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The Effect of Mutant Superoxide Dismutase1 (SOD1) on Cognitive Behavior in the Transgenic Model of Amyotrophic Lateral Sclerosis (ALS)

By Peregrine L. Murphy

A dissertation submitted to the Graduate Faculty in Psychology in partial fulfillment of the requirements for the degree of Doctor of Philosophy, The City University of New York

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Abstract

The Effect of Mutant Superoxide Dismutase1 (SOD1) on Cognitive Behavior in the Transgenic Model of Familial Amyotrophic Lateral Sclerosis (FALS)

By

Peregrine L. Murphy

Advisor: Professor Victoria Luine

The primary goal of this study was to determine if mutations in Superoxide dismutase 1 (SOD1) affected cognition before first physical symptom onset in the transgenic (Tg) animal model of familial amyotrophic lateral sclerosis (FALS). A secondary aim of the study was to identify how neurochemical activity was affected in brain regions known for cognition and memory function. The Tg line of mice carrying the mutant gene for SOD1 (MSOD1 Tg+) and the Tg line of mice over-expressing normal human SOD1 (WT SOD1 Tg+) and both lines non-transgenic littermates (Tg-) were evaluated with the Eight-Arm Radial Maze (RAM), a frontal cortex and hippocampal dependent memory task. Results showed: 1) younger MSOD1 mice performed better than older MSOD1 mice; and 2) Tg+ mice carrying the SOD1 gene, whether mutated or over-expressed, recorded more errors on the RAM when compared with Tg- littermates. Amino acids and monoamines were significantly different between the two lines of mice and differences were influenced by the presence of the transgene, gender, and the age of the mice when euthanized.

Preface

In 1988, I met my first patient diagnosed with amyotrophic lateral sclerosis (ALS). I was completing my second unit of Pastoral Care at The Presbyterian Hospital in New York City. Presbyterian Hospital located within the Columbia Presbyterian Medical Center (CPMC) in Washington Heights carried the astute reputation of being one of the more challenging hospitals within New York City--the staff greatly accomplished, the patients terribly ill and the environment severely limited. I had spent a hot Washington Heights summer, along with the rest of the medical staff, in a cramped Emergency Room attempting to meet the needs of a growing population. I was looking forward to returning to my academic work at General Theological Seminary. The oldest Episcopal Seminary in the United States presented an environment that too was limited. However, as the architecture of the Seminary was Victorian the limitations associated with the gentile antiquated Close were nothing more than passing inconveniences. The unique demands presented by Presbyterian Hospital were overwhelming. I felt that my reservoir to address even the simplest need was tapped dry. I was now in my last few weeks of the clinical unit and a request arrived at the Department of Pastoral Care: "Could they please send over a Chaplain to help lead a support group for patients diagnosed with ALS?"

I was known for having a strong interest in psychology and religion and over the prior two summers had developed a fascination with disorders of the nervous system; I was sent. I did not know what ALS was and furthermore, had never heard of the disorder. I did not know what to expect and was frankly, anxious. I arrived at the appointed time to

the Parkinson's disease Conference Room filled with people. The room was dark and carried a style reminiscent of the 1960's. I made my way over to the conference table to sit down. Once I was seated, I looked at the people in the room more closely. I could see that some of the people sitting around the table were in wheelchairs and their arms frail and thin. The group facilitator began the educational forum, which lasted approximately one hour. The second hour was spent with families and patients sharing challenges, joys and heartbreaks. Those hours were captivating and as I listened to stories of those noble families struggling with the demands of this disease, I felt a strong desire growing within me: I wanted to find someway to be of service--even in the smallest way.

The summer ended. I returned to Seminary and continued my coursework. I found myself agreeing to complete an extended unit of Pastoral Care. I spent Monday afternoons in the Muscular Dystrophy Clinic in order to learn more about ALS. The interdisciplinary nature of the clinic afforded the opportunity to talk with patients in greater depth to gain an understanding of their needs. I continued helping with the ALS Support Group. At the end of 1989, I was supposed to graduate from General Seminary, but instead added a fourth year in order to complete a residency in Pastoral Care. I requested a specialty in ALS. There were concerns expressed by the Board of Pastoral Care, however in the end, the request was granted.

By November, the clinical research nurse for ALS had accepted another appointment. I was well into my residency in Pastoral Care but invited to consider a professional appointment with the ALS Research Team. When I telephoned my Bishop

the Rt. Rev. G. P. Mellick Belshaw and explained how this invitation came about he replied that: "...this could only be a sign of the Spirit and that I should accept the position!" Therefore, I did

Acknowledgements

I am deeply appreciative of the guidance provided by committee members: Drs. Victoria Luine (Chair), Stefan Baumrin, Peter Moller, Serge Przedborski, Lewis P. Rowland, and John B. Willett -- may I be as gracious with others as they have been with me.

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Finally, my ability to persevere is inspired by my faith and guidance offered through: Bishops Richard Grein, Catherine Roskam, Mark Sisk, the Rev.'s James Burns, Carol Pinkham-Oak, Carl Sword, OHC, the Rev. Deacon Dr. Minka Sprague and the communities of St. Mary's of Mohegan Lake and Iglesia Memorial de San Andres of Yonkers in the Episcopal Diocese of New York; and, in the Archdiocese of New York by G. Simon Harak, SJ and Bishop James F. McCarthy.

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Chapter 1

INTRODUCTION

I had the pleasure and privilege of serving as Clinical Researcher for Neuromuscular Disease in the Department of Neurology at Columbia Presbyterian Medical Center from 1989 until 1996 under the direction of Dr. Lewis P. Rowland, who was at that time Chairman. Even though Dr. Jean Martin Charcot identified the neuropathological hallmarks of ALS in 1869, Dr. Rowland forged his career with clinical milestones laid on behalf of patients diagnosed with ALS. Pivotal in defining what the disease was not, he provided diagnostic guidelines (and hope) for neurologists striving to treat patients diagnosed with ALS (Rowland, 1998, 2000; Rowland & Shneider, 2001).

After diagnostic confirmation of ALS, the patient was referred to me for counseling and support and possible entry into a clinical research trial. From diagnosis until death, I watched patients and family members struggle with the emotional devastation that frequently accompanies a terminal diagnosis--but with ALS every struggle seemed much more profound. I observed tremendous indecision and anxiety regarding even the most basic decisions of medical care. While we would counsel the benefit of a pro-active medical plan of care few patients and families could truly be pro-active.

Disease management influences tremendously how patients experience their disease. Ultimately, the management of symptoms, psychological health, and family functioning influence long-term prognosis. To some extent, hesitancy in the incorporation

of recommended medical care is expected when decisions are complex: for example, the insertion of a feeding tube such as, one for percutaneous endoscopic gastrostomy (PEG) when a patient is at risk for aspiration. Hesitancy may also be expected with the initiation of non-invasive assisted ventilation (Bi-Pap), or tracheotomy, when respiratory failure is imminent. However, hesitancy was seen in non-complex decision making such as the signing of a health care proxy, obtaining education about the disease process, or seeking adjunct therapies, such as physical therapy, speech therapy, psychological counseling. Often, patient's decision-making was fraught with inactivity and forgetfulness, which could be the result of stress, but may also be due to other factors. The difficulties in decision-making not only affected medical care, but the functioning of the individual and the family. I observed tremendous stress in patients and families with this decision-making process. It is difficult to know if this behavior is indicative of the psychological trauma associated with a terminal diagnosis or underlying psycho neurobiological functioning. The clinical diagnosis of ALS is confirmed at autopsy; therefore, any assessment of this sort is a challenge.

In 1993, mutations in the gene that encodes superoxide dismutase 1 (SOD1) were identified in patients diagnosed with familial amyotrophic lateral sclerosis (FALS), an inherited subgroup of the disorder comprising approximately 5-10% of all ALS cases (Deng et al., 1993). A year later, a transgenic mouse model of ALS was created expressing a mutant form of SOD (Gurney et al., 1994). Dr. Serge Przedborski, also at Columbia, seeking an understanding of neurodegeneration, particularly apoptosis (programmed cell death) graciously included me in his work on ALS. Dr. Przedborksi's research with the murine model of ALS inspired me to propose an investigation of

cognitive behavior in the mouse model. I left the Department of Neurology in 1996 to pursue that investigation and this Thesis presents the culmination of that activity, as well as other bio-medical ethical aspects of scientific investigation that have become increasingly important to the research community.

Distinctive Features of This Study

The primary aims of this study are to investigate if mutations in SOD1 affect cognitive functioning and whether there is any change in cognitive functioning prior to first physical symptom onset. Therefore, the transgenic line of mice carrying the mutant gene for SOD1 (MSOD1 Tg+) will be compared with their non-transgenic littermates, MSOD1 mice that do not carry the mutant gene (MSOD1 Tg-). In order to delineate if changes in cognition are due to the mutation in SOD1 or the over-expression of the enzyme MSOD1 mice will be compared with the transgenic line of mice that over-expresses normal human SOD1, Wild Type (WT SOD1 Tg+) mice and their littermates (WT SOD1 Tg-). Cognitive functioning will be assessed with an Eight-Arm Radial Arm Maze (RAM), a behavioral tool that measures performance indicative of hippocampal and frontal lobe cognitive function.

Secondary aims of this study are to investigate the way in which monoamines such as dopamine (DA), norepinephrine (NE), and serotonin (5-HT), their metabolites 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA) and 5-hydroxyindoleacetic acid (5-HIAA), and amino acids, glutamate and γ-aminobutyric acid (GABA) are affected in cognitive function. Concentrations of these neurochemicals are

measured in the frontal cortex and the hippocampus, neuroanatomical regions of the brains involved with memory and cognitive processing.

Given these aims there are four distinctive features of this study and four hypotheses:

1) The MSOD1 Tg+ mice while examined for motor function (Gurney, Cutting et al., 1996) have not been assessed cognitively. Therefore, a distinctive feature of this study is the examination of cognitive function in the MSOD1 mouse model of ALS before paralysis.

2) The Eight-Arm RAM is a cognitive measure used extensively with rats but little with mice. Research involving the RAM will be a useful addition to literature involving behavioral and cognitive functioning of transgenic mice.

3) Ethical dilemmas regarding research with mice have increased. There is academic and public concern regarding the continued use of mice and a need for justification of research. This study supports that need.

4) Finally, husbandry of mice has received some criticism. Husbandry of transgenic mice, that are sometimes more frail than their non-transgenic littermates, requires refinement. This study affirms husbandry methods that have enhanced the quality of life of mice used in experimental protocols.

Hypotheses

H_1: Younger MSOD1 mice will show an enhanced ability in learning a spatial memory task when compared with older MSOD1 mice.

H_2: WT SOD1 mice will demonstrate enhanced ability at learning a spatial memory task and their performance may be influenced by the presence of the transgene, gender, or age when compared with MSOD1 mice.

H_3: As elevated levels of glutamate have been shown in the spinal cord of patients diagnosed with ALS and excitotoxicity is suspected to be associated with mutations SOD1 it is suspected that WT SOD1 mice will show decreased concentrations of glutamate in brain regions associated with cognitive function, which may be influenced by the presence of the transgene, gender, or age when compared with MSOD1 mice.

H_4: As monoamines are involved with cognitive functioning, it is suspected that there will be differences between the two transgenic lines and they may be influenced by the presence of the transgene, gender, or age.

Overview of This Study

The Thesis is organized into five chapters: Introduction, Literature Review, Research Design, Research Findings, and Research Limitations and Conclusions. Appendix I contains an example of the record of the Generalized Least Squares Regression Model to which I fitted behavioral data obtained from the SOD1 mice and a record of an example of the Ordinary Least Squares Regression Model to which I fitted the neurochemistry data. The Bibliography for this research study follows the Appendix.

Chapter II

LITERATURE REVIEW

Overview

This review of the literature encompasses five areas: 1) a description of the epidemiological, clinical and biochemical features relative to both human ALS and FALS in the transgenic mouse model; 2) human and murine neuropathology of ALS; 3) cognitive function associated with ALS; 4) neurobiological correlates of cognitive function; and, 5) ethical concerns relative to this study.

Epidemiological, Clinical and Biochemical Features of ALS

Epidemiology

Amyotrophic lateral sclerosis (ALS), also known as Charcot's disease, or motor neuron disease in England and Lou Gehrig's disease in the United States, is a neurodegenerative disorder. "Amyotrophic" refers to the atrophy, weakness, wasting and fasciculations that are neurological signs of the lower motor neuron. "lateral sclerosis" suggests hardening of the lateral columns of the spinal cord, which follows gliosis (proliferation of connective tissue, such as astrocytes or microglia) after neuronal cell death (Rowland & Shneider, 2001).

The World Federation of Neurology developed El Escorial Criteria criteria for diagnosing ALS in 1994 to facilitate entry into clinical trials. Those guidelines have been revised further, and offer community neurologists parameters, which may be accessed easily (Brooks, 1998). Despite increasing knowledge about ALS, misdiagnosis of ALS has been noted with devestating consequences for patients and family members

(Rowland, 1998). Therefore, consensus regarding diagnosis is key to understanding the epidemiological features of the disease.

The diagnosis of ALS is confirmed with the presence of both upper and lower motor neuron signs (Rowland, 1998). If only upper motor neuron (UMN) signs are present, the diagnosis is primary lateral sclerosis; and if lower motor neuron (LMN) signs are evident, the diagnosis is progressive spinal muscular atrophy (Rowland & Shneider, 2001). This distinction is an important one as both of the two diseases have courses that are not as severe as ALS and individuals who develop these disorders live longer.

Onset of ALS is rare before age 20 except in the juvenile form of the disorder. ALS customarily affects individuals in their fifth and sixth decade. Men are diagnosed with the disease more often than women (ratio 1.7:1.0). However, after age 65 the ratio approaches 1:1 with women protracting the disease as often as men (Appel & Smith, 2001). Rowland (1998) illuminates that more male celebrities are known for having the disorder as the best known figures are likely to be men in societies where leadership opportunities are limited for women.

The incidence of ALS is 1/100,000 and the prevalence is 3-5/100,000. Ninety percent of ALS cases are classic sporadic ALS and 10% are familial, an inherited form of the disorder (Appel & Smith, 2001). Etiologic hypotheses have varied: environmental toxins, viral antigens, physical trauma, and vigorous physical exercise. However, findings to support these hypotheses have been inconclusive (Nelson, 1996).

Interestingly, the population affected with ALS features a preponderance of thin people and many have competed athletically (Rowland, 1998). The average duration of the illness is two to four years and approximately 20% of patients survive longer than

five years (Rowland, Lange & Murphy, 1997). The course of the illness is rapid and fatal. Without ameliorative technology, death in ALS is most often due to respiratory impairment (Belsh, 1996) or other intercurrent illnesses (Rowland, 1998).

There are essentially four forms of ALS. Only ten percent of cases are genetically inherited, however, neurogenetics has recently attributed ALS with a variety of gene loci (Wong, Price & Subramanian, 2001).

- Classic sporadic ALS, which accounts for the majority of patients (90%) and includes a subgroup of patients diagnosed with progressive bulbar palsy:
    - a small number of classic sporadic patients have presented with mutations in the gene which encodes a subunit of neurofilament (NFH) (Al-Chalabi, Andersen et al., 1999);
- FALS an autosomal dominant disorder, which affects about 5-10% of patients:
    - 20% of FALS patients carry mutations of the gene encoding SOD1 (Rosen et al., 1993);
    - Dominant X-linked ALS has been associated with chromosome Xp11-q12 (Hong, Brooks, Hung, Siddique & Rimmler, 1998); and,
    - Patients diagnosed with both FALS and frontotemporal dementia (FTD) have been linked to chromosome 9q21-q22 (Hosler et al., 2000).
- Juvenile ALS:
    - automsomal dominant has been linked with chromosome 9q34 (Chance et al., 1998);
    - automsomal recessive has been associated with chromosome 15q15-q22 (Hentati, Ouahchi, Pericak-Vance et al., 1998); and,

- chromosome 2q33-q35 (Hentati, Bejaoui, Pericak-Vance et al., 1994).

- Guamanian ALS, which may also have either parkinsonism or dementia or both and is found in the Western Pacific (Hirano, Kurland, Krooth, & Lessel, 1961).

Familial Amyotrophic Lateral Sclerosis. The association of mutations of the SOD1 gene with FALS (Rosen et al., 1993) and chromosome 21q22.1 as the locus for FALS (Siddique & Deng, 1996) have been pivotal in our understanding of ALS. After linkage was established, 5-20% patients with autosomal dominant FALS were identified with mutations of the gene that encodes SOD1 (Deng et al., 1993; Tu, Gurney, Julien, Lee, & Trojanowski, 1997). Heterozygous mutations were identified in 17 of 49 FALS families. When SOD enzymatic activity was measured in the red blood cells of members of FALS families, mutant SOD1 activity was less than half (41%) of normal controls (Deng et al., 1993). Since that time, many more mutations have been identified with SOD1 (Cudkowicz et al., 1997).

Mouse model of ALS developed from mutations found on SOD1. Gurney and colleagues (1994) developed a line of transgenic mice expressing one of the mutations associated SOD1. The G93A transgenic mice (Gly93 → Ala mutation) express the largest amount of MSOD. Since then, many lines of transgenic mice with mutations in the SOD1 transgene have been produced and develop motor neuron disease. The motor neuron disease that develops in mice appears to correlate with the expression of MSOD1 (number of gene copies) rather than enzymatic activity. Therefore, when fewer copies of the SOD1 transgene are expressed, motor neuron disease does not develop in mice (Tu, Gurney, Julien, Lee, & Trojanowski, 1997).

Mouse model developed from overexpressing WT SOD1. A transgenic mouse was created overexpressing SOD1 as a model for Down syndrome (DS). DS is thought to result from the overexpression of genes on chromosome 21 because trisomic (cells containing three chromosomes instead of the usual two) contain almost 1.5 times as much SOD1 as normal cells (Epstein et al., 1987). It has been suggested that overexpression of SOD1 may disrupt the equilibrium of the cell and may result in disorders such as DS or other forms of mental retardation or diseases of cognitive function (Epstein et al., 1987). The limitations posed with research on humans, and particularly as those individuals affected with mental retardation are protected under research guidelines, a transgenic model of DS that overexpresses WT SOD1 was developed (Epstein et al., 1987).

Epstein and colleagues (1987) created multiple lines of transgenic mice expressing one to twelve copies of the WT SOD1 gene. Electrophoresis assessed levels of Cu/Zn SOD expression, which varied in tissues within a given line but remained constant in successive generations of transgenic mice. The SF-218 transgenic mouse line showed normal phenotypical development, subtle pathogenic changes and over-expression of SOD1.

SOD may affect cognitive development and may assume a contributory role in DS, but its role in FALS is unknown. The common locus of chromosome 21 and the possible association of DS and ALS provides rational for comparative investigations of MSOD and WT SOD1 transgenic mice.

