomly distributed throughout the kennels three times a week. All animal procedures were approved by the University of Florida Institutional Animal Care and Use Committee and were performed in accordance with the NIH *Guide for the Care and Use of Laboratory Animals*.

Behavioral Testing

Animals were removed from their home cages and placed singly into Plexiglas testing cages (22 × 15 × 28 cm) two hours prior to the beginning of their dark cycle. Food, water, and bedding were provided, and the mice were left undisturbed for 2 hours to allow for habituation to the new environment and recovery from the stress of handling. Stereotypic behavior was quantified using an automated testing apparatus (Columbus Instruments, Columbus, OH) designed to register stereotypic motor activity (e.g., repetitive jumping or backward somersaulting) as photobeam breaks. All testing sessions were recorded using a digital video surveillance system (GeoVision Inc., Taipei, Taiwan) and checked in order to verify the accuracy of the automated counts. Data was collected for two dark cycles, and the stereotypy score for each animal was calculated as the average number of photobeam interruptions per hour of testing. The stereotypy scores for all animals tested were rank ordered, and animals belonging to the highest and lowest tercile of stereotypy scores were designated as high (HS, greater than 600 photobeam breaks/hr) and low stereotypy (LS, less than 400 photobeam breaks/hr) animals,

respectively. Animals with intermediate stereotypy scores between 400 and 600 photobeam breaks/hr were not used for analysis of FosB/ Δ FosB expression.

Western Blotting

Levels of FosB (45 kDa) and Δ FosB (35-37 kDa) in the striatum, motor cortex, sensory cortex, and hippocampus were determined using immunoblotting analysis. Animals (n = 6-7 per group) were sacrificed by cervical dislocation. The brains were rapidly removed, snap frozen in isopentane, and sliced into 300 μ m thick sections on a cryostat at -20°C. Tissue samples from the regions of interest were microdissected by punch method and sonicated in a homogenization buffer containing 20 mM Tris, 150 mM NaCl, 5 mM EDTA, 5 mM EGTA, 1% Triton X-100, 10 μ M DTT, and a protease inhibitor tablet (Roche). Samples were then placed on ice and vortexed every 15 min for 1 hr before clarification via centrifugation at 16,000g for 15 min. The supernatants were collected, and protein concentrations were determined using the DC Protein Assay kit (Bio-Rad Laboratories, Hercules, CA) following manufacturer's instructions. Equal amounts of total protein from each sample were loaded onto 12% polyacrylamide gels and separated by SDS-PAGE before transfer to polyvinylidene difluoride membranes. Membranes were blocked with 5% nonfat milk in Tris-buffered saline and 0.05% Tween (TBST) for 1 hr before incubation with the primary antibody (1:1,000 anti-FosB, Santa Cruz Biotechnology) overnight at 4°C. The next day, blots were washed in TBST for 15 min before incubation with a horseradish-peroxidase-conjugated anti-rabbit secondary antibody (1:5,000) for 1hr at room temperature. After washing with TBST, blots were developed using enhanced chemiluminescence and exposed to CL-XPosure film (Pierce Biotechnology, Rockford, IL) for visualization of protein bands. To control for variations in sample loading, blots were then incubated in stripping buffer (62.5 mM Tris-HCl, 100 mM β -mercaptoethanol, 2% SDS) for 30

min at 50°C before being reprobbed for α -tubulin (1:1,500, BioDesign, Saco, ME). Quantification of immunoreactive protein bands was done by densitometry using ImageJ software (version 1.37, NIH, Bethesda, MD). The densitometric values for Δ FosB bands were normalized relative to those of the loading control and are expressed as relative optical densities (ROD).

Double-Label Immunohistochemistry

Following behavioral testing, animals (n = 5 per group) were deeply anesthetized via isoflurane inhalation and transcardially perfused with 0.9% saline followed by ice-cold 4% paraformaldehyde. The brains were removed and post-fixed in the same fixative overnight at 4°C before equilibration in a cryoprotectant solution of 30% sucrose in 0.01 M phosphate-buffered saline (PBS). Forty-micron thick coronal sections were collected through the striatum, and sequential dual-antigen immunohistochemical staining was performed on free-floating sections in PBS using antibodies raised against FosB (1:300, Santa Cruz Biotechnology) and μ -opioid receptor-1 (MOR-1, 1:2,500, ImmunoStar, Hudson, WI). First, endogenous peroxidase activity was quenched by incubating the sections in 0.5% H₂O₂ in 10% methanol for 15 min. Sections were then rinsed in PBS before blocking in 10% Normal Donkey Serum (NDS) in 0.3% Triton X-100 for 1 hr. Subsequently, sections were incubated with the primary antibody in 1% NDS in 0.3% Triton X-100 overnight at 4°C. The next day, tissues were rinsed in PBS before incubation with the appropriate biotinylated secondary antibody in 1% NDS in 0.3% Triton X-100 for 1 hr. Sections were then rinsed again before being incubated in an avidin-biotin-peroxidase complex solution (Vectastain Elite ABC kit, Vector Laboratories, Burlingame, CA) for 1 hr. FosB-immunoreactive nuclei were first visualized by reaction with 3, 3'-diaminobenzidine (DAB) enhanced with 0.05% nickel ammonium sulfate and 0.05% cobalt chloride, which yielded a dark

blue-black stain, before the sections were processed for MOR-1-rich striosomes using DAB, which yielded a light orange-brown stain.

Image Analysis

For each animal, three equally spaced sections corresponding to coronal planes ranging from approximately 1.2 mm to 0.48 mm anterior to bregma (Paxinos and Watson, 1998) were analyzed. The number of FosB/ Δ FosB-immunoreactive nuclei in the striatum was estimated using the optical fractionator method (West et al., 1991). The contours of the striatum were outlined, and analysis was initiated from a random starting position within the striatum. The striatum was then systematically sampled by 250 μ m stepwise movements of the stage in the x and y directions. FosB/ Δ FosB-immunoreactive nuclei were counted when they came into focus, following the unbiased counting rules for the optical disector (Gundersen et al., 1988). The dimensions of the counting frame were set at 50 \times 50 μ m, and the disector height was 15 μ m with the guard volume offset 3 μ m from the tissue surface. This sampling scheme was designed to yield a coefficient of error (Glaser and Wilson, 1998) of less than 10% for each estimate.

Point counting stereology was used to estimate the density of FosB/ Δ FosB-immunoreactive nuclei in the striosome and matrix compartments as well as the volume fraction of the striatum occupied by striosomes. For this analysis, a 10 \times 10 μ m grid probe was superimposed over the counting frame, and FosB/ Δ FosB-immunoreactive nuclei were counted when they intersected the grid. Additionally, individual points were also identified as being striosome-positive where they co-localized with MOR-1-immunoreactive patches.

Statistical Procedures

All data are presented as mean \pm standard error of the mean (SEM). An independent samples t-test (two-tailed) was used to determine the effect of housing condition on stereotypy

levels (SPSS, Chicago, IL). One-way analyses of variance (ANOVAs) were used to analyze behavioral data and FosB/ Δ FosB densities with post hoc analysis of significant effects accomplished using Newman-Keuls test. Correlations between FosB/ Δ FosB activation and stereotypy scores were calculated according to the method of Pearson. The minimum level of significance was set at $p < 0.05$ for all analyses.

Results

Behavioral Data

Consistent with our previous findings (Powell et al., 1999; Powell et al., 2000), environmental enrichment resulted in a marked attenuation of stereotypic behavior ($t(97) = -4.089, p < 0.001$). The interaction of housing condition with stereotypy yielded our four experimental groups: environmentally enriched, low stereotypy (EE-LS, $n = 12$); environmentally enriched, high stereotypy (EE-HS, $n = 11$); standard caged, low stereotypy (SC-LS, $n = 11$); and standard caged, high stereotypy (SC-HS, $n = 12$) mice. Stereotypy scores for the experimental groups are shown in Table 5-1. As expected, significant differences ($F(3,42) = 75.4, p < 0.001$) were found in the frequency of stereotypic behavior between the high stereotypy groups (EE-HS and SC-HS) and the low stereotypy groups (EE-LS and SC-LS). Stereotypy scores for the low stereotypy groups were not significantly different from each other. Likewise, stereotypy scores also did not significantly differ between the high stereotypy groups.

Δ FosB Expression in Selected Brain Regions

In order to determine if the long-term effects of environmental enrichment and motor stereotypy would localize to certain brain regions, we first examined FosB and Δ FosB expression in the striatum, motor cortex, sensory cortex, and hippocampus using immunoblotting analysis (Table 5-2). These regions were selected based on their involvement in motor function (striatum and motor cortex) or their demonstrated sensitivity (Rampon et al., 2000; van Praag et

al., 2000) to the effects of environmental enrichment (hippocampus and cortical areas). Levels of FosB (45 kDa) in many samples were too low to allow for quantitative analysis. In contrast, the chronic Δ FosB isoform (35-37 kDa) demonstrated measurable levels of expression throughout all regions examined and was also the predominant isoform expressed in the striatum (Figure 5-1). No differences were found in Δ FosB levels between groups in the motor cortex ($F(3,22) = 0.79$, $p = 0.512$) or hippocampus ($F(3,22) = 0.317$, $p = 0.813$). However, Δ FosB induction in the sensory cortex was significantly higher ($F(3,22) = 3.93$, $p = 0.022$) in EE-LS mice than in EE-HS, SC-LS, or SC-HS mice. A significant difference ($F(3,22) = 4.54$, $p = 0.013$) in Δ FosB activation was also found in the striatum. Post hoc analysis revealed that this was due to significantly greater ($p < 0.01$) striatal concentrations of Δ FosB in SC-HS mice when compared to SC-LS mice.

Regional Distribution of Striatal FosB/ Δ FosB Expression

The Western blotting results indicated that, for standard caged mice, increased stereotypy was associated with increased Δ FosB accumulation within the striatum. Given the functional and anatomical heterogeneity of the striatum, we next used immunohistochemistry to more precisely characterize the anatomical distribution of FosB/ Δ FosB expression. This was particularly important because our previous studies (Turner et al., 2002; Turner and Lewis, 2003; Turner et al., 2003) have indicated that the beneficial effects of environmental enrichment are specific to the dorsolateral striatum so there may have been a dilution effect in examining the entire striatum.

To characterize the topographical distribution of FosB/ Δ FosB activation, the striatum was subdivided along its dorsoventral and mediolateral axes into dorsolateral (DLS), dorsomedial (DMS), ventrolateral (VLS), and ventromedial (VMS) quadrants (Figure 5-2). EE-LS mice

showed significantly greater ($F(3,16) = 28.5, p < 0.001$) levels of FosB/ Δ FosB in the dorsolateral striatum when compared to the other groups and significantly higher ($F(3,16) = 4.28, p = 0.021$) levels of FosB/ Δ FosB in the dorsomedial striatum when compared to the SC-LS group (Figure 5-4). FosB/ Δ FosB expression in both the ventrolateral ($F(3,16) = 4.75, p = 0.015$) and ventromedial ($F(3,16) = 4.59, p = 0.017$) regions of the striatum were significantly greater in the EE-LS, EE-HS, and SC-HS groups than in the SC-LS group.

Correlations between the estimated number of FosB/ Δ FosB-immunoreactive nuclei and stereotypy scores in the four subregions were also calculated separately for enriched, standard caged, and all animals (Table 5-3). When the animals were analyzed altogether, no significant correlations were found between FosB/ Δ FosB expression and stereotypy levels in any of the striatal subregions examined. However, when the data was segregated according to housing conditions, a distinct pattern emerged. For standard caged animals, increasing stereotypy was associated with increasing FosB/ Δ FosB expression in all striatal subregions, whereas, for enriched animals, a significant correlation was found only in the dorsolateral striatum (Figure 5-5). Furthermore, in contrast to the standard caged animals, increasing stereotypy was associated with decreased FosB/ Δ FosB induction in the enriched animals.

Compartmental Distribution of Striatal FosB/ Δ FosB Expression

We next examined the distribution of FosB/ Δ FosB expression in the striosome and matrix compartments of the striatum (Figure 5-3). For this analysis, we calculated an index of striosome to matrix predominance (ISMP) value, defined as the ratio of the density of FosB/ Δ FosB-immunoreactive nuclei in the striosomes to the density of FosB/ Δ FosB-immunoreactive nuclei in the matrix. The data revealed a significant difference in ISMP values ($F(3,16) = 10.1, p = 0.001$) between high (EE-HS and SC-HS) and low (EE-LS and SC-LS) stereotypy animals with high

stereotypy animals demonstrating significantly higher ISMP values (Figure 5-6) than low stereotypy animals. The relative distribution of compartmental FosB/ Δ FosB activation, as measured by the ISMP value, also more closely paralleled changes in stereotypy than the density of FosB/ Δ FosB expression in each compartment alone (Figure 5-7). These differences in compartmental FosB/ Δ FosB activation did not appear to be result from differences in overall striosome volumes between groups (data not shown).

Discussion

In this study, we mapped the regional distribution of Δ FosB induction in order to identify the neuronal systems which are activated by persistent, spontaneous stereotypy and long-term exposure to an enriched environment. The data demonstrate that, for standard caged mice, levels of Δ FosB accumulate specifically in the striatum with prolonged stereotypic motor activity. As determined by immunoblotting analysis, levels of Δ FosB were significantly higher in standard caged, high stereotypy mice when compared to standard caged, low stereotypy mice.

Immunohistochemical analysis of FosB/ Δ FosB expression further indicated that increasing stereotypy in standard caged animals was correlated with increasing FosB/ Δ FosB induction in all striatal subregions. In contrast, the effects of enrichment on stereotypy were localized only to the dorsolateral region of the striatum. Also, in contrast to the standard caged animals, increasing stereotypy in enriched animals was associated with decreasing levels of FosB/ Δ FosB expression.

Once established, stereotypies appear to become entrenched within the behavioral repertoire of deer mice and become increasingly intractable in response to further environmental manipulations (Hadley et al., 2006). Likewise, clinical stereotypies are also noted for being extremely refractory to treatment. Striatal accumulation of Δ FosB may mediate some of the neuroadaptations which lead to the continued persistence and maintenance of stereotypic

behaviors. In support of this hypothesis, a role for Δ FosB has also been implicated in other repetitive movement disorders.

In animal models of Parkinson's disease, the appearance of abnormal involuntary movements (i.e., AIMs or dyskinesias) following chronic L-DOPA treatment correlates temporally with a gradual increase in Δ FosB levels (Andersson et al., 1999). Several lines of evidence have suggested that Δ FosB mediates the development of these behaviors.

First, increases in Δ FosB levels are only observed in animals that develop dyskinesias. A subset of animals treated with L-DOPA do not develop dyskinesias; these animals show levels of Δ FosB comparable to those of control (i.e., saline treated) animals (Andersson et al., 1999).

Second, experimental manipulations which decrease Δ FosB expression also decrease dyskinetic symptoms. Intrastratial injection of antisense FosB inhibits Δ FosB expression and also attenuates dyskinetic symptoms (Chen et al., 2006). Likewise, pharmacological downregulation of Δ FosB has also been shown to result in a reduction in dyskinetic symptoms (Westin et al., 2007).

Third, the regional pattern of Δ FosB induction has been shown to be somatotopically related to the types of abnormal involuntary movements observed. For example, locomotor AIMs are preferentially associated with increased Δ FosB expression in the medial striatum, whereas axial, limb, and orolingual AIMs are selectively associated with increased Δ FosB expression in the lateral striatum (Andersson et al., 1999).

The results of this study suggest that the beneficial effects of environmental enrichment on stereotypy are associated with long-term alterations in the functioning of the dorsolateral striatum. Environmentally enriched, low stereotypy mice showed significantly higher levels of Δ FosB in the dorsolateral striatum than all other groups. Furthermore, a significant correlation

between stereotypy and Δ FosB activation was only observed in the dorsolateral striatum. These results are congruent with previous findings in our lab which have localized enrichment-related differences in metabolic activity (Turner et al., 2002), dendritic morphology (Turner et al., 2003), and neurotrophin expression (Turner and Lewis, 2003) to the dorsolateral striatum of environmentally enriched, low stereotypy mice. These results are also functionally significant because the dorsolateral striatum receives projections from the sensorimotor cortex which are responsible for mediating forelimb, trunk, and hindlimb movements (McGeorge and Faull, 1989; Ebrahimi et al., 1992). Thus, the striatal subregion which is the most sensitive to the effects of enrichment is also the subregion which is involved in producing the types of stereotypies (i.e., repetitive jumping, backflipping) which our mice express.

In addition to the changes observed in the dorsolateral striatum, environmentally enriched, low stereotypy mice also showed higher levels of Δ FosB in the sensory cortex than the other groups. Changes in the organizational features of somatosensory cortex have been shown to occur following environmental enrichment (Coq and Xerri, 1998). Indeed, active interaction with an enriched environment is required to produce the neurobiological effects of enrichment (Ferchmin and Bennett, 1975). Some of our animals do not benefit from the ameliorative effects of enrichment on stereotypy (i.e., the enriched, high stereotypy mice); these animals also do not show differences in Δ FosB activation when compared to standard caged animals. This increase in Δ FosB gene expression in the sensory cortex of enriched, low stereotypy mice might reflect their increased engagement with the environment and also contribute to some of the changes in Δ FosB expression observed in the dorsolateral striatum.

Another goal of this study was to characterize the effects of environmental enrichment and stereotypy on the compartmental (striosome/matrix) distribution of Δ FosB expression. Our data

indicates that stereotypy is associated with enhanced Δ FosB activation in the striosomes relative to activation in the matrix regardless of housing condition. Furthermore, we also found that the relative activation more closely paralleled changes in stereotypy across housing conditions than activation in either of the compartments alone, suggesting coordinate regulation of the striatal compartments. These results are consistent with research performed using models of psychostimulant-induced stereotypy (Canales and Graybiel, 2000; Capper-Loup et al., 2002; Saka et al., 2004). Predominant striosomal Δ FosB expression has also been observed in conjunction with the development of AIMs following chronic L-DOPA administration (Cenci et al., 1999; Saka et al., 1999). These findings suggest that behavioral stereotypy may originate as a consequence of a functional imbalance in the regulation of striosome and matrix compartments. Since the striosomes are thought to mediate reinforcement-related behaviors (White and Hiroi, 1998), this change in the balance of gene activation with increasing stereotypy may indicate a shift towards more motivationally-directed behaviors in high stereotyping animals.

Altogether, these data demonstrate that chronic stereotypy and environmental enrichment induce regionally and compartmentally specific patterns of Δ FosB activation and that Δ FosB can mediate both adaptive and maladaptive behavioral plasticity depending on the form of chronic stimulation (stereotypic motor activity or environmental enrichment) experienced. This latter finding, in particular, suggests further investigation.

The functional consequences of Δ FosB induction on motor behavior can differ diametrically depending on the striatal cell population activated. The neuropeptides, dynorphin and enkephalin, are selectively expressed in the medium spiny neurons comprising the direct and indirect pathways, respectively. Activation of the direct pathway augments motor output, whereas activation of the indirect pathway attenuates motor output. Transgenic mice

overexpressing Δ FosB in dynorphin-containing striatal neurons show increased wheel-running behavior relative to controls, whereas mice overexpressing Δ FosB in enkephalin-containing striatal neurons show less wheel-running behavior (Werme et al., 2002). Similarly, Δ FosB activation in dynorphin-positive neurons is associated with L-DOPA-induced dyskinesias (Andersson et al., 1999; Pavon et al., 2006), whereas a selective increase in Δ FosB expression in enkephalin-containing neurons following the chronic administration of neuroleptic drugs is correlated with the appearance of extrapyramidal side effects. Our past research has indicated that stereotypy may be linked to imbalances in the activity of the direct and indirect pathways (Presti et al., 2003; Presti et al., 2004a; Presti and Lewis, 2005); therefore, Δ FosB may mediate different forms of neural plasticity, in part, through cell-specific expression.

Δ FosB could also exert its opposing effects through differential gene expression. In studies of chronic drug abuse, it has been shown that Δ FosB activation following short- and long-term exposure to cocaine results in the transcription of different target genes (McClung and Nestler, 2003). Furthermore, these differences in gene expression produced different behavioral effects: gene expression induced by short-term Δ FosB activation reduced the rewarding effects of cocaine, whereas long-term Δ FosB activation heightened drug reward. Thus, it is possible that, even though high levels of Δ FosB were observed in both standard caged, high stereotypy and environmentally enriched, low stereotypy animals, different subsets of target genes were activated to produce differing behavioral effects. Future studies will be necessary to determine which of these mechanisms Δ FosB utilizes to induce different forms of behavioral plasticity.

Table 5-1. Stereotypy scores for experimental groups.

Group	<i>n</i>	Stereotypy Scores
EE-LS	12	206 ± 26
EE-HS	11	815 ± 40
SC-LS	11	246 ± 34
SC-HS	12	989 ± 69

Values represent mean stereotypy scores (photobeam breaks/hr) ± SEM.

Table 5-2. Levels of Δ FosB expression in selected brain regions.

Group	Striatum	Motor Cortex	Sensory Cortex	Hippocampus
EE-LS	0.94 ± 0.07	0.89 ± 0.07	0.75 ± 0.05^b	0.78 ± 0.05
EE-HS	0.84 ± 0.08	0.82 ± 0.08	0.57 ± 0.06	0.80 ± 0.04
SC-LS	0.71 ± 0.07	0.76 ± 0.08	0.51 ± 0.05	0.74 ± 0.05
SC-HS	1.11 ± 0.09^a	0.74 ± 0.07	0.53 ± 0.06	0.75 ± 0.05

Values shown are expressed as mean relative optical densities ± standard error of the mean. Significant values are shown in boldface. a indicates $p < 0.01$ vs SC-LS, and b indicates $p < 0.05$ vs EE-HS, SC-LS, and SC-HS.

Table 5-3. Correlations between FosB/ Δ FosB in striatal quadrants and stereotypy scores.

Subregion	EE	SC	All
DLS	-0.87^b	0.789^b	-0.326
DMS	-0.506	0.897^a	0.064
VLS	-0.091	0.893^a	0.417
VMS	-0.29	0.7^c	0.258

Values represent correlation coefficients (r_p). Significant values are shown in boldface. a, b, and c indicate $p < 0.001$, $p < 0.01$, and $p < 0.05$, respectively.



Figure 5-1. Representative immunoblot for the detection of FosB isoforms in the striatum (top panel). The same blot was re-probed for α -tubulin (bottom panel) as a loading control. The blots show that the predominant FosB isoform expressed in the striatum in all of the experimental groups is the chronic Δ FosB (35-37 kDa) isoform. ns indicates non-specific bands.

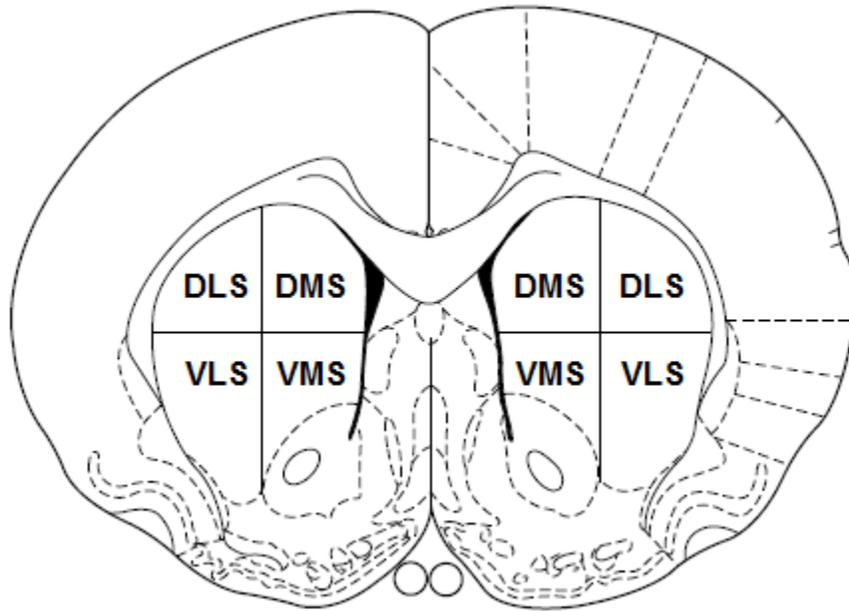


Figure 5-2. Schematic illustrating striatal subregions. The striatum was subdivided along its dorsoventral and mediolateral axes into the dorsolateral (DLS), dorsomedial (DMS), ventrolateral (VLS), and ventromedial (VMS) quadrants.

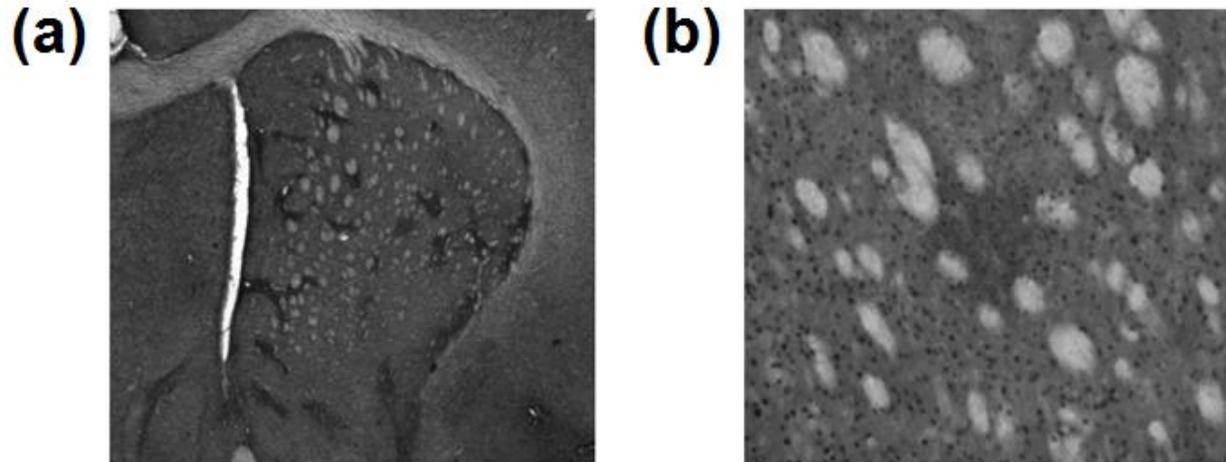


Figure 5-3. Photomicrographs of sections double stained for FosB/ Δ FosB nuclei (black dots) and MOR-1-rich striosomes (gray patches) at (a) low and (b) high magnification. Representative sections are from a SC-HS animal.

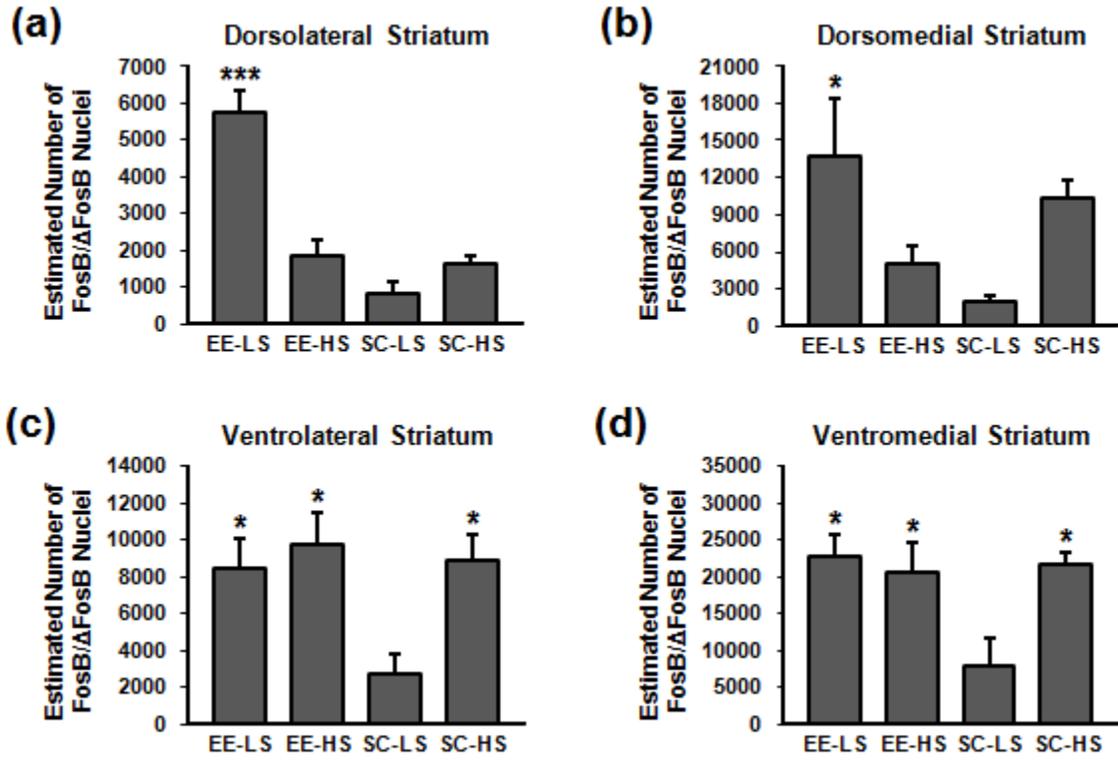


Figure 5-4. Subregional distribution of FosB/ΔFosB-immunoreactive nuclei in EE-LS, EE-HS, SC-LS, and SC-HS animals.

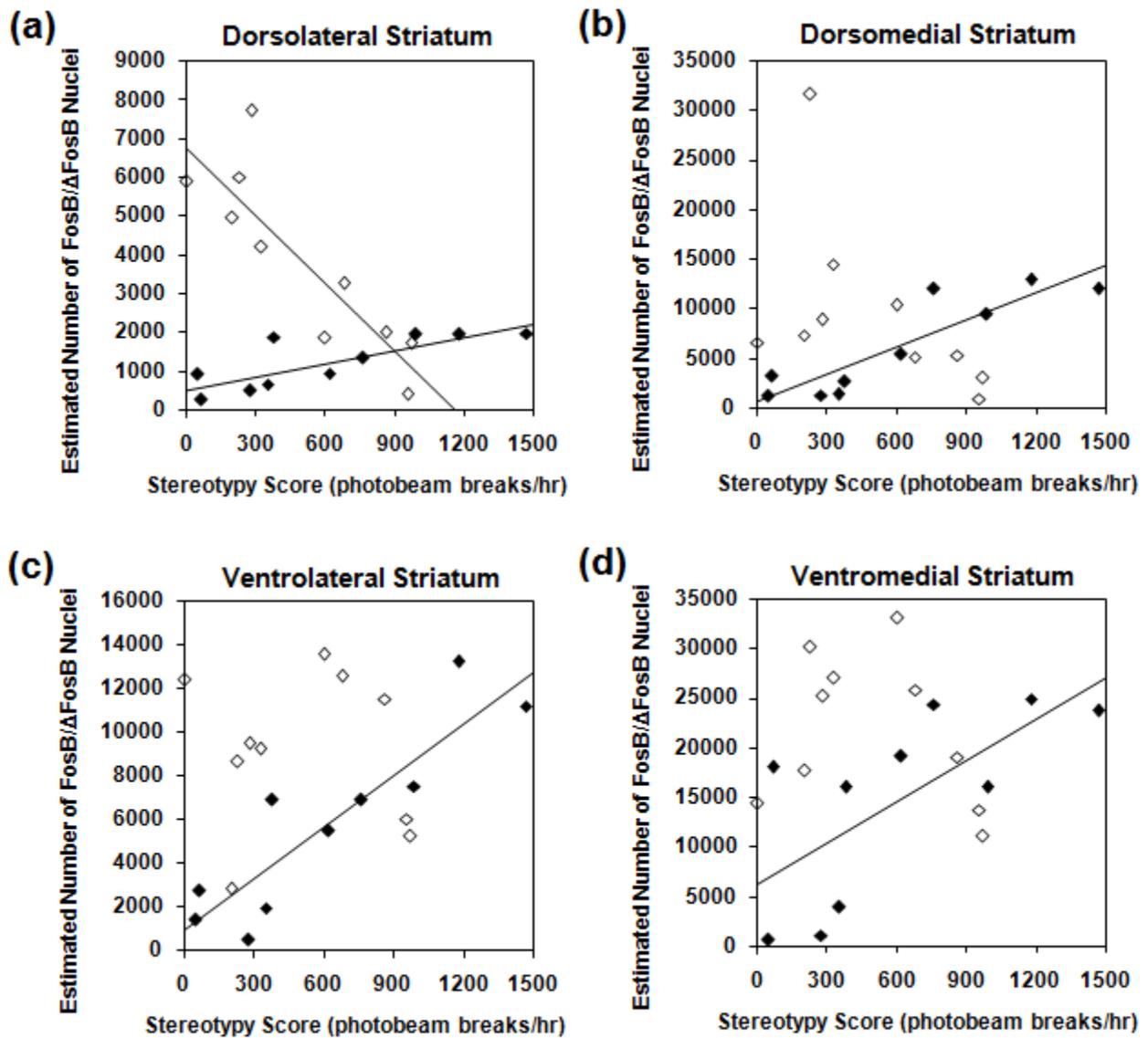


Figure 5-5. Correlations between the estimated number of FosB/ Δ FosB-immunoreactive nuclei and stereotypy scores in the (a) dorsolateral, (b) dorsomedial, (c) ventrolateral, and (d) ventromedial quadrants of the striatum. Trendlines indicate significant correlations. Open symbols (\diamond) indicate environmentally enriched animals. Closed symbols (\blacklozenge) indicate standard caged animals.

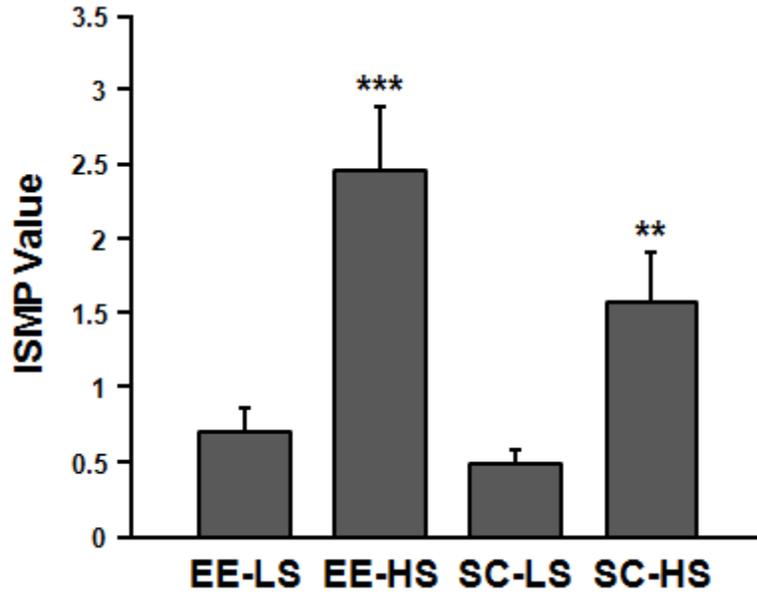


Figure 5-6. Distribution of FosB/ Δ FosB induction in the striosome and matrix compartments of the striatum. The index of striosome to matrix predominance (ISMP) value corresponds to the ratio of FosB/ Δ FosB density in the striosomes to FosB/ Δ FosB density in the matrix. ** and *** indicate $p < 0.01$ and $p < 0.001$ versus EE-LS and SC-LS, respectively.

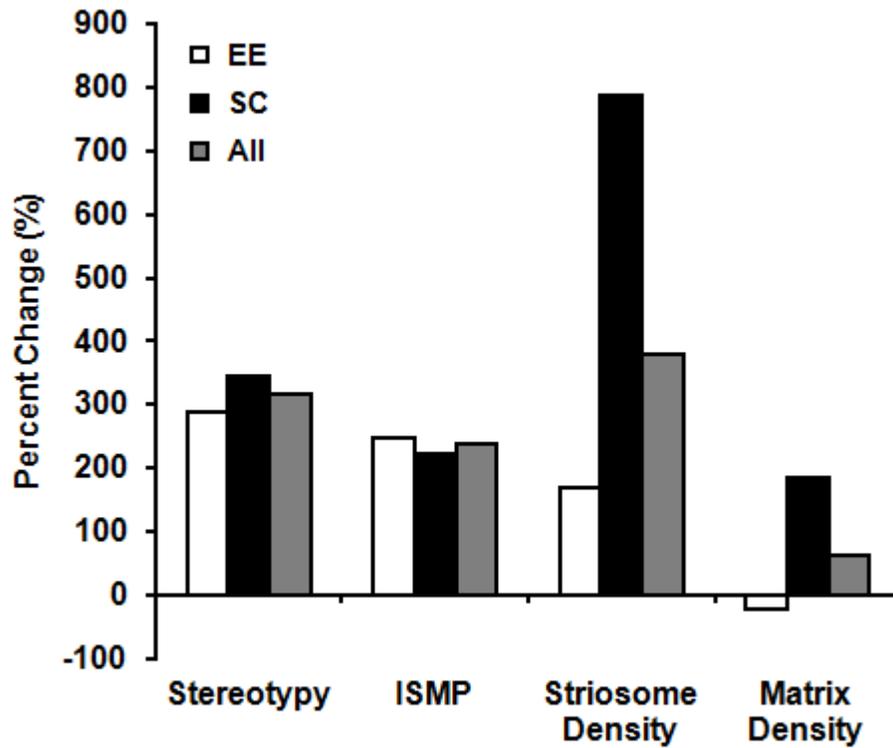


Figure 5-7. Changes in ISMP value more closely parallel changes in stereotypy than changes in the density of FosB/ Δ FosB expression in the striosome or matrix alone. Values shown are for high stereotypy animals normalized relative to low stereotypy animals belonging to the same group.

CHAPTER 6
EFFECTS OF ENVIRONMENTAL ENRICHMENT AND ABNORMAL STEREOTYPED
MOTOR BEHAVIOR ON SYNAPTIC PROTEIN EXPRESSION

Introduction

Stereotypies refer to abnormal repetitive motor behaviors which are characterized by topographical invariance and a lack of obvious purpose or function (Lewis and Baumeister, 1982; Berkson, 1983; Mason, 1991). Although stereotypies normally occur in typically developing human infants and have, in fact, been hypothesized to enhance the early development of motor skills (Thelen, 1979), the persistence of these behaviors later in childhood and into adulthood is most often associated with neuropsychiatric and developmental disorders, such as autism and mental retardation. In these cases, stereotypies (e.g., body-rocking, head-rolling, repetitive hand movements) are considered to be clinically relevant because they can become behaviorally dominant and interfere with more purposeful activities. Indeed, stereotypic individuals often show deficits in social interaction (Matson et al., 2006) and are impaired in the acquisition of new skills (Koegel and Covert, 1972) as well as the performance of established skills (Morrison and Rosales-Ruiz, 1997), including those necessary for normal adaptive functioning (Matson et al., 1997).

Although absent in the wild, stereotypies are also frequently observed in animals kept in captivity, especially those housed in barren environments with limited opportunities to engage in species-typical behaviors. Because they are thought to represent an animal's response to suboptimal housing conditions, the high occurrence of stereotypies in captive populations poses a major animal welfare concern. As environmental impoverishment has been demonstrated to induce stereotypic behaviors in a wide range of animal species (Cronin and Wiepkema, 1984; Mason, 1993; Würbel et al., 1996; Bashaw et al., 2001; Garner et al., 2003b; Mallapur and Choudhury, 2003; Vickery and Mason, 2004; Elzanowski and Sergiel, 2006), attempts to provide

an “enriched” environment have been employed as potential strategies to prevent or reduce these behaviors (Swaigood and Shepherdson, 2005). In these experiments, it was discovered that even modest enhancements to an animal’s environment (e.g., larger cage size, the addition of nesting materials, etc.) yielded appreciable decreases in stereotypy.

Although these observations indicate a strong role for environmental regulation in the development and expression of stereotypic behaviors, the specific pathophysiological mechanisms which give rise to motor stereotypies have yet to be clearly elucidated. In order to resolve the etiological factors more definitively, some researchers have utilized its contextual-dependence to generate animal models of stereotypy based on environmental restriction. Our lab has adopted one such model. Despite its species-specific nature, the abnormal repetitive motor behaviors (e.g., repetitive jumping, backwards somersaulting, and patterned running) exhibited by deer mice (*Peromyscus maniculatus*) mimic the fundamental characteristics observed in clinical populations; i.e., these invariant and apparently functionless behaviors emerge early in development and persist throughout the lifespan of the animal. Moreover, our lab has established that, while these stereotypies occur spontaneously under standard laboratory housing, their development may be delayed and their frequency reduced by rearing the mice in an enriched environment (Powell et al., 1999; Powell et al., 2000). Based on the premise that it might ultimately lead to the derivation of new therapeutic strategies for clinical stereotypies, our previous line of research was aimed at deciphering the neural mechanisms underlying these beneficial effects of environmental enrichment.

The effects of environmental enrichment on brain structure and function are well-documented. Environmental enrichment increases brain weight (Bennett et al., 1969), cortical thickness (Diamond et al., 1976), dendritic branching (Greenough et al., 1973) and length

(Wallace et al., 1992), dendritic spine density (Turner and Greenough, 1985), and synapse number and size (Sirevaag and Greenough, 1987). Exposure to an enriched environment also promotes neurogenesis (Kempermann et al., 2002) and reduces spontaneous apoptosis (Young et al., 1999). Additionally, increases in measures of non-neuronal plasticity, such as angiogenesis and gliogenesis, have also been reported following enrichment (Dong and Greenough, 2004; Markham and Greenough, 2004). Previous work from our lab has linked enrichment-related attenuation of stereotypy to changes in neuronal metabolic activity (Turner et al., 2002), dendritic morphology (Turner et al., 2003), and neurotrophin expression (Turner and Lewis, 2003). These changes were not only specific to environmentally enriched, low stereotypy animals, but they were also regionally specific for areas involved in the control of motor behavior.

The goal of this study was to determine whether region-specific modifications in synaptic efficacy would represent an additional mechanism contributing to the effects of environmental enrichment on stereotypy. To this end, we evaluated concentrations of the synaptic proteins, synaptophysin and neural cell adhesion molecule (NCAM), in various brain regions.

Synaptophysin and NCAM are both considered reliable markers of synaptic density (Walaas et al., 1988; Jorgensen, 1995). Furthermore, increases in the expression of both proteins have been documented following enrichment (Koo et al., 2003; Nithianantharajah et al., 2004). Thus, we hypothesized that analysis of these synaptic proteins would serve to index the molecular changes which occur as a consequence of enrichment-related effects on stereotypy.

Materials and Methods

Animals

Deer mice (*Peromyscus maniculatus bairdii*) were obtained from a breeding colony maintained at the University of Florida. The mice were housed in a room kept at 25°C,

maintained on a 16:8 light:dark cycle (lights off at 2200), and provided with access to food and water *ad libitum*. At weaning (3 weeks of age), mice were randomly assigned to either standard cage (SC, n = 39) or environmentally enriched (EE, n = 40) housing conditions. Standard caged animals were housed in standard laboratory rodent cages (29 × 33 × 15 cm) with two other same-sex weanlings, while environmentally enriched animals were housed in groups of five or six in modified large dog kennels (122 × 81 × 89 cm). These enrichment kennels were fitted with galvanized wire mesh to create multiple interconnected levels and equipped with bedding, a running wheel, and assorted ceramic and plastic objects varying in color, shape, and size. Throughout the 2 month experimental period, enrichment items were removed and replaced with novel objects on a weekly basis. To encourage foraging behaviors, approximately 50 grams of birdseed were also randomly distributed throughout the kennels three times a week. All animal procedures were approved by the University of Florida Institutional Animal Care and Use Committee and were performed in accordance with the NIH *Guide for the Care and Use of Laboratory Animals*.

Behavioral Testing

Animals were tested for stereotypy after 2 months in their respective housing conditions. Mice were removed from their home cages and placed singly into Plexiglas testing cages (22 × 15 × 28 cm) two hours prior to the beginning of their dark cycle. Food, water, and bedding were provided, and the mice were left undisturbed for 2 hours to allow for habituation to the new environment and recovery from the stress of handling. Stereotypic behaviors were quantified using an automated testing apparatus (Columbus Instruments, Columbus, OH) designed to register stereotypic motor activity (e.g., repetitive jumping or backward somersaulting) as photobeam breaks. All testing sessions were recorded using a digital video surveillance system

(GeoVision Inc., Taipei, Taiwan) and checked in order to verify the accuracy of the automated counts. Data were collected for two dark cycles, and the stereotypy score for each animal was calculated as the average number of photobeam interruptions per hour of testing. Following behavioral testing, the stereotypy scores for all animals tested were rank ordered, and animals belonging to the highest and lowest third of stereotypy scores were designated as high stereotypy (HS, greater than 600 photobeam breaks/hr) and low stereotypy (LS, less than 400 photobeam breaks/hr) animals, respectively. Animals with intermediate stereotypy scores between 400 and 600 photobeam breaks/hr were not used for analysis of synaptic protein levels.

Western Blotting

Levels of synaptophysin and neural cell adhesion molecule (NCAM) in the cortex, dorsolateral striatum, dorsomedial striatum, and hippocampus (Figure 6-1) were determined using Western blotting. Animals were sacrificed by cervical dislocation. The brains were rapidly removed, snap-frozen in isopentane, and stored at -80°C until processed for protein extraction. Briefly, 300 μm thick coronal sections were collected in a cryostat held at -20°C . Next, tissue samples from the regions of interest were microdissected by punch method and sonicated in a homogenization buffer containing 20 mM Tris, 150 mM NaCl, 5 mM EDTA, 5 mM EGTA, 1% Triton X-100, 10 μM DTT, and a protease inhibitor tablet (Roche). Samples were then placed on ice and vortexed every 15 min for 1 hr before clarification via centrifugation at 16,000g for 15 min. Subsequently, the supernatant was collected, and the protein concentrations were determined using the DC Protein Assay kit (Bio-Rad Laboratories, Hercules, CA), following manufacturer's instructions. Equal amounts (20 μg) of total protein from each sample were loaded onto 12% polyacrylamide gels and separated by SDS-PAGE before transfer to polyvinylidene difluoride membranes. Afterwards, gels and blots were stained with Coomassie blue and Ponceau S (Sigma), respectively, in order to verify equal sample loading. The

membranes were then washed to remove excess stain and blocked with 5% nonfat milk in Tris-buffered saline and 0.05% Tween (TBST) for 1 hr before incubation with the primary antibody (1:50,000 synaptophysin or 1:100 anti-NCAM; both antibodies from Chemicon, Temecula, CA) overnight at 4°C. The next day, blots were washed in TBST for 15 min before incubation with the appropriate horseradish peroxidase-conjugated secondary antibody (1:5,000; Santa Cruz Biotechnology, Santa Cruz, CA) for 1 hr at room temperature. Blots were then developed using enhanced chemiluminescence and exposed to CL-XPosure film (Pierce Biotechnology, Rockford, IL) for visualization of protein bands. Finally, quantification of immunoreactive protein bands was done by densitometry using ImageJ software (version 1.37, NIH, Bethesda, MD).

Statistical Analysis

All data are expressed as mean \pm standard error of the mean (SEM). Independent samples t-tests (two-tailed) were used to determine the effect of housing condition on levels of stereotypy (SPSS, Chicago, IL). Two-way (2×2) factorial analyses of variance (ANOVAs) with housing condition (EE or SC) and stereotypy (LS or HS) as the two factors were used to evaluate differences in synaptic protein levels. Correlations between protein concentrations and stereotypy scores were calculated according to the method of Pearson. The minimum level of significance was set at $p < 0.05$ for all analyses.

Results

Behavioral Data

Consistent with our previous findings (Powell et al., 1999; Powell et al., 2000), environmental enrichment resulted in a marked attenuation of stereotypic behavior ($t(77) = -2.36$, $p = 0.02$). Because we hypothesized neurobiological changes would be more apparent in phenotypically distinct groups, only animals testing within the highest and lowest third of

stereotypy scores were used for analysis of synaptic protein concentrations. The average stereotypy score for high stereotypy (HS, $n = 31$) animals was 906 ± 52 photobeam breaks/hr, and the average stereotypy score for low stereotypy (LS, $n = 23$) animals was 258 ± 17 photobeam breaks/hr. Of these animals, the average stereotypy score for EE animals ($n = 26$) was 534 ± 65 photobeam breaks/hr, and the average stereotypy score for SC animals ($n = 28$) was 719 ± 81 photobeam breaks/hr. Stereotypy scores were not significantly different between these two housing conditions ($t(52) = -1.76$, $p = 0.08$).

Effects of Housing Condition and Stereotypy on Synaptic Protein Expression

Levels of synaptophysin and neural cell adhesion molecule (NCAM) were evaluated in the cortex, dorsolateral striatum, dorsomedial striatum, and hippocampus (Figure 6-1). These areas were selected based on their demonstrated sensitivity (Rampon et al., 2000; van Praag et al., 2000) to the effects of environmental enrichment (cortex and hippocampus) or their role in the control of motor behavior (striatum). Additionally, the dorsolateral and dorsomedial subregions of the striatum were analyzed separately based on evidence suggesting anatomical and functional dissociation of the two regions (Voorn et al., 2004; Yin and Knowlton, 2006).

Synaptophysin migrated as a single 38 kDa band, whereas NCAM was expressed as three isoforms, with molecular weights of 120, 140, and 180 kDa, in all regions examined. Optical densities were calculated separately for each NCAM isoform. Significant main effects of housing condition were found for synaptophysin levels in the dorsolateral striatum ($F(1,50) = 4.86$, $p = 0.031$, Figure 6-2a) and NCAM-140 levels in the cortex ($F(1,50) = 5.14$, $p = 0.028$, Figure 6-3a) with EE animals demonstrating higher levels of the synaptic proteins than SC animals. Although there were no significant main effects of stereotypy on synaptic protein levels, correlational analysis did reveal significant negative correlations between stereotypy scores and synaptophysin concentrations in the dorsolateral striatum ($r(52) = -0.381$, $p = 0.005$, Figure 6-2b) and between

stereotypy scores and NCAM-140 concentrations in the cortex ($r(52) = -0.273$, $p = 0.046$, Figure 6-3b). There were no differences in synaptic protein expression as a consequence of either housing condition or stereotypy in the dorsomedial striatum or hippocampus. No significant housing condition \times stereotypy interactions were found for any of the regions examined.

Discussion

In this study, we examined the role of synaptic changes in the environmental regulation of stereotypy. Our results show that experience-dependent plasticity was associated with region-specific changes in synaptic protein content localized to the dorsolateral striatum and cortex. Specifically, environmental enrichment increased the expression of synaptophysin in the dorsolateral striatum and NCAM-140 in the cortex. Increased levels of synaptophysin in the dorsolateral striatum and NCAM-140 in the cortex were also associated with decreasing stereotypy. We also analyzed synaptic protein concentrations in the dorsomedial striatum and hippocampus; however, no differences in synaptophysin or NCAM expression were found as a result of either housing condition or motor stereotypy in these areas.

This regional pattern of effects is consistent with our previous research. We have demonstrated increases in neuronal metabolic activity (Turner et al., 2002), dendritic spine density (Turner et al., 2003), and brain-derived neurotrophic factor (BDNF) expression (Turner and Lewis, 2003) in cortical and striatal regions following enrichment. This same regional distribution of increased neural plasticity was also evident when we compared low stereotypy animals to high stereotypy animals. Furthermore, these changes were regionally selective as no effects of enrichment or stereotypy were found in any of the other areas examined, such as the hippocampus.

Although we did not systematically differentiate between the dorsolateral and dorsomedial striatum in our previous studies, we analyzed the striatal subregions separately in this study in

order to more precisely delineate the regions involved in mediating the effects of environmental enrichment on stereotypy. Our results showed that synaptic modifications only occurred in the dorsolateral subregion of the striatum. Whereas the dorsomedial striatum generally receives projections from association cortices, the dorsolateral striatum primarily receives afferent projections from the sensorimotor cortex, especially those involved in controlling hindlimb, trunk, and forelimb movements (McGeorge and Faull, 1989; Ebrahimi et al., 1992). Thus, this finding suggests that the effects of environmental enrichment and stereotypy on synaptic plasticity were targeted specifically to the corticostriatal regions responsible for the control of motor behavior.