Clinical Features of Human ALS

The diagnosis of ALS is confirmed with the presence of upper and lower motor neuron signs. UMN signs are hyperreflexia (increased tendon reflexes), Babinski reflex (the toe moves upward, when the bottom of the foot is stroked, which is sometimes present in newborns), Hoffman reflex (flipping the nail of the second, third or fourth finger which results in flexion of these fingers and thumb), and clonus (alternating muscular contractions and relaxations). LMN signs are weakness and wasting in the limbs and sometimes the tongue, with fasciculations (muscle twitching) present throughout.

El Escorial Criteria state that confirmation of the diagnosis of ALS is with clinical, electrophysiological and neuropathologic examination, UMN degeneration is confirmed by clinical examination, and with observation that there is a progressive spread of symptoms. These signs must be coupled with electrophysiological, pathological, and neuroimaging evidence that there are no other disease processes present (Brooks, 1998).

Electromyography (EMG) will show normal nerve conduction studies and signs of neuronal denervation, fibrillation, and positive sharp waves, large motor unit potentials, reduced interference pattern with rates higher than 10 Hz and unstable motor unit potentials (Brooks, 1998).

Neuroimaging excludes abnormalities of the skull or spinal cord. Clinical laboratory tests are also exclusionary, and, if abnormal, identify syndromes that may mimic ALS: autoantibodies, hormonal abnormalities, lymphoma, evidence of infection, lead in either blood or urine, and hexoseaminidase A/B deficiency (Brooks, 1998).

Pathological signs that confirm the disease are UMN and LMN degeneration and hardening of the spinal columns, which are found at autopsy.

Symptoms of the disease vary with each patient but usually begin with painless asymmetrical weakness of the limbs. Conversely, slurred speech and difficulty swallowing or breathing are also symptoms of ALS. A frequently described first symptom by many patients is: difficulty in turning a key when opening a lock. Regardless of symptom presentation, weakness continues (unless intercurrent illness occurs first) until all limbs are affected except for the eyes. Bladder, bowel, and sexual functioning are not typically affected in ALS (Siddique, Sufit & Siddique, 1996).

Clinical Features of the MSOD1 Mouse

The transgenic line that develops the most severe form of motor neuron disease is the G93A line, which has more than four times SOD1 activity when compared to their nontransgenic littermates and more than 18 copies of the MSOD1 gene (Gurney et al., 1994). A syndrome resembling human motor neuron disease develops in mice at age three to four months (Gurney et al., 1994). First signs of the clinical disease in the MSOD1 Tg+ mouse are observed at 91 $\pm$ 14 days of age with a tremor in one or more limbs. Passive movement of the hind limbs with tremor reveals increased resistance and spasticity. When the mice are tapped on the knee or ankle they are hyperreflexive. MSOD1 Tg+ mice have a shorter stride at an average of 125 + 11 days of age when compared with controls; males had a longer stride than females, however, clinical onset did not differ (Chiu et al., 1995). Paralysis develops between three and four months of age and death by approximately 187 days of age. Lines of mice that express lower levels

of the MSOD1 develop the disease and die much later. (Dal Canto & Gurney, 1995). As such, the G93A line has been useful in testing drugs or treatments that may affect or stop the disease.

In one study, antioxidants vitamin E and selenium were administered; although they did not affect survival, both onset and progression of the illness were delayed (Gurney, Cutting et al., 1996). Similar findings were discovered when transgenic G93A mice were administered riluzole (2-amino-6-trifluromethoxy-benzothiazole) (Gurney, Fleck, Himes, & Hall, 1998b), creatine (Klivenyi et al., 1999) ginseng root (Jiang, DeSilva & Turnbull, 2000) and caspase inhibition (Li et al., 2000). Onset was also delayed when the G93A FALS transgenic mice were crossed with hemizygote transgenic *bcl-2* mice. *Bcl-2* mice over express the *Bcl-2* protein, which normally modulates cell death by inhibiting apoptosis (Kostic, Jackson-Lewis, de Bilbao, Dubois-Dauphin, & Przedborski, 1997a). These findings suggest that reactive oxygen species and apoptosis contributed to the pathogenesis of the disease.

Clinical Features of the WT SOD1 Mouse

Dal Canto & Gurney (1995) noted that the WT SOD1 mouse did not demonstrate clinical signs of ALS at 300 days of age. Chiu et al., (1995) confirmed that signs of ALS were still not present at one year of age.

Biochemical Features of ALS.

The cause of the neurodegenerative process has not been identified in ALS. It is suspected that there is an interaction between genetics, oxidative stress, and the excitatory

effect of glutamate on motor neurons (Morrison & Morrison, 1999; Shaw, 1999). Cleveland (1999) identified four primary hypotheses currently under exploration:

- Oxidative damage;
- Axonal strangulation from neurofilament disorganization;
- Toxicity arising from intercellular aggregates; and,
- Excitoxicity from glutamate.

Oxidative Damage. Researchers suspect that there is a relationship between FALS associated mutations and toxic properties in SOD1 (Przedborski, Donaldson et al., 1996; Price, Sisodia, & Borchelt, 1998). SOD1 is actually an endogenous free radical scavenger enzyme, which functions as a dismutase. A dismutase acts on two molecules: one molecule is oxidized and the other reduced (Thomas, 1989). SOD1 is thought to be present in all eukaryotic cells and part of a family of three SOD enzymes: manganese-dependent mitochondrial SOD (SOD2), which maps to chromosome 6q25; and, copper-zinc extracellular SOD (SOD3), which is located on chromosome 4p15.2. Neither SOD2 or SOD3 are linked to FALS (Brown, 1995; Siddique & Deng, 1996).

SOD1 is a homodimer, which has an active site containing one atom of copper and one atom of zinc. SOD1 catalyzes the conversion of superoxide (O_2^-) and water (H_2O) to oxygen (O_2) and hydrogen peroxide (H_2O_2). In the conversion, O_2^- is guided to the molecule's active site. Amino acids allow the negatively charged O_2^- to dock close to the copper atom and the dismutase reaction occurs rapidly (Gurney, Liu, Althaus, Hall & Becker, 1998a). H_2O_2 is detoxified to water by either catalase or glutathione peroxidase. However, H_2O_2 is not a free radical. The compound diffuses through biological

membranes and initiates excitotoxicity at distant sites. When H_2O_2 functions in the presence of copper and iron, which accumulate in regions within the brain, hydroxyl radicals are produced at accelerated rates (Facchinetti, Dawson, & Dawson, 1998). When the protein that incorporates copper into SOD1 was disrupted, levels of SOD1 were decreased but not eliminated (Wong, Waggoner, Subramaniam, (2000).

Pathogenic processes involving the MSOD1 are still not well understood. It is thought that mutations may induce conformational changes and molecules that are customarily excluded may have easier access to this site (Siddique, Sufit, & Siddique, 1999). MSOD1 may also affect the equilibrium in the cell by disturbing the intracellular homeostasis of copper and zinc, which are both potential neurotoxins (Siddique & Deng, 1996). When copper is bound to SOD in the presence of low zinc content, nitric oxide (NO) is produced by either WT or mutant SOD and induces apoptosis in cultured motor neurons (Estevez et. al., 1999). Therefore, structural changes may lead to depressed enzymatic activity, which in turn, leads to an accumulation of superoxide anions. An increase in superoxide anions reacting with NO may produce the highly reactive oxygen species (ROS), peroxynityrite, which could have a deleterious effect on motor neurons (Beckman, Carson, Smith, & Koppenol, 1993) and other cellular processes. For example, oxidative damage was found in the mitochondria of presymptomatic MSOD1 mice as early as 25 weeks of age (Warita, Hayashi, Murakami, Manabe & Abe, 2001).

The association of SOD1 mutant properties with pathological changes and how the changes occur have yet to be determined. Mice deficient in SOD1 (knock-out mice) developed normally and autopsy revealed a paucity of pathology; however, as the mice aged they showed increased sensitivity to facial nerve axotomy (Reaume, 1996),

hindlimb denervation at two months of age and reduction of stride at six months of age (Food et al., 1999). Therefore, onset and progression of ALS is now viewed as a "gain" of an adverse function or toxic property (Tu, Gurney, Julien, Lee, & Trojanowski, 1997; Gurney, Liu, Althaus, Hall, & Becker, 1998c). However, what ALS is, when it develops, and where it derives from is unknown.

Axonal strangulation from neurofilament disorganization. Overabundance of NFT's are pathological signs associated with ALS, which affect axonal transport. Recently developed transgenic mice generated up to twice the amount of protein required for heavy neurofilament production and developed neurological deficits and abnormal neurofilamentous swellings similar to those found in ALS (Cote, Collard & Julien, 1993). Alternatively, mice not expressing light neurofilaments were delayed in disease onset by approximately 40 days (Williamson et al., 1998).

Toxicity arising from intracellular aggregates. As stated, deficits in axonal transport due to heavy concentration of NFT's have been noted in MSOD1 Tg+ mice. Decreased transport precedes disease onset by as much as five months (Williamson & Cleveland, 1999). Therefore, it is difficult to determine which factors actually contribute to reduced axonal flow. Increased NFT's and accumulations of protein aggregates have both been observed in several neurodegenerative diseases (Cleveland, 1999). Protein aggregates are characteristic of MSOD1 and are immunoreactive to antibodies to SOD1. Aggregate accumulation may arise either through disequilibrium or the loss of essential components in SOD1 and ultimately, result in increased toxicity.

Excitoxicity from glutamate. Neuronal death occurs when there is an increase in intracellular calcium induced by the binding of glutamate on receptors of the motor

neurons. Glutamate affects the neuron by binding to two receptors (membrane spanning proteins that influence the opening and closing of ion channels in the neuron): the glutamate gated N-methyl-D-aspartic acid (NMDA) receptor; and, the α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptor. Permeability of AMPA receptors varies according to its molecular composition. If one of the subunits, GluR2 is present the cell repels calcium and when it is not present the cell is permeable to calcium (Sommer, Kohler, Sprengel & Seeburg, 1991).

Glutamate is released from the excitatory neurons in the descending corticospinal pathways and innervate neurons that project to muscle spindles and tendon organs. This process, when imbalanced, may lead to the generation of free radicals, and activation of proteases, as well as the initiation of transcriptional cell death programs (Shaw, 1999). Removal of glutamate from the synaptic cleft occurs through excitatory amino acid transporter (EAAT) proteins, which are located on the perisynaptic astrocytes (Rothstein, Martin, Levey et al., 1994). However, 60-70% of patients with ALS have reduced astrocytic glutamate transporter protein EAAT2 in the motor cortex and spinal cord (Rothstein, Van Kammen, Levey, Martin, & Kuncl, 1995), which results from a defect in a messenger RNA (mRNA) splicing regulatory factor (Lin et al., 1998).

Structural changes in motor neurons were induced by IgG from patients diagnosed with ALS (Appel, Smith, Alexiano, Engelhardt, & Stefani, 1995). Offen et al., (1998) reported that 79% of patients diagnosed with ALS were found to have serum IgG that blocks L-type calcium channels. However, Drachman et al., (1995) disagree with the autoimmune theory and were unable to reproduce the IgG findings. Although the

excitatory neurotoxic hypothesis offers a plausible explanation for neurodegeneration, findings are not conclusive.

Biochemistry of Down Syndrome. Commonalities between ALS, DS and other neurodegenerative have been increasingly recognized (Iannello & Kola, 2001). Specifically, DS is linked to both chromosome 21 and SOD1 and is associated with premature aging and progressive mental retardation. As some individuals with DS grow into adulthood, progressive dementia similar to Alzheimers develops and neuropathology reveals accumulations of senile plaques, Aβ amyloid deposits and neurofibrillary tangles (Brugge et al., 1994).

Apparently, cortical neuritic plaques are present in both demented and non-demented DS patients. The plaque core is composed of insoluble beta amyloid. The gene for amyloid β precursor protein (βAPP) has also been mapped to chromosome 21. βAPP has many functions and is expressed in both neural and non-neural cells (Caselli & Boeve, 1999). In DS, it is suspected that an overproduction of β-peptide is followed by increased cellular oxidation and neuronal degeneration. Loss of cellular function and damage to macromolecules occurs through the Fenton reaction (which is the production of hydroxyl radicals through the interaction of H_2O_2 with iron).

Odetti et al., (1998) measured thiobarbituric acid reactive substances and 4-hydroxynonenal in DS fetal brains and found that both by-products of lipid peroxidation were increased when compared to controls. These mechanisms support findings that: SOD increases H_2O_2,. The increase in H_2O_2 can lead to the generation of hydroxy radicals. Increased concentration of soluble Aβ in the brain can produce ROS. Therefore,

comparative studies between FALS and DS may further understanding of either one or both diseases.

Neuropathology of ALS

Classical Sporadic ALS

The neuropathology of classical ALS is distinct: loss and degeneration of the anterior horn cells of the spinal cord and the lower cranial motor nuclei of the brainstem, striated muscles and gliosis (proliferation of the connective tissue of the nervous system such as astrocytes, oligodendroglias, and microglia). However, motor neurons in the occulomotor neuron system as well as Oneuf's nucleus are usually spared. A primary UMN finding is the loss of Betz cells in the motor cortex. Common neuropathological LMN signs are: synaptophysin, phosphorylated neurofilaments, ubiquitin, abnormal Golgi apparati, Bunina bodies and superoxide dismutase (Hirano, 1996).

In patients with ALS, synaptophysin (membrane protein in synaptic vesicles that may regulate release) expression is decreased in the presynaptic terminals of the anterior horn cells. The reduction of synaptophysin parallels the severity of the neuronal and dendritic loss (Kato, Hirano & Donnenfeld, 1987). There are also distinct phosphorylated neurofilaments (NFT's) present in the soma, which may influence impaired axonal transport (Carpenter, 1968). Ubiquitin-positive skein-like inclusions have been identified in sporadic, familial and Guamanian ALS, which have also been observed in other multi-system disorders of degeneration (Matsumoto et al., 1993). The fragmentation of the Golgi apparatus occurs early in the pathogenesis of ALS (Gonatas et al., 1992). The Golgi apparatus is involved in the processing and transport of plasma membrane,

lysosomal, secreted proteins as well as, axonal plasmic flow of fast moving macromolecules. Bunina bodies, small eosinophilic granules in the anterior horn cell of ALS, are also present (Bunina, 1962 as cited in Hirano, 1996). Finally, other filamentous hyaline inclusions in some of the surviving neurons similar to Lewy body-like inclusions, which are characteristic of some forms of FALS (Hirano, Kurland & Sayre, 1967), stain positively for neurofilaments (Mizusawa et al., 1989), ubiquitin (Matsumoto et al., 1993) and SOD1(Shibata et al., 1996).

Familial ALS

Neuropathology of FALS is not uniform. Some of the cases of FALS present with the same alterations as classical ALS. However, FALS associated with SOD1 mutations involves multi-system alterations. The spinocerebellar tracts, as well as the posterior column, are affected in FALS. The anterior horn cells of patients with FALS show Lewy body-like hyalin inclusions (Hirano, Kurland & Sayre, 1967), which are strongly positive for ubiquitin (Matsumoto et al., 1993), neurofilaments (Mizusawa et al., 1989) and SOD1 antibodies (Shibata et al., 1993).

Neurons in Clark's column are similarly affected as with the anterior horn cell. On the other hand, UMN's in FALS are remarkable preserved (Hirano, 1996).

Guamanian ALS

Neuropathology of Guamanian ALS is comparable to classical ALS. However, Alzheimer's neurofibrillary changes are observed and are similar to those found in Parkinson's dementia complex (Yoshida, Murakami & Hashizume, 1996).

MSOD1 Transgenic Mice

Pathological studies of the ventral motor root show loss of large, myelinated axons and in the dorsal root, large scattered swollen axons. Macrophages were present at all levels of the spinal cord. Similar to FALS, afferent sensory fibers within the dorsal column of the spinal cord are affected. However, less than 10% of muscle fibers show the characteristic angular clustering of denervated fibers (Dal Canto & Gurney, 1995).

Mice sacrificed at 70 days of age, before the onset of clinical symptoms, showed lesions in the large motor neurons of the spinal anterior horns. These lesions are small tightly packed cytoplasmic vacuoles, which encompassed the entire neuronal body, but do not affect the nuclei. The posterior columns are normal or minimally involved. There are decreased axons in the anterior root; surviving axons were swollen with vacuolated axoplasm. Vacuolar changes appear to be characteristic of mice sacrificed between 73 and 163 days of age (Dal Canto & Gurney, 1995).

Mice sacrificed between 101 and 163 days of age also show vacuoles, and examination of the ventral portion of the anterior horns reveal swollen axons packed with fibrillary material. Neurons contain eosinophilic hyaline inclusions. The anterior roots are severely affected and macrophages are extensive. There is a severe loss of motor neurons particularly in the anterior and lateral columns, and mild involvement of the posterior column and roots. Pathological processes are present in the brain stem. The pathological pattern of the older mice is the severe loss of neurons of the anterior horn and the presence of hyaline intracytoplasmic inclusions (Dal Canto & Gurney, 1995).

In addition, the Golgi apparatus show pathology and disorganization. The endoplasmic reticulum and the mitochondria are affected and reflect disorganization. At end stages, mitochondria are completely unraveled into a series of flat or curled membranes. Changes in white matter are noted at four months (Dal Canto & Gurney, 1995).

WT SOD1 Transgenic Mice

Subtle pathological changes have been noted in WT SOD1 mice even though there is no association with clinical developments of the disease. Pathological changes were seen in the anterior horn and in the motor fibers traversing the anterior columns. Vacuolated dendrites and swollen motor fibers are filled with NFT's and vacuoles. Finally, mitochondrial fragmentation has been observed in the WT SOD1 mice similar to the mitochondrial unraveling found in the MSOD1 transgenic mice (Dal Canto & Gurney, 1995).

Cognitive Function Associated with ALS

Cognitive behavioral deficits have been difficult to document in ALS. Pathogenesis is not always certain (Caselli & Boeve, 1999). In the mid 1980's, fragmented Golgi, now known to be associated with ALS, was noted in one case of ALS and dementia (Horoupian et al., 1984). In brain regions known for cognitive function, Nakano (1993) has observed loss of neurons and gliosis in the medial cortex between the CA1 regions and the subiculum of the hippocampus. Similarly, ubiquinated intracytoplasmic

inclusions have been found in the granule cells of the dentate gyrus of the hippocampus and the cortical neurons of the frontal lobe (Wightman et al., 1992).

However, findings associated with ALS and dementia are still being documented. Recently, Wilson, Grace, Munoz, He & Strong, (2001) noted that intraneuronal ubiquitin positive inclusions and neuritis were present in both cognitively impaired and cognitively intact patients with ALS; density was greater in cognitively impaired patients and greatest in the cingulate gyrus. Findings suggested that cognitive impairment in ALS was increasingly associated with a greater representation of neuropathological features suggesting a disease continuum. The possibility of genetic susceptibility was raised after patients diagnosed with ALS and incidence of ALS, dementia and Parkinson's disease in first degree relatives, were noted to be higher when compared with controls (Majoor-Krakauer, Ottman, Johnson & Rowland, 1994).

In patients without marked dementia, some cerebral cortical degeneration is present at autopsy; but degeneration is more obvious in patients with known dementia (Hudson, 1981). Dementia is more commonly associated with a subtype of ALS found in the Western Pacific Islands, ALS with parkinsonism (Hirano, Kurland, Krooth, & Lessel, 1961), as well as with inherited ALS (Kurland & Mulder, 1955). In other cases of ALS, patterns of dementia have varied as had neuropsychological assessment (Abrahams, Goldstein, Lloyd, Brooks, & Leigh, 1995; Abrahams et al., 1996; Boyar & Decker, 1984; Mitsuyama, 1984; David & Gillham, 1986; Iwaskaki, Kinoshita, Ikeda, Takamiya & Shiojima, 1990; Iwasaki, Kinoshita, Ikeda, Takamiya & Shiojima, 1990; Ludolph et al., 1992; Ferrer et al., 1993; Hartikainen, Helkala, Soininen & Riekkinen, Sr., 1993; Kew et

al., 1993; Cavalleri & DeRenzi, 1994; Abrahams, Goldstein, Kew et al., 1996; Abrahams, Goldstein, Al-Chalabi et al., 1997; Massman et al., 1996, Mitsuyama, 2000).

As pathological signs associated with dementia are not observable until after death, positron emission tomography (PET) has enabled researchers to establish a relationship between neuropsychological functioning and cerebral blood flow. Tanaka et al., (1993) found that cerebellar blood perfusion and oxygen metabolism were reduced in patients diagnosed with ALS with known dementia when compared with patients not known to have dementia. Other studies have supported those findings (Abrahams, Goldstein, Kew, Brooks, Lloyd, Frith, et al., 1996) while others have observed reduced cerebral blood flow in ALS patients with normal indicators of neuropsychological function (Kew et al., 1993). A prospective study of cognitive impairment in bulbar ALS found that deficits in neuropsychological function became worse over time, which coincided with neuronal loss in the anterior cingulate gyrus as demonstrated by MR spectroscopy (Strong et al., 1999). Reduced fluorodopa uptake was found in the caudate nucleus, putamen or both in five of 14 FALS patients (Przedborski, Dhawan et al., 1996). However, none of these patients showed symptoms of cognitive deficit; and, neuropsychological assessment was not conducted. Moreover, seven of FALS patients carried the mutation for SOD1 and only one out of these patients showed reduced uptake.

FALS associated with the mutations of SOD1 has not been identified with dementia. However, FALS patients without mutations of SOD1 have been associated with Frontal Temporal Lobe Dementia (FTD) and with chromosome 9q21-22 (Hosler et al., 2000). FTD is a neurodegenerative disorder, distinct from the malady described by Hosler and colleagues, which features clinical neuromuscular signs of ALS.

FTD has been linked with chromosome 17 (Lynch et al., 1994). In a study of one family with FTD, neuropathological examination of brains at autopsy showed atrophy of the frontal and temporal lobes, spongiform changes and neuronal loss in the substantia nigra. Cell loss was also observed in the amygdala with accompanying astrocytosis. The cerebral white matter and cerebellum were normal. Plaques, tangles or Lewy bodies were not present (Lynch et al., 1994). In contrast, one ALS patient at autopsy did reveal signs of both plaques and neurofibrillary tangles (Muler, Vieregge, Reusche & Ogomori, 1993). Therefore, while dementia with ALS still remains difficult to diagnosis, FTD has been linked chromosome to 17, FALS and FTD with chromosome 9, FALS associated with chromosome 21, and chromosome 21 identified with DS and SOD.

Neurobiological Correlates of Cognitive Behavior

The ability of rodents to demonstrate awareness of memory is known through the work of Olton and colleagues (1976; 1978; 1981). As demonstrated in these studies, the ability to acquire and recall behavioral tasks associated with spatial and non-spatial memory has been indicative of learning, motivation, and short- and long-term memory processes. Alternatively, impairment of these abilities has correlated with physiological and biochemical changes and pathology. Some physiological changes are due to hormonal restructuring, such as with development and aging (Luine, Bowling & Hearns 1990; Luine & Rodriguez, 1994; Luine, 1994; 1997; Wickelgren, 1997; Luine, Richards, Wu & Beck, 1998; Wilson, Puolivali, Heikkinen & Riekkinem, 1999). Other changes occur because of stresses: internally driven, from the environment, or both (Luine,

Spencer & McEwen, 1993; Luine, 1994; 1997; Wilson, Puolivali, Heikkinen & Riekkinen, 1999; Kneavel, 2000).

The Eight-Arm RAM has been a useful tool to capture these aspects of spatial and non-spatial memory as well as changes in cognitive functioning. Changes that may be due to developmental, biological or environmental processes-for example, lesions to the hippocampus impair performance on the RAM (Becker, Walker & Olton, 1980). The RAM is remarkably adaptive. Olton & Feustle (1981) proposed modification of spatial characteristics associated with the RAM in order to enhance understanding of the neural processes involved with non-spatial memory. In this study, the arms of the RAM were rotated around the center platform after the rat returned to the center from traversing the arm of the maze. This paradigm of non-spatial memory and cognitive mapping encouraged the rat to use extra-maze stimuli and stimuli located inside of each arm of the maze.

Although rats have contributed to investigations of cognition and memory because of their ease in handling, hardiness, and large neuroanatomical brain regions, cognition and behavioral assessment of mice is currently being pursued with fervor (Azar, 2000). The development of transgenic mouse models has prompted investigations of simple and complex behaviors in order to enhance understanding of the molecular genetics underlying these functions (Crawley, Belknap, Collins et al., 1997).

However, there are strains of transgenic and mutant mice that are fragile and must be housed in pathogen free environments. Other strains must have their environment strictly controlled: for example, one strain is so sensitive that mice will seize even if they hear the clap of hands (Malakoff, 2000). Therefore, some transgenic models are

phenotypically more "hardy" and may perform more effectively on one behavioral measure as opposed to another; other transgenic models are frail and have difficulty completing any task (Crawley, Belknap, Collins et al., 1997). Reliability and validity in test results are important as with any research study. These concerns are particularly important when reviewing rodent findings and considering behavioral paradigms to ascertain performance in mice. Levy, Kluge & Elsmore, (1983) chose the RAM to assess cognitive behavior in mice. Aside from being economical, convenient, and easy to interpret, it is ecological and builds on the natural foraging behaviors of the rodent. Mice are by nature inquisitive, and generally more active and exploratory than rats (Crawley & Paylor, 1997). Thus, the RAM is a natural complement to these ethologically driven behaviors.

In response to concerns raised by the research community about the ability of mice to perform on the RAM, investigators incorporated a modified food deprivation schedule (approximately 80 mg of chow per day) and a six-arm RAM. RAM acquisition, the effects of atropine sulfate on memory, and the role of the cholinergic system in memory processes in mice were investigated (Levy, Kluge, & Elsmore, 1983). Similarly, Whishaw & Tomie (1996) demonstrated that the performance of mice equal rats when tested with the RAM, but not with the water maze. Many other studies have supported the notion that mice have the ability to adapt and perform on the RAM (Ammassari-Teule et al., 1990; Ammassari-Teule, Hoffman & Rossi-Arnaud, 1993; Ammassari-Teule, Fagioli & Rossi-Arnaud, 1994). Therefore, the RAM is a useful and adaptive measure to assess cognitive function in mice, as well as rats.

However, paradigms may need to vary in order to capture strain differences (Reinstein, DeBoissiere, Robinson & Wurtman, 1983; Hyde, Hoplight & Denenberg, 1998). In an early study, the RAM was modified with plastic guillotine doors that were raised when the mouse returned to the center platform (Reinstein, DeBoissiere, Robinson & Wurtman, 1983). Ammassari-Teule, Hoffman & Rossi-Arnaud (1993) discovered that interstrain differences emerged when the RAM was used with an eight arm baited paradigm. In another study, interstrain differences were captured best with a water version of the RAM (Hyde, Hoplight & Denenberg, 1998). Food deprivation, among other factors have been viewed as disadvantageous in obtaining optimal outcomes. Ongoing debate surrounds research measures assessing cognitive function, factors governing their administration and use of one measure over another, which varies from laboratory to laboratory.

For example, the Morris Water Maze was used to measure cognitive impairment in the murine model for Alzheimer's disease. However, it did not reveal deficits in cognitive functioning in mice over-expressing mutant human β-amyloid precursor protein until the mice were 9-10 months of age (Hsiao et al., 1996). Bach, Hawkins, Osman, Kandel & Mayford, (1995) used the Barnes Circular Maze to assess spatial memory in another line of transgenic mice -- CaMKII Asp-286. When these mice were trained on the Barnes maze for 45 days, wild type mice (89%) acquired the task much faster than the transgenics (6%). The Barnes Circular Maze was chosen over the Morris Water Maze because it was felt that the Morris Water Maze was too taxing for subjects.

Aside from behavioral observations of cognition, mechanisms of neurochemistry associated with cognition have also been investigated. Luine, Villegas, Martinez &

McEwen (1994) assessed how amino acids as well as the monoaminergic system mediated cognitive performance. After rats experienced 21 days of stress, a decrement in performance was found on the RAM. However, when rats were administered phenytoin (which blocks amino acid release and transmission) and tianeptine (which enhances serotonin uptake and ameliorates depression) the performance of stressed rats and control rats were comparable. In a non-spatial task of cognition, Beck & Luine (1999) found that food deprivation was the mediating factor of stress and attenuated cognitive impairment. Changes in amino acid levels were due to stress and/or food deprivation. Increased serotonergic activity in the hippocampus and the prefrontal cortex was noted in rats that were food deprived. Thus, the effects of food deprivation modulated the effects of stress.

Another study found that endogenous septal GABAergic interneurons indirectly mediated mediated cholinergic septo-hippocampal activity (Durkin, 1992). GABA is one of two primary transmitters that activate inhibitory receptors in the central nervous system. However, the GABA agonist, muscimol, and the GABA antagonist, bicuculline, promoted significant inhibition of septo-hippocampal cholinergic activity in mice. Although diverse effects in transmission has been noted with GABA, further investigations of how amino acids, particularly GABA, are implicated in memory processes are sought.

The monoaminergic system has also been associated with cognitive processing and aging. Luine, Bowling & Hearns, (1990) found that performance on the RAM was significantly impaired in aged rats as compared to younger rats. Significant decreases of DOPAC in the hippocampus correlated with the impaired performance of aged rats. Interestingly, 5-HT was significantly reduced in the hippocampus of aged females

(Luine, Bowling, & Hearns, 1990). Activation of cholinergic activity in the cortex and the hippocampus was also associated with corresponding improvement on the RAM (Toumane, Durkin, Marighetto, Galey & Jaffard, 1988). However, differences in performance on the RAM were not found between aged and younger rats when they were water deprived as opposed to food deprived. Choline acetyltransferase (ChAT) activity in the cingulate cortex was positively correlated with choice accuracy, but negatively correlated when activity was measured from the sensorimotor cortex (Bernstein, Olton, Ingram et al., 1984). These inter-relationships between amino acid and monoaminergic pathways support the notion that biochemical processes following excitatory input to the hippocampus may be modulated by these neurochemicals, but these processes need further clarification.

Murine models for DS have long been examined for learning and behavior deficits. Comparative chromosomal mapping has revealed shared genetic homology between human chromosome 21 and mouse chromosome 16. Reeves et al., (1995) developed a mouse model that demonstrated behavioral abnormalities consistent with DS: spontaneous locomotor activity and deficits in memory as demonstrated by performance on the Morris water maze. Deficits in cognition were also shown in another mouse model of DS (Sago et al., 1998).

A study assessing scavenging effects of SOD1 resulted in the production of double transgenic mice expressing the SOD1/S100β genes (Gahtan, Auerbach, Groner, & Segal, 1998). The hippocampus expressing the product of S100β, a calcium binding protein, resulted in mice deficient in spatial memory. Hippocampal slices demonstrated normal synaptic physiology but long-term potentiation (exogenous stimulation that

increases the size of the postsynaptic potential and enhances memory) was impaired. Investigators suspected that increased H_2O_2 led to diminished LTP and affected cognitive behavior.

Levin et al. (1998) found that mice overexpressing extracellular superoxide dismutase (EC-SOD), showed impairment of long-term but not short-term memory. The EC-SOD over expressing mice were resistant to the cognitive effects of L-NAME (N^G-nitro-L-arginine methyl ester hydrochloride), a nitric oxide synthase (NOS) inhibitor. These findings suggest that decreased NO may have countered the effects of NOS inhibition by L-NAME (Levin et al. 1998). In another study of transgenic mice overexpressing extracellular SOD, synaptic plasticity was either blocked or attenuated depending upon the LTP induction paradigm (Thiels et al. 2000). Again, learning deficits were relegated to long-term memory consolidation.

Although the MSOD1 transgenic mouse model of FALS has not been assessed cognitively, Kostic, Gurney et al. (1997b) have established that monoaminergic systems are affected in the transgenic model of SOD1. DA levels in the caudate-putamen and nucleus accumbens are significantly lower in transgenic MSOD1 mice when compared with other non-mutant transgenic mice. Overall, clarification is needed on the human aspects of cognition and dementia in ALS, the association of SOD and cognitive deficits of DS, the potential relationship between FALS, DS and chromosome 21, and cognitive processes before symptomatic disease onset. Therefore, cognitive assessment of the MSOD1 transgenic mouse model of FALS is timely.

Ethical Concerns Relative to This Study

Inside and outside of the medical community, we are faced with a quandary. We depend on animal experimentation in order to gain understanding of complex disease processes; and, in conducting such research are unable to resolve ontological moral dilemmas of beneficence: is the good that one hopes to accomplish by reducing suffering in human beings worth the pain that is caused with animal experimentation? When opposing positions such as these present epistemology may be our best hope—in defining the meaning of "beneficence", in becoming aware of how research with animals is actually conducted, and in understanding the needs of the research community as well as society's. Increased information or shared dialogue in neutral settings offered by researchers, and others, inside and outside of the medical community, aids our understanding of the constitution of research and creates common ground. Specifically, how research has added to our quality of life, indeed our survival. For some researchers, and individuals outside of the research community, understanding what occurs in the research setting may be enough. Discussion will further dialogue and resolution of the quandary is achieved. For other individuals, dialogue is not enough. These researchers wish to have practical solutions to aid them in resolving the ethical dilemmas associated with the suffering of animals. The ontological essence of the dilemma may not change but at the very least, researchers can be assured that they have developed their experimental design in such a way that ethical dilemmas are addressed. It is the latter position that I wish to support by affirming a few practical recommendations that may be accommodated into experimental designs involving mice. In considering these factors and justifying beneficence, a brief survey of the ethical framework and the social,

economic and medical factors impacting animal experimentation may help clarify why accommodation of experimental design is necessary at all.

Tannenbaum & Rowan (1995) examined the ethical positions influencing the debate on animal research, which are: 1) ethical skepticism and relativism (there are no moral claims or right or wrong only facts); 2) absolute dominionism (animals may be used as humans see fit); 3) anthropocentric consequentialism (our care of animals is viewed indirectly as our care of humankind); 4) reverence of life (all life has a will to live and injury is unavoidable); 5) humane beneficence (animals experience pain and distress, which humans must not cause unnecessarily, and yet, animals may be killed painlessly for legitimate human purposes); and, 6) utilitarianism (of which there are two positions -- passive utilitarianism holds that the pain to animals is outweighed by the alleviation of pain to human beings or other animals and restrictive utilitarianism claims that animal research causes more pain than benefit).

Epistemological frameworks, such as these, aid our understanding of alternate views especially those of influential advocates and ultimately, the formulation and justification of our own position. In developing dialogue, some positions are difficult to understand and interpret for example, ethicists who insist that moral rights are not granted to either animals or humans. Tannenbaum & Rowan (1995) explain that this is the position that Peter Singer holds. As a "restrictive utilitarian," Singer believes that moral rights are not naturally granted to humans or animals. Quoting Singer, Tannenbaum & Rowan (1995, page 35) state that most research has produced little benefit for humans and " significant pain, distress, and discomfort for the animals."

While Singer is restrictive, the utilitarian approach offered by Bernard and Kaufman (1997) appears more moderate. To begin, the ethicists use language common to both the research and ethical communities. They raise concerns about research with animals but while doing so encourage one to consider answers. The variables may be controllable through accommodations of experimental design; here, dialogue appears possible. Bernard & Kaufman (1997) find that research with animals is wasteful. Experimental variables, such as handling of the animals, confinement, isolation, and food deprivation confound data and produce misleading results and furthermore, that some studies have even shown that findings differ when data obtained from animals are applied to humans.

However, it is difficult to understand which ethical perspective is posed by extreme animal welfare activists and wonder if dialogue is desired as violent deeds preclude an ability to share common ground. For instance, researchers in Great Britain have experienced mail and car bombings, razor blade letters, arsons, and violent demonstrations in front of their homes (Trull, 1999). These incidents have occurred in the United States. Trull (1999, page 1477) in a letter to the Editor of Science commented on how disturbing it was that when 87 medical researchers received letters with razor blades, Ingrid Newkirk, president of People for the Ethical Treatment of Animals responded, "'Perhaps the mere idea of receiving a nasty missive will allow animal researchers to empathize with their victims for the first time in their lousy careers'" (Letter to the editor of the *Atlanta Journal and Constitution*, 1 November 1999, p. A10).

In order for any ethical understanding to be reached or accommodation to become common ground, Conn & Parker (1998) suggest that the research community must

compassionately educate the public. For both researchers have observed college-educated citizens testify before public agencies about the advancement of health, and have heard these individuals state that advancement is solely dependent upon epidemiology, cell culture and computer simulation. Further support for increased knowledge is given by Roger Caras, president of the American Society for the Prevention of Cruelty to Animals who was quoted as stating "'most people just don't understand what health researchers do. Many are not old enough to remember how devastating childhood diseases used to be...Hearing allegations of cruelty to animals in research settings, they are perplexed that anyone would deliberately harm an animal" (Conn & Parker, 1998, page 1417).