Synaptic changes in the cortex and dorsolateral striatum were differentially indexed by the two synaptic proteins; i.e., synaptophysin was a better marker of enrichment- and stereotypy-related changes in the dorsolateral striatum, whereas NCAM-140 was better able to detect experience-dependent synaptic modifications in the cortex. This suggests that different mechanisms may have been operative in inducing synaptic change in these two regions.

Synaptophysin is an integral membrane component of presynaptic vesicles (Wiedenmann and Franke, 1985). As a specific marker of presynaptic terminals (Hiscock et al., 2000), synaptophysin is generally used to index synaptic density (Walaas et al., 1988). However, changes in synaptophysin expression have also been shown to reflect changes in neurotransmitter-release not related to changes in synapse number (Alder et al., 1992; Alder et al., 1995; Nakamura et al., 1999). Therefore, the changes in synaptophysin levels observed in the dorsolateral striatum may indicate changes in synaptogenesis and/or presynaptic neurotransmission.

NCAMs have also been validated as quantitative markers of synaptic density (Jorgensen, 1995). Of the three major NCAM isoforms expressed in the brain, only differences in cortical NCAM-140 levels were detected when comparing experimental groups. This isoform-specificity is important because the different isoforms demonstrate different localizations and functions. For example, whereas NCAM-120 is found in glia and NCAM-180 is expressed primarily on postsynaptic membranes, NCAM-140 is expressed on both pre- and post-synaptic membranes (Noble et al., 1985; Persohn et al., 1989). As such, NCAM-140 can exert its effects on cell-cell adhesion through either homophilic or heterophilic binding to stabilize synaptic connections (Schachner, 1997). In addition, NCAM-140 also plays a major role in altering neuronal morphology by inducing neuritogenesis through its interactions with different signal transduction pathways (Niethammer et al., 2002).

Regardless of the specific mechanisms involved, these changes in synaptic protein concentrations suggest increased synaptic reorganization. On a functional level, these structural alterations likely translate to changes in synaptic properties. Improved synaptic functioning, as mediated through greater synaptic efficacy, could contribute to the greater behavioral flexibility observed in low stereotypy mice.

Although we did not find a significant interaction between housing condition and stereotypy on synaptic protein levels, higher levels of synaptophysin and NCAM-140 were found in the dorsolateral striatum and cortex, respectively, of both enriched and low stereotypy mice. Thus, even though we cannot attribute enrichment-related decreases in stereotypy to the synaptic changes in these regions, the fact that both enrichment and decreasing stereotypy levels demonstrate identical patterns of region- and protein-specific effects is still suggestive of an

association between the effects of environmental enrichment on synaptic plasticity and the effects of environmental enrichment on stereotypy.

Here, we employed immunoblotting techniques to assay synaptic protein concentrations due to the large sample size and multiple regions involved in our study. Although this method is useful for high-throughput analysis, changes in synaptic structure can only be inferred from changes in protein levels. Therefore, future studies may be important to determine the exact morphological changes induced by environmental enrichment and motor stereotypy.

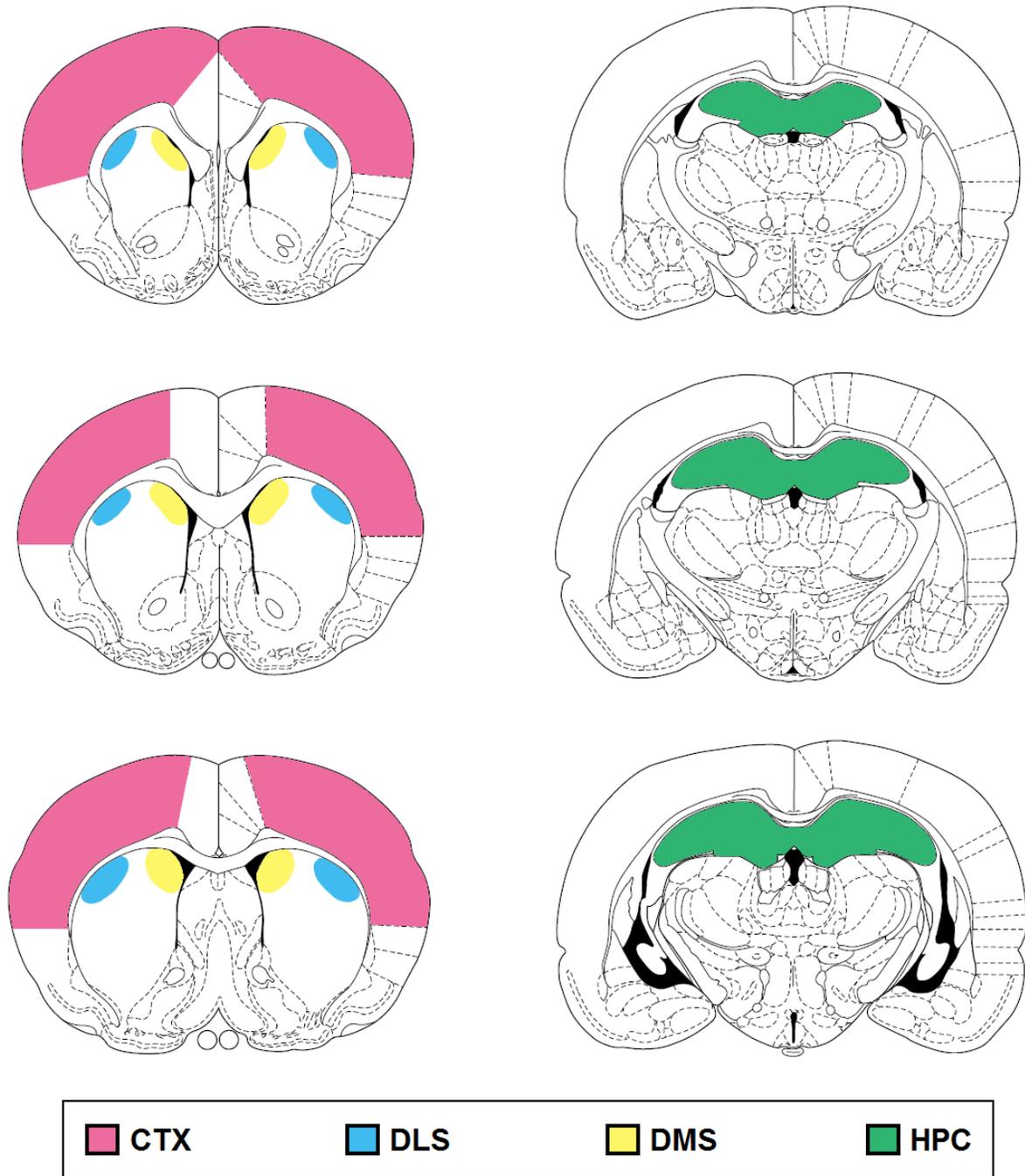


Figure 6-1. Diagrammatic representation of the dissection procedure. Coronal sections shown are adapted from the atlas of Paxinos and Watson (1998). CTX, cortex; DLS, dorsolateral striatum; DMS, dorsomedial striatum; and HPC, hippocampus.

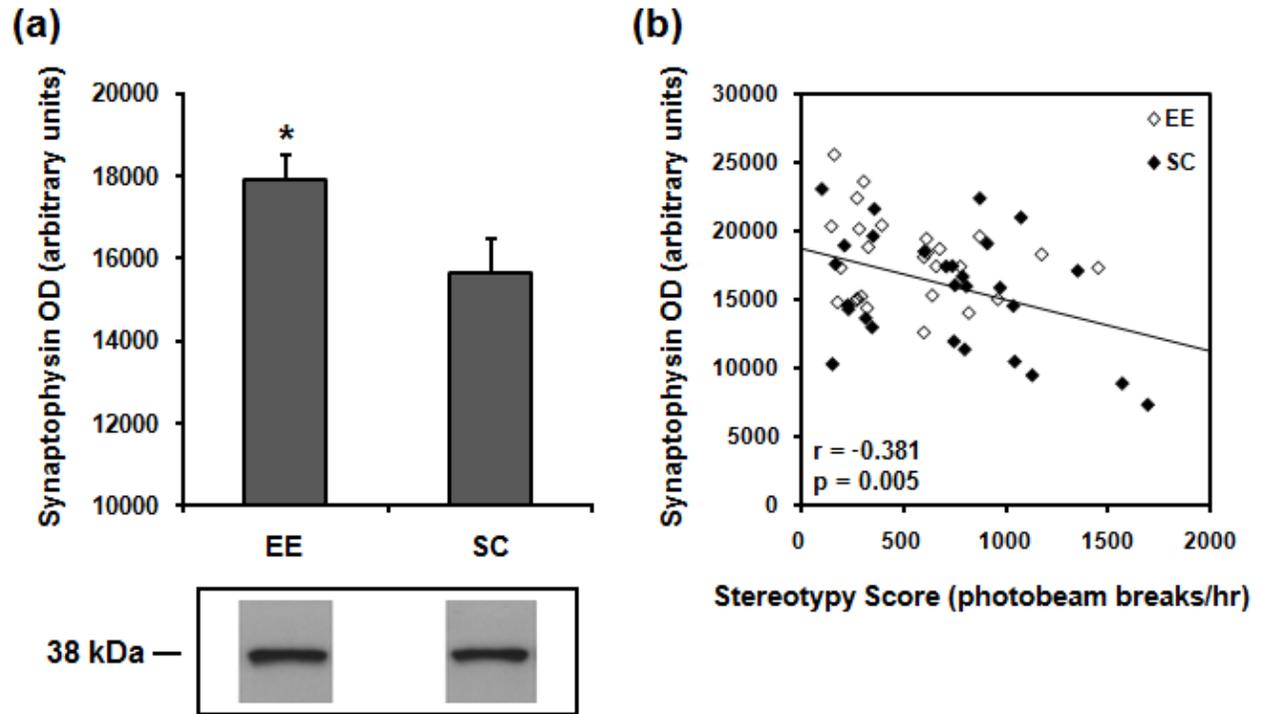


Figure 6-2. Effects of (a) environmental enrichment and (b) motor stereotypy on synaptophysin protein expression in the dorsolateral striatum. Representative blots of synaptophysin (38 kDa) are shown in the lower panel. * indicates $p < 0.05$. Open symbols (\diamond) indicate environmentally enriched (EE) animals. Closed symbols (\blacklozenge) indicate standard caged (SC) animals.

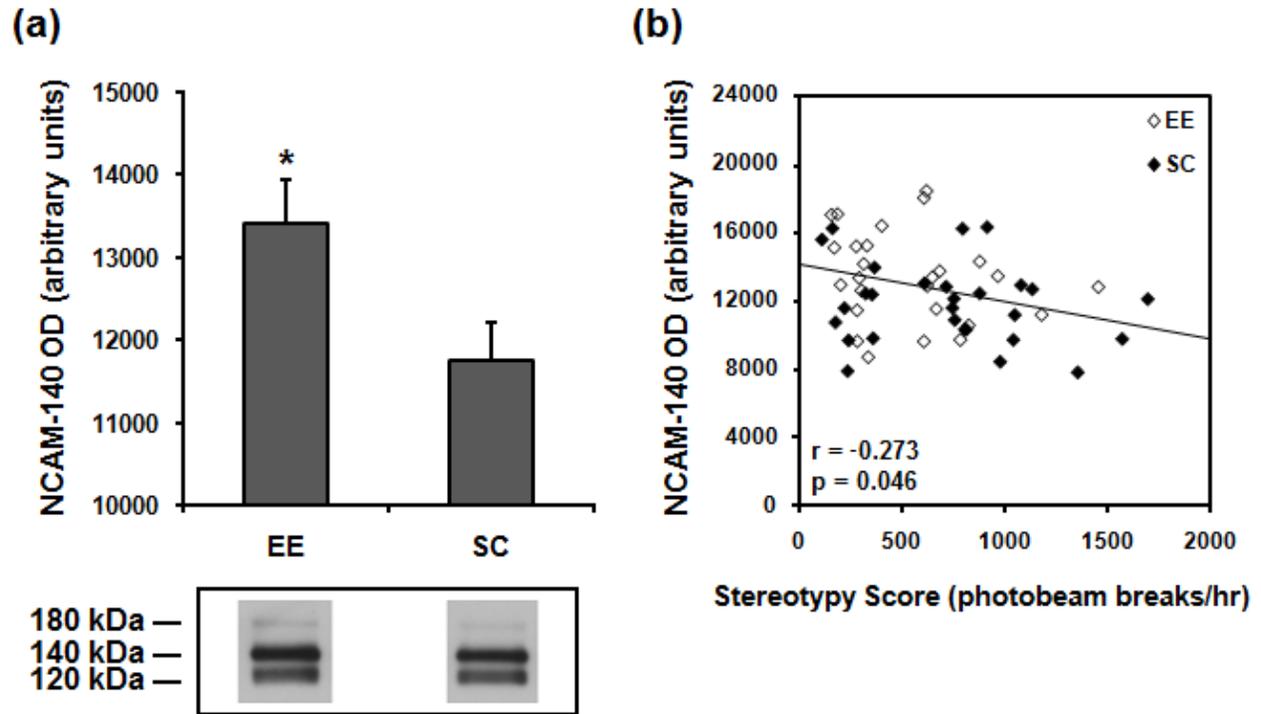


Figure 6-3. Effects of (a) environmental enrichment and (b) motor stereotypy on NCAM-140 protein expression in the cerebral cortex. Representative blots of NCAM (expressed as three isoforms with apparent molecular weights of 120, 140, and 180 kDa) are shown in the lower panel. * indicates $p < 0.05$. Open symbols (\diamond) indicate environmentally enriched (EE) animals. Closed symbols (\blacklozenge) indicate standard caged (SC) animals.

CHAPTER 7
EFFECTS OF ENVIRONMENTAL ENRICHMENT ON SPATIAL AND STIMULUS-
RESPONSE LEARNING DEFICITS ASSOCIATED WITH ABNORMAL STEREOTYPED
MOTOR BEHAVIOR

Introduction

The multiple systems theory of memory organization is based on the hypothesis that different kinds of information are processed and stored in different parts of the brain. According to this hypothesis, the dorsal striatum mediates a form of learning and memory in which stimulus-response associations are incrementally acquired (Packard and Knowlton, 2002), while the hippocampus processes information about the relationships among stimuli or events. Initial evidence for this theory was based on observations that damage to a neuroanatomical structure selectively impaired the acquisition of some learning tasks while sparing others. In these experiments, it was noted that lesions to the hippocampal formation selectively impaired the acquisition of tasks requiring the use of “cognitive” spatial learning, whereas lesions to the neostriatum impaired the acquisition of tasks requiring the use of stimulus-response “habit” or procedural learning (Packard et al., 1989; Packard and McGaugh, 1992; McDonald and White, 1994). Since then, additional studies demonstrating the task-dependent effects of intracerebral drug injections have provided further support that learning and memory abilities can be dissociated into independent neural systems (Packard and White, 1991; Packard et al., 1994; Packard and McGaugh, 1996; Packard and Teather, 1997a, b; Packard, 1999; Packard et al., 2001; Schroeder et al., 2002). More recently, at the molecular and cellular levels of analysis, the localization of learning-induced changes in gene transcription (Colombo et al., 2003; Teather et al., 2005; Gill et al., 2007) and neurochemistry (Chang and Gold, 2003; Pych et al., 2005) to specific brain regions continues to suggest that specialized regions of the brain subserve different mnemonic functions.

Dissociation methodology has also been used to demonstrate the existence of multiple memory systems in humans. Patients with damage to the medial temporal lobe (e.g., amnesiacs and Alzheimer's patients) show impairments on neuropsychological tasks which require an intact declarative memory system but perform normally on procedural learning tasks (Knowlton et al., 1994; Eldridge et al., 2002). In contrast, patients with diseases affecting the basal ganglia (e.g., Parkinson's disease, Huntington's disease, Tourette's syndrome) are impaired on tasks which require the ability to probabilistically associate cues with specific task outcomes but are able to answer explicit, factual questions about the task (Knowlton et al., 1996a; Knowlton et al., 1996b; Marsh et al., 2004; Marsh et al., 2005). In the present study, we utilize an analogous experimental approach to further investigate the behavioral functions of the striatum.

Our lab has characterized an animal model of striatal-based neuropathology. We have observed frequent and intense motor stereotypies dominating the behavioral repertoire of prairie deer mice (*Peromyscus maniculatus bairdii*). Neurobiological studies conducted using this model have implicated striatal dysfunction in the pathophysiology of these behaviors (Presti et al., 2002; Presti et al., 2003; Presti et al., 2004a; Presti et al., 2004b; Presti and Lewis, 2005), indicating that stereotypy may be a behavioral sign of striatal pathology. The stereotypies expressed by these mice are notable in that they are extremely context-dependent: phenotypically distinct groups of mice, i.e., low stereotypy and high stereotypy, can be generated by differentially housing deer mice in either environmentally enriched or environmentally impoverished conditions, respectively (Powell et al., 1999; Powell et al., 2000). Previous findings from our lab have demonstrated that enrichment-related attenuation of stereotypy in these animals is associated with increases in measures of synaptic plasticity localized to the striatum (Turner et al., 2002; Turner and Lewis, 2003; Turner et al., 2003), thereby suggesting

that the beneficial effects of environmental enrichment on stereotypy are linked to improvements in striatal function.

This experiment was designed to accomplish three goals. First, because stereotypy is associated with striatal dysfunction and striatal dysfunction also interferes with stimulus-response learning and memory abilities, we wanted to determine whether stereotypy would be selectively associated with impairments in stimulus-response learning. To this end, we tested deer mice in a striatum-dependent cued Morris water maze task. As a control, we also evaluated performance in a hippocampus-dependent spatial Morris water maze task. Our second goal was to determine whether the beneficial effects of environmental enrichment on stereotypy, and therefore presumably striatal functioning, would also extend to behavioral performance in the water maze tasks. Thus, the mice were reared in either standard cage or environmentally enriched housing conditions. Finally, our last goal was to determine whether differences in task performance would be associated with regionally specific changes in neuroplasticity. Therefore, we analyzed levels of the synaptic proteins, synaptophysin and neural cell adhesion molecule (NCAM), following water maze training. We selected these proteins as markers of synaptic plasticity based on previous research demonstrating experience-dependent changes in their levels of expression following both enrichment (Frick and Fernandez, 2003; Koo et al., 2003; Gresack and Frick, 2004; Nithianantharajah et al., 2004; Lambert et al., 2005; Bennett et al., 2006; Nithianantharajah et al., 2007) and learning (Sandi et al., 2005; Venero et al., 2006; Derksen et al., 2007).

Materials and Methods

Animals

Deer mice (*Peromyscus maniculatus bairdii*) were obtained from a breeding colony maintained at the University of Florida. The mice were housed in a room kept at 25°C,

maintained on a 16:8 light:dark cycle (lights off at 2200), and provided with access to food and water *ad libitum*. At weaning (3 weeks of age), mice were randomly assigned to either standard cage (SC, n = 104) or environmentally enriched (EE, n = 112) housing conditions. Standard caged mice were housed in standard laboratory cages (29 × 33 × 15 cm) with two other same-sex weanlings, while environmentally enriched animals were housed in groups of five or six in modified large dog kennels (122 × 81 × 89 cm). These enrichment kennels were fitted with galvanized wire mesh to create multiple interconnected levels and equipped with bedding, a running wheel, and assorted ceramic and plastic objects varying in color, shape, and size. Throughout the 10 wk differential housing period, enrichment items were removed and replaced with novel objects on a weekly basis. To encourage foraging behaviors, approximately 50 grams of birdseed were also randomly distributed throughout the kennels three times a week. All animal procedures were approved by the University of Florida Institutional Animal Care and Use Committee and were performed in accordance with the NIH *Guide for the Care and Use of Laboratory Animals*.

Quantification of Stereotypic Behavior

Animals were tested for stereotypy after 8 weeks in their respective housing conditions (see Figure 7-1 for a detailed timeline of experimental events). Mice were removed from their home cages and placed singly into Plexiglas testing cages (22 × 15 × 28 cm) two hours prior to the beginning of their dark cycle. Food, water, and bedding were provided, and the mice were left undisturbed for 2 hours to allow for habituation to the new environment and recovery from the stress of handling. Stereotypic behaviors were quantified using an automated testing apparatus (Columbus Instruments, Columbus, OH) designed to register stereotypic motor activity (e.g., repetitive jumping or backwards somersaulting) as photobeam breaks. All testing sessions

were also recorded using a digital video surveillance system (GeoVision Inc., Taipei, Taiwan) and checked in order to verify the accuracy of automated counts. Data were collected for two dark cycles, and the stereotypy score for each animal was calculated as the average number of photobeam interruptions per hour of testing. Mice with stereotypy scores above 600 photobeam breaks/hr were designated as High Stereotypy (HS) animals, whereas mice with stereotypy scores below 400 photobeam breaks/hr were designated as Low Stereotypy (LS) animals. These values correspond to approximately the top and bottom third of stereotypy scores for all animals tested. Animals with intermediate stereotypy scores between 400 and 600 photobeam breaks/hr were not used for Morris water maze testing or Western analysis of synaptic protein concentrations.

Morris Water Maze (MWM) Testing

The water maze was a black circular pool (94 cm in diameter and 37 cm high). Four starting positions (N, S, E, and W) were equally spaced around the perimeter of the pool, dividing it into four quadrants. The pool was filled with water maintained at 25°C and made opaque with the addition of non-toxic white tempera paint. An escape platform (a 10 cm diameter Plexiglas disk covered with sheer nylon hose), which the mice can mount to escape the water, was submerged 2 cm below the surface of the water. Behavioral testing occurred during the light cycle between 0900 and 1100. For both the spatial and cued tasks, training consisted of 8 trials per day over the course of 7 consecutive days. In each trial, the animal was released, facing the wall of the maze, from one of the four starting positions and allowed to escape onto the hidden or cued platform. The position of the tester remained constant after placement of the animal into the maze, and each starting position was used twice during each 8 trial training session. After mounting the platform, the animal was allowed to remain on it for 10 sec before being placed into a holding cage for an intertrial interval of approximately 10 min. Three 60 sec

probe trials were conducted: the first probe trial was conducted midway through training, the second probe trial was conducted on the last day of training, and the final probe trial was conducted 3 days post-training.

Spatial MWM Task

In the spatial version of the water maze task, the escape platform was always located in the center of the NW quadrant. The animals were required to navigate to the submerged platform using the extra-maze spatial cues present in the room which included a computer, a video tracking system, a sink, and cabinets. The escape platform was removed during the probe trials. Animals were released from three different starting positions for each of the three probe trials.

Cued MWM Task

In the cued version of the water maze task, a white foam cross (7×7 cm) was always suspended 10 cm above the submerged platform during training. The location of the escape platform was varied from trial to trial in order to prevent the use of a spatial strategy. Each of the four quadrants contained the platform on two of the 8 trials, and the platform was placed so that the distance (i.e., proximal or distal) and direction (i.e., left or right) of the platform relative to the starting point was counterbalanced across each daily block of 8 trials. During the probe trials, the escape platform was removed from the maze; however, the cue remained suspended in the center of a maze quadrant located distal to the starting position. The cue was placed in three different quadrants for each of the three probe trials.

Western Blotting

Levels of synaptophysin and neural cell adhesion molecule (NCAM) in the dorsolateral striatum, dorsomedial striatum, hippocampus, and neocortex were determined using Western blotting. Tissue samples from control animals were obtained from a previous study. Animals trained in MWM tasks were sacrificed by cervical dislocation following the final probe trial. The

brains were rapidly removed, snap-frozen in isopentane, and stored at -80°C until processed for protein extraction. Briefly, 300 μm thick coronal sections were collected on a cryostat held at -20°C . Tissue samples from the regions of interest were microdissected by punch method and sonicated in a homogenization buffer containing 20 mM Tris, 150 mM NaCl, 5 mM EDTA, 5 mM EGTA, 1% Triton X-100, 10 μM DTT, and a protease inhibitor tablet (Roche). Samples were then placed on ice and vortexed every 15 min for 1 hr before clarification via centrifugation at 16,000g for 15 min. Subsequently, the supernatant was collected, and the protein concentrations were determined using the DC Protein Assay kit (Bio-Rad Laboratories, Hercules, CA), following manufacturer's instructions. Equal amounts (20 μg) of total protein from each sample were loaded onto 12% polyacrylamide gels and separated by SDS-PAGE before transfer to polyvinylidene difluoride membranes. Loading of each gel was counterbalanced according to condition and group as much as possible. Gels and blots were also routinely stained with Coomassie blue and Ponceau S (Sigma), respectively, in order to verify equal sample loading. The membranes were then washed to remove excess stain and blocked with 5% nonfat milk in Tris-buffered saline and 0.05% Tween (TBST) for 1 hr before incubation with the primary antibody (1:50,000 synaptophysin or 1:100 anti-NCAM; both antibodies from Chemicon, Temecula, CA) overnight at 4°C . The next day, blots were washed in TBST for 15 min before incubation with the appropriate horseradish peroxidase-conjugated secondary antibody (1:5,000; Santa Cruz Biotechnology, Santa Cruz, CA) for 1 hr at room temperature. Blots were then developed using enhanced chemiluminescence and exposed to CL-XPosure film (Pierce Biotechnology, Rockford, IL) for visualization of protein bands. Finally, quantification of immunoreactive protein bands was done by densitometry using ImageJ software (version 1.37, NIH, Bethesda, MD).

Data Analysis

The Ethovision Pro (version 2.3, Noldus Information Technology, the Netherlands) video tracking system was used to acquire and calculate maze performance parameters (e.g., escape latencies, path length to platform, swim velocities, thigmotaxis, quadrant time, platform crossings, etc.). Wintrack (version 2.4) (Wolfer et al., 2001) was used to generate plots of group search preferences during the final probe trial (Figures 7-3 and 7-5). For this analysis, the water maze was subdivided into 20×20 square tiles, and the time spent in each tile, relative to the amount of time expected according to chance, was averaged for each group with the darker tiles indicating greater time spent in an area of the water maze.