Therefore, more information about the ethical arguments assumed by those inside and outside of the research community would aid the public, as well as researchers, in understanding and assuming their own ethical position. Additionally, increased information about economic factors influencing the development of medical research is also helpful, and can be frankly, surprising even for individuals already involved in research. Economic growth and rising costs often correlate with private and personal need, limited resources, and justifications in argument. For example, over a short period, animal models have proved useful to the medical community and some animals have become increasingly useful, which has prompted new regulations. Specifically, the care and use of mice, rats and birds has not been protected by outside federal agencies. Recently the US Department of Agriculture has agreed to regulate use of these animals (Malakoff, 2000). Biomedical researchers express concern regarding these regulations because of the added economic burden to academic and research settings, which already struggle with overwhelming and rising costs. Costs of husbandry of mice will guide

researchers even in choosing one academic appointment over another (Vogel, 2000). One researcher reported that Stanford University required $800,000 to $1 million dollars a year to maintain 2,000 to 3,000 cages of mice. Another researcher at a leading mouse supplier reported that despite generous salaries and state of the art laboratories offered by various academic centers, none have been able to match the low cost of husbandry offered by her current institution (Vogel, 2000).

Thus, development of transgenic models of mice has spurred growth and accordingly, views of disease process, which influences economic factors affecting future research and ultimately, the provision of medical care. The research community has been able to investigate biochemical alterations, pathogenic mechanisms, and the character and evolution of pathologies in ways they have not been able to before (Price, Sisodia & Borchelt, 1998). Understandings of the neurodegenerative process and disease pathology have changed. The emerging view is that neurodegenerative diseases have occurred because of the presence of mutant proteins, which are improperly folded or aggregated, that either directly or indirectly trigger pathogenic biochemical cascades effecting the physiology of subsets of neural cells before (Price, Sisodia & Borchelt, 1998). This view is possible because of research findings that are only available through the use of transgenic animal models of the disease (Green, 1999).

Despite these advances in research, and justification for an ethical position of beneficence, the medical community admits that current research methods and practices need improvement (Lauerman, 1999). Therefore, one could offer that we need practical solutions in meeting federal guidelines and expansion of humane alternatives. As early as the 1950's William M. S. Russel and Rex L. Burch published The Principles of Humane

Experimental Technique and suggested that researchers follow the "3 R's". The three R's proposed by Russel and Burch were: 1) replacement of animals by *in vitro* or test-tube methods, 2) reduction of numbers of subjects through statistical techniques, and 3) refinement of the experiment so as to cause less suffering (Russel & Burch, 1959 in Mukerjee, 1997). These principles were incorporated into federal regulations and are standard expectations of academic Institutional Animal Care and Use Committees (IACUC). Yet, clarification of how to practically implement these guidelines is frequently needed by research communities (Dr. Cheryl Harding, Chair, Hunter College of the City University of New York, IACUC, April 26, 2000, personal communication).

I believe that Russel and Burch (1959) proposed moral principles that were complementary to one another and complementary to an ethical position of human beneficence. For replacement, internet access to medical and psychological journal libraries, such as "Pubmed" (the electronic library for the National Institutes of Health) has influenced the way in which background literature is reviewed before experimentation. Interconnected electronic libraries facilitate searches for prior experiments, as well as new statistical models, and influence the ways in which medical and research communities approach new projects. Animals may not always be the best and least costly alternative.

With respect to reduction of numbers, the second point raised by Russel & Burch (1959), increasingly sophisticated statistical approaches have changed the way in which research findings are presented to research communities as well as the public. The research community seeks new statistical approaches; as increasingly complex experimentation has necessitated the development of multi-variate designs. Some new

statistical approaches have reduced or eliminated the need for balanced designs and large numbers of subjects in order to achieve statistical power. High statistical power requires large numbers of datapoints. However, provided appropriate analytical techniques are applied the large numbers can be obtained by repeated measurement on a smaller number of subjects over time. (The statistical analysis used with this project incorporated such an approach.)

However, of the three R's proposed by Russel & Burch (1959), it is reduction of suffering in animals that has proved to be one of the more difficult challenges researchers face. The United States Department of Agriculture requested help in defining the word "distress" under the Animal Welfare Act when considering prospective legislation and received 2600 responses over a four-month period (Holden, 2000). To summarize it was suggested that distress should be viewed as a state in which an animal cannot escape from resulting in negative effects on its well being (Holden, 2000). Having a working definition does not mean that the problem is resolved. There are no simple measures that indicate when an animal who is stressed is now distressed (Donnelley, 1989; Holden, 2000). Most researchers maintain that set points where suffering is experienced may differ for each animal across species and furthermore, within the same species. Two identical animals in different environmental climates will respond differently to identical stimuli (Obrink & Rehbinder, 1999). For instance, stimuli such as, light, sound, nutrition, husbandry, caging, and bedding material can all be potentially stressful for some animals.

Practical solutions that will reduce suffering and aid researchers in addressing ethical dilemmas posed by experimental design were proposed by a researcher of the European academic community. However, this investigator was concerned with research

results as opposed to beneficence in animal experimentation. Nonetheless, Poole's (1996) findings are worthy of consideration. He proposed that an animal's state of mind has direct bearing on research results and cites three factors known to contribute to an animal's psychological well-being: 1) social factors, 2) physical environment, and, 3) the handling of the animal. A discussion of how each factor can enhance experimental design follows.

With respect to social welfare, laboratory mice are usually housed in cages in groups and form dominance hierarchies. Females are more tolerant of this organization than males. Data has shown that subordinates exhibit physiological signs of stress; and yet, single housing is considered potentially distressful. Poole (1996) draws upon the work of Hucklebridge et al. (1976) who demonstrated that hormonal profiles of singly housed males match dominants. Therefore, singly housed animals promote optimal well-being in mice. A second factor suggested by Poole (1996) was the capability of the animals to control their physical environment. Potential stressors for animals were such things as loud or ultrasonic noises (as with computers), continuous illumination, food and water deprivation, living in a soiled cage, changing cages too often, frequent movement, and being tested during times when nocturnal animals habitually sleep. Thus, environments that are species specific are of benefit. The third factor affecting the psychological well-being of animals is the way in which animals are handled by investigators. Optimally, the same investigator should conduct all experiments, and gently. Poole (1997) states that most laboratory mammals and birds recognize humans and strangers make them nervous. A handler who is unfamiliar to the animal may cause additional stress. Poole (1997, page 121) summarizes: "Good handling and training

animals to cooperate, not only improves the quality of the relationship between carer (sic) and animal, but also allows the animal to exercise its intelligence".

Therefore, it is possible to assume a position of humane beneficence while equally committed to scientific achievement. Both positions depend on the investigator's ability to manipulate and control variables in the environment. Therefore, if husbandry is enhanced, research is enhanced, ethical dilemmas addressed, and the pain and distress in research subjects reduced. Moreover, practical solutions of husbandry aid researchers so they are better prepared morally to address ethical dilemmas of reducing suffering in animals while also reducing suffering in human life. (These factors were incorporated into this experimental project and are explained in "Methods," Chapter Three, under the sections of "Husbandry" and "Behavioral Evaluation.")

In assuming an ethical position of human beneficence, Tannenbaum & Rowan (1985) suggest that there are three justifications to consider: benefits to humans; benefits to other animals; and, the value of science to research and knowledge. The authors suggest that the dilemma is in balancing the futile belief that each research project is potentially of great benefit and to what end one should ultimately pursue knowledge. The deciding point for Tannenbaum & Rowan (1995, page 42), and I would offer for anyone assuming a position of humane beneficence is, "'how much pain, distress, suffering, or killing is justified in the service of these aims.'"

Therefore, in justifying humane beneficence: the devastating aspects of ALS demand that experimental investigations be pursued with rigor. ALS is a neurodegenerative disease, a tragic disorder ultimately fatal (Rowland, 1998). The emotional and psychological devastation to the patient and family burdens them and

changes the way in which they view their lives. These burdens may or may not be supported by psychological and spiritual frameworks, which impact decisions of medical care and outcomes (Murphy, Albert, Weber, Del Bene & Rowland, 2000) Economic issues abound as well, and ultimately, affect the provision of medical care. When these burdens become overwhelming for patients and family members, physicians often end up treating multiple diseases and multiple family members. Stress on the medical team also increases as they struggle to support patients and families. Resources, such as medical expertise, become limited as medical professionals are increasingly stretched to meet these overwhelming demands. Therefore, research with animal models of ALS is essential. There is no other method available to the scientific community at this point in time. Given the tremendous humanitarian benefit the animal model of ALS must be used for research in order to discover a cure, identify potential treatment for ALS, as well as lessen ethical, medical, psychological, spiritual and economic burdens affecting the patient, family, physician, interdisciplinary treatment team, and indeed all of society.

Chapter III

RESEARCH DESIGN

Overview

This chapter presents the experimental design used to test the four hypotheses of this dissertation which are:

H_1: Younger MSOD1 mice will show an enhanced ability in learning a spatial memory task when compared with older MSOD1 mice.

H_2: WT SOD1 mice will demonstrate enhanced ability at learning a spatial memory task and their performance may be influenced by the presence of the transgene, gender or age when compared with MSOD1 mice.

H_3: As elevated levels of glutamate have been shown in the spinal cord of patients diagnosed with ALS and excitotoxicity is suspected to be associated with mutations SOD1 it is suspected that WT SOD1 mice will show decreased concentrations of glutamate in brain regions associated with cognitive function, which may be influenced by the presence of the transgene, gender, or age when compared with MSOD1 mice.

H_4: As monoamines are involved with cognitive functioning, it is suspected that there will be differences between the two transgenic lines and they may be influenced by the presence of the transgene, gender, or age.

The chapter is divided into five sections that describe: 1) the sample of subjects in the study; 2) the procedures that were followed during the evaluation period; 3) the

measures that were used in ascertaining outcomes, predictions, and controls; 4) a discussion of the statistical analyses conducted; and 5) comments on the reporting of statistical findings.

Subjects

The experimental protocol was conducted according to the guidelines of the National Institutes of Health for the use of live animals and was approved by the Columbia University Institutional Animal Care and Use Committee. A copy of the approved protocol was also reviewed by the Chairman of the Hunter College Institutional Animal Care and Use Committee.

Two transgenic lines of mice were evaluated for this study:

1) MSOD1, G93A transgenic mice carry more than eighteen copies of the MSOD1 gene and is one of the animal models of familial amyotrophic lateral sclerosis; and,
2) WT SOD1, SF-218 transgenic mice one of the early animal models of DS, carry at least eight copies of the non-mutated or WT SOD1 gene.
3) Control subjects were littermates (Tg-): mice born in each litter of both lines that do not carry either mutated or human SOD1 protein.

Table 1 details the total number of mice used for the experiment, by the classes of measure administered in the study (behavioral and neurochemical) and the neuroanatomical regions examined during the study (frontal cortex and hippocampus), age at which the mice entered into testing, were euthanized and gender.

Table 1
Total Number of Transgenic Mice Used in Experiment By Transgenic Line, Measure, Neuroanatomical Region, Age and Gender

	Numbers of Mice					
	Behavior		Neurochemistry			
			Frontal Cortex	Frontal Cortex	Hippocampus	Hippocampus
Transgenic Line	6-12 Wks	8-15 Wks	8 Wks	15 Wks	8 Wks	15 Wks
Mutant SOD1 (MSOD1 Tg+)	6 Females	8 Females	9 Females	8 Females	10 Females	7 Females
	6 Males	10 Males	9 Males	10 Males	10 Males	10 Males
MSOD1 Littermates (MSOD1 Tg-)	6 Females	9 Females	8 Females	9 Females	7 Females	9 Females
	6 Males	12 Males	12 Males	12 Males	13 Males	12 Males
Wild Type SOD1 (WT SOD1 Tg+)		6 Females	7 Females	6 Females	7 Females	6 Females
		14 Males	11 Males	13 Males	12 Males	12 Males
WT SOD1 Littermates (WT SOD1 Tg-)		7 Females	3 Females	7 Females	5 Females	6 Females
		9 Males	9 Males	7 Males	9 Males	9 Males

Mice were evaluated in three groups: the first group of MSOD1 mice entered into testing at six weeks of age, (i.e., young mutants, YMSOD1 mice); the second group of MSOD1 mice entered into testing at eight weeks of age, (i.e., old mutants, OMSOD1 mice); and, a third group, WT SOD1 mice that entered into testing at eight weeks of age, (as there were no young WT SOD1 mice entered into testing the acronym identifying WT SOD1 mice entered into testing at eight weeks of age will be WT SOD1 mice). The young mutants were evaluated behaviorally, but not neurochemically. The old mutants and old Wild Types were evaluated behaviorally and neurochemically, and were matched with eight week control mice that were not evaluated behaviorally.

Nine mice were either euthanized or expired before the evaluation period ended: three Tg- littermates expired before testing began (one MSOD1 female, one MSOD1 male and one WT SOD1 male); three Tg- littermates (two MSOD1 females and one WT SOD1 male) were consistently agitated and could not be tested; one MSOD1 Tg- littermate female became pregnant; and, one MSOD1 Tg+ male developed leg weakness at 15 weeks. In total, 172 mice were evaluated for this experiment.

Experimental Procedures

Transgenic Mice

The G93A transgenic line or MSOD1 mice are produced by mating hemizygote (carriers of human SOD1 transgene which are inserted into the chromosome in a random manner and displayed in a characteristic tandem fashion) transgenic males (C57bl/6 x SJL; Jackson Laboratory, Bar Harbor, ME strain name - B6SJL-TgN(SOD1-G93A)1Gur) carrying the point mutation glycine for alanine (Gly →Ala) at codon 93 of the human

SOD1 gene with hybrid females (C57BL/6JF x SJL/J M; Jackson Laboratory strain name - B6SJLF1/J). Off spring carry more than 18 copies of the MSOD1 gene and express elevated levels of SOD1 activity (more than fourfold) when compared to nontransgenic littermates (Gurney, 1994).

Hybrid females from the Jackson Laboratory (B6SJLF1) also produced the second line of transgenic mice evaluated for this experiment. The SF-218 transgenic line was originally generated by Epstein et al. (1987) as an animal model for DS. SF-218 transgenic males (C57bl/6 x SJL/J) carry eight copies of the WT SOD1 gene. Heterozygous off spring, as well as successive generations, showed elevated levels of SOD activity in the brain, heart, lung, liver and red blood cells (Epstein et al. 1987). Both mouse lines were maintained in colonies at Columbia University under the direction of Serge Przedborski, M.D., Ph.D.

Both of the transgenic lines of mice evaluated in this experiment were developed through the manipulation of the mouse genome. DNA was integrated into chromosomes after microinjection into embryos, which were then implanted into pseudopregnant female mice. Offspring were screened to identify carriers of the transgene. When a transgene encoding a native protein is incorporated into a cell that already expresses the protein, the gene is overexpressed. Alternatively, if the transgene encodes a mutated or modification of that protein onto the transgene expressing the normal protein the animal model expresses a modified version of that protein, which affects the animal in either a "gain-of-function" or a "loss-of-function" fashion. This latter process mimics a mutation found in human disease (Williams & Wagner, 2000).

Genotyping

Progeny of both lines of mice were genotyped on postnatal day 14 by polymerase chain reaction (PCR) on purified genomic DNA (QIAamp, Qiagen, Chatsworth, CA), extracted from 1 cm of tail. Genotyping was performed at least twice for each mouse pup and after confirmation of genotype, each pup was assigned an arbitrary code and labeled male or female. The Investigator was "Blinded" to all genomic factors influencing study outcomes. Therefore, the Przedborski laboratory maintained all genomic information and delivered the "Unblinded Code" to the Blinded Investigator for statistical analysis after completion of all behavioral testing and euthanasia.

Husbandry

Location. Approximately one week before testing mice were moved from the Columbia University barrier facility to an animal-testing cubicle located in the animal facility. The YMSOD1 mice were maintained and tested in an isolated animal cubicle located on a floor that housed moderate and large animals. The MSOD1 and WT SOD1 were maintained and tested in a sterile area of the animal facility that housed smaller animals. Incorporating Poole's (1996) suggestions in addressing the animals' need for social welfare, individual plastic cages with filter tops housed each mouse inside the animal-testing cubicle until completion of the experimental trial. The cages were located on aluminum shelving that lined one wall of the animal-testing cubicle.

Physical Environment. Each plastic cage contained beta (shavings) and two nestlets (woven bedding material) for warmth and comfort. Nestlets protected the mice against ranging temperatures and provided material with which they could control their

environment (Van de Weerd, Van Loo, Zutphen, Koolhaas & Baumans, 1997). Nestlets also enriched the mouse's environment. Animals from enriched environments are physiologically and psychologically more stable and "better representatives of their species" (Van de Weerd, Van Loo, Zutphen, Koolhaas & Baumans, 1997, page 133). Sherwin (1997) adds that nest building is spontaneous and innate; therefore, when nestlets are added to cages mice are motivated in expressing natural behaviors.

The physical environment also included the noise and movement transmitted throughout the Animal Facility. As such, the protocol required procedures that addressed these techniques of husbandry as well. For example, the Animal Facility's technicians clean cubicles daily most often between the hours of 8:00 AM and 2:00 P.M.. However, some mammals mark their home range and to be placed in clean unfamiliar cages on a daily basis can be highly stressful for the animal (Poole, 1997). Therefore, the protocol requested that the cages for the SOD1 mice were to be cleaned after 12 P.M. on Fridays and therefore, after completion of testing measures for the week. Additionally, the Animal Facility's technicians check all animals daily. After receiving approval from the Veterinarian supervising the Animal Facility, a sign was posted on the outside of the cubicle door stating that technicians should not enter the SOD1 animal cubicle until after 12 P.M. Therefore, environmental noise was modified by the technicians entering the animal cubicle before 12 P.M. and the cages of the SOD1 mice were changed once a week on Friday. Regardless of these controls, all three groups of mice were exposed to the noise generated by the technicians in the animal room, movement associated with cleaning of other cages from other cubicles in the animal facility, and the noise generated by the movement of carts carrying animals for testing to other laboratories located on

other floors of the building. All three groups of mice were also exposed to the noise generated by larger animals located in other areas of the Animal Facility, e.g., occasional barking of dogs, etc. However, after 2:00 P.M. the Animal Facility was for the most part quiet.

Lighting and temperature of the animal cubicle were also controlled. Overhead fluorescent lighting was set on a reverse light/dark cycle (lights on at 12 P.M., lights off at 12 A.M.). Illuminance from the middle of the maze under fluorescent lighting conditions was 150 lux (approximately three feet from the light source, United Detector Technology, 40 A Opto-Meter). As mice are nocturnal conditions were simulated by providing dim red lighting during testing (Philips, Colortone Light Bulb, 25 Watts). The YMSOD1 experienced dim red lighting during behavioral testing. The OMSOD1 and WT SOD1 mice were exposed to continual dim red lighting. Lights were not turned off after testing was completed. Illuminance from the middle of the maze under these conditions was measured at .5 lux (approximately four feet from the light source, United Detector Technology, 40 A Opto-Meter). Room temperature was recorded daily by the Animal Facility technician and optimally maintained at 70 $\pm$ 2° F.

Food and Water. Mice retained free access to water at all times. Food (Rodent Chow) was available ad lib from Friday afternoon until Sunday afternoon. On Sunday afternoon the food was removed from the mouse's cage. Mice were food controlled until testing was completed at weeks end. Monday through Friday, after the testing period, mice were fed approximately 2 – 4.5 grams of food depending upon weight gain or loss when they returned to their home cage.

Rodent behavioral protocols customarily food deprive subjects until they are approximately 85-90% of their body weight. However, as testing occurred during a period associated with growth and development and food deprivation has been related to stress (Beck & Luine, 1999), mice were not food deprived. Access to food was limited and weight was maintained during evaluation as food restriction has been associated with increased life span in mice (Silberberg, Jarrett & Silberberg, 1961). Free access to food was available on weekends and during rest periods. Rest periods for OMSOD1 mice and WT SOD1 mice occurred when mice were ten, 11, 13 and 14 weeks of age. Despite attempts to maintain consistent caloric levels of food, some mice demonstrated more activity than others did. Thus, on Monday mornings, an occasional mouse would show signs of lethargy (e.g., weakness or hypotaxia). If a mouse was lethargic, food was immediately placed in the bottom of the mouse's cage near the mouse's snout. After the mouse consumed the food, signs of lethargy in the mouse dissipated (the mouse was observed running around inside the cage) and testing commenced.

Evaluation Procedures and Measures

Evaluations of the mice were ascertained behaviorally and neurochemically. The RAM measured cognitive function; and, high-performance liquid chromatography (HPLC) quantified monoamines (DA, NE, and 5-HT, their metabolites DOPAC, HVA and 5-HIAA, and amino acids (glutamate and GABA).

Behavioral Evaluation

Mice are nocturnal animals. Therefore, they were evaluated for cognitive performance Monday through Friday approximately six hours after their dark cycle was initiated and during their most active period inside the animal-testing cubicle. The cage was removed from the aluminum shelf and placed upon a chair, the top taken off, and the mouse encouraged to move to back of the hand of the Blinded Investigator. The Blinded Investigator placed her hand in the center of the RAM and encouraged the mouse to move to the platform. When the mouse completed the trial or demonstrated that it was unable to complete the trial (by not moving on the maze or by repeating errors past the time allotted for the trial) the Blinded Investigator followed the same procedure in returning the mouse to home cage. Mice were tested in reverse sequence every other day in order to reduce the likelihood of confounding olfactory cues on the maze. On almost all occasions, the Blinded Investigator evaluated all mice.

Weights of the mice were recorded Monday through Friday after they had completed the RAM. The mice were placed on the scale in the same manner in which they had been moved to the RAM. An open bowl was added to the scale and balanced. Most often, mice would sit inside of the bowl for a period of time while the weight was being recorded (Polder scale, set for grams, weighed the first three litters of mice; a Scout Electronic Balance [Ohaus Corporation], also set for grams, weighed the remaining nine litters).

Modifications to Enhance Animal Husbandry. In summary, modifications to the experimental design included: 1) individual housing; 2) acclimating the mouse to the testing cubicle before testing; 3) testing the mouse inside the housing cubicle; 4)

modifying the food regime, food maintenance as opposed to food deprivation; 5) environmental enrichment, by providing material for nesting; 6) changing cages once a week, after the completion of testing and allowing acclimation to clean cages over the weekend; 7) maintaining mice under constant dim red light conditions; 8) testing animals during their nocturnal and most active period; 9) handling mice gently and moving from one apparatus to another on the back of the Blinded Investigator's hand; and, 10) when possible having the same Blinded Investigator conduct all testing and procedures involving mouse care, i.e., weighing.

Behavioral Measures

An eight-arm RAM was used to obtain behavioral measures of cognitive outcomes in the mice. The RAM was constructed from clear Plexiglas, which locked into a flat base covered with light brown contact paper that prevented visibility of stimuli from below. The arms of the maze were 37.3 cm long and 7.7 cm wide with 9.5 cm walls. The center was octagonal with a 23 cm radius. The maze rested approximately 92 cm off the ground on a stationary plastic cart.

Mice have demonstrated discrimination to visual cues, particularly patterns demonstrating brightness, contrast and variability (Hyde & Denenberg, 1999). Hence, a mixture of maze cues differing in brightness and pattern facilitated spatial orientation for the mice: the north wall featured a large blue door; the south wall black and white checkered contact paper; the west wall houses a slated vent and is white; and, the east wall held the rack of aluminum shelving. The scale that was used to weigh the mice and husbandry supplies were located on one shelf. The remaining shelves held mice caging.

On the first day of testing, slivers of peanuts were placed along each arm of the maze: one-third, two-thirds and at the ends of each arm near a black Plexiglas food partition. From the fourth and subsequent days of testing, a sliver of the peanut rested behind the Plexiglas partition near the end of the arm of the maze.

Each trial on the RAM was initiated by placing the mouse in the center of the maze and the mouse removed from the maze after five minutes or after the mouse had visited all eight arms randomly at least once. If the mouse had not visited all eight arms of the maze within five minutes, the mouse was returned to its cage and the trial was not recorded. A visit was recorded if the mouse traveled approximately half way down the arm of the maze, time was recorded with the last choice after the front two paws and half of the mouse's body entered the arm of the maze, and an error was counted if the mouse visited a previously visited arm. If a mouse indicated that it was going to visit a third arm on the maze in succession (response stereotypy) an associative noise (such as a foot stomp) was made, which in some cases was successful at deterring the mouse. If the mouse continued with choosing the third arm in succession, the mouse was returned to home cage, and another trial was attempted later that morning.

The Blinded Investigator stood in the same location during periods of evaluation and wore an examination gown and gloves. The RAM was washed with a mild detergent after the completion of each trial and between mice to remove olfactory cues. At the end of the evaluation period mice, were euthanized by rapid decapitation in a separate room.

Outcomes, Control and Prediction Measures

Outcomes. There were four outcomes of the behavioral task: where the mouse made their first error on the maze (First Error); the total correct number of choices on the maze (Total Correct); the total number of errors on the maze (Total Errors); and, the total amount of time that passed until the mouse visited the eight arms of the RAM (Duration).

Controls. In all analysis, I controlled for the mouse's age on entry into the study and because the behavioral data were longitudinal, with repeated measurement on each mouse, I also included the number of days that the mouse has practiced the behavioral task (DP) as a predictor in my regression analysis.

Predictors. My principal question predictors were: mouseline (described by variables MSOD1, YMSOD1, OMSOD1 and WT SOD1), presence of transgene (described by Tg+), and gender (described by Male). However, in subsequent regression analysis, I concluded that mouseline and age of mouse on entry were collinear ($r=0.4635$). Plots of entry-age against transgenic line revealed three principal groupings of mice: MSOD1 mice at 42-43 days of age; MSOD1 mice 53-60 days of age; and WT SOD1 53-60 days of age. Therefore, new predictors for transgenic line and entry-age were created accordingly: "young mutants," YMSOD1 mice 42-43 days of age upon entry; "old mutants," OMSOD1 mice 53-60 days of age upon entry; and, old "Wild Type," WT SOD1 mice 53-60 days of age upon entry.

Neurochemical Measures

HPLC assessed neurochemical activity in the frontal cortex and hippocampus. Amino acids were measured in the frontal cortex; monoamines and their metabolites were measured in both brain regions.

Following rapid decapitation the mouse's brain was quickly removed from the cranial cavity and submerged in finely chopped dry ice. Frozen whole brains were stored in a Revco freezer at -80°C. For dissection, brains were removed from the Revco freezer and partially thawed on an ice-cold Petri dish. The frontal cortex and hippocampus were dissected out freehand, weighed, and placed in 1.5 ml Eppendorf tubes. The tubes were stored in the Revco freezer at -80°C until transported on dry ice to Dr. Luine's laboratory at Hunter College.

Monoamine analysis

In the Hunter Laboratory, frontal cortex and hippocampus tissue samples were transferred from the 1.5 ml Eppendorf tubes to new 1.5 Eppendorf ml tubes with 60 µl of sodium acetate buffer containing α-methyldopamine (as an internal standard). After freeze-thawing and centrifugation, the supernatant was removed and 2 µl of 1 mg/ml ascorbate oxidase solution (Sigma) was added to each sample. Forty microliters were injected into a Waters 2690 Separation Module chromatographic system with an ESA Coulocomb 5100A electrochemical detector with the screening electrode set at +0.018 V and the detecting electrode at +0.4 V. Referencing standards using peak integration with a computer assisted Waters Millenium system calculated concentrations of neurotransmitters and metabolites. A sample run averaged 40-50 minutes. Concentrations

of monoamines and metabolites in the frontal cortex and hippocampus are expressed in pg/mg of tissue weight.

Amino Acid analysis

Quantification of amino acids were obtained following derivatization of an aliquot of the supernatant with o-phthalaldehyde and β-mercaptoethanol, which was injected into a Waters 717 automated refrigerated injected system using a Waters 590 pump. The mobile phase (12% methanol and 5% acetonitrile) was pumped through a C-18 reverse phase column (Waters Nova-Pak) then an ESA 5011 analytical cell. An ESA 5100A Coulochem detector was set at +0.20 V to oxidize and remove derivatization contaminants and +0.40 to oxidize and detect amino acids. Sample runs averaged between 60 and 90 minutes in order to separate glutamate and GABA. Picograms of glutamate and GABA were calculated by comparing the ratio of the integrated peak areas of the transmitters and the internal standard, homoserine, in sample chromatograms with the same ratio of peak areas in chromatograms from external standards. Concentrations of glutamate and GABA are expressed in ng/mg of tissue weight.

Outcomes, Control and Prediction Measures

Outcomes. There were eight outcomes in the neurochemical analysis: concentrations of six monoamines (DA, NE, DOPAC, HVA, 5-HT, and 5-HIAA) from brain regions associated with cognition (frontal cortex and hippocampus) and concentrations of two amino acids (glutamate and GABA) from the frontal cortex.

Controls. Study mice were euthanized at 15 weeks of age and were matched with eight week old control mice that had not been exposed to the RAM. The age of the mouse when euthanized and whether they had been exposed to the RAM were controlled statistically in the prediction of outcomes.

Predictors. My principal question predictors were: mouseline (described by variables MSOD1), presence of transgene (described by Tg+), and gender (described by Male).

Statistical Analyses

Data obtained from behavioral outcome measures were entered into a spreadsheet created by StatSoft, Inc., (2000), Statistica for Windows, Tulsa, OK., and transferred into Intercooled Stata 7.0 for Windows 98/95 NT, copyright 1985-2001, Stata Corporation, College Station, TX, using Stat/Transfer, copyright 1986-2001 Circle Systems, Inc., Seattle, WA. Data for neurochemical analysis of monoamines and amino acids were entered into a Microsoft Excel spreadsheet and pasted into Statistica for Windows, and transferred to Stata Statistical Software: Release 7.0 for data cleaning and statistical analysis using Stat/Transfer. Data cleaning and statistical analyses for all outcome measures were conducted using Stata Statistical Software: Release 7.0.

Coefficients obtained from the regression analysis conducted by Stata 7.0 were entered into another Excel spreadsheet. The estimation of regression coefficients were then transferred to PowerPoint, (Microsoft), and used to recover predicted trajectories for prototypical mice. The fitted trajectories are therefore predictors from the fitted

regression models and use the data on all mice, on all occasions, to generate the best estimate of learning of particular classes of ("prototype") mice.

Cleaning of Data-Behavioral Outcomes

RAM trials that did not meet study criteria were removed from statistical analysis. Statistical significance was not found with OLS regression analyses on the RAM trials that were removed. Out of 2,035 RAM trials: 177 were not included in data analysis due to sequencing ($p<0.387$); 189 trials were removed because they were not completed within the allotted time period ($p<0.147$); five trials were not recorded because the mice were highly agitated and subsequently euthanized ($p<0.575$); and, 15 trials were not included because the mouse was lethargic ($p<0.167$).

Once these trials were omitted, the four behavioral outcomes were assessed for normality, symmetry, and peakedness using univariate plots (normal probability plots, histograms and stem leaf).

The distribution of Total Errors had a long upper tail, which was successfully reduced by a square root transformation. The transformed variable was used in subsequent analysis. The distributions of the remaining three outcomes were not normal (First Error and Total Correct were asymmetric, and Duration was platykurtic). I could not resolve these problems with transformation and analyzed them subsequently untransformed.

Preliminary OLS regression analyses were used to identify atypical datapoints, using the Cook's D statistic (Hamilton as cited in Stata Reference Manual, 2001). Eight observations fell below tolerance and were removed from the data set.

Cleaning of Data–Weights of Mice

The distribution of weights for the mice were assessed for normality, symmetry, and peakedness in a similar manner. The distribution of Weight had a long upper tail.

However, before statistical tests for normality were conducted, 72 values were inputed (added) by averaging observations of the mouse's weight preceding and following the missing observations.

Again, preliminary OLS regression analysis was used to identify atypical datapoints, and using Cook's D statistic one extreme datapoint was removed. After removal, I took the square root of "Weight" in order to diminish the long upper tail. The transformed value was used in subsequent analysis.

Cleaning of Data–Neurochemical Outcomes

Nine samples for HPLC analysis of monoamines from the frontal cortex and three samples from the hippocampus were not included with the data assessed for normality because the Eppendorf tubes were either mislabeled or difficult to read after undergoing analysis by the HPLC.

The remaining data were examined with plots of normality, stem leaf and bar graphs and all reflected long upper tails. Data were modified by mathematical square root to retract the long upper tails of the curves.

OLS regression analyses were used to identify atypical datapoints in the concentrations of neurochemicals, using Cook's D statistic. Transformation did not resolve the problems of normality and the distribution of the outcomes for concentrations

of DA and DOPAC in the frontal cortex and 5-HT in the hippocampus. These values remained non-parametrically distributed despite transformation. Data ranges before and after modifications are noted in Table 2 as well as the numbers of atypical datapoints that were removed from each concentration.

Cleaning of Data – Amino Acid Outcomes

Concentrations of Amino Acids from the frontal cortex were also examined with plots of normality, stem leaf and bar graphs. Both data sets reflected long upper tails and were subsequently mathematically square rooted.

OLS regression analyses were used to identify atypical datapoints using Cook's D statistic. Data ranges before and after modifications are noted in Table 2. Two extreme datapoints were removed from GABA and the remaining transformed variables were used in subsequent analysis.

Table 2

SOD1 Neurochemical Data Ranges Before and After Robust Regression Cook's D to Identify Extreme Measures, and Square Root to Introduce Curve Normality

	RANGES OF DATA					
Variables	**Observations**	**Minimum**	**Maximum**	**Observations**	**Min Sq Root**	**Max Sq Root**
Frontal Cortex						
DA	140	0.9	1920.9	132	0.9	6.9
NE	140	2.7	260.4	138	1.7	13.4
DOPAC	140	1.8	1983.7	132	1.3	11.1
HVA	140	2.7	566.8	137	1.7	12.1
HVA/DA	140	0.3	51.3	139	0.5	5.9
5-HT	140	0.8	300.8	132	0.9	16.1
5-HIAA	140	17.1	334.6	140	4.1	18.3
5-HIAA/5-HT	140	0.6	60.8	136	0.8	5.8
Glutamate	149	8.1	566.4	149	2.8	23.8
GABA	149	16.9	493.2	147	4.1	17.6
Hippocampus						
DA	144	0.8	910.2	140	0.9	17.9
NE	144	11.9	1286.9	139	5.7	14.4
DOPAC	144	1.2	1154.1	143	1.1	25.2
HVA	144	1.9	250.8	144	1.4	15.8
HVA/DA	144	0.2	32.7	140	0.4	3.4
5-HT	144	14.1	958.6	137	4.3	20.7
5-HIAA	144	16.3	711.1	141	6.6	17.3
5-HIAA/5-HT	144	0.4	7.5	142	0.6	2

Longitudinal Analysis

In order to handle the unbalanced nature of my research design and the repeated measures present for each mouse in the dataset, I used individual growth modeling and generalized least-squares (GLS) regression analysis to analyze my behavioral data. The two hypotheses analyzed with GLS regression analysis were:

H_1: Younger MSOD1 mice will show an enhanced ability in learning a spatial memory task when compared with older MSOD1 mice.

H_2: WT SOD1 mice will demonstrate enhanced ability at learning a spatial memory task and their performance may be influenced by the presence of the transgene, gender or age when compared with MSOD1 mice.

For each behavioral outcome, my regression strategy was similar. In each case, I began by fitting the most complex model feasible. This model contained my control and question predictors as main effects, as well as two-way, three-way and four-way effects. Then, in a series of model comparisons I eliminated unnecessary higher order terms in order to produce a final, more parsimonious model that adequately explained variations in the outcome.

The statistical process used to analyze the behavioral outcomes in this study is best understood by describing my analysis of "First Error." (In include the series of models fitted in Appendix I). In the most complex GLS model fitted, main effects were: YMSOD1, WT SOD1, Tg+, M and DP. There were nine potential two-way effects

(YMSOD1 x DP [YMSDP], WT SOD1 x DP [WTSDP], Tg+ x DP [TDP], M x DP [MDP], YMSOD1 x Tg+ [YMST], WT SOD1 x Tg+ [WTST], YMSOD1 x M [YMSM], WT SOD1 x M [WTSM], and Tg+ x M [TM]), seven possible three-way effects (YMSOD1 x Tg+ x M [YMTM], WT SOD1 x Tg+ x M [WTTM], YMSOD1 x Tg+ x DP [YMTDP], WT SOD1 x Tg+ x DP [WTTDP], YMSOD1 x M x DP [YMMDP] WT SOD1 x M x DP [WTMDP] and Tg+ x M x DP [TMDP]) and two four-way effects (YMSOD1 x Tg+ x M x DP [YMTMDP] and WT SOD1 x Tg+ x M x DP [WTTMDP]). Outcome for First Error was referenced on all these main effects and interactions using GLS. A global test was used to assess the significance of higher order effects was conducted for each group of the preceding interactions in order to conserve Type I Error. The first global test revealed that the four-way interactions were not significant. These interactions were removed from the model. The revised model was refitted. Another Global test then ascertained that the seven three-way interactions were not statistically significant and they too were removed from the model. The analysis continued in the assessment of the nine two-way interactions. The final model contained only the main effects. A similar process was undertaken with each of the remaining behavioral outcomes.