Statistical analyses were performed using SPSS software (Chicago, IL). All data are presented as mean \pm standard error of the mean (SEM). Morris water maze performance was analyzed using two-way repeated-measures analyses of variance (ANOVAs) with either Housing, Stereotypy, or Group as the independent variable and Training Day as the repeated measure. Separate one-way ANOVAs without repeated measures were used to compare Group performance within the same Training Day. One-way ANOVAs without repeated measures were also used to determine differences in probe trial and synaptic proteins data. Post hoc analysis of significant effects was accomplished using Newman-Keuls test. Correlations between MWM performance and synaptic protein levels were calculated according to Pearson's method. Any other tests used are as indicated in the text. The minimum level of significance was set at $p < 0.05$ for all tests.

Results

Subjects

The interaction of housing condition and stereotypy yielded four experimental groups of mice: environmentally enriched, low stereotypy (EE-LS); environmentally enriched, high

stereotypy (EE-HS); standard caged, low stereotypy (SC-LS); and standard caged, high stereotypy (SC-HS). Because previous studies have suggested that behavioral flexibility may be impaired in stereotypic animals (Garner and Mason, 2002; Garner et al., 2003b; Garner et al., 2003a), separate sets of the four groups were tested on spatial and cued versions of the MWM task. For analysis of synaptic marker proteins, a control condition, consisting of animals not exposed to water maze training, was also used. Stereotypy scores did not differ significantly between the same groups assigned to different conditions. One mouse was unable to swim and was removed from MWM testing after the first trial. Two mice also failed to find the escape platform in any of the 56 training trials and were eliminated from behavioral analysis as non-learners. The final sample sizes and stereotypy data for each condition (spatial MWM, cued MWM, and control) are shown in Table 7-1.

Morris Water Maze Testing

The mean escape latency, total distance moved, average swim velocity, thigmotaxis, and percent time spent in the platform quadrant were used as measures of water maze task acquisition. For the probe trials, several measures of performance (e.g., quadrant time, platform crossings, mean proximity to the platform, etc.) were calculated; however, the average number of platform crossings proved to be the most sensitive measure at detecting group differences for both tasks.

Spatial MWM Task

For the spatial MWM task, all groups learned to find the hidden platform as suggested by a significant ($p < 0.001$) main effect of Training Day for all parameters examined. However, significant differences in escape latency ($F(1,42) = 9.21, p = 0.004$), the total distance moved ($F(1,42) = 8.79, p = 0.005$), and NW (i.e., platform) quadrant time ($F(1,42) = 6.18, p = 0.017$) were found for the effect of Stereotypy with HS animals performing more poorly than LS

animals. There were no significant Stereotypy \times Training Day interactions. A main effect of Housing was found for thigmotaxis ($F(1,42) = 6.12, p = 0.017$) with SC mice showing significantly greater thigmotaxis than EE animals. Significant Housing \times Training Day interactions were also found for the total distance moved ($F(6,252) = 2.28, p = 0.037$) and time spent in the NW quadrant ($F(6,252) = 3.19, p = 0.005$). Group comparisons (Figure 7-2) revealed significant differences in escape latency ($F(3,40) = 5.60, p = 0.003$), distance ($F(3,40) = 3.90, p = 0.016$), and thigmotaxis ($F(3,40) = 3.37, p = 0.028$) between groups with the SC-HS group performing more poorly than the EE-LS, EE-HS, and SC-LS groups which did not differ significantly from each other. One-way ANOVAs, comparing group performance within individual training days, revealed that significant differences between the SC-HS animals and the other groups occurred primarily during the last few days of training. No significant Group \times Training Day interactions were found. No differences in swim velocities were found as a function of Housing ($F(1,42) = 0.766, p = 0.386$), Stereotypy ($F(1,42) = 1.69, p = 0.201$), or Group ($F(3,40) = 1.43, p = 0.249$).

No group differences ($F(3,40) = 0.085, p = 0.968$) in the number of platform crossings were observed during the first spatial probe trial which was conducted midway through training (Figure 7-3). However, by the last day of training (Probe Trial 2), all groups, except for the SC-HS group, demonstrated significant improvements in performance. EE-LS, EE-HS, and SC-LS mice performed significantly better ($F(3,40) = 8.83, p < 0.001$) than SC-HS mice during the final probe trial (Probe Trial 3) which was conducted 3 days post-training. There was also a significant main effect of Housing (Student's *t*-test: $t(42) = 4.39, p < 0.001$) on performance during the third probe trial with EE animals showing significantly more platform crossings than SC animals. The performance of the EE-LS and EE-HS groups did not differ between the second

and third probe trials, whereas SC-LS mice showed significantly fewer platform crossings ($p < 0.05$) during the final probe trial when compared to the second probe trial.

Cued MWM Task

For the cued MWM task, all groups learned to find the cued platform as suggested by a significant ($p < 0.001$) main effect of Training Day for all parameters examined. There was a highly significant main effect of Stereotypy on escape latency ($F(1,43) = 42.6, p < 0.001$), distance moved ($F(1,43) = 27.4, p < 0.001$), thigmotaxis ($F(1,43) = 26.8, p < 0.001$), and cued quadrant time ($F(1,43) = 28.4, p < 0.001$) with HS animals performing more poorly than LS animals on all measures. Group comparisons (Figure 7-4) further confirmed this pattern of results: the EE-HS and SC-HS groups performed more poorly than the EE-LS and SC-LS groups on the measures of escape latency ($F(3,41) = 17.2, p < 0.001$), total distance moved ($F(3,41) = 10.5, p < 0.001$), thigmotaxis ($F(3,41) = 10.1, p < 0.001$), and cued quadrant time ($F(3,41) = 12.7, p < 0.001$). Significant Stereotypy \times Training Day ($F(6,258) = 4.56$ to $10.7, p < 0.001$) and Group \times Training Day ($F(18,246) = 2.11$ to $4.66, p < 0.01$) interactions were also found for all parameters examined except velocity. Although the EE groups appeared to perform consistently better than their SC counterparts, this trend failed to reach significance, and no significant main effects of Housing were found for any of the measures examined. There were also no significant Housing \times Training Day interactions. As with the spatial task, there were no systematic differences in swim velocities due to Housing ($F(1,43) = 0.001, p = 0.982$), Stereotypy ($F(1,43) = 1.10, p = 0.300$), or Group ($F(3,41) = 0.638, p = 0.595$).

Analysis of cued probe trial performance (Figure 7-5) revealed that the EE-LS group performed significantly better ($F(3,41) = 9.13, p < 0.001$) than all other groups in the first probe trial and significantly better ($F(3,41) = 6.49, p = 0.001$) than the EE-HS and SC-HS groups in the final probe trial. All groups showed similar levels of performance on the last day of training

(Probe Trial 2). When compared across probe trials, the EE-LS group also showed significantly more platform crossings ($F(2,33) = 4.71$, $p = 0.016$) in the first probe trial than in the second probe trial.

Synaptic Proteins

Synaptophysin and neural cell adhesion molecule (NCAM) levels were evaluated in the dorsolateral striatum, dorsomedial striatum, hippocampus, and neocortex using Western blotting analysis. The dorsal striatum and hippocampus were chosen based on their roles in stimulus-response and spatial learning, respectively. The medial and lateral subregions of the dorsal striatum were analyzed separately based on evidence suggesting functional dissociation with regard to learning and memory (Yin and Knowlton, 2006), and the neocortex was selected based on previous studies suggesting its importance in mediating enrichment-related effects on synaptic plasticity and learning and memory (Frick and Fernandez, 2003). Synaptophysin migrated as a single 38 kDa band, while NCAM was expressed as 3 isoforms, with apparent molecular weights of 120, 140, and 180 kDa, in all regions examined (Figure 7-6).

Learning-induced changes in synaptic protein expression

To determine learning-induced changes in synaptic plasticity, the synaptic protein concentrations for mice trained in the MWM were first normalized relative to the corresponding group of control animals not exposed to water maze training. One-way ANOVAs were then used to make comparisons between different Groups (EE-LS, EE-HS, SC-LS, SC-HS) within the same Condition (spatial MWM, cued MWM, control) and between different Conditions within the same Group (Table 7-2). The results show that the EE-LS group trained in the cued MWM had significantly higher levels of synaptophysin in the dorsolateral striatum than all other groups trained in the cued MWM ($F(3,41) = 9.35$, $p < 0.001$), EE-LS mice trained in the spatial MWM ($F(2,34) = 5.52$, $p = 0.008$), and control EE-LS mice ($F(2,34) = 5.52$, $p = 0.008$). Levels of

NCAM-180 were higher in the hippocampus of EE-HS mice trained in the spatial MWM than in EE-HS cued MWM or EE-HS control mice ($F(2,32) = 6.07, p = 0.006$). SC-LS mice trained in the spatial MWM also demonstrated higher hippocampal concentrations of NCAM-180 ($F(2,28) = 3.53, p = 0.043$) when compared to SC-LS cued MWM and SC-LS control mice. EE-LS mice trained in the spatial MWM showed significantly greater levels of NCAM-180 in the neocortex ($F(2,34) = 6.32, p = 0.005$) than either EE-LS mice trained in the cued MWM or control EE-LS mice. Similarly, EE-HS mice trained in the spatial MWM also showed significantly higher ($F(2,32) = 4.74, p = 0.016$) levels of NCAM-180 in the neocortex than EE-HS control animals.

Relationships between MWM performance and synaptic protein levels

Correlational analyses were performed to determine the relationship between overall synaptic protein levels and water maze performance. There were no significant correlations between synaptic protein levels and spatial MWM performance. In contrast, for the cued MWM, synaptophysin levels in the dorsolateral striatum were positively correlated with improved performance during both training and probe trials as indicated by a negative correlation ($r(43) = -0.553, p < 0.001$) between synaptophysin levels and the mean escape latency averaged over all 7 training days and a positive correlation ($r(43) = 0.581, p < 0.001$) between synaptophysin levels and the number of platform crossings during the final probe trial (Figure 7-7). The converse was true for NCAM-120 levels in the hippocampus which were inversely correlated with cued MWM performance: hippocampal NCAM-120 levels were positively correlated ($r(43) = 0.467, p = 0.001$) with the average escape latency during training and negatively correlated ($r(43) = -0.495, p = 0.001$) with the number of platform crossings during the final probe trial (Figure 7-8).

Discussion

Our data demonstrate that stereotypy is associated with deficits in both spatial and stimulus-response learning and memory. This was indicated by the performance of the high

stereotypy groups relative to the low stereotypy groups in the spatial and cued versions of the Morris water maze task, which required spatial and stimulus-response learning, respectively. Standard caged, high stereotypy mice performed more poorly than all other groups in the spatial task, and both groups of high stereotypy mice performed more poorly than the low stereotypy mice in the cued task. Although these findings appear to be contrary to our initial hypothesis that stereotypy would be selectively associated with deficits in only stimulus-response learning, and not spatial learning, these results are not incongruent with a specific role for striatal dysfunction in the pathophysiology of stereotypy.

Functional heterogeneity exists within the striatum. With respect to the role of the dorsal striatum in learning and memory, one theory suggests that the medial and lateral subregions support different mnemonic functions. According to this theory, the dorsolateral striatum selectively mediates stimulus-response learning, whereas the dorsomedial striatum may also be involved in spatial learning. Previous studies have demonstrated that damage to the medial, but not lateral, portions of the dorsal striatum disrupts spatial learning in hidden platform Morris water maze tasks (Whishaw et al., 1987; Devan et al., 1999) and results in a preference for response strategies in tasks designed to simultaneously evaluate place- versus cue-guided behaviors (Devan et al., 1999; Devan and White, 1999; Yin and Knowlton, 2004). Given experimental evidence supporting a further dissociation of learning functions in the dorsal striatum, one interpretation of the present data is that the striatal pathology associated with stereotypy in our model encompasses both the dorsolateral and dorsomedial regions of the striatum with dysfunction of the dorsolateral striatum contributing to deficits in stimulus-response learning and dysfunction of the dorsomedial striatum contributing to deficits in spatial learning. This interpretation is consistent with previous neurobiological studies from our lab. We

have demonstrated differences in synaptic plasticity between high and low stereotypy mice localized to both the dorsolateral and dorsomedial regions of the striatum (Turner et al., 2002). In addition, our research has indicated that these differences are regionally specific for areas involved in motor control as no differences in synaptic structure or function were found in limbic areas, such as the hippocampus (Turner and Lewis, 2003; Turner et al., 2003).

Another explanation for these results may pertain to possible interactions between the striatum and hippocampus during learning. Although once thought to be relatively independent, it is increasingly appreciated that complex interactions exist between memory systems. These interactions may be either cooperative or competitive. Thus, if the striatum and hippocampus normally cooperate during spatial learning, striatal pathology may also negatively impact spatial learning ability, thereby explaining the spatial learning deficit associated with stereotypy. Indeed, it has been shown that lesions to the striatum can interfere with spatial MWM task performance in some cases (Block et al., 1993; Devan et al., 1999; Mura and Feldon, 2003). It should also be noted that this interpretation is not mutually exclusive with the aforementioned role for the dorsomedial striatum in spatial learning. In fact, the presence of direct anatomical connections between the posterior dorsomedial striatum and entorhinal cortex (Totterdell and Meredith, 1997) suggests that the dorsomedial striatum and hippocampus may influence each other to produce behavioral output, providing support for both interpretations.

The spatial and stimulus-response learning deficits associated with stereotypy did not appear to result from motor, sensory, or motivational components. There were no systematic differences in swim velocities between groups in either of the water maze tasks to indicate motor impairment or differences in swimming ability. For both tasks, all groups also showed increased time spent in the platform quadrant with training. Increases in this measure both require the

ability to perceive extra-maze and intra-maze cues and indicate motivation to escape the water maze. Furthermore, we have also conducted other water maze studies using a fixed, visible escape platform. In these studies, standard caged, high stereotypy mice (i.e., the group that performed most poorly in both tasks) did not perform significantly differently from other groups (unpublished observations).

Environmental enrichment alone was sufficient to counteract the observed deficits in spatial learning: environmentally enriched, high stereotypy mice performed significantly better than standard caged, high stereotypy mice in the spatial water maze task at levels similar to the low stereotypy groups. Moreover, enrichment also promoted the retention of spatial memory 3 days post-training; i.e., the number of platform crossings shown by the enriched, low stereotypy and enriched, high stereotypy groups did not decrease between the probe trials conducted during the last day of training and 3 days later. However, enrichment was unable to compensate for deficits in stimulus-response learning since both groups of high stereotypy mice, enriched and standard caged, performed more poorly than the low stereotypy groups in the cued task. Thus, there was a dissociation in the effect of environmental enrichment on performance in the two water maze tasks, suggesting that the central structures which mediate spatial and stimulus-response learning (i.e., the hippocampal formation and dorsal striatum, respectively) may have been differentially sensitive to the beneficial effects of environmental enrichment.

Previous studies have demonstrated that the effects of environmental enrichment on learning and memory are highly dependent on the form of enrichment provided. For example, the effects of social and inanimate stimulation on spatial and reversal learning are dissociable, suggesting that they may act on different neural substrates (Schrijver et al., 2004). Certain individual elements of enrichment, such as voluntary exercise, have also been shown to

contribute more greatly to the memory-enhancing effect of enrichment than other parameters (Lambert et al., 2005; Harburger et al., 2007b). Similarly, variations in the duration of enrichment also impact performance on learning tasks (Bennett et al., 2006). Consistent with the results of this experiment, other studies have also demonstrated that enrichment improves performance specifically in spatial but not cued water maze tasks (Frick and Fernandez, 2003; Frick et al., 2003). Our enrichment paradigm incorporated many of the experimental parameters typically used in enrichment studies, including increased spatial density, increased opportunities for social interactions, increased inanimate stimulation via the provision of “toys”, and access to running wheels for exercise. Therefore, it is difficult to determine which of these parameters may have been responsible for the improvements seen in spatial task performance. However, environmental novelty was also a key aspect of our enrichment protocol: enriched mice were regularly exposed to different enrichment items arranged in different configurations. We speculate that this might have provided them with additional cognitive stimulation with respect to the formation of spatial maps, thereby enhancing their spatial learning abilities.

The patterns of learning-induced changes in synaptic protein expression were different following training in the two MWM tasks. However, the changes were generally localized to regions thought to mediate a specific learning task with the groups showing superior levels of task performance generally showing increased expression of the synaptic proteins. This suggests that lasting modifications in synaptic function occurred in the animals which performed well. For example, environmentally enriched, low stereotypy mice far outperformed the other groups in the cued water maze task. This group also had the highest levels of synaptophysin in the dorsolateral striatum when compared to the other groups trained in the cued task, environmentally enriched, low stereotypy mice trained in the spatial task, and environmentally

enriched, low stereotypy control mice. Generally considered to be a reliable marker of synaptic density (Walaas et al., 1988; Eastwood et al., 2006), learning-induced increases in levels of synaptophysin in the dorsolateral striatum of environmentally enriched, low stereotypy mice, therefore, suggest that improved performance in the cued MWM task was related to increased synaptogenesis within the dorsolateral striatum. Because synaptophysin is an integral membrane component of neurotransmitter-containing presynaptic vesicles (Wiedenmann and Franke, 1985), these results further suggest that an increase in the efficacy of synaptic transmission within the dorsolateral striatum may have contributed to better task performance. The overall positive correlation between cued MWM performance and synaptophysin levels in the dorsolateral striatum for all groups trained in the cued task also supports this conclusion.

In the spatial water maze task, all groups, except for the standard caged, high stereotypy group, demonstrated improvements in spatial memory with training. Similarly, all groups, except for the standard caged, high stereotypy group, showed significant increases in NCAM-180 levels in either the hippocampus or neocortex when compared to their corresponding control group. Improved performance in the spatial MWM task requires the formation of spatial memory for the hidden platform. Experimental data support a role for neural cell adhesion molecules in long-term memory storage (Welzl and Stork, 2003; Sandi, 2004). Several studies have demonstrated that interfering with NCAM function impairs performance in spatial learning tasks (Cremer et al., 1994; Arami et al., 1996; Cambon et al., 2003), whereas administration of synthetic peptides which mimic NCAM action improves spatial learning (Cambon et al., 2004; Klementiev et al., 2007). NCAM-180, in particular, has been shown to play a crucial role in synapse formation and stabilization, especially during the synaptic remodeling processes which accompany long-term potentiation (Schuster et al., 1998; Dityatev et al., 2000; Sandi et al., 2005). Therefore, these

results suggest that enhanced synaptic connectivity, as mediated through increased NCAM-180 levels, in the hippocampus and neocortex may have contributed to the improved performance of the enriched and standard caged, low stereotypy groups in the spatial MWM task.

Although superior task performance generally corresponded to increases in synaptic protein expression, one notable exception was the inverse relationship between cued water maze performance and hippocampal NCAM-120 levels. As mentioned previously, within the conceptual framework of the multiple memory systems theory, interactions between memory systems can be competitive as well as cooperative. This finding indicates that increased synaptic plasticity in one region (i.e., the hippocampus) was detrimental to the performance mediated by another system (i.e., the dorsolateral striatum) and may represent a neural substrate of competitive interference.

Altogether, these results demonstrate that the striatal dysfunction associated with stereotypy in deer mice is also linked to deficits in both spatial and stimulus-response learning. Environmental enrichment alone was sufficient to counteract deficits in spatial learning and even enhanced the retention of spatial memory post-training. However, enrichment was unable to compensate for deficits in stimulus-response learning. Finally, the regional patterns of learning-induced changes in synaptic protein expression were task-specific and related to levels of performance.

Table 7-1. Stereotypy scores of experimental groups.

Group	Spatial MWM		Cued MWM		Control	
	<i>n</i>	Stereotypy Score	<i>n</i>	Stereotypy Score	<i>n</i>	Stereotypy Score
EE-LS	12	209 ± 35	12	212 ± 31	13	264 ± 20
EE-HS	11	824 ± 47	11	783 ± 45	13	804 ± 72
SC-LS	10	166 ± 35	11	247 ± 36	10	250 ± 29
SC-HS	11	1087 ± 82	11	1034 ± 108	18	980 ± 71

Values represent mean stereotypy scores (photobeam breaks/hr) ± SEM.

Table 7-2. Immunoblotting analysis of synaptic marker proteins.

Synaptic Protein	Group	Dorsolateral Striatum		Dorsomedial Striatum		Hippocampus		Neocortex	
		Spatial MWM	Cued MWM	Spatial MWM	Cued MWM	Spatial MWM	Cued MWM	Spatial MWM	Cued MWM
Synaptophysin	EE-LS	98.3 ± 5.4	120.5 ± 4.5^a	101.8 ± 5.6	103.7 ± 5.3	97.5 ± 6.8	107.7 ± 6.1	99.3 ± 4.1	101.9 ± 4.6
	EE-HS	101.9 ± 3.6	95.7 ± 2.8	102.4 ± 5.8	101.7 ± 5.5	100.4 ± 6.7	98.2 ± 5.4	99.5 ± 4.0	101.3 ± 3.7
	SC-LS	103.4 ± 3.1	100.0 ± 5.0	99.2 ± 7.6	102.3 ± 4.8	97.9 ± 8.8	98.0 ± 7.5	101.0 ± 3.1	101.2 ± 3.8
	SC-HS	104.0 ± 5.4	93.2 ± 3.9	99.3 ± 5.4	102.1 ± 5.3	100.4 ± 11.2	97.9 ± 7.3	99.9 ± 3.7	97.7 ± 3.1
NCAM-120	EE-LS	98.9 ± 4.9	96.1 ± 7.6	103.4 ± 9.0	103.6 ± 6.0	101.5 ± 8.9	89.8 ± 8.1	101.2 ± 7.4	103.9 ± 6.9
	EE-HS	101.1 ± 6.0	105.7 ± 4.8	101.7 ± 8.7	103.6 ± 7.6	100.8 ± 6.9	112.8 ± 5.6	102.6 ± 5.5	104.2 ± 4.3
	SC-LS	98.2 ± 7.4	98.2 ± 7.6	103.7 ± 6.6	104.6 ± 7.6	103.8 ± 9.4	98.1 ± 7.6	104.0 ± 5.0	104.4 ± 4.4
	SC-HS	98.9 ± 7.2	102.2 ± 4.9	102.0 ± 8.5	103.6 ± 7.3	105.1 ± 12.2	107.3 ± 5.7	100.1 ± 4.3	97.9 ± 4.9
NCAM-140	EE-LS	101.5 ± 3.8	100.2 ± 6.1	103.7 ± 7.5	103.4 ± 6.5	112.4 ± 5.7	102.2 ± 5.5	100.8 ± 5.7	99.6 ± 4.9
	EE-HS	98.9 ± 4.3	100.1 ± 3.8	103.0 ± 7.4	103.3 ± 6.4	123.9 ± 8.4	111.3 ± 6.7	100.7 ± 6.6	100.4 ± 5.6
	SC-LS	96.8 ± 5.3	99.6 ± 3.7	103.0 ± 8.4	103.0 ± 8.7	116.3 ± 9.3	97.6 ± 4.8	101.0 ± 7.1	100.8 ± 6.9
	SC-HS	99.4 ± 6.7	98.5 ± 6.3	101.9 ± 7.1	102.7 ± 6.8	111.8 ± 11.2	104.2 ± 5.6	99.3 ± 6.1	100.2 ± 8.1
NCAM-180	EE-LS	103.0 ± 7.8	102.6 ± 6.2	114.0 ± 7.4	103.2 ± 6.6	119.6 ± 8.5	100.5 ± 11.4	138.1 ± 8.4^d	102.7 ± 7.7
	EE-HS	102.2 ± 6.3	101.9 ± 4.9	110.0 ± 7.9	102.1 ± 9.0	146.7 ± 8.4^b	107.9 ± 12.0	139.2 ± 9.9^e	106.1 ± 7.9
	SC-LS	102.3 ± 8.0	103.0 ± 3.9	110.0 ± 7.3	102.9 ± 9.5	142.3 ± 10.3^c	107.6 ± 11.0	122.3 ± 9.5	105.3 ± 7.9
	SC-HS	102.6 ± 9.2	100.8 ± 6.8	104.5 ± 6.1	104.0 ± 6.8	115.2 ± 10.8	103.7 ± 7.0	123.2 ± 9.5	97.9 ± 9.9

Densitometric values are normalized to the corresponding control group and are expressed as mean (% control) ± SEM. Significant values are shown in boldface.

Symbols

a, indicates p < 0.001 compared to EE-HS, SC-LS, and SC-HS animals trained in the cued MWM and p < 0.05 compared to EE-LS control and EE-LS spatial MWM animals.

b, indicates p < 0.01 compared to EE-HS control and p < 0.05 compared to EE-HS cued MWM animals.

c, indicates p < 0.05 compared to SC-LS control and SC-LS cued MWM animals.

d, indicates p < 0.01 compared to EE-LS control and EE-LS cued MWM animals.

e, indicates p < 0.05 compared to EE-HS control animals.

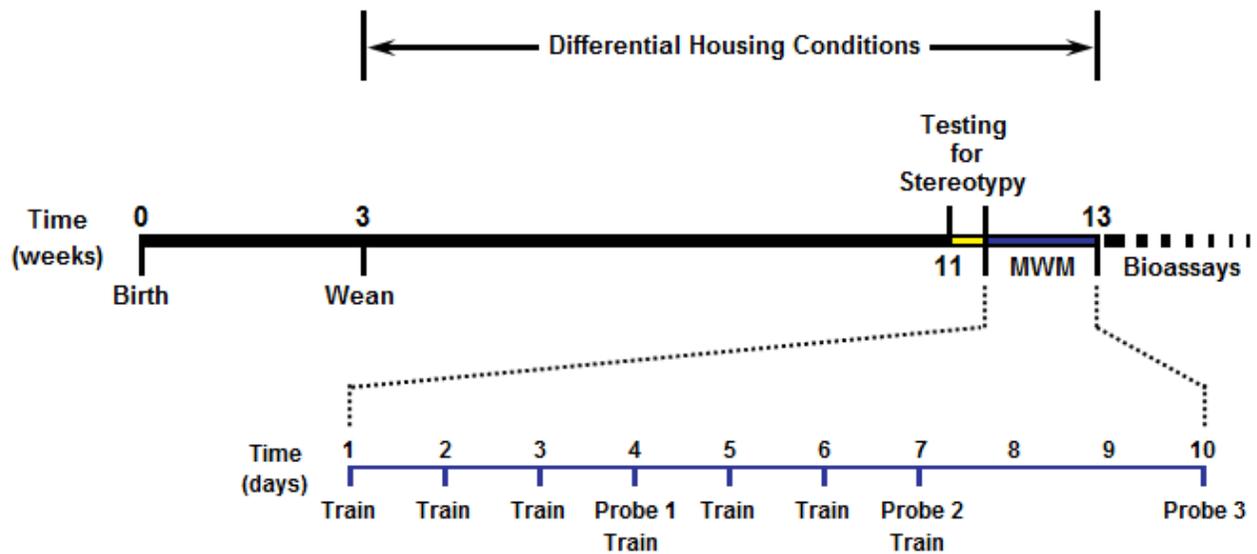


Figure 7-1. Illustration depicting the timeline of experimental events. Animals were housed under either enriched or standard cage conditions starting from the time of weaning (3 wks) to the conclusion of Morris water maze (MWM) testing (13 wks). At 11 wks, animals were evaluated for stereotypy prior to training in either spatial or cued versions of the MWM task. The training schedule was identical for both tasks. Animals were trained for 7 days with probe trials conducted prior to the 4th and 7th days of training and 3 days following the final day of training. Numbers indicate the time in weeks (top line) and days (bottom line).

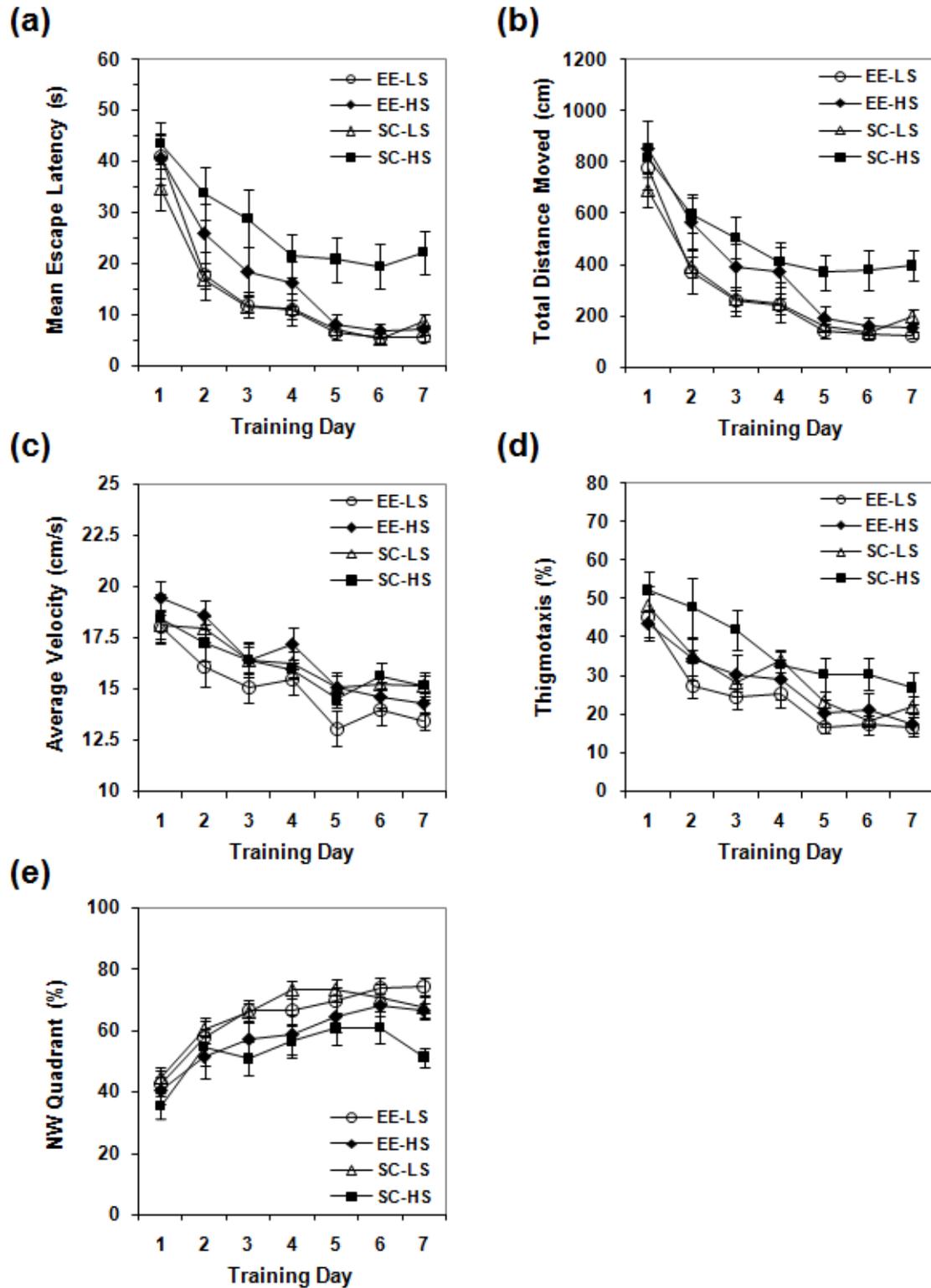


Figure 7-2. Performance during spatial MWM task acquisition as measured by (a) mean escape latency, (b) total distance moved, (c) average velocity, (d) thigmotaxis, and (e) percent time spent in the NW (platform) quadrant.

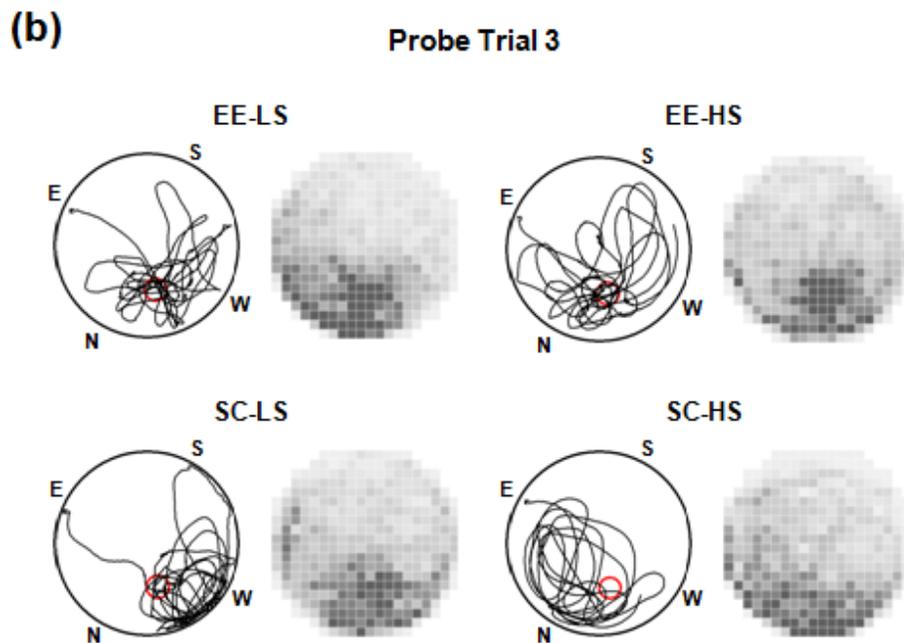
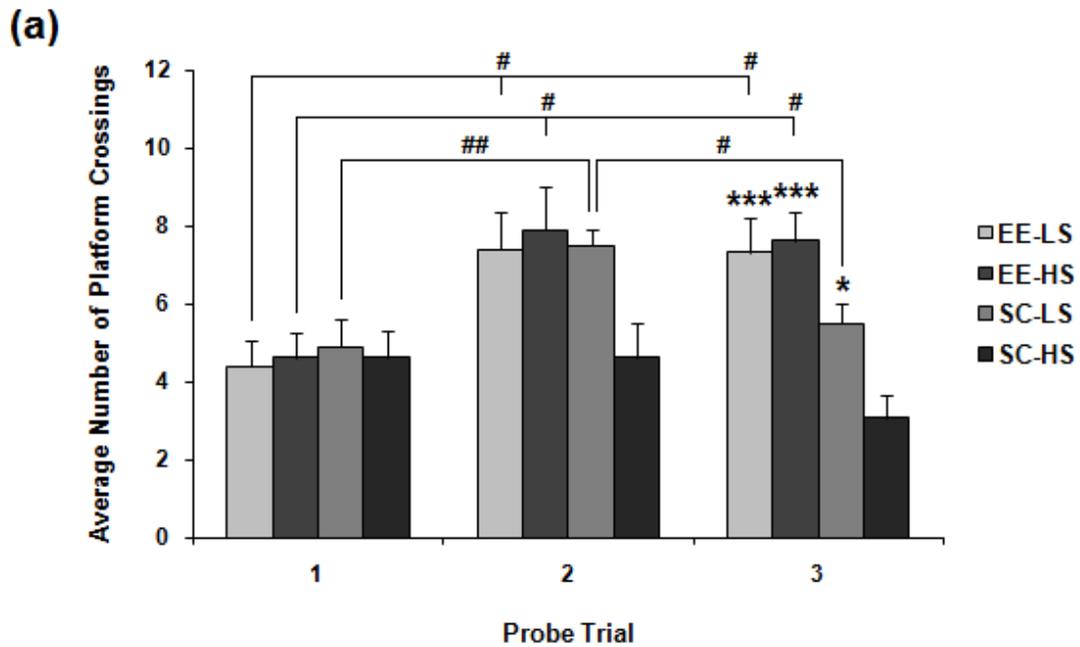


Figure 7-3. Performance during spatial MWM probe trials as measured by (a) the average number of platform crossings. Probe trials were conducted mid-way through training (Probe Trial 1), on the last day of training (Probe Trial 2), and 3 days post-training (Probe Trial 3). For the final probe trial, (b) a representative swim path (left) and the time each group spent searching in different areas of the water maze (right) are also shown. Darker tiles indicate more time spent within an area of the water maze. ## and # indicate significant differences of $p < 0.01$ and $p < 0.05$, respectively. *** and * indicate $p < 0.001$ and $p < 0.05$ vs SC-HS, respectively.

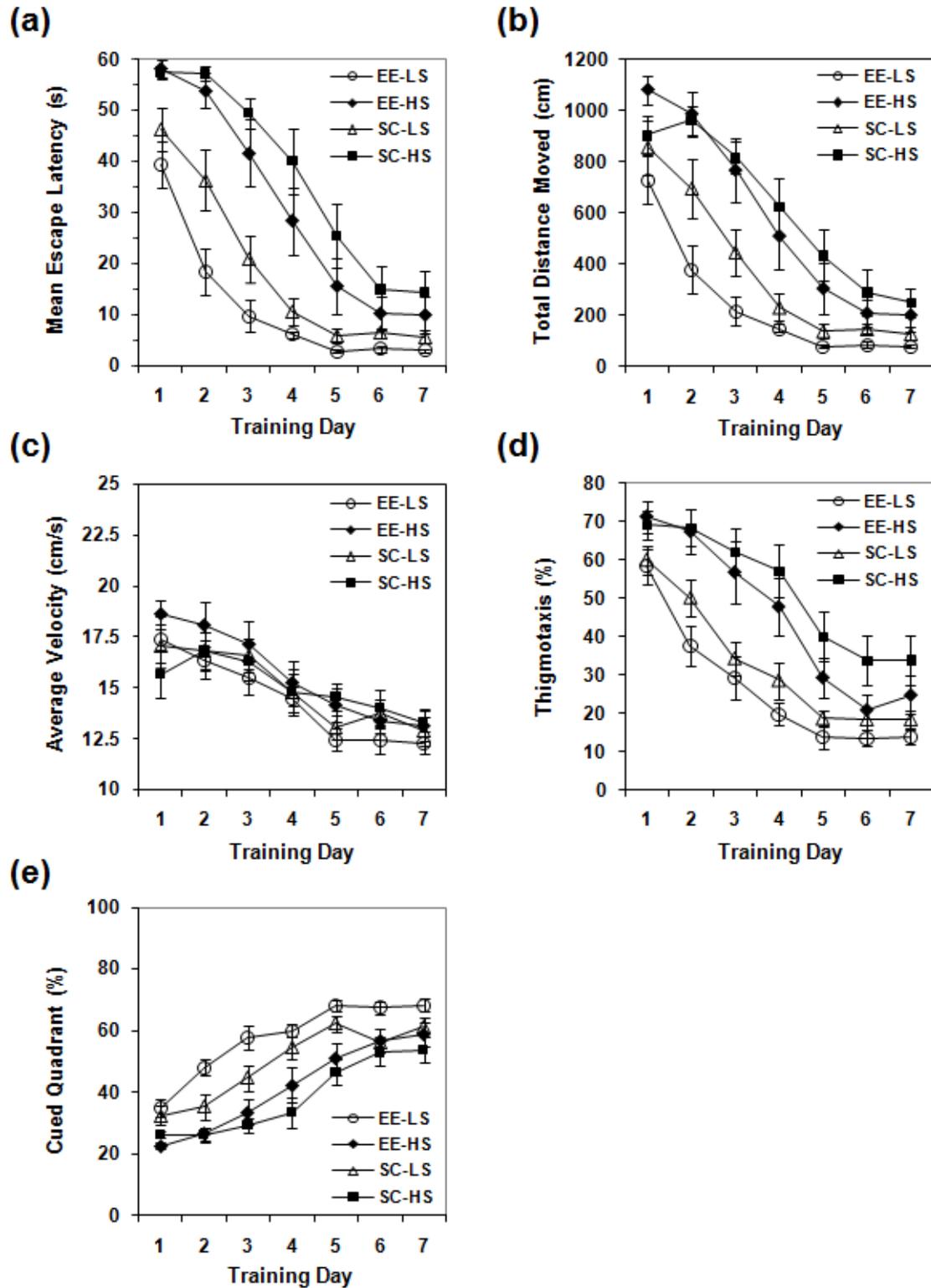


Figure 7-4. Performance during cued MWM task acquisition as measured by (a) mean escape latency, (b) total distance moved, (c) average velocity, (d) thigmotaxis, and (e) percent time spent in the cued (platform) quadrant.

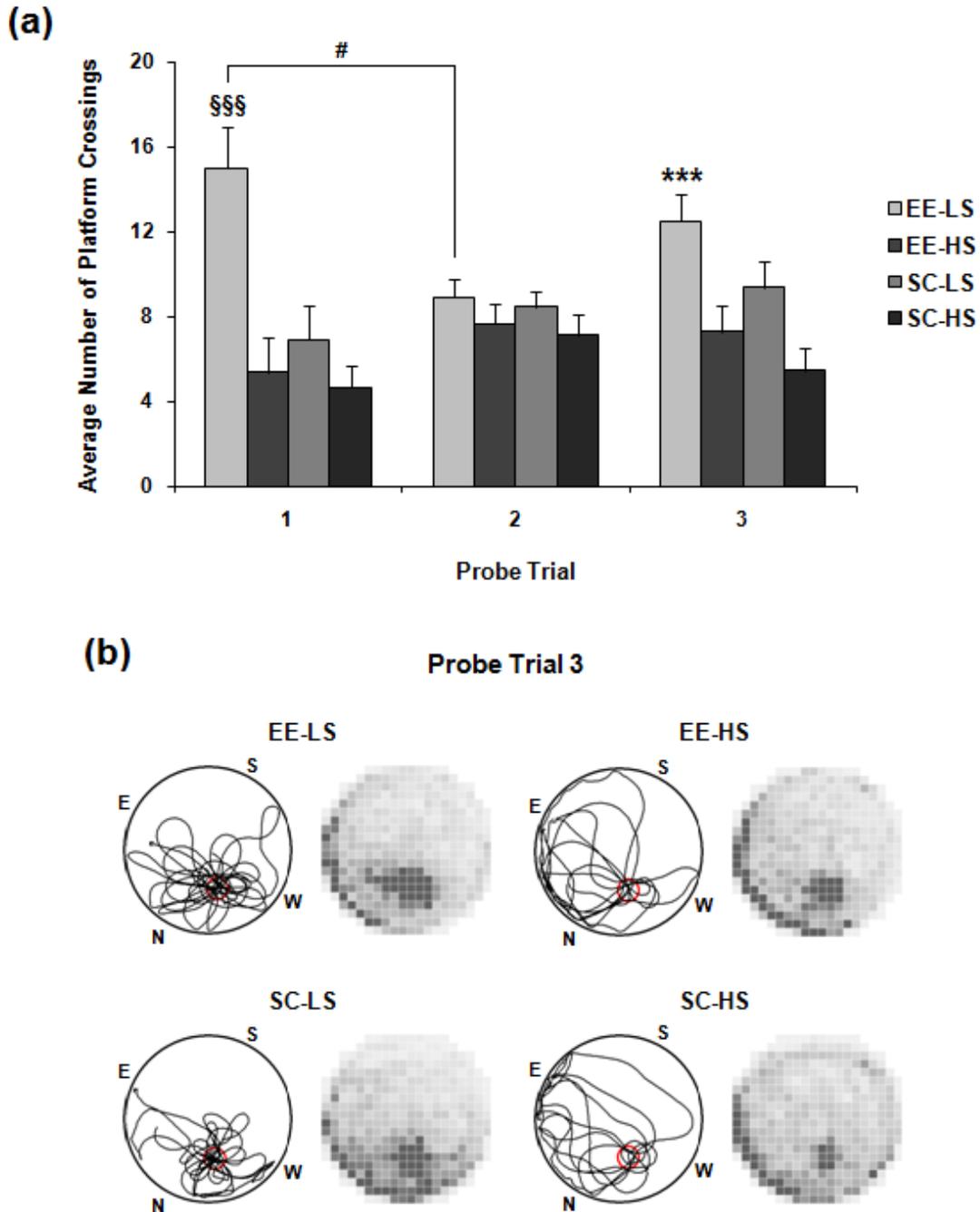


Figure 7-5. Performance during cued MWM probe trials measured by (a) the average number of platform crossings. Probe trials were conducted mid-way through training (Probe Trial 1), on the last day of training (Probe Trial 2), and 3 days post-training (Probe Trial 3). For the final probe trial, (b) a representative swim path (left) and the time each group spent searching in different areas of the water maze (right) are also shown. Darker tiles indicate more time spent within an area of the water maze. # indicates a significant difference of $p < 0.05$. \$\$\$ indicates $p < 0.001$ vs EE-HS, SC-LS, and SC-HS. *** indicates $p < 0.001$ vs SC-HS and $p < 0.05$ vs EE-HS.

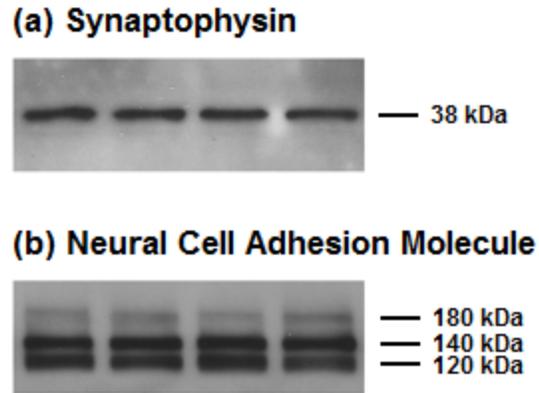


Figure 7-6. Representative immunoblots of the synaptic proteins (a) synaptophysin and (b) neural cell adhesion molecule (NCAM). Synaptophysin migrated as a single 38 kDa band. NCAM was expressed as 3 isoforms with molecular weights of 120, 140, and 180 kDa.

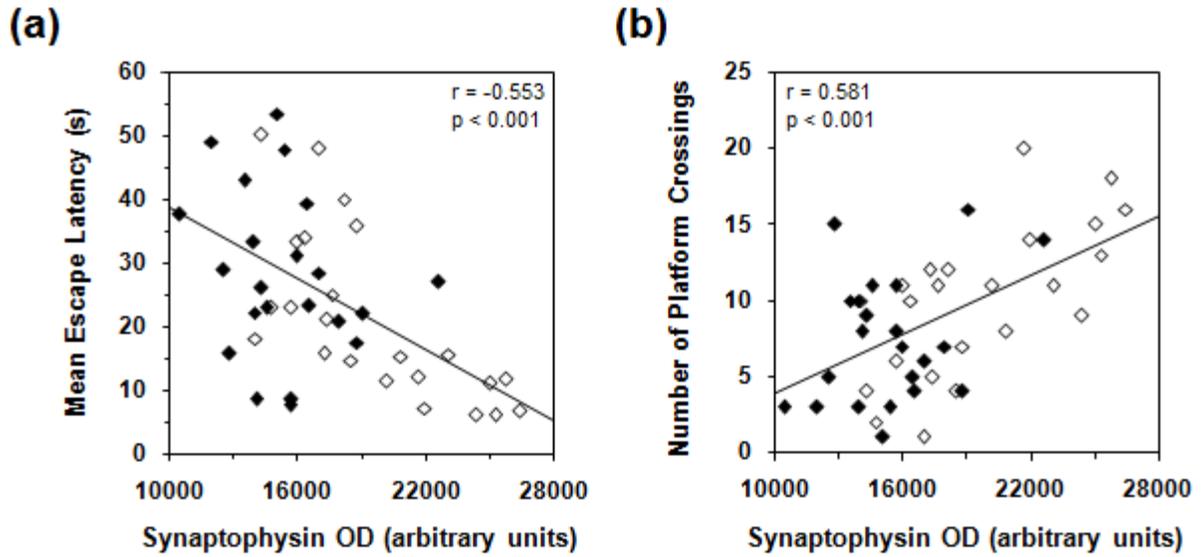


Figure 7-7. Relationship between synaptophysin levels in the dorsolateral striatum and cued MWM performance as measured by (a) the mean escape latency averaged over all training days and (b) the number of platform crossings during the final probe trial. Higher levels of synaptophysin in the dorsolateral striatum were associated with better performance in the cued MWM task. Open symbols (\diamond) indicate environmentally enriched animals. Closed symbols (\blacklozenge) indicate standard caged animals.

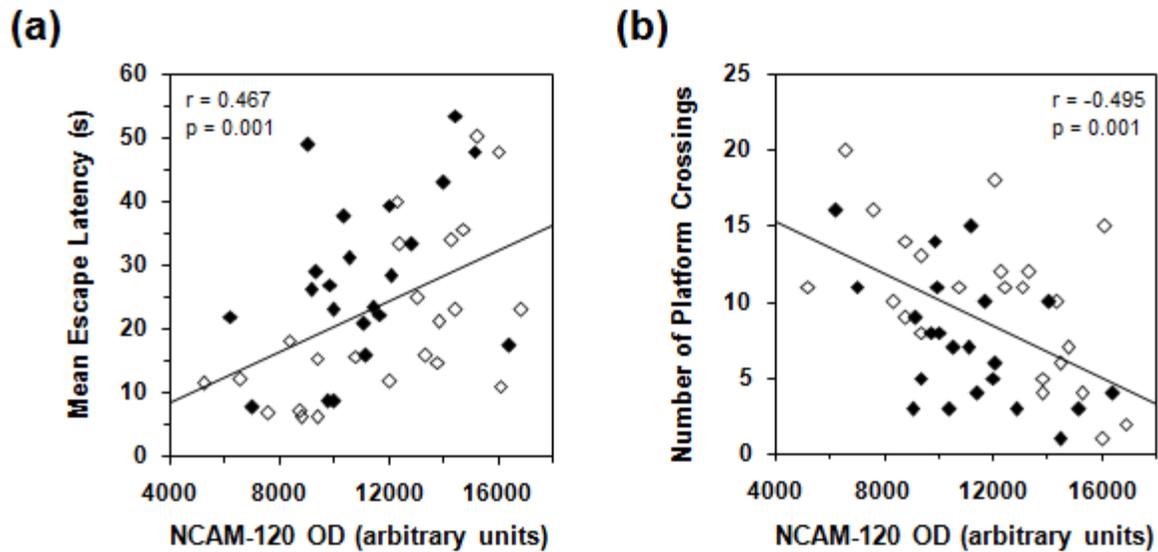


Figure 7-8. Relationship between NCAM-120 levels in the hippocampus and cued MWM performance as measured by (a) the mean escape latency averaged over all training days and (b) the number of platform crossings during the final probe trial. Higher levels of NCAM-120 in the hippocampus were associated with poorer performance in the cued MWM task. Open symbols (\diamond) indicate environmentally enriched animals. Closed symbols (\blacklozenge) indicate standard caged animals.

CHAPTER 8 CONCLUSIONS

Summary of Findings

Previous work in our lab has consistently demonstrated the ameliorative effects of environmental enrichment on stereotypy (Powell et al., 1999; Powell et al., 2000; Turner et al., 2002; Turner and Lewis, 2003; Turner et al., 2003; Hadley et al., 2006). The goal of this research was to identify the mechanisms which contribute to the impact of environmental manipulations on stereotypy.

As reviewed previously (Chapter 1), the involvement of the nigrostriatal dopamine system in the production of motor stereotypies is well established. Given the importance of dopamine function in the mediation of stereotypic behaviors, our initial characterization of this model included neurobiological studies targeted at detecting differences in dopamine receptor density or endogenous dopamine content. Although these early studies failed to find differences in the density of striatal D₁ or D₂ dopamine receptors or in the concentration of striatal dopamine and its metabolites as a function of either housing condition or stereotypy level (Powell et al., 1999), subsequent studies from our lab have since demonstrated that differences in the response to dopamine do exist between high and low stereotypy animals (Presti et al., 2003; Presti and Lewis, 2005). The results of our first study (Chapter 4) reconciled these findings by demonstrating that alterations in dopamine signaling represent one mechanism for the observed differences in dopamine sensitivity between high and low stereotypy animals. Our data demonstrate that stereotypy is inversely related to the proportion of DARPP-32 phosphorylated at Thr75 (phospho-Thr75-DARPP-32/total DARPP-32). Because phosphorylation of DARPP-32 at Thr75 converts it into an inhibitor of PKA, these results suggest that decreased stereotypy is linked to lower tonic activation of the cAMP/PKA signaling cascade in striatal neurons, whereas

increased stereotypy is associated with a release in PKA inhibition. We also found significantly lower levels of total DARPP-32 protein specifically in environmentally enriched, low stereotypy animals, therefore, suggesting that enrichment-related decreases in stereotypy may involve synergistic regulation of DARPP-32 at both the transcriptional and post-translational levels.