Reporting of Results of the Regression Model. By substitution into the final fitted "First Error" regression model, I can obtain predicted values of First Error for each kind of mouse (transgenic line, transgene, gender) on all possible occasions of measurement (day of practice). These predicted values of First Error can then be plotted against days of practice to provide an estimated learning trajectory for First Error for each "prototypical" class of mouse (e.g., one trajectory for MSOD1 Tg+ male mice, one for WT SOD1 Tg-

female mice, etc.). By inspection of these fitted trajectories I can elucidate the First Error findings by describing interesting features and differences in, and away from the trajectory. I plotted similar trajectories for each behavioral outcome and they can all be found in Chapter 5.

Cross-Sectional Analysis

Neurochemical analysis quantified concentrations of monoamines and amino acids in the frontal cortex and hippocampus from OMSOD1 mice and WT SOD1 mice, which with their Tg- littermates controls were matched for genotype, gender and age. As the dataset contained single observations of each animal and compared mice from different time points, the analysis was cross-sectional. Ordinary Least Squares (OLS) regression analysis was chosen for analysis. Parameter slope estimates and approximate p-values are found in Tables 5, 6, 7, 8 and 9. The two hypotheses examined with OLS are:

H_3: As elevated levels of glutamate have been shown in the spinal cord of patients diagnosed with ALS and excitotoxicity is suspected to be associated with mutations SOD1, it is suspected that WT SOD1 mice will show decreased concentrations of glutamate in brain regions associated with cognitive function, which may be influenced by the presence of the transgene, gender, or age when compared with MSOD1 mice.

H_4: As monoamines are involved with cognitive functioning, it is suspected that there will be differences between the two transgenic lines and they may be influenced by the presence of the transgene, gender, or age.

My regression strategy for each neurochemical outcome was similar to analysis that I used with the behavioral outcomes. As before, I began by fitting the most complex model feasible. My model contained control and question predictors as well as main effects, two-way, three-way and four-way effects. With model comparisons, unnecessary higher order terms were eliminated in order to produce a final parsimonious model that explained variations in the outcome.

For example, the process involving the analysis of NE from the frontal cortex involved the following (the series of models fitted to the concentrations of NE are found in Appendix I). The most complex OLS regression model that fitted the main effects were: WT SOD1, Tg+ , M and B. There were six potential two-way effects (WT SOD1 x B [WTB], Tg+ x B [TB], M x B [MB], WT SOD1 x Tg+ [WTT], WT SOD1 x M [WTM], Tg+ x M [TM]), four possible three-way effects (WT SOD1 x Tg+ x M [WTTM], WT SOD1 x Tg+ x B [WTTB], WT SOD1 x M x B [WTMB], Tg+ x M x B [TMB]), and one four-way effect (WT SOD1 x Tg+ x M x B [WTTMB]).

Again to reduce Type I error, a Global test assessed the significance of one four-way effect and four three-way effects. The test was significant and the one four-way interaction was removed from the model. The four three-way interactions were analyzed and the Global test was significant at the p=0.01 level. The interaction with the highest "p" value was removed, which was TMB. The data were again fitted to the regression

model, parsimony sought and the highest order terms retained. In the case of NE, the final model retained two three-way effects and the six two-way effects: WTB, TB, MB, WTT, WTM, TM, WTTM and WTMB.

Reporting of Results of the Regression Model. Similar to the process outlined for the behavioral outcome of First Error, by substitution into the final fitted "NE" regression model, I have predicted values of NE for each kind of mouse (transgenic line, transgene and gender) at eight and fifteen weeks of age. The predicted values for NE can be plotted against the age of the mouse when euthanized, before or after behavioral testing providing an estimated level of concentration for each prototypical class of mouse (e.g., one estimated level for MSOD1, Tg+ male mice at eight weeks of age, one for WT SOD1 Tg- female mice at 15 weeks of age, etc.). Through elucidation findings can describe interesting features or differences in the levels of concentrations. Similar points were plotted for each concentration level and they can be found in Chapter 5.

The Plots may be read in this fashion: the Y axis is the dependent variable or the concentration level of the monoamine or amino acid; and, the X-axis is the independent variable, the findings for each prototypical mouse. Gender differences are noted: arrows indicate values for males; and circles indicate the values for females. The panel in the upper left quadrant displays an estimated concentration level for a MSDO1 mouse at eight weeks of age, before behavioral testing. The lower left quadrant features the estimated value for the MSOD1 mouse at 15 weeks of age, after behavioral testing. The upper right and lower quadrants feature WT SOD1 mice organized in a similar manner according to age mice were euthanized.

Reporting of Statistical Findings

With both GLS and OLS regression analyses levels of statistical significance are reported at 0.1~, 0.05*, 0.01**, and 0.001***. Study findings are displayed in tables detailing slope parameter estimates (coefficients) and approximate p-values for each study outcome.

Chapter IV

STUDY HYPOTHESES AND RESEARCH FINDINGS

Overview

Primary objectives for this study were to determine if: 1) mutant properties associated with SOD1 affect cognitive functioning; and, 2) whether the effect of mutant SOD1 on cognitive performance is present prior to disease onset.

Secondary objectives of the study examined the effects of neurochemical functioning.

Study Hypotheses and Research Findings are organized into five sections: 1) a comparison of the weights of the mice during the experimental period, 2) study hypotheses and findings related to behavioral outcomes of the study, 3) a summary of the behavioral findings, 4) study hypotheses and findings reflecting the association of neurochemical and cognitive function, and 5) a summary of neurochemical findings.

Weight

Mice were weighed daily during behavioral testing. Parameter estimates and standard errors are noted in Table 3 and fitted trajectories of weight gain for the MSOD1 and WT SOD1 mice are displayed in Figure 1. All mice gained weight at the same rate ($p<0.001$). At the beginning of the evaluation period, YMSOD1 mice weighed significantly less than OMSOD1 ($p<0.05$). Weight was much higher in males compared to females ($p<0.001$).

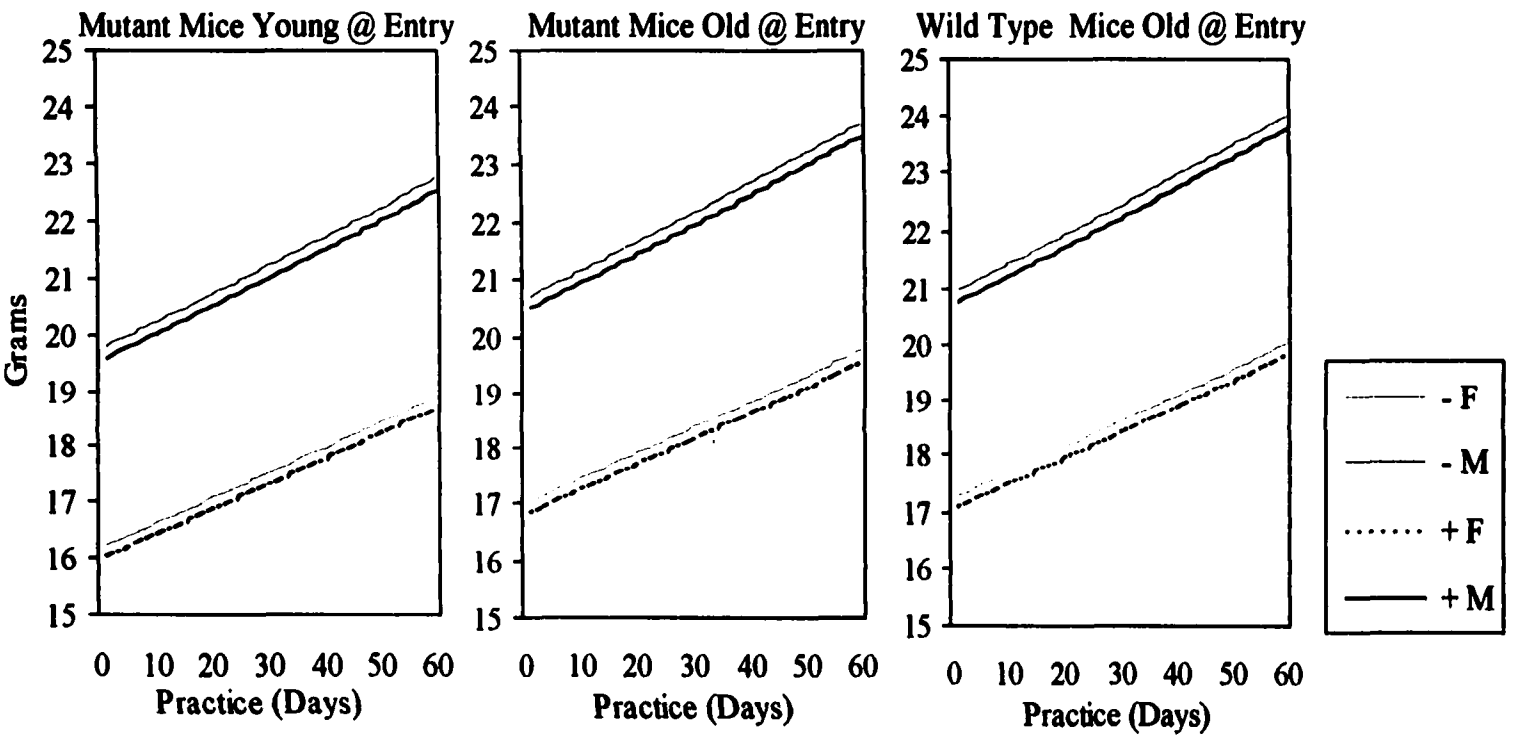
Table 3
Parameter Slope Estimates (and approximate p-values) from the GLS Regression of Weight Gain and Days of Practice for MSOD1 and WT SOD1 Mice

	OUTCOME
Predictors	**Sq Rt Weight Gain**
Intercept	4.1236***
Young Mutant SOD1 (YMSOD1)	-0.1007*
Mutant SOD1 (MSOD1)	-0.0292
Days Entered into Practice (DP)	0.0054***
Positive for Transgene (Tg+)	-0.023
Male (M)	0.4224***
R^2 ***within***	0.3086
R^2 ***between***	0.5934

~ $p < .10$
* $p < .05$
** $p < .01$
*** $p < .001$

Figure 1

Fitted Trajectories of Weight as a function of days of practice, for MSOD1 and WT SOD1 Mice who were "young" and "old" entry, by gender and presence of transgene



All mice significantly increased in weight over the study period ($p<0.001$). YMSOD1 mice gained significantly more weight than OMSOD1 mice ($p<0.001$) and males more than females ($p<0.001$).

Behavioral Findings

Two of the four research questions investigated questions concerning cognitive behavior.

Hypotheses One

H_1: Younger MSOD1 mice will show enhanced ability in learning a spatial memory task when compared with older MSOD1 mice.

Results

H_1: The YMSOD1 mice perform significantly better on all measures of cognitive outcome on the RAM when compared to the OMSOD1 mice: they make their first error later ($p<0.001$, Figure 2), choose more correct arms on the RAM during their first eight choices ($p<0.01$, Figure 3), and record fewer errors on the RAM ($p<0.001$, Figure 4). With respect to task duration, while YMSOD1 mice initially require more time in learning the task ($p<0.05$, Figure 5) with more experience on the RAM the YMSOD1 mice complete the task more quickly as shown in a two-way interaction and the dramatically declining slope of the trajectory ($p<0.001$, Figure 5). Therefore, H_1 is retained and H_{O1} is rejected. YMSOD1 mice do demonstrate enhanced ability in learning a spatial memory task when compared with OMSOD1 mice.

Hypothesis Two

H_2: WT SOD1 mice will demonstrate enhanced ability at learning a spatial memory task when compared with the MSOD1 mice and performance may be influenced by the presence of the transgene, gender or age when compared with MSOD1 mice.

Results

H_2: As reflected in the Predictors noted in Table 4 and displayed in Figures 2, 3, and 4 there are no differences in the behavioral outcomes of First Error, Total Correct and Total Errors on the RAM when comparing the OMSOD1 mice with the WT SOD1 mice. However, the presence of the transgene also significantly affects the number of errors that are made on the RAM by the SOD1 mice ($p<0.101$, Figure 4). The SOD1 mice carrying the positive transgene make significantly more errors on the RAM when compared with their non-transgenic littermates regardless of whether the protein is mutant or Wild Type ($p<0.1$, Figure 4). A number of two-way interactions show that there are statistically significant ways in which the MSOD1 transgenic line differs from the WT SOD1 line. Specifically, the WT SOD1 mice spend significantly less time in completing the RAM task when compared with the MSOD1 mice ($p<0.1$, Figure 5), particularly the Tg+ male mice. However, this finding is reversed when considering the performance of WT SOD1 Tg+ females ($p<0.1$, Figure 5). Over the study period, the WT SOD1 Tg+ females spend more time completing the maze than the Tg+ males. Therefore, H_2 is retained and the H_{O2} is rejected.

Table 4

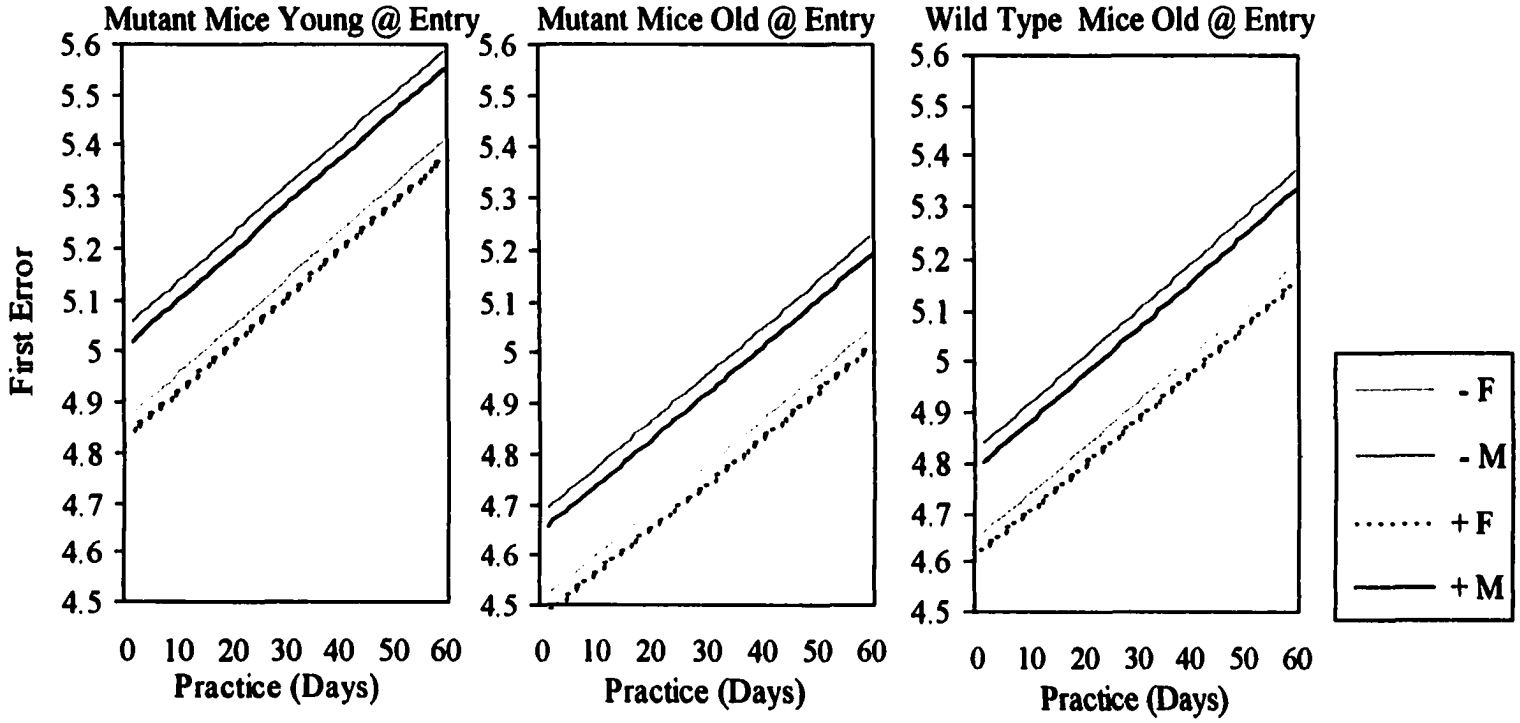
Parameter Slope Estimates (and approximate p-values) from the GLS Regression of Choice Accuracy on days of practice, transgene and gender for MSOD1 and WT SOD1 Mice controlling for Age of Entry

	OUTCOME			
Predictors	**First Error**	**Total Correct**	**Sq Rt Total Errors**	**Duration**
Intercept	4.51***	5.46***	3.04***	141.76***
Young Mutant SOD1 (YMSOD1)	0.36***	0.214**	-0.272***	16.97*
Old Mutant SOD1 (MSOD1)	-0.143	-0.05	-0.011	-6.53
Days Entered into Practice (DP)	0.009***	0.006***	-0.005***	-0.766***
Positive for Transgene (Tg+)	-0.036	-0.011	0.107~	17.03**
Male (M)	0.178*	0.101~	-0.155**	29.75***
YMSOD1*DP				-1.078***
MSOD1*DP				0.298~
MSOD1*M				21.577*
Tg+*M				-16.339~
R^2 ***within***	0.0098	0.0135	0.0066	0.1166
R^2 ***between***	0.1111	0.1035	0.1565	0.1782

~ p < .10
* p < .05
** p < .01
*** p < .001

Figure 2

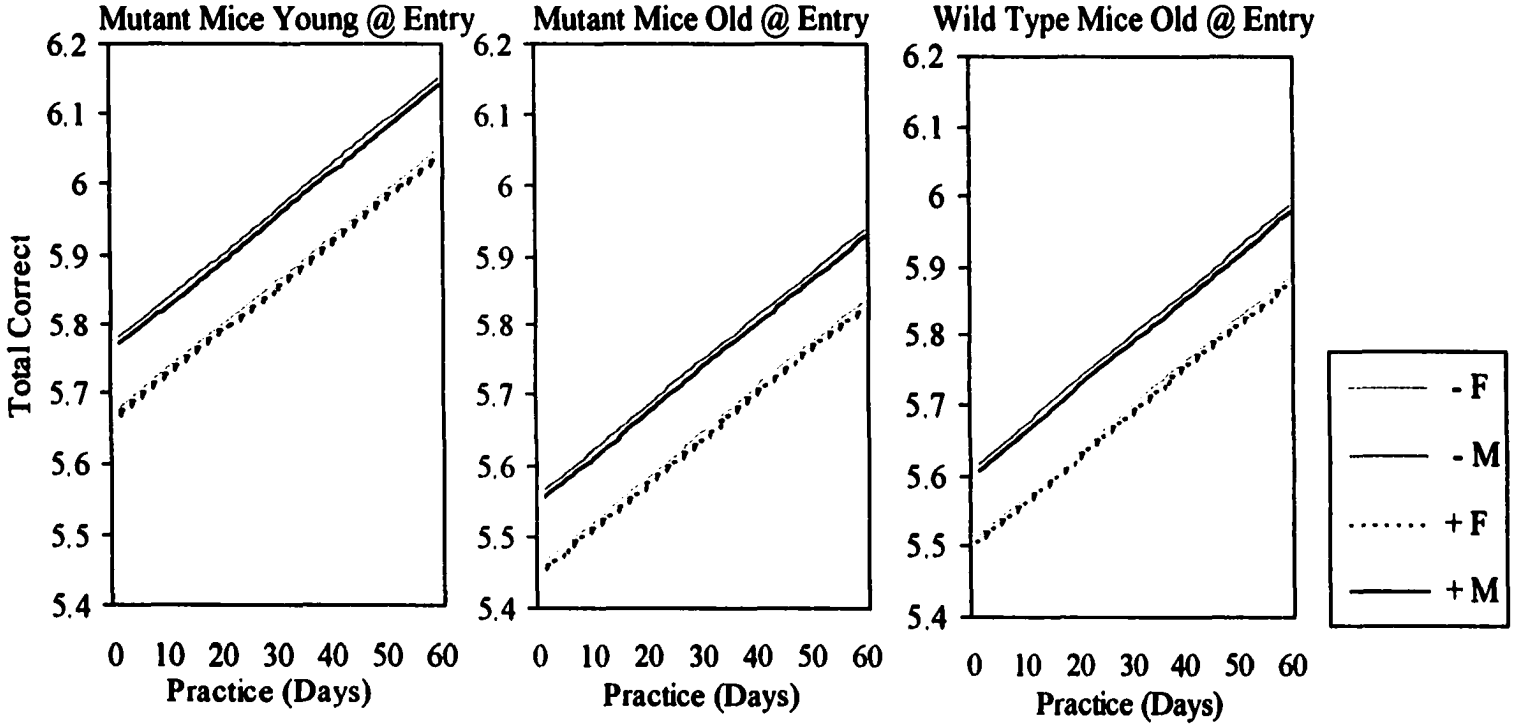
Fitted Trajectories of First Error on the Radial Arm Maze as a function of days of practice, for MSOD1 and WT SOD1 Mice who were "young" and "old" entry, by gender and presence of transgene



YMSOD1 mice made their First Error significantly later than OMSOD1 and WT SOD1 mice on the RAM ($p<0.001$), males made their First Error later than females ($p<0.05$), and all mice significantly improved with more days of practice ($p<0.001$).