Our second study (Chapter 5) examined the pattern of long-term changes in neuronal activation, as indexed by Δ FosB induction, following chronic stereotypy and environmental enrichment. Initial immunoblotting studies indicated that levels of Δ FosB accumulate specifically in the striatum with prolonged stereotypic activity. Further immunohistochemical analysis of striatal subregions showed that, for standard caged animals, increasing stereotypy was correlated with increased FosB/ Δ FosB expression throughout the entire striatum. In contrast, the effects of enrichment on stereotypy were localized only to the dorsolateral region of the striatum, and, also in contrast to the standard cage controls, increasing stereotypy in enriched animals was associated with a decrease in FosB/ Δ FosB expression. Analysis of the compartmental (striosome/matrix) distribution of FosB/ Δ FosB expression demonstrated that the degree to which gene activation in the striosomes exceeded that in the matrix was a reliable indicator of behavioral stereotypy regardless of housing condition. These findings suggest that Δ FosB can mediate both maladaptive and adaptive forms of long-term, experience-dependent neuroplasticity depending on the nature of the chronic stimulation (motor stereotypy or environmental enrichment) experienced.

The goal of our third study (Chapter 6) was to determine whether these differences in gene expression would translate to changes in synaptic structure and function. To this end, we evaluated levels of the synaptic proteins, synaptophysin and NCAM, in the cerebral cortex, dorsolateral striatum, dorsomedial striatum, and hippocampus. Our results show that enrichment

increased concentrations of synaptophysin in the dorsolateral striatum and NCAM-140 in the cortex, whereas stereotypy decreased synaptophysin levels in the dorsolateral striatum and NCAM-140 levels in the cortex. No significant effects were found in the dorsomedial striatum or hippocampus. These results provide additional evidence that the effects of environmental enrichment and stereotypy on neuroplasticity are localized to the corticostriatal neurocircuitry responsible for the control of motor behaviors.

Finally, our last study (Chapter 7) investigated whether stereotypy would be associated with deficits in other behaviors mediated by the striatum and whether the beneficial effects of environmental enrichment would extend to these deficits. The results indicated that stereotypy was associated with deficits in both spatial and stimulus-response learning and memory. Furthermore, we found that the effects of environmental enrichment on these impairments in water maze performance were dissociable: enrichment alone was sufficient to counteract deficits in spatial learning and even enhanced spatial memory but did not have a significant effect on stimulus-response learning. In order to determine whether these differences in behavior were mediated through experience-dependent changes in synaptic plasticity, we again evaluated the synaptic proteins, synaptophysin and NCAM. The results of this analysis indicated that learning-induced changes in synaptic protein expression were task-specific and generally corresponded to levels of performance.

Future Directions

Ultimately, the studies described herein strive to gain a more complete picture of how the brain is dynamically sculpted by complex environmental stimuli on a molecular level so as to exploit the therapeutic potential of this knowledge. In order for enrichment studies to guide the development of novel treatments, the mechanisms which underlie experience-dependent plastic changes in the nervous system must first be identified so that approaches can be developed to

enhance them when they play a beneficial role and downregulate them when they are maladaptive. We have sought to better elucidate the molecular substrates for the beneficial effects of environmental enrichment on stereotypy. However, much still remains to be learned.

A recurring theme in many of these studies was the search for regional specificity. Based on our previous research, our focus has been on the dorsolateral striatum. Indeed, many of the findings in these studies recapitulate the results of our previous work. Nevertheless, the heterogeneous composition of the striatum, both architectonically and functionally, deserves additional consideration. The striatum exhibits complex organization on several levels only some of which we have begun to systematically examine. For example, the topographical organization of connections to and from the striatum varies along all of its dimensional axes (i.e., rostro-caudal, dorso-ventral, and medio-lateral). The striosome and matrix compartments of the striatum can also be further functionally segregated into their core/periphery and matrisome subdivisions. Striatal cell subpopulations, especially those comprising the direct and indirect pathways, are also open to investigation. Future studies might also include other brain regions, especially those which interact with the striatum.

Despite the robust effects of early environmental enrichment on stereotypy, there also appears to be a critical period beyond which the impact of environmental manipulations steadily begins to diminish. One approach that could be used to determine this sensitive period would be to incorporate a longitudinal design into our studies and examine deer mice at various developmental timepoints.

Future studies could also continue to build upon the findings of these studies. For example, the effects of environmental enrichment and motor stereotypy on downstream effectors of DARPP-32 and Δ FosB action could be characterized. Because many of these proteins interact

with or indirectly influence each other, a clearer idea of the molecular and cellular processes which participate in the environmental regulation of stereotypic behaviors could gradually emerge. Additionally, histological analysis could also be performed to confirm and clarify the synaptic changes implied by changing synaptophysin and NCAM-140 levels. Finally, as different forms of learning are functionally and anatomically dissociable, other learning and memory tasks could also be evaluated to determine if they are also impacted by the neuropathology associated with stereotypy. By constructing a more comprehensive picture of the neurobiological processes which are involved in the pathogenesis of stereotypy as well as the beneficial effects of environmental enrichment on reducing stereotypy, the insights gained from these studies may eventually help to guide the development of therapeutic approaches for the treatment of human repetitive behavior disorders.

LIST OF REFERENCES

- Alder J, Xie ZP, Valtorta F, Greengard P, Poo M (1992) Antibodies to synaptophysin interfere with transmitter secretion at neuromuscular synapses. *Neuron* 9:759-768.
- Alder J, Kanki H, Valtorta F, Greengard P, Poo MM (1995) Overexpression of synaptophysin enhances neurotransmitter secretion at *Xenopus* neuromuscular synapses. *J Neurosci* 15:511-519.
- American Psychiatric Association (1994) Diagnostic and statistical manual of mental disorders (4th ed.). Washington, DC, US: American Psychiatric Publishing, Inc.
- Andersson M, Hilbertson A, Cenci MA (1999) Striatal fosB expression is causally linked with l-DOPA-induced abnormal involuntary movements and the associated upregulation of striatal prodynorphin mRNA in a rat model of Parkinson's disease. *Neurobiol Dis* 6:461-474.
- Andin J, Hallbeck M, Mohammed AH, Marcusson J (2007) Influence of environmental enrichment on steady-state mRNA levels for EAAC1, AMPA1 and NMDA2A receptor subunits in rat hippocampus. *Brain Res* 1174:18-27.
- Arami S, Jucker M, Schachner M, Welzl H (1996) The effect of continuous intraventricular infusion of L1 and NCAM antibodies on spatial learning in rats. *Behav Brain Res* 81:81-87.
- Arendash GW, Garcia MF, Costa DA, Cracchiolo JR, Wefes IM, Potter H (2004) Environmental enrichment improves cognition in aged Alzheimer's transgenic mice despite stable beta-amyloid deposition. *Neuroreport* 15:1751-1754.
- Arnt J (1985) Antistereotypic effects of dopamine D-1 and D-2 antagonists after intrastriatal injection in rats. Pharmacological and regional specificity. *Naunyn Schmiedebergs Arch Pharmacol* 330:97-104.
- Aubert I, Guigoni C, Hakansson K, Li Q, Dovero S, Barthe N, Bioulac BH, Gross CE, Fisone G, Bloch B, Bezard E (2005) Increased D1 dopamine receptor signaling in levodopa-induced dyskinesia. *Ann Neurol* 57:17-26.
- Barbelivien A, Herbeaux K, Oberling P, Kelche C, Galani R, Majchrzak M (2006) Environmental enrichment increases responding to contextual cues but decreases overall conditioned fear in the rat. *Behav Brain Res* 169:231-238.
- Bashaw MJ, Tarou LR, Maki TS, Maple TL (2001) A survey assessment of variables related to stereotypy in captive giraffe and okapi. *Appl Anim Behav Sci* 73:235-247.
- Bashaw MJ, Kelling AS, Bloomsmith MA, Maple TL (2007) Environmental effects on the behavior of zoo-housed lions and tigers, with a case study of the effects of a visual barrier on pacing. *J Appl Anim Welf Sci* 10:95-109.

- Benaroya-Milshtein N, Hollander N, Apter A, Kukulansky T, Raz N, Wilf A, Yaniv I, Pick CG (2004) Environmental enrichment in mice decreases anxiety, attenuates stress responses and enhances natural killer cell activity. *Eur J Neurosci* 20:1341-1347.
- Bennett EL, Rosenzweig MR, Diamond MC (1969) Rat brain: effects of environmental enrichment on wet and dry weights. *Science* 163:825-826.
- Bennett JC, McRae PA, Levy LJ, Frick KM (2006) Long-term continuous, but not daily, environmental enrichment reduces spatial memory decline in aged male mice. *Neurobiol Learn Mem* 85:139-152.
- Berardi N, Braschi C, Capsoni S, Cattaneo A, Maffei L (2007) Environmental enrichment delays the onset of memory deficits and reduces neuropathological hallmarks in a mouse model of Alzheimer-like neurodegeneration. *J Alzheimers Dis* 11:359-370.
- Berkson G (1983) Repetitive stereotyped behaviors. *American Journal of Mental Deficiency* 88:239-246.
- Berkson G, Rafaeli-Mor N, Tarnovsky S (1999) Body-rocking and other habits of college students and persons with mental retardation. *Am J Ment Retard* 104:107-116.
- Berridge KC, Aldridge JW, Houchard KR, Zhuang X (2005) Sequential super-stereotypy of an instinctive fixed action pattern in hyper-dopaminergic mutant mice: a model of obsessive compulsive disorder and Tourette's. *BMC Biol* 3:4.
- Bibb JA, Yan Z, Svenningsson P, Snyder GL, Pieribone VA, Horiuchi A, Nairn AC, Messer A, Greengard P (2000) Severe deficiencies in dopamine signaling in presymptomatic Huntington's disease mice. *Proc Natl Acad Sci U S A* 97:6809-6814.
- Bibb JA, Chen J, Taylor JR, Svenningsson P, Nishi A, Snyder GL, Yan Z, Sagawa ZK, Ouimet CC, Nairn AC, Nestler EJ, Greengard P (2001) Effects of chronic exposure to cocaine are regulated by the neuronal protein Cdk5. *Nature* 410:376-380.
- Bibb JA, Snyder GL, Nishi A, Yan Z, Meijer L, Fienberg AA, Tsai LH, Kwon YT, Girault JA, Czernik AJ, Haganir RL, Hemmings HC, Jr., Nairn AC, Greengard P (1999) Phosphorylation of DARPP-32 by Cdk5 modulates dopamine signalling in neurons. *Nature* 402:669-671.
- Bishop SL, Richler J, Lord C (2006) Association between restricted and repetitive behaviors and nonverbal IQ in children with autism spectrum disorders. *Child Neuropsychol* 12:247-267.
- Black JE, Sirevaag AM, Greenough WT (1987) Complex experience promotes capillary formation in young rat visual cortex. *Neurosci Lett* 83:351-355.

- Black JE, Zelazny AM, Greenough WT (1991) Capillary and mitochondrial support of neural plasticity in adult rat visual cortex. *Exp Neurol* 111:204-209.
- Block F, Kunkel M, Schwarz M (1993) Quinolinic acid lesion of the striatum induces impairment in spatial learning and motor performance in rats. *Neurosci Lett* 149:126-128.
- Bodfish JW, Powell SB, Golden RN, Lewis MH (1995) Blink rate as an index of dopamine function in adults with mental retardation and repetitive behavior disorders. *Am J Ment Retard* 99:335-344.
- Bodfish JW, Parker DE, Lewis MH, Sprague RL, Newell KM (2001) Stereotypy and motor control: differences in the postural stability dynamics of persons with stereotyped and dyskinesic movement disorders. *Am J Ment Retard* 106:123-134.
- Borgkvist A, Fisone G (2007) Psychoactive drugs and regulation of the cAMP/PKA/DARPP-32 cascade in striatal medium spiny neurons. *Neurosci Biobehav Rev* 31:79-88.
- Bredy TW, Zhang TY, Grant RJ, Diorio J, Meaney MJ (2004) Peripubertal environmental enrichment reverses the effects of maternal care on hippocampal development and glutamate receptor subunit expression. *Eur J Neurosci* 20:1355-1362.
- Brenes JC, Fornaguera J (2008) Effects of environmental enrichment and social isolation on sucrose consumption and preference: associations with depressive-like behavior and ventral striatum dopamine. *Neurosci Lett* 436:278-282.
- Brenes JC, Rodríguez O, Fornaguera J (2008) Differential effect of environment enrichment and social isolation on depressive-like behavior, spontaneous activity and serotonin and norepinephrine concentration in prefrontal cortex and ventral striatum. *Pharmacology Biochemistry and Behavior* 89:85-93.
- Brown J, Cooper-Kuhn CM, Kempermann G, Van Praag H, Winkler J, Gage FH, Kuhn HG (2003) Enriched environment and physical activity stimulate hippocampal but not olfactory bulb neurogenesis. *Eur J Neurosci* 17:2042-2046.
- Bruel-Jungerman E, Laroche S, Rampon C (2005) New neurons in the dentate gyrus are involved in the expression of enhanced long-term memory following environmental enrichment. *Eur J Neurosci* 21:513-521.
- Cambon K, Venero C, Berezin V, Bock E, Sandi C (2003) Post-training administration of a synthetic peptide ligand of the neural cell adhesion molecule, C3d, attenuates long-term expression of contextual fear conditioning. *Neuroscience* 122:183-191.
- Cambon K, Hansen SM, Venero C, Herrero AI, Skibo G, Berezin V, Bock E, Sandi C (2004) A synthetic neural cell adhesion molecule mimetic peptide promotes synaptogenesis, enhances presynaptic function, and facilitates memory consolidation. *J Neurosci* 24:4197-4204.

- Canales JJ, Graybiel AM (2000) A measure of striatal function predicts motor stereotypy. *Nat Neurosci* 3:377-383.
- Capper-Loup C, Canales JJ, Kadaba N, Graybiel AM (2002) Concurrent activation of dopamine D1 and D2 receptors is required to evoke neural and behavioral phenotypes of cocaine sensitization. *J Neurosci* 22:6218-6227.
- Cenci MA, Tranberg A, Andersson M, Hilbertson A (1999) Changes in the regional and compartmental distribution of FosB- and JunB-like immunoreactivity induced in the dopamine-denervated rat striatum by acute or chronic L-dopa treatment. *Neuroscience* 94:515-527.
- Chang Q, Gold PE (2003) Switching memory systems during learning: changes in patterns of brain acetylcholine release in the hippocampus and striatum in rats. *J Neurosci* 23:3001-3005.
- Chapillon P, Manneche C, Belzung C, Caston J (1999) Rearing environmental enrichment in two inbred strains of mice: 1. Effects on emotional reactivity. *Behav Genet* 29:41-46.
- Chen J, Kelz MB, Hope BT, Nakabeppu Y, Nestler EJ (1997) Chronic Fos-related antigens: stable variants of deltaFosB induced in brain by chronic treatments. *J Neurosci* 17:4933-4941.
- Chen Z, Guan Q, Cao X, Xu Y, Wang L, Sun S (2006) Effect of antisense FosB and CREB on the expression of prodynorphin gene in rats with levodopa-induced dyskinesias. *J Huazhong Univ Sci Technolog Med Sci* 26:542-544.
- Colombo PJ, Brightwell JJ, Countryman RA (2003) Cognitive strategy-specific increases in phosphorylated cAMP response element-binding protein and c-Fos in the hippocampus and dorsal striatum. *J Neurosci* 23:3547-3554.
- Comery TA, Shah R, Greenough WT (1995) Differential rearing alters spine density on medium-sized spiny neurons in the rat corpus striatum: evidence for association of morphological plasticity with early response gene expression. *Neurobiol Learn Mem* 63:217-219.
- Comery TA, Stamoudis CX, Irwin SA, Greenough WT (1996) Increased density of multiple-head dendritic spines on medium-sized spiny neurons of the striatum in rats reared in a complex environment. *Neurobiol Learn Mem* 66:93-96.
- Coq JO, Xerri C (1998) Environmental enrichment alters organizational features of the forepaw representation in the primary somatosensory cortex of adult rats. *Exp Brain Res* 121:191-204.

- Costa DA, Cracchiolo JR, Bachstetter AD, Hughes TF, Bales KR, Paul SM, Mervis RF, Arendash GW, Potter H (2007) Enrichment improves cognition in AD mice by amyloid-related and unrelated mechanisms. *Neurobiol Aging* 28:831-844.
- Creese I, Iversen SD (1972) Amphetamine response in rat after dopamine neurone destruction. *Nat New Biol* 238:247-248.
- Cremer H, Lange R, Christoph A, Plomann M, Vopper G, Roes J, Brown R, Baldwin S, Kraemer P, Scheff S, et al. (1994) Inactivation of the N-CAM gene in mice results in size reduction of the olfactory bulb and deficits in spatial learning. *Nature* 367:455-459.
- Cronin GM, Wiepkema PR (1984) An analysis of stereotyped behaviour in tethered sows. *Ann Rech Vet* 15:263-270.
- Cross HA, Harlow HF (1965) Prolonged and progressive effects of partial isolation on the behavior of macaque monkeys. *Journal of Experimental Research in Personality* 1:39-49.
- Dahlqvist P, Ronnback A, Bergstrom SA, Soderstrom I, Olsson T (2004) Environmental enrichment reverses learning impairment in the Morris water maze after focal cerebral ischemia in rats. *Eur J Neurosci* 19:2288-2298.
- Del Arco A, Segovia G, Canales JJ, Garrido P, de Blas M, Garcia-Verdugo JM, Mora F (2007) Environmental enrichment reduces the function of D1 dopamine receptors in the prefrontal cortex of the rat. *J Neural Transm* 114:43-48.
- DeLorey TM, Handforth A, Anagnostaras SG, Homanics GE, Minassian BA, Asatourian A, Fanselow MS, Delgado-Escueta A, Ellison GD, Olsen RW (1998) Mice lacking the beta3 subunit of the GABAA receptor have the epilepsy phenotype and many of the behavioral characteristics of Angelman syndrome. *J Neurosci* 18:8505-8514.
- Derksen MJ, Ward NL, Hartle KD, Ivanco TL (2007) MAP2 and synaptophysin protein expression following motor learning suggests dynamic regulation and distinct alterations coinciding with synaptogenesis. *Neurobiol Learn Mem* 87:404-415.
- Devan BD, White NM (1999) Parallel information processing in the dorsal striatum: relation to hippocampal function. *J Neurosci* 19:2789-2798.
- Devan BD, McDonald RJ, White NM (1999) Effects of medial and lateral caudate-putamen lesions on place- and cue-guided behaviors in the water maze: relation to thigmotaxis. *Behav Brain Res* 100:5-14.
- Diamond MC, Lindner B, Raymond A (1967) Extensive cortical depth measurements and neuron size increases in the cortex of environmentally enriched rats. In, pp 357-364.

- Diamond MC, Lindner B, Johnson R, Bennett EL, Rosenzweig MR (1975) Differences in occipital cortical synapses from environmentally enriched, impoverished, and standard colony rats. *J Neurosci Res* 1:109-119.
- Diamond MC, Ingham CA, Johnson RE, Bennett EL, Rosenzweig MR (1976) Effects of environment on morphology of rat cerebral cortex and hippocampus. *J Neurobiol* 7:75-85.
- Diamond MC, Law F, Rhodes H, Lindner B, Rosenzweig MR, Krech D, Bennett EL (1966) Increases in cortical depth and glia numbers in rats subjected to enriched environment. *J Comp Neurol* 128:117-126.
- Dityatev A, Dityateva G, Schachner M (2000) Synaptic strength as a function of post- versus presynaptic expression of the neural cell adhesion molecule NCAM. *Neuron* 26:207-217.
- Dong WK, Greenough WT (2004) Plasticity of nonneuronal brain tissue: roles in developmental disorders. *Ment Retard Dev Disabil Res Rev* 10:85-90.
- Dourish CT, Hutson PH, Curzon G (1985) Low doses of the putative serotonin agonist 8-hydroxy-2-(di-n-propylamino) tetralin (8-OH-DPAT) elicit feeding in the rat. *Psychopharmacology* 86:197-204.
- Duffy SN, Craddock KJ, Abel T, Nguyen PV (2001) Environmental enrichment modifies the PKA-dependence of hippocampal LTP and improves hippocampus-dependent memory. *Learn Mem* 8:26-34.
- Dunlap G, Dyer K, Koegel RL (1983) Autistic self-stimulation and intertrial interval duration. *Am J Ment Defic* 88:194-202.
- Eastwood SL, Weickert CS, Webster MJ, Herman MM, Kleinman JE, Harrison PJ (2006) Synaptophysin protein and mRNA expression in the human hippocampal formation from birth to old age. *Hippocampus* 16:645-654.
- Ebrahimi A, Pochet R, Roger M (1992) Topographical organization of the projections from physiologically identified areas of the motor cortex to the striatum in the rat. *Neurosci Res* 14:39-60.
- Edwards MJ, Dale RC, Church AJ, Trikouli E, Quinn NP, Lees AJ, Giovannoni G, Bhatia KP (2004) Adult-onset tic disorder, motor stereotypies, and behavioural disturbance associated with antibasal ganglia antibodies. *Mov Disord* 19:1190-1196.
- Eldridge LL, Masterman D, Knowlton BJ (2002) Intact implicit habit learning in Alzheimer's disease. *Behav Neurosci* 116:722-726.
- Ellinwood EH, Jr. (1967) AMPHETAMINE PSYCHOSIS: I. DESCRIPTION OF THE INDIVIDUALS AND PROCESS. *Journal of Nervous and Mental Disease* 144:273-283.

- Ellinwood EH, Jr., Sudilovsky A, Nelson LM (1973) Evolving behavior in the clinical and experimental amphetamine (model) psychosis. *Am J Psychiatry* 130:1088-1093.
- Elzanowski A, Sergiel A (2006) Stereotypic behavior of a female Asiatic elephant (*Elephas maximus*) in a zoo. *J Appl Anim Welf Sci* 9:223-232.
- Ernst AM, Smelik PG (1966) Site of action of dopamine and apomorphine on compulsive gnawing behaviour in rats. *Experientia* 22:837-838.
- Evans AH, Katzenschlager R, Paviour D, O'Sullivan JD, Appel S, Lawrence AD, Lees AJ (2004) Punding in Parkinson's disease: its relation to the dopamine dysregulation syndrome. *Mov Disord* 19:397-405.
- Faherty CJ, Kerley D, Smeyne RJ (2003) A Golgi-Cox morphological analysis of neuronal changes induced by environmental enrichment. *Brain Res Dev Brain Res* 141:55-61.
- Fazzi E, Lanners J, Danova S, Ferrarri-Ginevra O, Gheza C, Luparia A, Balottin U, Lanzi G (1999) Stereotyped behaviours in blind children. *Brain Dev* 21:522-528.
- Ferchmin PA, Bennett EL (1975) Direct contact with enriched environment is required to alter cerebral weights in rats. *J Comp Physiol Psychol* 88:360-367.
- Fibiger HC, Fibiger HP, Zis AP (1973) Attenuation of amphetamine-induced motor stimulation and stereotypy by 6-hydroxydopamine in the rat. *Br J Pharmacol* 47:683-692.
- Fields JZ, Drucker GE, Wichlinski L, Gordon JH (1991) Neurochemical basis for the absence of overt "stereotyped" behaviors in rats with up-regulated striatal D2 dopamine receptors. *Clin Neuropharmacol* 14:199-208.
- Fienberg AA, Greengard P (2000) The DARPP-32 knockout mouse. *Brain Res Brain Res Rev* 31:313-319.
- Fienberg AA, Hiroi N, Mermelstein PG, Song W, Snyder GL, Nishi A, Cheramy A, O'Callaghan JP, Miller DB, Cole DG, Corbett R, Haile CN, Cooper DC, Onn SP, Grace AA, Ouimet CC, White FJ, Hyman SE, Surmeier DJ, Girault J, Nestler EJ, Greengard P (1998) DARPP-32: regulator of the efficacy of dopaminergic neurotransmission. *Science* 281:838-842.
- Floeter MK, Greenough WT (1979) Cerebellar plasticity: modification of Purkinje cell structure by differential rearing in monkeys. *Science* 206:227-229.
- Fog R (1969) Stereotyped and non-stereotyped behaviour in rats induced by various stimulant drugs. *Psychopharmacologia* 14:299-304.

- Fog R (1972) On stereotypy and catalepsy: studies on the effect of amphetamines and neuroleptics in rats. *Acta Neurol Scand Suppl* 50:3-66.
- Forgays DG, Forgays JW (1952) The nature of the effect of free-environmental experience in the rat. *J Comp Physiol Psychol* 45:322-328.
- Franklin KBJ, Paxinos G (1997) *The mouse brain in stereotaxic coordinates*. San Diego: Academic Press.
- Frick KM, Fernandez SM (2003) Enrichment enhances spatial memory and increases synaptophysin levels in aged female mice. *Neurobiol Aging* 24:615-626.
- Frick KM, Stearns NA, Pan JY, Berger-Sweeney J (2003) Effects of environmental enrichment on spatial memory and neurochemistry in middle-aged mice. *Learn Mem* 10:187-198.
- Friske JE, Gammie SC (2005) Environmental enrichment alters plus maze, but not maternal defense performance in mice. *Physiol Behav* 85:187-194.
- Frith CD, Done DJ (1983) Stereotyped responding by schizophrenic patients on a two-choice guessing task. *Psychol Med* 13:779-786.
- Fry JP, Sharman DF, Stephens DB (1981) Cerebral dopamine, apomorphine and oral activity in the neonatal pig. *J Vet Pharmacol Ther* 4:193-207.
- Fuchs JL, Montemayor M, Greenough WT (1990) Effect of environmental complexity on size of the superior colliculus. *Behav Neural Biol* 54:198-203.
- Garner JP (2005) Stereotypies and other abnormal repetitive behaviors: potential impact on validity, reliability, and replicability of scientific outcomes. *Ilar J* 46:106-117.
- Garner JP, Mason GJ (2002) Evidence for a relationship between cage stereotypies and behavioural disinhibition in laboratory rodents. *Behav Brain Res* 136:83-92.
- Garner JP, Meehan CL, Mench JA (2003a) Stereotypies in caged parrots, schizophrenia and autism: evidence for a common mechanism. *Behav Brain Res* 145:125-134.
- Garner JP, Mason GJ, Smith R (2003b) Stereotypic route-tracing in experimentally caged songbirds correlates with general behavioural disinhibition. *Animal Behaviour* 66:711-727.
- Garner JP, Meehan CL, Famula TR, Mench JA (2006) Genetic, environmental, and neighbor effects on the severity of stereotypies and feather picking in Orange-winged Amazon parrots (*amazona amazonica*): An epidemiological study. *Applied Animal Behaviour Science* 96:153-168.