Figure 3

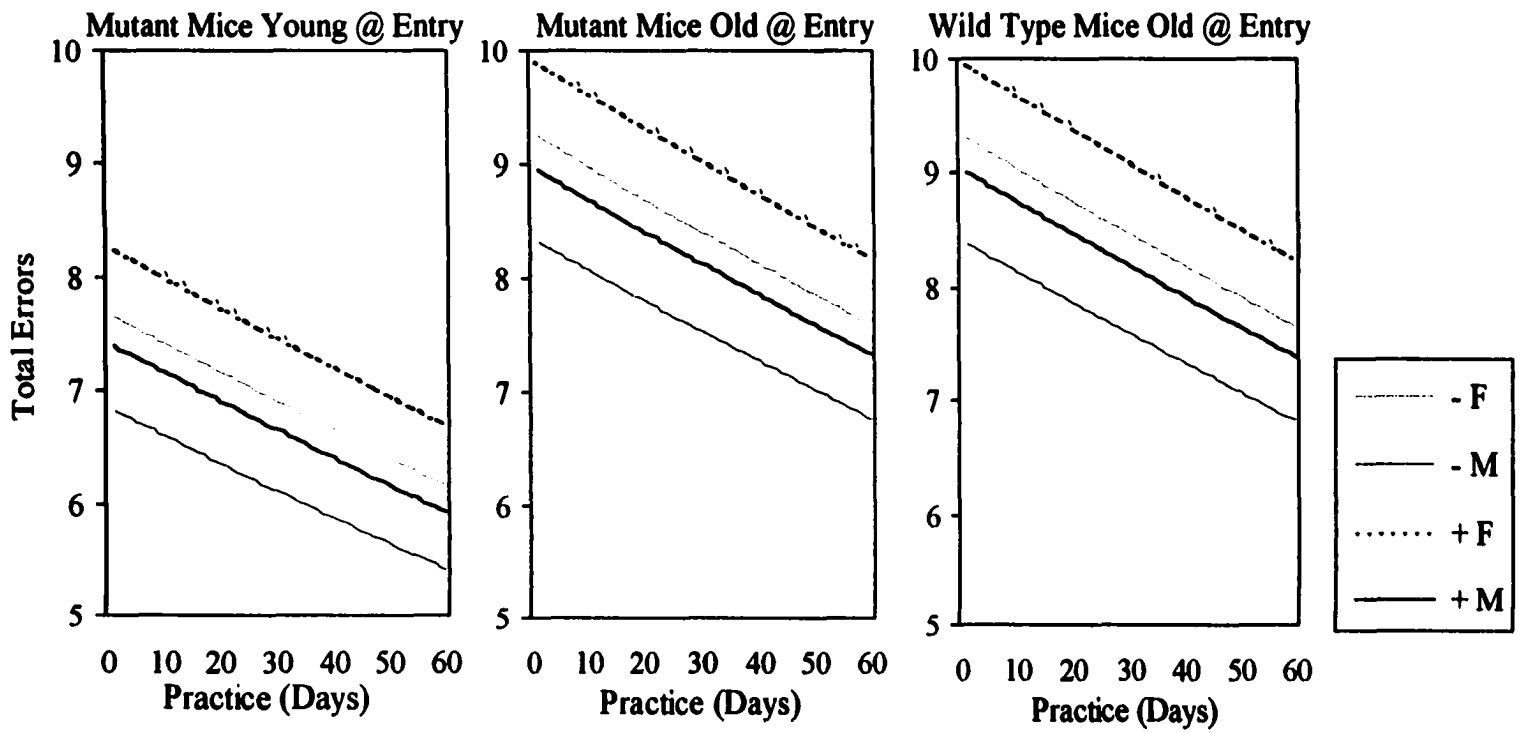
Fitted Trajectories of Total Correct In First Eight Choices on the Radial Arm Maze as a function of days of practice, for MSOD1 and WT SOD1 Mice who were "young" and "old" entry, by gender and presence of transgene



YMSOD1 mice chose more Total Correct in Eight Choices on the RAM than OMSOD1 mice ($p<0.01$), males made more correct choices than females ($p<0.1$), and all mice significantly improved in performance with more days of practice on the RAM ($p<0.001$).

Figure 4

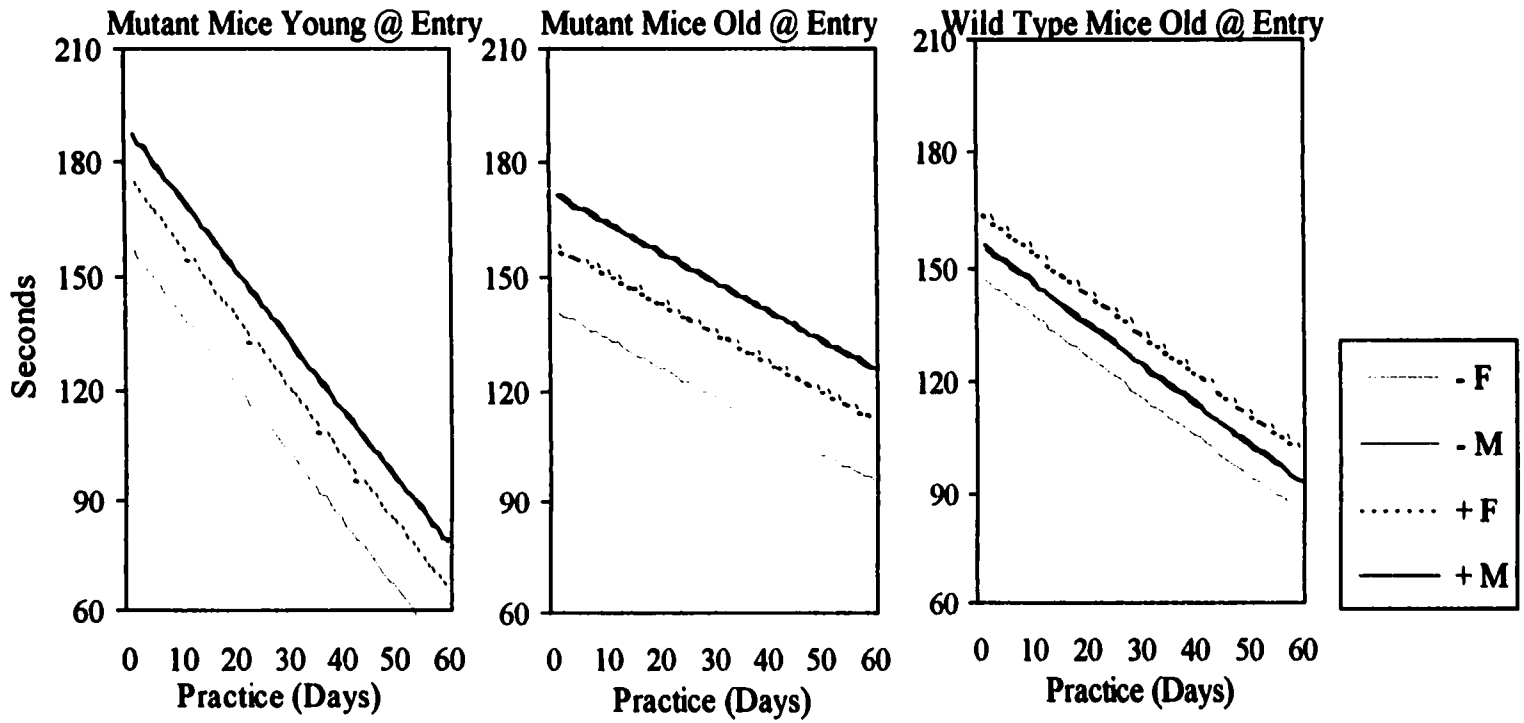
Fitted Trajectories of Total Errors on Radial Arm Maze as a function of days of practice, for MSOD1 and WT SOD1 Mice who were "young" and "old" entry, by gender and presence of transgene



YMSOD1 mice made significantly fewer errors than OMSOD1 mice ($p<0.001$), Tg+ mice made significantly more errors than Tg- mice ($p<0.1$), females made significantly more errors than males ($p<0.01$), and all mice made fewer errors as days of practice increased ($p<0.001$).

Figure 5

Fitted Trajectories of Duration Until All Arms of the Radial Arm Maze Are Visited as a function of days of practice, for MSOD1 and WT SOD1 Mice who were "young" and "old" entry, by gender and presence of transgene



Interactions of: transgenic line and days of practice results in a significant decrease in RAM duration for the YMSOD1 mice ($p<0.001$) and the WT SOD1mice ($p<0.1$), transgenic line and gender produce significant decreases in duration in male MSOD1 mice ($p<0.05$), and genotype and gender results in increased duration for the MSOD1 Tg+ male mice and the WT SOD1 Tg+ female mice ($p<0.1$).

Summary of Hypotheses One and Two

Both transgenic lines and younger and older mice are able to learn the spatial memory task and improve over time. Notably, YMSOD1 mice record their first error later, choose more correct in the first eight choices, and record fewer errors during completion of the task. However, there are no significant differences in the performance of the MSOD1 mice and WT SOD1 mice of the RAM. However, this finding is mediated with the presence of the transgene. SOD1 Tg- mice record fewer errors than SOD1 Tg+ mice. The presence of the transgene affects the mouse's performance. Also, male mice perform better than female mice on the RAM except in the amount of time required to complete the task. Two two-way interactions result in Tg- females requiring the least amount of time to complete the RAM. However, that finding is reversed in the two transgenic lines with Tg+ males in the MSOD1 line and the Tg+ females in the WT SOD1 line spend the most amount of time to complete the RAM.

Neurochemical Findings

The remaining two research questions assess neurochemical activity in brain regions known for cognitive behavior. Tables 5-9 and Figures 6-23b present the findings of the neurochemical analysis.

Hypotheses Three

H_3: As elevated levels of glutamate have been shown in the spinal cord of patients diagnosed with ALS and excitotoxicity is suspected to be associated with mutations of SOD1 it is suspected that WT SOD1 mice will show decreased concentrations of glutamate in brain regions associated with cognitive function, which may be influenced by the presence of the transgene, gender, or age when compared with MSOD1 mice.

Results.

H_3: Glutamate is significantly low in the frontal cortex of the MSOD1 mice when compared with the WT SOD1 mice ($p<0.1$, Figure 6) and significantly low in SOD1 mice that carry the transgene ($p<0.01$, Figure 6). Both transgenic lines reveal significantly elevated levels of glutamate at 15 weeks of age when compared with mice at eight weeks of age ($p<0.1$, Figure 6). In addition to these findings involving glutamate, another amino acid, GABA is also significantly low in MSOD1 mice when compared with WT SOD1 mice ($p<0.01$, Figure 7). Therefore, H_3 is rejected and H_{O3} is retained.

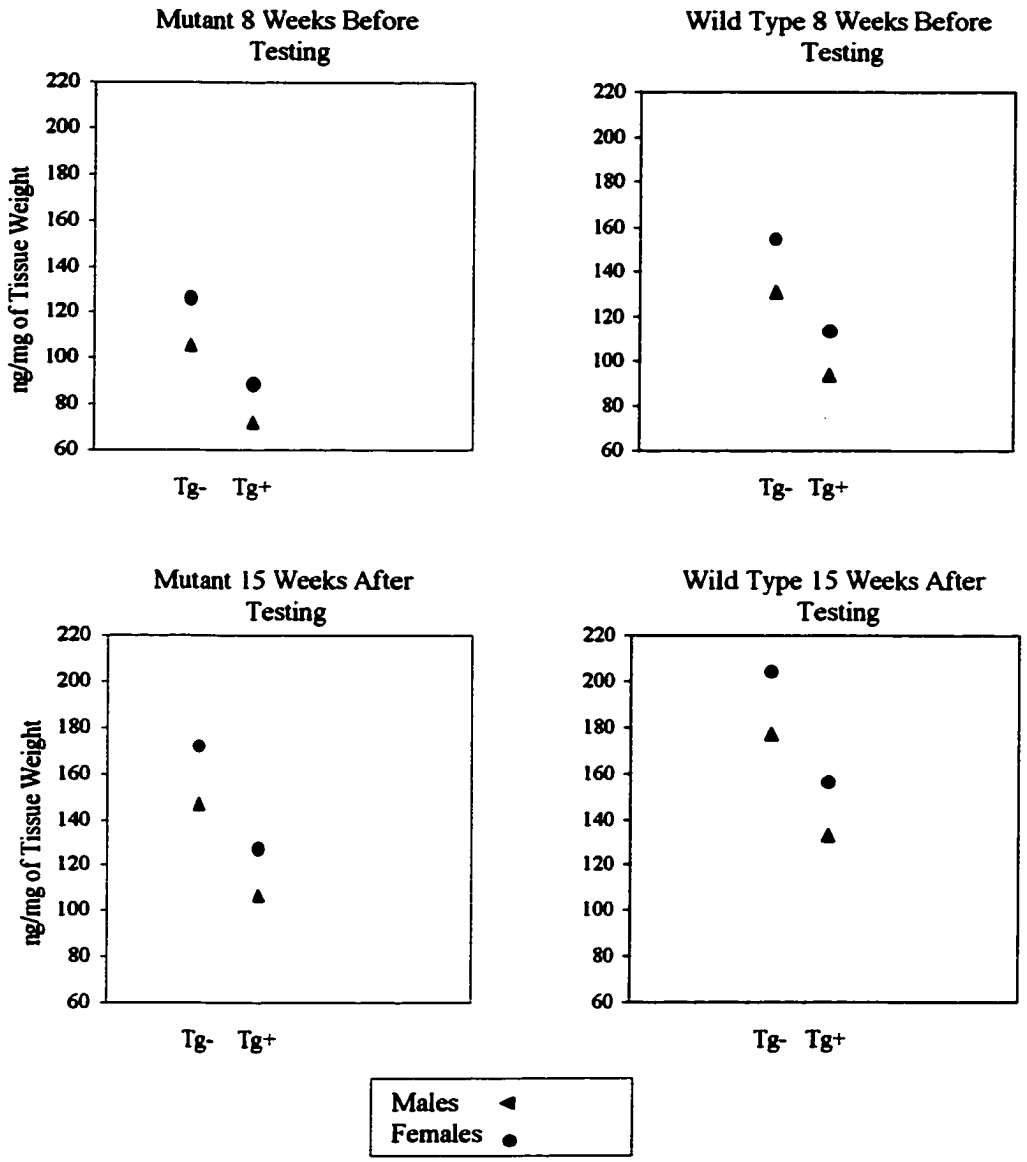
Table 5

Parameter Slope Estimates (and approximate p-values) from the OLS Regression of Amino Acids from the Frontal Cortex on Transgene, Gender, and Behavioral Testing for MSOD1 and WT SOD1 Mice

	OUTCOME	
Predictors	**Sq Rt Glutamate**	**Sq Rt GABA**
Intercept	11.2352***	10.4246***
Mutant SOD1 (MSOD1)	-1.1817*	-1.1091**
Positive for Transgene (Tg+)	-1.7905**	-0.5061
Male (M)	-0.9541	-0.5511
Mice 15 Weeks after Behavioral Testing (B)	1.8527**	0.267
R^2 between	0.1395	0.0814