- Gill KM, Bernstein IL, Mizumori SJ (2007) Immediate early gene activation in hippocampus and dorsal striatum: effects of explicit place and response training. *Neurobiol Learn Mem* 87:583-596.
- Glaser EM, Wilson PD (1998) The coefficient of error of optical fractionator population size estimates: a computer simulation comparing three estimators. *J Microsc* 192:163-171.
- Greenough WT, Volkmar FR (1973) Pattern of dendritic branching in occipital cortex of rats reared in complex environments. *Exp Neurol* 40:491-504.
- Greenough WT, Volkmar FR, Juraska JM (1973) Effects of rearing complexity on dendritic branching in frontolateral and temporal cortex of the rat. *Exp Neurol* 41:371-378.
- Greenough WT, West RW, DeVoogd TJ (1978) Subsynaptic plate perforations: changes with age and experience in the rat. *Science* 202:1096-1098.
- Greenough WT, McDonald JW, Parnisari RM, Camel JE (1986) Environmental conditions modulate degeneration and new dendrite growth in cerebellum of senescent rats. *Brain Res* 380:136-143.
- Gresack JE, Frick KM (2004) Environmental enrichment reduces the mnemonic and neural benefits of estrogen. *Neuroscience* 128:459-471.
- Guilarte TR, Toscano CD, McGlothlan JL, Weaver SA (2003) Environmental enrichment reverses cognitive and molecular deficits induced by developmental lead exposure. *Ann Neurol* 53:50-56.
- Gundersen HJ, Bagger P, Bendtsen TF, Evans SM, Korbo L, Marcussen N, Moller A, Nielsen K, Nyengaard JR, Pakkenberg B, et al. (1988) The new stereological tools: disector, fractionator, nucleator and point sampled intercepts and their use in pathological research and diagnosis. *Apmis* 96:857-881.
- Hadley C, Hadley B, Ephraim S, Yang M, Lewis MH (2006) Spontaneous stereotypy and environmental enrichment in deer mice (*Peromyscus maniculatus*): Reversibility of experience. *Applied Animal Behaviour Science* 97:312-322.
- Haemisch A, Voss T, Gartner K (1994) Effects of environmental enrichment on aggressive behavior, dominance hierarchies, and endocrine states in male DBA/2J mice. *Physiol Behav* 56:1041-1048.
- Hakansson K, Lindskog M, Pozzi L, Usiello A, Fisone G (2004) DARPP-32 and modulation of cAMP signaling: involvement in motor control and levodopa-induced dyskinesia. *Parkinsonism Relat Disord* 10:281-286.

- Hamm RJ, Temple MD, O'Dell DM, Pike BR, Lyeth BG (1996) Exposure to environmental complexity promotes recovery of cognitive function after traumatic brain injury. *J Neurotrauma* 13:41-47.
- Hannigan JH, Berman RF (2000) Amelioration of fetal alcohol-related neurodevelopmental disorders in rats: exploring pharmacological and environmental treatments. *Neurotoxicol Teratol* 22:103-111.
- Hannigan JH, Berman RF, Zajac CS (1993) Environmental enrichment and the behavioral effects of prenatal exposure to alcohol in rats. *Neurotoxicol Teratol* 15:261-266.
- Harburger LL, Lambert TJ, Frick KM (2007a) Age-dependent effects of environmental enrichment on spatial reference memory in male mice. *Behav Brain Res*.
- Harburger LL, Nzerem CK, Frick KM (2007b) Single enrichment variables differentially reduce age-related memory decline in female mice. *Behav Neurosci* 121:679-688.
- Harlow HF, Dodsworth RO, Harlow MK (1965) Total social isolation in monkeys. *Proc Natl Acad Sci U S A* 54:90-97.
- Hebb DO (1949) *The organization of behavior; a neuropsychological theory*. New York: Wiley.
- Helmeste DM, Seeman P, Coscina DV (1981) Relation between brain catecholamine receptors and dopaminergic stereotypy in rat strains. *Eur J Pharmacol* 69:465-470.
- Hemmings HC, Jr., Greengard P, Tung HY, Cohen P (1984) DARPP-32, a dopamine-regulated neuronal phosphoprotein, is a potent inhibitor of protein phosphatase-1. *Nature* 310:503-505.
- Hiscock JJ, Murphy S, Willoughby JO (2000) Confocal microscopic estimation of GABAergic nerve terminals in the central nervous system. *J Neurosci Methods* 95:1-11.
- Hockly E, Cordery PM, Woodman B, Mahal A, van Dellen A, Blakemore C, Lewis CM, Hannan AJ, Bates GP (2002) Environmental enrichment slows disease progression in R6/2 Huntington's disease mice. *Ann Neurol* 51:235-242.
- Hoffman AN, Malena RR, Westergom BP, Luthra P, Cheng JP, Aslam HA, Zafonte RD, Kline AE (2007) Environmental enrichment-mediated functional improvement after experimental traumatic brain injury is contingent on task-specific neurobehavioral experience. *Neurosci Lett*.
- Hogan LA, Tribe A (2007) Prevalence and cause of stereotypic behaviour in common wombats (*Vombatus ursinus*) residing in Australian zoos. *Applied Animal Behaviour Science* 105:180-191.

- Hollander E, Anagnostou E, Chaplin W, Esposito K, Haznedar MM, Licalzi E, Wasserman S, Soorya L, Buchsbaum M (2005) Striatal volume on magnetic resonance imaging and repetitive behaviors in autism. *Biol Psychiatry* 58:226-232.
- Homanics GE, DeLorey TM, Firestone LL, Quinlan JJ, Handforth A, Harrison NL, Krasowski MD, Rick CE, Korpi ER, Makela R, Brilliant MH, Hagiwara N, Ferguson C, Snyder K, Olsen RW (1997) Mice devoid of gamma-aminobutyrate type A receptor beta3 subunit have epilepsy, cleft palate, and hypersensitive behavior. *Proc Natl Acad Sci U S A* 94:4143-4148.
- Hornig M, Weissenbock H, Horscroft N, Lipkin WI (1999) An infection-based model of neurodevelopmental damage. *Proc Natl Acad Sci U S A* 96:12102-12107.
- Hymovitch B (1952) The effects of experimental variations on problem solving in the rat. *J Comp Physiol Psychol* 45:313-321.
- Ingram JL, Peckham SM, Tisdale B, Rodier PM (2000) Prenatal exposure of rats to valproic acid reproduces the cerebellar anomalies associated with autism. *Neurotoxicol Teratol* 22:319-324.
- Ishmael JE, Franklin PH, Murray TF (1998) Dextrorotatory opioids induce stereotyped behavior in Sprague-Dawley and Dark Agouti rats. *Psychopharmacology (Berl)* 140:206-216.
- Iwamoto ET, Way EL (1977) Circling behavior and stereotypy induced by intranigral opiate microinjections. *J Pharmacol Exp Ther* 203:347-359.
- Jadavji NM, Kolb B, Metz GA (2006) Enriched environment improves motor function in intact and unilateral dopamine-depleted rats. *Neuroscience* 140:1127-1138.
- Jankowsky JL, Melnikova T, Fadale DJ, Xu GM, Slunt HH, Gonzales V, Younkin LH, Younkin SG, Borchelt DR, Savonenko AV (2005) Environmental enrichment mitigates cognitive deficits in a mouse model of Alzheimer's disease. *J Neurosci* 25:5217-5224.
- Jenny S, Schmid H (2002) Effect of feeding boxes on the behavior of stereotyping amur tigers (*Panthera tigris altaica*) in the Zurich Zoo, Zurich, Switzerland. In, pp 573-584.
- Jeppesen LL, Heller KE, Bildsoe M (2004) Stereotypies in female farm mink (*Mustela vison*) may be genetically transmitted and associated with higher fertility due to effects on body weight. *Applied Animal Behaviour Science* 86:137-143.
- Johansson BB (1996) Functional outcome in rats transferred to an enriched environment 15 days after focal brain ischemia. *Stroke* 27:324-326.
- Jones RS, Wint D, Ellis NC (1990) The social effects of stereotyped behaviour. *J Ment Defic Res* 34 (Pt 3):261-268.

- Jones TA, Greenough WT (1996) Ultrastructural evidence for increased contact between astrocytes and synapses in rats reared in a complex environment. *Neurobiol Learn Mem* 65:48-56.
- Jones TA, Klintsova AY, Kilman VL, Sirevaag AM, Greenough WT (1997) Induction of multiple synapses by experience in the visual cortex of adult rats. *Neurobiol Learn Mem* 68:13-20.
- Jorgensen OS (1995) Neural cell adhesion molecule (NCAM) as a quantitative marker in synaptic remodeling. *Neurochem Res* 20:533-547.
- Juraska JM, Kopcik JR (1988) Sex and environmental influences on the size and ultrastructure of the rat corpus callosum. *Brain Res* 450:1-8.
- Kalivas PW, Stewart J (1991) Dopamine transmission in the initiation and expression of drug- and stress-induced sensitization of motor activity. *Brain Res Brain Res Rev* 16:223-244.
- Kanner L (1943) Autistic disturbances of affective contact. *Nervous Child* 2:217-250.
- Karson CN (1983) Spontaneous eye-blink rates and dopaminergic systems. *Brain* 106 (Pt 3):643-653.
- Kates WR, Lanham DC, Singer HS (2005) Frontal white matter reductions in healthy males with complex stereotypies. *Pediatr Neurol* 32:109-112.
- Kempermann G, Kuhn HG, Gage FH (1997) More hippocampal neurons in adult mice living in an enriched environment. *Nature* 386:493-495.
- Kempermann G, Gast D, Gage FH (2002) Neuroplasticity in old age: sustained fivefold induction of hippocampal neurogenesis by long-term environmental enrichment. *Ann Neurol* 52:135-143.
- Kennedy CH, Schroeder SR, Oster-Granite ML, Thompson T (2002) Evolution of stereotypy into self-injury. In: *Self-injurious behavior: Gene-brain-behavior relationships.*, pp 133-143: American Psychological Association.
- Keyvani K, Sachser N, Witte OW, Paulus W (2004) Gene expression profiling in the intact and injured brain following environmental enrichment. *J Neuropathol Exp Neurol* 63:598-609.
- Klein SL, Lambert KG, Durr D, Schaefer T, Waring RE (1994) Influence of environmental enrichment and sex on predator stress response in rats. *Physiol Behav* 56:291-297.
- Klementiev B, Novikova T, Novitskaya V, Walmod PS, Dmytriyeva O, Pakkenberg B, Berezin V, Bock E (2007) A neural cell adhesion molecule-derived peptide reduces

- neuropathological signs and cognitive impairment induced by Abeta25-35. *Neuroscience* 145:209-224.
- Kline AE, Wagner AK, Westergom BP, Malena RR, Zafonte RD, Olsen AS, Sozda CN, Luthra P, Panda M, Cheng JP, Aslam HA (2007) Acute treatment with the 5-HT(1A) receptor agonist 8-OH-DPAT and chronic environmental enrichment confer neurobehavioral benefit after experimental brain trauma. *Behav Brain Res* 177:186-194.
- Knowlton BJ, Squire LR, Gluck MA (1994) Probabilistic classification learning in amnesia. *Learn Mem* 1:106-120.
- Knowlton BJ, Mangels JA, Squire LR (1996a) A neostriatal habit learning system in humans. *Science* 273:1399-1402.
- Knowlton BJ, Squire LR, Paulsen JS, Swerdlow NR, Swenson M (1996b) Dissociations within nondeclarative memory in Huntington's disease. *Neuropsychology* 10:538-548.
- Kobayashi S, Ohashi Y, Ando S (2002) Effects of enriched environments with different durations and starting times on learning capacity during aging in rats assessed by a refined procedure of the Hebb-Williams maze task. *J Neurosci Res* 70:340-346.
- Koegel RL, Covert A (1972) The relationship of self-stimulation to learning in autistic children. *J Appl Behav Anal* 5:381-387.
- Koh S, Chung H, Xia H, Mahadevia A, Song Y (2005) Environmental enrichment reverses the impaired exploratory behavior and altered gene expression induced by early-life seizures. *J Child Neurol* 20:796-802.
- Koo JW, Park CH, Choi SH, Kim NJ, Kim HS, Choe JC, Suh YH (2003) The postnatal environment can counteract prenatal effects on cognitive ability, cell proliferation, and synaptic protein expression. *Faseb J* 17:1556-1558.
- Krech D, Rosenzweig MR, Bennett EL (1962) Relations between chemistry and problem-solving among rats raised in enriched and impoverished environments. *J Comp Physiol Psychol* 55:801-807.
- Lambert TJ, Fernandez SM, Frick KM (2005) Different types of environmental enrichment have discrepant effects on spatial memory and synaptophysin levels in female mice. *Neurobiol Learn Mem* 83:206-216.
- Lawrence AJ, Blackwell AD, Barker RA, Spagnolo F, Clark L, Aitken MR, Sahakian BJ (2007) Predictors of punding in Parkinson's disease: Results from a questionnaire survey. *Mov Disord*.

- Lazarov O, Robinson J, Tang YP, Hairston IS, Korade-Mirnic Z, Lee VM, Hersh LB, Sapolsky RM, Mirnic K, Sisodia SS (2005) Environmental enrichment reduces Abeta levels and amyloid deposition in transgenic mice. *Cell* 120:701-713.
- Leggio MG, Mandolesi L, Federico F, Spirito F, Ricci B, Gelfo F, Petrosini L (2005) Environmental enrichment promotes improved spatial abilities and enhanced dendritic growth in the rat. *Behav Brain Res* 163:78-90.
- Lewis MH, Baumeister AA (1982) Stereotyped Mannerisms in Mentally-Retarded Persons - Animal-Models and Theoretical Analyses. *International Review of Research in Mental Retardation* 11:123-161.
- Lewis MH, Bodfish JW (1998) Repetitive behavior disorders in autism. *Mental Retardation and Developmental Disabilities Research Reviews* 4:80-89.
- Lewis MH, Gluck JP, Beauchamp AJ, Keresztury MF, Mailman RB (1990) Long-term effects of early social isolation in *Macaca mulatta*: changes in dopamine receptor function following apomorphine challenge. *Brain Res* 513:67-73.
- Lewis MH, Bodfish JW, Powell SB, Wiest K, Darling M, Golden RN (1996) Plasma HVA in adults with mental retardation and stereotyped behavior: biochemical evidence for a dopamine deficiency model. *Am J Ment Retard* 100:413-418.
- Li C, Niu W, Jiang CH, Hu Y (2007) Effects of enriched environment on gene expression and signal pathways in cortex of hippocampal CA1 specific NMDAR1 knockout mice. *Brain Res Bull* 71:568-577.
- Li S, Tian X, Hartley DM, Feig LA (2006) The environment versus genetics in controlling the contribution of MAP kinases to synaptic plasticity. *Curr Biol* 16:2303-2313.
- Linder JC, Klemfuss H, Groves PM (1987) Acute ultrastructural and behavioral effects of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) in mice. *Neuroscience Letters* 82:221-226.
- Lindskog M, Svenningsson P, Pozzi L, Kim Y, Fienberg AA, Bibb JA, Fredholm BB, Nairn AC, Greengard P, Fisone G (2002) Involvement of DARPP-32 phosphorylation in the stimulant action of caffeine. *Nature* 418:774-778.
- Lopez BR, Lincoln AJ, Ozonoff S, Lai Z (2005) Examining the relationship between executive functions and restricted, repetitive symptoms of Autistic Disorder. *J Autism Dev Disord* 35:445-460.
- Lutz C, Well A, Novak M (2003) Stereotypic and self-injurious behavior in rhesus macaques: a survey and retrospective analysis of environment and early experience. *Am J Primatol* 60:1-15.

- MacLean WE, Jr., Lewis MH, Bryson-Brockmann WA, Ellis DN, Arendt RE, Baumeister AA (1985) Blink rate and stereotyped behavior: evidence for dopamine involvement? *Biol Psychiatry* 20:1321-1325.
- Mallapur A, Choudhury BC (2003) Behavioral abnormalities in captive nonhuman primates. *J Appl Anim Welf Sci* 6:275-284.
- Maraganore DM, Lees AJ, Marsden CD (1991) Complex stereotypies after right putaminal infarction: a case report. *Mov Disord* 6:358-361.
- Marashi V, Barnekow A, Ossendorf E, Sachser N (2003) Effects of different forms of environmental enrichment on behavioral, endocrinological, and immunological parameters in male mice. *Horm Behav* 43:281-292.
- Markham JA, Greenough WT (2004) Experience-driven brain plasticity: beyond the synapse. *Neuron Glia Biol* 1:351-363.
- Marsh R, Alexander GM, Packard MG, Zhu H, Peterson BS (2005) Perceptual-motor skill learning in Gilles de la Tourette syndrome. Evidence for multiple procedural learning and memory systems. *Neuropsychologia* 43:1456-1465.
- Marsh R, Alexander GM, Packard MG, Zhu H, Wingard JC, Quackenbush G, Peterson BS (2004) Habit learning in Tourette syndrome: a translational neuroscience approach to a developmental psychopathology. *Arch Gen Psychiatry* 61:1259-1268.
- Martin LA, Ashwood P, Braunschweig D, Cabanlit M, Van de Water J, Amaral DG (2008) Stereotypies and hyperactivity in rhesus monkeys exposed to IgG from mothers of children with autism. *Brain Behav Immun*.
- Martin LJ, Spicer DM, Lewis MH, Gluck JP, Cork LC (1991) Social deprivation of infant rhesus monkeys alters the chemoarchitecture of the brain: I. Subcortical regions. *J Neurosci* 11:3344-3358.
- Martinez-Cue C, Baamonde C, Lumbreras M, Paz J, Davisson MT, Schmidt C, Dierssen M, Florez J (2002) Differential effects of environmental enrichment on behavior and learning of male and female Ts65Dn mice, a model for Down syndrome. *Behav Brain Res* 134:185-200.
- Mason GJ (1991) Stereotypies: A critical review. *Animal Behaviour* 41:1015-1037.
- Mason GJ (1993) Age and context affect the stereotypies of caged mink. *Behaviour* 127:191-229.
- Mason GJ, Latham NR (2004) Can't stop, won't stop: is stereotypy a reliable animal welfare indicator? *Animal Welfare* 13:S57-s69.

- Mason GJ, Leipoldt A, de Jonge G (1995) Why do female mink with high stereotypy levels have slow-growing offspring? In: Proceedings of the 29th Congress of the International Society for Applied Ethology (Rutter SM, Rushen J, Randle HD, Eddison JC, eds), pp 133-134. Exeter, UK: The International Society for Applied Ethology.
- Matson JL, Kiely SL, Bamburg JW (1997) The effect of stereotypes on adaptive skills as assessed with the DASH-II and Vineland Adaptive Behavior Scales. *Res Dev Disabil* 18:471-476.
- Matson JL, Minshawi NF, Gonzalez ML, Mayville SB (2006) The relationship of comorbid problem behaviors to social skills in persons with profound mental retardation. *Behav Modif* 30:496-506.
- McBride SD, Hemmings A (2005) Altered mesoaccumbens and nigro-striatal dopamine physiology is associated with stereotypy development in a non-rodent species. *Behav Brain Res* 159:113-118.
- McClung CA, Nestler EJ (2003) Regulation of gene expression and cocaine reward by CREB and DeltaFosB. *Nat Neurosci* 6:1208-1215.
- McDonald RJ, White NM (1994) Parallel information processing in the water maze: evidence for independent memory systems involving dorsal striatum and hippocampus. *Behav Neural Biol* 61:260-270.
- McGeorge AJ, Faull RL (1989) The organization of the projection from the cerebral cortex to the striatum in the rat. *Neuroscience* 29:503-537.
- McNair K, Broad J, Riedel G, Davies CH, Cobb SR (2007) Global changes in the hippocampal proteome following exposure to an enriched environment. *Neuroscience* 145:413-422.
- Meehan CL, Garner JP, Mench JA (2004) Environmental enrichment and development of cage stereotypy in Orange-winged Amazon parrots (*Amazona amazonica*). *Dev Psychobiol* 44:209-218.
- Moretti P, Bouwknecht JA, Teague R, Paylor R, Zoghbi HY (2005) Abnormalities of social interactions and home-cage behavior in a mouse model of Rett syndrome. *Hum Mol Genet* 14:205-220.
- Morrison K, Rosales-Ruiz J (1997) The effect of object preferences on task performance and stereotypy in a child with autism. *Res Dev Disabil* 18:127-137.
- Mura A, Feldon J (2003) Spatial learning in rats is impaired after degeneration of the nigrostriatal dopaminergic system. *Mov Disord* 18:860-871.
- Naka F, Narita N, Okado N, Narita M (2005) Modification of AMPA receptor properties following environmental enrichment. *Brain Dev* 27:275-278.

- Nakamura H, Kobayashi S, Ohashi Y, Ando S (1999) Age-changes of brain synapses and synaptic plasticity in response to an enriched environment. *J Neurosci Res* 56:307-315.
- Nestler EJ (2001) Molecular basis of long-term plasticity underlying addiction. *Nat Rev Neurosci* 2:119-128.
- Nicol C (1999) Understanding equine stereotypies. *Equine Vet J Suppl*:20-25.
- Niethammer P, Delling M, Sytnyk V, Dityatev A, Fukami K, Schachner M (2002) Cosignaling of NCAM via lipid rafts and the FGF receptor is required for neuritegenesis. *J Cell Biol* 157:521-532.
- Nilsson M, Perfilieva E, Johansson U, Orwar O, Eriksson PS (1999) Enriched environment increases neurogenesis in the adult rat dentate gyrus and improves spatial memory. *J Neurobiol* 39:569-578.
- Nishi A, Bibb JA, Snyder GL, Higashi H, Nairn AC, Greengard P (2000) Amplification of dopaminergic signaling by a positive feedback loop. *Proc Natl Acad Sci U S A* 97:12840-12845.
- Nistico G, Stephenson JD, Montanini S, Marmo E (1981) Behavioral and electrocortical effects of beta-endorphin after intracerebral infusion in rats. *J Med* 12:463-474.
- Nithianantharajah J, Levis H, Murphy M (2004) Environmental enrichment results in cortical and subcortical changes in levels of synaptophysin and PSD-95 proteins. *Neurobiol Learn Mem* 81:200-210.
- Nithianantharajah J, Barkus C, Murphy M, Hannan AJ (2007) Gene-environment interactions modulating cognitive function and molecular correlates of synaptic plasticity in Huntington's disease transgenic mice. *Neurobiol Dis*.
- Noble M, Albrechtsen M, Moller C, Lyles J, Bock E, Goridis C, Watanabe M, Rutishauser U (1985) Glial cells express N-CAM/D2-CAM-like polypeptides in vitro. *Nature* 316:725-728.
- Odberg FO (1986) The jumping stereotypy in the bank vole (*Clethrionomys glareolus*). *Biology of Behaviour* 11:130-143.
- Odberg FO, Kennes D, De Rycke PH, Bouquet Y (1987) The effect of interference in catecholamine biosynthesis on captivity-induced jumping stereotypy in bank voles (*Clethrionomys glareolus*). *Arch Int Pharmacodyn Ther* 285:34-42.
- Ödberg FO (1987) The influence of cage size and environmental enrichment on the development of stereotypies in bank voles (*Clethrionomys glareolus*). *Behavioural Processes* 14:155-173.

- Ohlsson AL, Johansson BB (1995) Environment influences functional outcome of cerebral infarction in rats. *Stroke* 26:644-649.
- Packard MG (1999) Glutamate infused posttraining into the hippocampus or caudate-putamen differentially strengthens place and response learning. *Proc Natl Acad Sci U S A* 96:12881-12886.
- Packard MG, White NM (1991) Dissociation of hippocampus and caudate nucleus memory systems by posttraining intracerebral injection of dopamine agonists. *Behav Neurosci* 105:295-306.
- Packard MG, McGaugh JL (1992) Double dissociation of fornix and caudate nucleus lesions on acquisition of two water maze tasks: further evidence for multiple memory systems. *Behav Neurosci* 106:439-446.
- Packard MG, McGaugh JL (1996) Inactivation of hippocampus or caudate nucleus with lidocaine differentially affects expression of place and response learning. *Neurobiol Learn Mem* 65:65-72.
- Packard MG, Teather LA (1997a) Double dissociation of hippocampal and dorsal-striatal memory systems by posttraining intracerebral injections of 2-amino-5-phosphonopentanoic acid. *Behav Neurosci* 111:543-551.
- Packard MG, Teather LA (1997b) Posttraining injections of MK-801 produce a time-dependent impairment of memory in two water maze tasks. *Neurobiol Learn Mem* 68:42-50.
- Packard MG, Knowlton BJ (2002) Learning and memory functions of the Basal Ganglia. *Annu Rev Neurosci* 25:563-593.
- Packard MG, Hirsh R, White NM (1989) Differential effects of fornix and caudate nucleus lesions on two radial maze tasks: evidence for multiple memory systems. *J Neurosci* 9:1465-1472.
- Packard MG, Cahill L, McGaugh JL (1994) Amygdala modulation of hippocampal-dependent and caudate nucleus-dependent memory processes. *Proc Natl Acad Sci U S A* 91:8477-8481.
- Packard MG, Vecchioli SF, Schroeder JP, Gasbarri A (2001) Task-dependent role for dorsal striatum metabotropic glutamate receptors in memory. *Learn Mem* 8:96-103.
- Pavon N, Martin AB, Mendiola A, Moratalla R (2006) ERK phosphorylation and FosB expression are associated with L-DOPA-induced dyskinesia in hemiparkinsonian mice. *Biol Psychiatry* 59:64-74.

- Paxinos G, Watson C (1998) The rat brain in stereotaxic coordinates. San Diego: Academic Press.
- Pena Y, Prunell M, Dimitsantos V, Nadal R, Escorihuela RM (2006) Environmental enrichment effects in social investigation in rats are gender dependent. *Behav Brain Res* 174:181-187.
- Persohn E, Pollerberg GE, Schachner M (1989) Immunoelectron-microscopic localization of the 180 kD component of the neural cell adhesion molecule N-CAM in postsynaptic membranes. *J Comp Neurol* 288:92-100.
- Picconi B, Centonze D, Hakansson K, Bernardi G, Greengard P, Fisone G, Cenci MA, Calabresi P (2003) Loss of bidirectional striatal synaptic plasticity in L-DOPA-induced dyskinesia. *Nat Neurosci* 6:501-506.
- Pinaud R, Penner MR, Robertson HA, Currie RW (2001) Upregulation of the immediate early gene arc in the brains of rats exposed to environmental enrichment: implications for molecular plasticity. *Brain Res Mol Brain Res* 91:50-56.
- Pinaud R, Tremere LA, Penner MR, Hess FF, Robertson HA, Currie RW (2002) Complexity of sensory environment drives the expression of candidate-plasticity gene, nerve growth factor induced-A. *Neuroscience* 112:573-582.
- Powell SB, Newman HA, Pendergast JF, Lewis MH (1999) A rodent model of spontaneous stereotypy: initial characterization of developmental, environmental, and neurobiological factors. *Physiol Behav* 66:355-363.
- Powell SB, Newman HA, McDonald TA, Bugenhagen P, Lewis MH (2000) Development of spontaneous stereotyped behavior in deer mice: effects of early and late exposure to a more complex environment. *Dev Psychobiol* 37:100-108.
- Presti MF, Lewis MH (2005) Striatal opioid peptide content in an animal model of spontaneous stereotypic behavior. *Behav Brain Res* 157:363-368.
- Presti MF, Powell SB, Lewis MH (2002) Dissociation between spontaneously emitted and apomorphine-induced stereotypy in *Peromyscus maniculatus bairdii*. *Physiol Behav* 75:347-353.
- Presti MF, Mikes HM, Lewis MH (2003) Selective blockade of spontaneous motor stereotypy via intrastriatal pharmacological manipulation. *Pharmacol Biochem Behav* 74:833-839.
- Presti MF, Gibney BC, Lewis MH (2004a) Effects of intrastriatal administration of selective dopaminergic ligands on spontaneous stereotypy in mice. *Physiol Behav* 80:433-439.

- Presti MF, Watson CJ, Kennedy RT, Yang M, Lewis MH (2004b) Behavior-related alterations of striatal neurochemistry in a mouse model of stereotyped movement disorder. *Pharmacol Biochem Behav* 77:501-507.
- Pych JC, Chang Q, Colon-Rivera C, Haag R, Gold PE (2005) Acetylcholine release in the hippocampus and striatum during place and response training. *Learn Mem* 12:564-572.
- Rafaeli-Mor N, Foster L, Berkson G (1999) Self-reported body-rocking and other habits in college students. *Am J Ment Retard* 104:1-10.
- Rampon C, Jiang CH, Dong H, Tang YP, Lockhart DJ, Schultz PG, Tsien JZ, Hu Y (2000) Effects of environmental enrichment on gene expression in the brain. *Proc Natl Acad Sci U S A* 97:12880-12884.
- Randrup A, Munkvad I (1967) Stereotyped activities produced by amphetamine in several animal species and man. *Psychopharmacologia* 11:300-310.
- Rasmuson S, Olsson T, Henriksson BG, Kelly PA, Holmes MC, Seckl JR, Mohammed AH (1998) Environmental enrichment selectively increases 5-HT1A receptor mRNA expression and binding in the rat hippocampus. *Brain Res Mol Brain Res* 53:285-290.
- Restivo L, Ferrari F, Passino E, Sgobio C, Bock J, Oostra BA, Bagni C, Ammassari-Teule M (2005) Enriched environment promotes behavioral and morphological recovery in a mouse model for the fragile X syndrome. *Proc Natl Acad Sci U S A* 102:11557-11562.
- Richman DM (2008) Early intervention and prevention of self-injurious behaviour exhibited by young children with developmental disabilities. *J Intellect Disabil Res* 52:3-17.
- Richman DM, Lindauer SE (2005) Longitudinal assessment of stereotypic, proto-injurious, and self-injurious behavior exhibited by young children with developmental delays. *Am J Ment Retard* 110:439-450.
- Ridley RM (1994) The psychology of perseverative and stereotyped behaviour. *Prog Neurobiol* 44:221-231.
- Robey KL, Reck JF, Giacomini KD, Barabas G, Eddy GE (2003) Modes and patterns of self-mutilation in persons with Lesch-Nyhan disease. *Dev Med Child Neurol* 45:167-171.
- Roebel AM, MacLean WE, Jr. (2007) Spontaneous eye-blinking and stereotyped behavior in older persons with mental retardation. *Res Dev Disabil* 28:37-42.
- Ronnback A, Dahlqvist P, Bergstrom SA, Olsson T (2005) Diurnal effects of enriched environment on immediate early gene expression in the rat brain. *Brain Res* 1046:137-144.

- Rosenzweig MR, Bennett EL (1996) Psychobiology of plasticity: effects of training and experience on brain and behavior. *Behav Brain Res* 78:57-65.
- Rosenzweig MR, Bennett EL, Hebert M, Morimoto H (1978) Social grouping cannot account for cerebral effects of enriched environments. *Brain Res* 153:563-576.
- Rylander G (1972) Psychoses and the punding and choreiform syndromes in addiction to central stimulant drugs. *Psychiatr Neurol Neurochir* 75:203-212.
- Saka E, Elibol B, Erdem S, Dalkara T (1999) Compartmental changes in expression of c-Fos and FosB proteins in intact and dopamine-depleted striatum after chronic apomorphine treatment. *Brain Res* 825:104-114.
- Saka E, Goodrich C, Harlan P, Madras BK, Graybiel AM (2004) Repetitive behaviors in monkeys are linked to specific striatal activation patterns. *J Neurosci* 24:7557-7565.
- Sandi C (2004) Stress, cognitive impairment and cell adhesion molecules. *Nat Rev Neurosci* 5:917-930.
- Sandi C, Woodson JC, Haynes VF, Park CR, Touyarot K, Lopez-Fernandez MA, Venero C, Diamond DM (2005) Acute stress-induced impairment of spatial memory is associated with decreased expression of neural cell adhesion molecule in the hippocampus and prefrontal cortex. *Biol Psychiatry* 57:856-864.
- Santini E, Valjent E, Usiello A, Carta M, Borgkvist A, Girault JA, Herve D, Greengard P, Fisone G (2007) Critical involvement of cAMP/DARPP-32 and extracellular signal-regulated protein kinase signaling in L-DOPA-induced dyskinesia. *J Neurosci* 27:6995-7005.
- Schachner M (1997) Neural recognition molecules and synaptic plasticity. *Curr Opin Cell Biol* 9:627-634.
- Scheel-Kruger J, Arnt J, Braestrup C, Christensen AV, Cools AR, Magelund G (1978) GABA-dopamine interaction in substantia nigra and nucleus accumbens--relevance to behavioral stimulation and stereotyped behavior. *Adv Biochem Psychopharmacol* 19:343-346.
- Scheggi S, Rauggi R, Gambarana C, Tagliamonte A, De Montis MG (2004) Dopamine and cyclic AMP-regulated phosphoprotein-32 phosphorylation pattern in cocaine and morphine-sensitized rats. *J Neurochem* 90:792-799.
- Schilling G, Savonenko AV, Coonfield ML, Morton JL, Vorovich E, Gale A, Neslon C, Chan N, Eaton M, Fromholt D, Ross CA, Borchelt DR (2004) Environmental, pharmacological, and genetic modulation of the HD phenotype in transgenic mice. *Exp Neurol* 187:137-149.

- Schmidt WJ (1986) Intrastriatal injection of DL-2-amino-5-phosphonovaleric acid (AP-5) induces sniffing stereotypy that is antagonized by haloperidol and clozapine. *Psychopharmacology (Berl)* 90:123-130.
- Schneider JS, Lee MH, Anderson DW, Zuck L, Lidsky TI (2001) Enriched environment during development is protective against lead-induced neurotoxicity. *Brain Research* 896:48-55.
- Schneider T, Przewlocki R (2005) Behavioral alterations in rats prenatally exposed to valproic acid: animal model of autism. *Neuropsychopharmacology* 30:80-89.
- Schneider T, Turczak J, Przewlocki R (2006) Environmental enrichment reverses behavioral alterations in rats prenatally exposed to valproic acid: issues for a therapeutic approach in autism. *Neuropsychopharmacology* 31:36-46.
- Schoenecker B, Heller KE (2000) Indication of a genetic basis of stereotypies in laboratory-bred bank voles (*Clethrionomys glareolus*). *Applied Animal Behaviour Science* 68:339-347.
- Schrijver NC, Pallier PN, Brown VJ, Wurbel H (2004) Double dissociation of social and environmental stimulation on spatial learning and reversal learning in rats. *Behav Brain Res* 152:307-314.
- Schroeder JP, Wingard JC, Packard MG (2002) Post-training reversible inactivation of hippocampus reveals interference between memory systems. *Hippocampus* 12:280-284.
- Schuster T, Krug M, Hassan H, Schachner M (1998) Increase in proportion of hippocampal spine synapses expressing neural cell adhesion molecule NCAM180 following long-term potentiation. *J Neurobiol* 37:359-372.
- Schwaibold U, Pillay N (2001) Stereotypic behaviour is genetically transmitted in the African striped mouse *Rhabdomys pumilio*. *Applied Animal Behaviour Science* 74:273-280.
- Scott L, Forsberg H, Aperia A, Diaz-Heijtjz R (2005) Locomotor effects of a D1R agonist are DARPP-32 dependent in adult but not weanling mice. *Pediatr Res* 58:779-783.
- Sears LL, Vest C, Mohamed S, Bailey J, Ranson BJ, Piven J (1999) An MRI study of the basal ganglia in autism. *Prog Neuropsychopharmacol Biol Psychiatry* 23:613-624.
- Segal DS, Weinberger SB, Cahill J, McCunney SJ (1980) Multiple daily amphetamine administration: behavioral and neurochemical alterations. *Science* 207:905-907.
- Shahbazian M, Young J, Yuva-Paylor L, Spencer C, Antalffy B, Noebels J, Armstrong D, Paylor R, Zoghbi H (2002) Mice with truncated MeCP2 recapitulate many Rett syndrome features and display hyperacetylation of histone H3. *Neuron* 35:243-254.
- Sharman DF, Mann SP, Fry JP, Banns H, Stephens DB (1982) Cerebral dopamine metabolism and stereotyped behaviour in early-weaned piglets. *Neuroscience* 7:1937-1944.

- Sirevaag AM, Greenough WT (1985) Differential rearing effects on rat visual cortex synapses. II. Synaptic morphometry. *Brain Res* 351:215-226.
- Sirevaag AM, Greenough WT (1987) Differential rearing effects on rat visual cortex synapses. III. Neuronal and glial nuclei, boutons, dendrites, and capillaries. *Brain Res* 424:320-332.
- Sirevaag AM, Greenough WT (1991) Plasticity of GFAP-immunoreactive astrocyte size and number in visual cortex of rats reared in complex environments. *Brain Research* 540:273-278.
- Sirevaag AM, Black JE, Shafron D, Greenough WT (1988) Direct evidence that complex experience increases capillary branching and surface area in visual cortex of young rats. *Brain Res* 471:299-304.
- Soffié M, Hahn K, Terao E, Eclancher F (1999) Behavioural and glial changes in old rats following environmental enrichment. *Behavioural Brain Research* 101:37-49.
- Sorensen G (1987) Stereotyped behaviour, hyperaggressiveness and 'tyrannic' hierarchy induced in bank voles (*Clethrionomys glareolus*) by a restricted cage milieu. *Progress in Neuro-Psychopharmacology & Biological Psychiatry* 11:9-21.
- South M, Ozonoff S, McMahon WM (2007) The relationship between executive functioning, central coherence, and repetitive behaviors in the high-functioning autism spectrum. *Autism* 11:437-451.
- Spires TL, Grote HE, Varshney NK, Cordery PM, van Dellen A, Blakemore C, Hannan AJ (2004) Environmental enrichment rescues protein deficits in a mouse model of Huntington's disease, indicating a possible disease mechanism. *J Neurosci* 24:2270-2276.
- Steiner B, Winter C, Hosman K, Siebert E, Kempermann G, Petrus DS, Kupsch A (2006) Enriched environment induces cellular plasticity in the adult substantia nigra and improves motor behavior function in the 6-OHDA rat model of Parkinson's disease. *Experimental Neurology* 199:291-300.
- Svendsen PM, Hansen BK, Malmkvist J, Hansen SW, Palme R, Jeppesen LL (2007) Selection against stereotypic behaviour may have contradictory consequences for the welfare of farm mink (*Mustela vison*). *Applied Animal Behaviour Science* 107:110-119.
- Svenningsson P, Tzavara ET, Liu F, Fienberg AA, Nomikos GG, Greengard P (2002) DARPP-32 mediates serotonergic neurotransmission in the forebrain. *Proc Natl Acad Sci U S A* 99:3188-3193.
- Svenningsson P, Nishi A, Fisone G, Girault JA, Nairn AC, Greengard P (2004) DARPP-32: an integrator of neurotransmission. *Annu Rev Pharmacol Toxicol* 44:269-296.

- Svenningsson P, Tzavara ET, Carruthers R, Rachleff I, Wattler S, Nehls M, McKinzie DL, Fienberg AA, Nomikos GG, Greengard P (2003) Diverse psychotomimetics act through a common signaling pathway. *Science* 302:1412-1415.
- Swaigood RR, Shepherdson DJ (2005) Scientific Approaches to Enrichment and Stereotypies in Zoo Animals: What's Been Done and Where Should We Go Next? *Zoo Biology* 24:499-518.
- Symons FJ, Butler MG, Sanders MD, Feurer ID, Thompson T (1999) Self-injurious behavior and Prader-Willi syndrome: behavioral forms and body locations. *Am J Ment Retard* 104:260-269.
- Szeligo F, Leblond CP (1977) Response of the three main types of glial cells of cortex and corpus callosum in rats handled during suckling or exposed to enriched, control and impoverished environments following weaning. *J Comp Neurol* 172:247-263.
- Tang YP, Wang H, Feng R, Kyin M, Tsien JZ (2001) Differential effects of enrichment on learning and memory function in NR2B transgenic mice. *Neuropharmacology* 41:779-790.
- Tarou LR, Bloomsmith MA, Maple TL (2005) Survey of stereotypic behavior in prosimians. *Am J Primatol* 65:181-196.
- Taylor JR, Morshed SA, Parveen S, Mercadante MT, Scahill L, Peterson BS, King RA, Leckman JF, Lombroso PJ (2002) An animal model of Tourette's syndrome. *Am J Psychiatry* 159:657-660.
- Teather LA, Packard MG, Smith DE, Ellis-Behnke RG, Bazan NG (2005) Differential induction of c-Jun and Fos-like proteins in rat hippocampus and dorsal striatum after training in two water maze tasks. *Neurobiol Learn Mem* 84:75-84.
- Temudo T, Oliveira P, Santos M, Dias K, Vieira J, Moreira A, Calado E, Carrilho I, Oliveira G, Levy A, Barbot C, Fonseca M, Cabral A, Dias A, Cabral P, Monteiro J, Borges L, Gomes R, Barbosa C, Mira G, Eusebio F, Santos M, Sequeiros J, Maciel P (2007) Stereotypies in Rett syndrome: analysis of 83 patients with and without detected MECP2 mutations. *Neurology* 68:1183-1187.
- Thelen E (1979) Rhythmical stereotypies in normal human infants. *Anim Behav* 27:699-715.
- Tiedtke PI, Bischoff C, Schmidt WJ (1990) MK-801-induced stereotypy and its antagonism by neuroleptic drugs. *J Neural Transm Gen Sect* 81:173-182.
- Toscano CD, McGlothan JL, Guilarte TR (2006) Experience-dependent regulation of zif268 gene expression and spatial learning. *Exp Neurol* 200:209-215.

- Totterdell S, Meredith GE (1997) Topographical organization of projections from the entorhinal cortex to the striatum of the rat. *Neuroscience* 78:715-729.
- Troster H, Brambring M, Beelmann A (1991) Prevalence and situational causes of stereotyped behaviors in blind infants and preschoolers. *J Abnorm Child Psychol* 19:569-590.
- Turner AM, Greenough WT (1985) Differential rearing effects on rat visual cortex synapses. I. Synaptic and neuronal density and synapses per neuron. *Brain Res* 329:195-203.
- Turner CA, Lewis MH (2003) Environmental enrichment: effects on stereotyped behavior and neurotrophin levels. *Physiol Behav* 80:259-266.
- Turner CA, Yang MC, Lewis MH (2002) Environmental enrichment: effects on stereotyped behavior and regional neuronal metabolic activity. *Brain Res* 938:15-21.
- Turner CA, Lewis MH, King MA (2003) Environmental enrichment: effects on stereotyped behavior and dendritic morphology. *Dev Psychobiol* 43:20-27.
- Turner CA, Presti MF, Newman HA, Bugenhagen P, Crnic L, Lewis MH (2001) Spontaneous stereotypy in an animal model of Down syndrome: Ts65Dn mice. *Behav Genet* 31:393-400.
- Turner M (1999) Annotation: Repetitive behaviour in autism: a review of psychological research. *J Child Psychol Psychiatry* 40:839-849.
- Turner M, Russell J (1997) Towards an executive dysfunction account of repetitive behaviour in autism. New York, NY, US: Oxford University Press.
- Valjent E, Pascoli V, Svenningsson P, Paul S, Enslen H, Corvol JC, Stipanovich A, Caboche J, Lombroso PJ, Nairn AC, Greengard P, Herve D, Girault JA (2005) Regulation of a protein phosphatase cascade allows convergent dopamine and glutamate signals to activate ERK in the striatum. *Proc Natl Acad Sci U S A* 102:491-496.
- van Dellen A, Blakemore C, Deacon R, York D, Hannan AJ (2000) Delaying the onset of Huntington's in mice. *Nature* 404:721-722.
- van Praag H, Kempermann G, Gage FH (2000) Neural consequences of environmental enrichment. *Nat Rev Neurosci* 1:191-198.
- van Praag H, Schinder AF, Christie BR, Toni N, Palmer TD, Gage FH (2002) Functional neurogenesis in the adult hippocampus. *Nature* 415:1030-1034.
- Vandebroek I, Ödberg FO (1997) Effects of apomorphine on the conflict-induced jumping stereotypy in bank voles. *Pharmacology, Biochemistry and Behavior* 57:863-868.

- Vandebroek I, Berckmoes V, Ödberg FO (1998) Dissociation between MK-801- and captivity-induced stereotypies in bank voles. *Psychopharmacology* 137:205-214.
- Venero C, Herrero AI, Touyarot K, Cambon K, Lopez-Fernandez MA, Berezin V, Bock E, Sandi C (2006) Hippocampal up-regulation of NCAM expression and polysialylation plays a key role on spatial memory. *Eur J Neurosci* 23:1585-1595.
- Vickery S, Mason G (2004) Stereotypic Behavior in Asiatic Black and Malayan Sun Bears. *Zoo Biology* 23:409-430.
- Volkmar FR, Greenough WT (1972) Rearing complexity affects branching of dendrites in the visual cortex of the rat. *Science* 176:1445-1447.
- Voorn P, Vanderschuren LJ, Groenewegen HJ, Robbins TW, Pennartz CM (2004) Putting a spin on the dorsal-ventral divide of the striatum. *Trends Neurosci* 27:468-474.
- Waiblinger E, nig B (2004) Refinement of gerbil housing and husbandry in the laboratory. *Animal Welfare* 13:229-235.
- Walaas SI, Jahn R, Greengard P (1988) Quantitation of nerve terminal populations: synaptic vesicle-associated proteins as markers for synaptic density in the rat neostriatum. *Synapse* 2:516-520.
- Wallace CS, Kilman VL, Withers GS, Greenough WT (1992) Increases in dendritic length in occipital cortex after 4 days of differential housing in weanling rats. *Behav Neural Biol* 58:64-68.
- Wallace CS, Withers GS, Weiler IJ, George JM, Clayton DF, Greenough WT (1995) Correspondence between sites of NGFI-A induction and sites of morphological plasticity following exposure to environmental complexity. *Brain Res Mol Brain Res* 32:211-220.
- Welch JM, Lu J, Rodriguiz RM, Trotta NC, Peca J, Ding JD, Feliciano C, Chen M, Adams JP, Luo J, Dudek SM, Weinberg RJ, Calakos N, Wetsel WC, Feng G (2007) Cortico-striatal synaptic defects and OCD-like behaviours in Sapap3-mutant mice. *Nature* 448:894-900.
- Welzl H, Stork O (2003) Cell adhesion molecules: key players in memory consolidation? *News Physiol Sci* 18:147-150.
- Werme M, Messer C, Olson L, Gilden L, Thoren P, Nestler EJ, Brene S (2002) Delta FosB regulates wheel running. *J Neurosci* 22:8133-8138.
- West MJ, Slomianka L, Gundersen HJ (1991) Unbiased stereological estimation of the total number of neurons in the subdivisions of the rat hippocampus using the optical fractionator. *Anat Rec* 231:482-497.

- Westin JE, Vercammen L, Strome EM, Konradi C, Cenci MA (2007) Spatiotemporal Pattern of Striatal ERK1/2 Phosphorylation in a Rat Model of L-DOPA-Induced Dyskinesia and the Role of Dopamine D1 Receptors. *Biol Psychiatry* 62:800-810.
- Whishaw IQ, Mittleman G, Bunch ST, Dunnett SB (1987) Impairments in the acquisition, retention and selection of spatial navigation strategies after medial caudate-putamen lesions in rats. *Behav Brain Res* 24:125-138.
- White NM, Hiroi N (1998) Preferential localization of self-stimulation sites in striosomes/patches in the rat striatum. *Proc Natl Acad Sci U S A* 95:6486-6491.
- Widman DR, Rosellini RA (1990) Restricted daily exposure to environmental enrichment increases the diversity of exploration. *Physiol Behav* 47:57-62.
- Wiedenmann B, Franke WW (1985) Identification and localization of synaptophysin, an integral membrane glycoprotein of Mr 38,000 characteristic of presynaptic vesicles. *Cell* 41:1017-1028.
- Wiedenmayer C (1996) Effect of cage size on the ontogeny of stereotyped behaviour in gerbils. *Applied Animal Behaviour Science* 47:225-233.
- Wiedenmayer C (1997a) Stereotypies resulting from a deviation in the ontogenetic development of gerbils. *Behavioural Processes* 39:215-221.
- Wiedenmayer C (1997b) Causation of the ontogenetic development of stereotypic digging in gerbils. *Animal Behaviour* 53:461-470.
- Wiedenmayer C, Brunner C (1993) Is stereotyped behaviour in gerbils determined by housing conditions? In: *International Congress on Applied Ethology*, pp 276–278. Berlin, Germany: Humboldt-Universität.
- Williams BM, Luo Y, Ward C, Redd K, Gibson R, Kuczaj SA, McCoy JG (2001) Environmental enrichment: effects on spatial memory and hippocampal CREB immunoreactivity. *Physiol Behav* 73:649-658.
- Winocur G (1998) Environmental influences on cognitive decline in aged rats. *Neurobiol Aging* 19:589-597.
- Wolfer DP, Madani R, Valenti P, Lipp HP (2001) Extended analysis of path data from mutant mice using the public domain software Wintrack. *Physiol Behav* 73:745-753.
- Wolff J (1989) Social behavior. In: *Advances in the study of Peromyscus (Rodentia)* (Kirkland GL, Jr., Layne JN, eds), pp 271–291. Lubbock, TX: Texas Tech University Press.
- Würbel H (2001) Ideal homes? Housing effects on rodent brain and behaviour. *Trends in Neurosciences* 24:207-211.

- Würbel H, Stauffacher M, von Holst D (1996) Stereotypies in laboratory mice: Quantitative and qualitative description of the ontogeny of 'wire-gnawing' and 'jumping' in Zur:ICR and Zur:ICR nu. *Ethology* 102:371-385.
- Yin HH, Knowlton BJ (2004) Contributions of striatal subregions to place and response learning. *Learn Mem* 11:459-463.
- Yin HH, Knowlton BJ (2006) The role of the basal ganglia in habit formation. *Nat Rev Neurosci* 7:464-476.
- Young D, Lawlor PA, Leone P, Dragunow M, During MJ (1999) Environmental enrichment inhibits spontaneous apoptosis, prevents seizures and is neuroprotective. *Nat Med* 5:448-453.
- Zachariou V, Sgambato-Faure V, Sasaki T, Svenningsson P, Berton O, Fienberg AA, Nairn AC, Greengard P, Nestler EJ (2006) Phosphorylation of DARPP-32 at Threonine-34 is required for cocaine action. *Neuropsychopharmacology* 31:555-562.
- Zhu J, Apparsundaram S, Bardo MT, Dwoskin LP (2005) Environmental enrichment decreases cell surface expression of the dopamine transporter in rat medial prefrontal cortex. *J Neurochem* 93:1434-1443.

BIOGRAPHICAL SKETCH

Linda Wen-Hua Lee received her bachelor's degrees in biomedical engineering, biological sciences (neurobiology), and psychology from Northwestern University and her doctoral degree in the medical sciences (neuroscience) from the University of Florida.