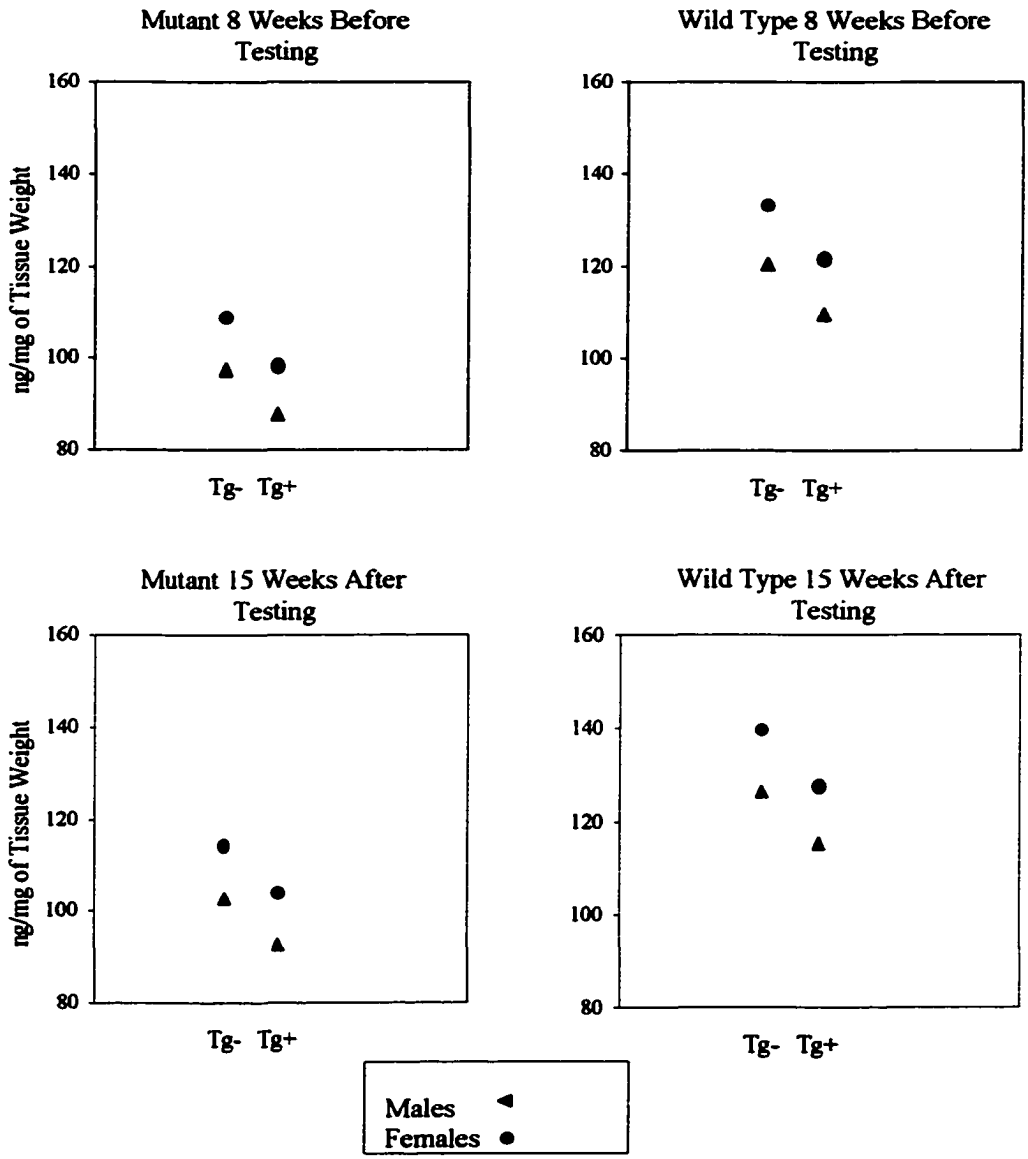
~ p < .10
* p < .05
** p < .01
*** p < .001

Figure 6
Fitted Concentrations of Glutamate in the Frontal Cortex of MSOD1 and WT SOD1 Mice



Significantly low concentrations of Glutamate are present in MSOD1 mice ($p<0.1$) and in mice carrying the transgene ($p<0.01$) and elevated concentrations of glutamate are found in both transgenic lines of mice at 15 weeks of age ($p<0.01$) when compared with mice at eight weeks of age.

Figure 7
Fitted Concentrations of γ-aminobutyric acid (GABA) in the Frontal Cortex of MSOD1 and WT SOD1 Mice



MSOD1 mice when compared with WTSOD1 show significantly low concentrations of GABA in the frontal cortex ($p<0.01$).

Hypothesis Four

H_4: As monoamines are involved with cognitive functioning it is suspected that there will be differences between the two transgenic lines and these neurochemicals may be influenced by the presence of the transgene, gender, or age.

Results

H_4: Concentrations of DA and NE in the frontal cortex of the MSOD1 mice are significantly elevated when compared to levels of monoamines in the WT SOD1 mice. However, when concentrations of monoamines from the hippocampus are analyzed significant findings are found in complex interactions.

A description of each monoamine and its metabolites follow:

Dopamine, DOPAC and HVA. The catecholamine, DA is significantly elevated in the frontal cortex of MSOD1 mice ($p<0.01$, see Figure 8). However, both transgenic lines show significantly elevations of DA at 15 weeks of age when compared with mice at eight weeks of age ($p<0.001$, Figure 8). Significant findings specific to age are also found in the hippocampus in an interaction involving transgenic line and age: MSOD1 mice at 15 weeks show significantly elevated levels of DA ($p<0.001$, Figure 8).

Table 6

Parameter Slope Estimates (and approximate p-values) from the OLS Regression of Monamines from the Frontal Cortex on Transgene, Gender, and Behavioral Testing for MSOD1 and WT SOD1 Mice

	OUTCOME				
Predictors	**Sq Rt DA**	**Sq Rt DOPAC**	**Sq Rt HVA**	**Sq Rt NE**	**Sq Rt HVA/DA**
Intercept	2.5781***	5.2504***	7.2913***	7.5682***	3.3973***
Mutant SOD1 Transgenic (MSOD1)	0.5447**	2.574***	2.932***	4.9786***	0.8286***
Positive for Transgene (Tg+)	-0.2964	1.529**	0.0468	0.2018	0.2551
Male (M)	-0.0267	0.3138	0.126	-0.6263	-0.2962
Mice 15 Weeks after Behavioral Testing (B)	1.6712***	-0.0778	-0.5953~	1.2244**	-2.0763***
MSOD1*B		-1.4289**	-3.0897***	-2.9813***	-1.4161***
M*B				1.7287**	0.6227~
Tg+*M		-1.2271*		-0.8667~	
MSOD1*M				-1.3956~	
MSOD1*M*B				2.6474**	
R^2 between	0.3978	0.3354	0.35	0.7389	0.3541

~ p < .10
* p < .05
** p < .01
*** p < .001

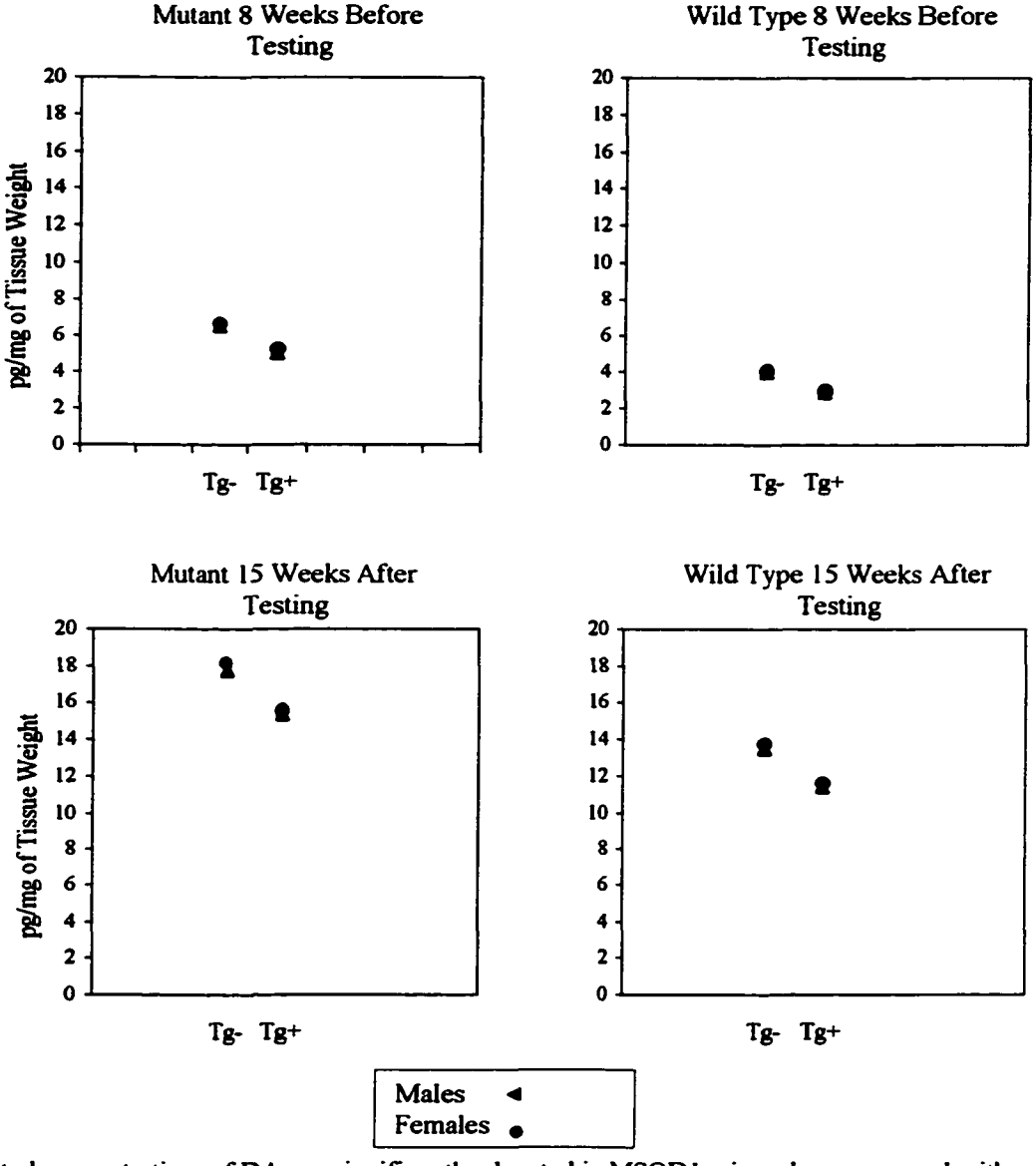
Table 7

Parameter Slope Estimates (and approximate p-values) from the OLS Regression of Monoamines from the Hippocampus on Transgene, Gender and Behavioral Testing for MSOD1 and WT SOD1 Mice

	OUTCOME				
Predictors	**Sq Rt DA**	**Sq Rt DOPAC**	**Sq Rt HVA**	**Sq Rt NE**	**Sq Rt HVA/DA**
Intercept	3.9611***	11.1947***	7.7683***	9.0472***	1.7531***
Mutant SOD1 (MSOD1)	0.1622	0.0374	0.3031	-0.1980	-0.332***
Positive for Transgene (Tg+)	0.5342	0.4681	0.5959	-1.1844***	0.1194
Male (M)	0.2705	0.1288	0.4171	-0.1228	0.0329
Mice 15 Weeks after Behavioral Testing (B)	5.0212***	-3.4133***	-0.8353~	2.3964***	-0.9485***
MSOD1*B	3.6097***			2.6475***	
MSOD1*Tg+				-2.1534***	
R^2 between	0.3400'	0.1320'	0.0421	0.3454	0.5692

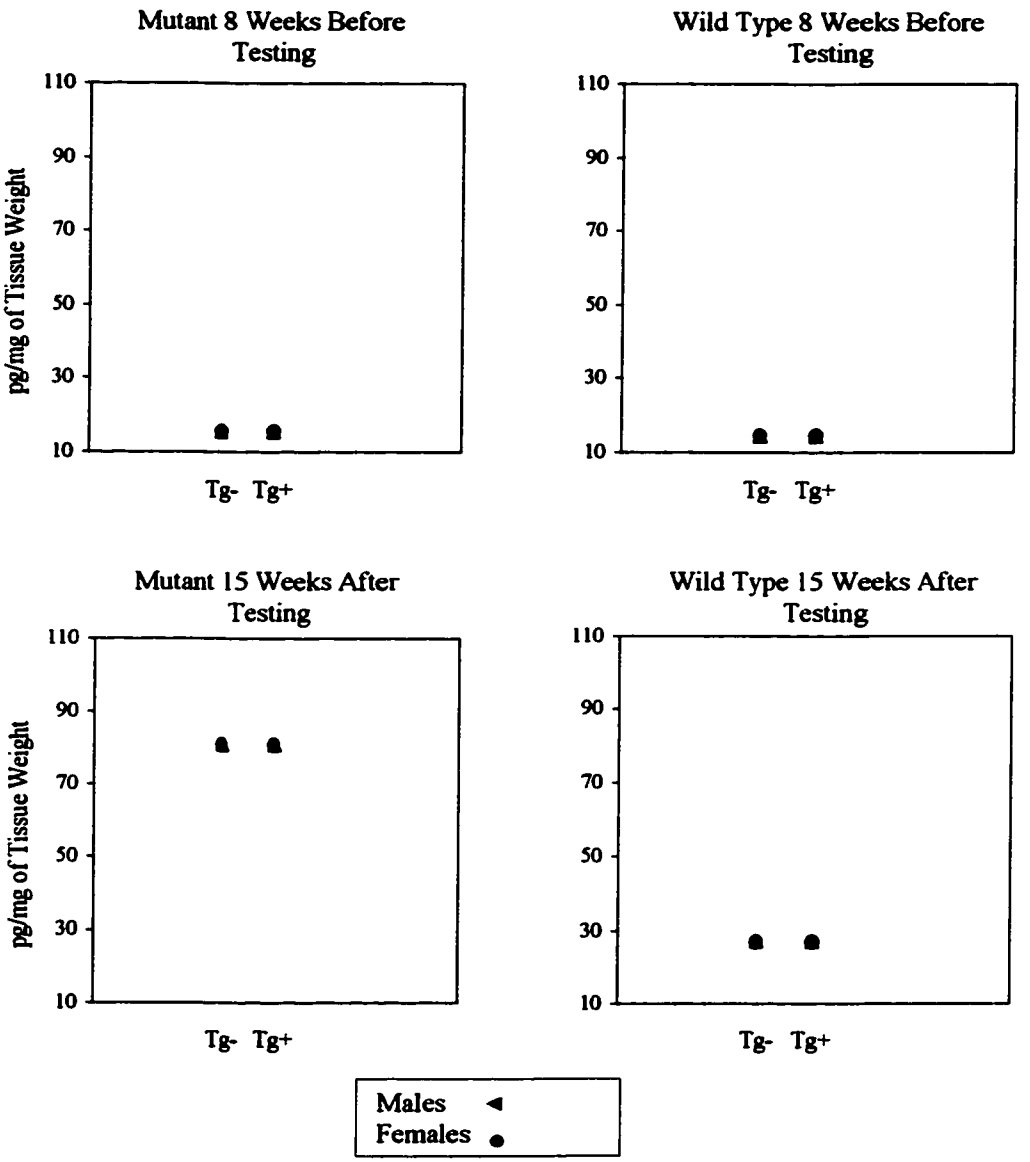
~ p < .10
* p < .05
** p < .01
*** p < .001

Figure 8
Fitted Concentrations of Dopamine (DA) in the Frontal Cortex of MSOD1 and WT SOD1 Mice



Fitted concentrations of DA are significantly elevated in MSOD1 mice when compared with WT SOD1 mice ($p<0.01$) and in mice evaluated at 15 weeks of age ($p<0.001$) when compared with mice at eight weeks of age.

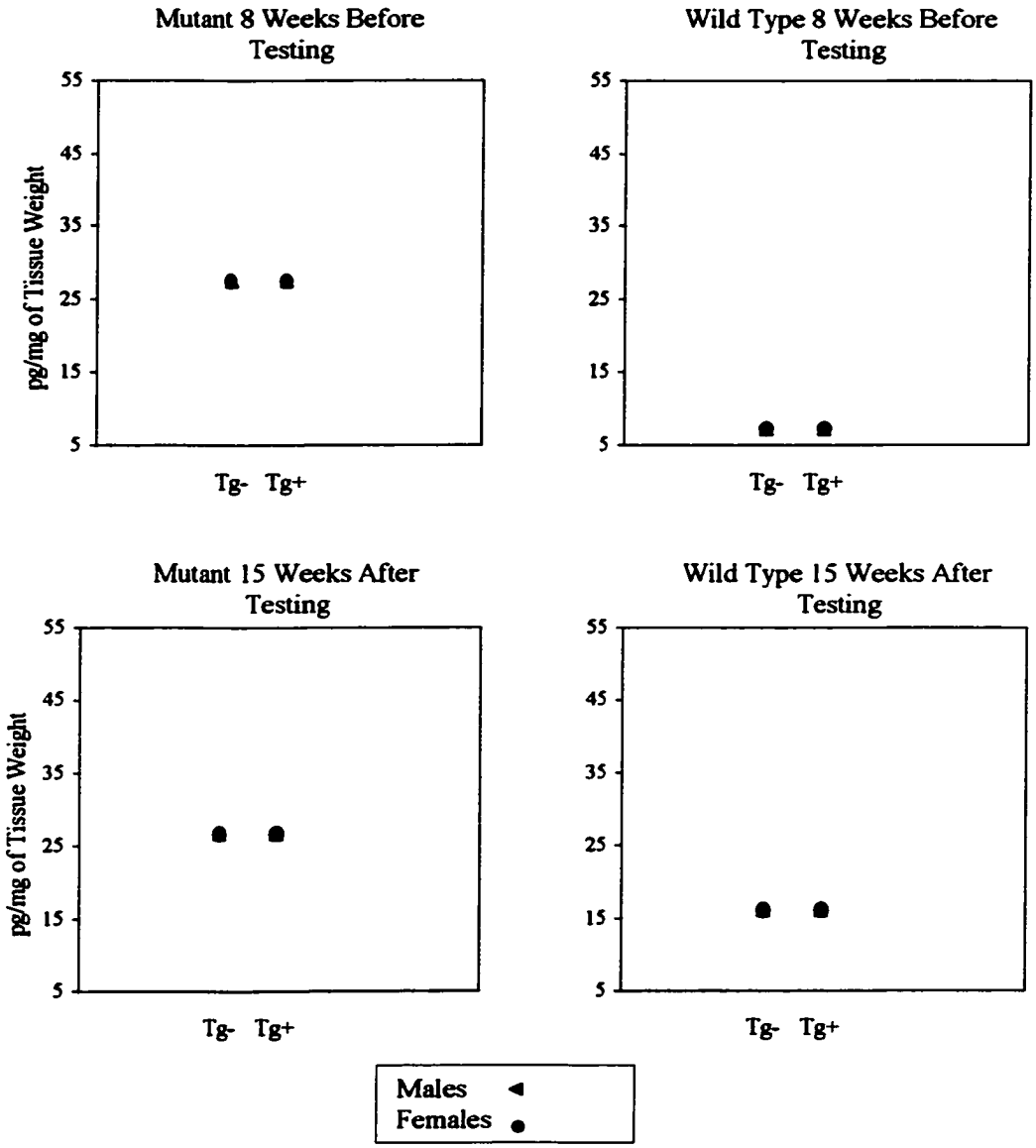
Figure 9
Fitted Concentrations of Dopamine (DA) in the Hippocampus of MSOD1 and WT SOD1 Mice



Significantly elevated concentrations of DA in the Hippocampus reflect an interaction of transgenic line and age as shown in the MSOD1 mice at 15 weeks of age ($p<0.001$).

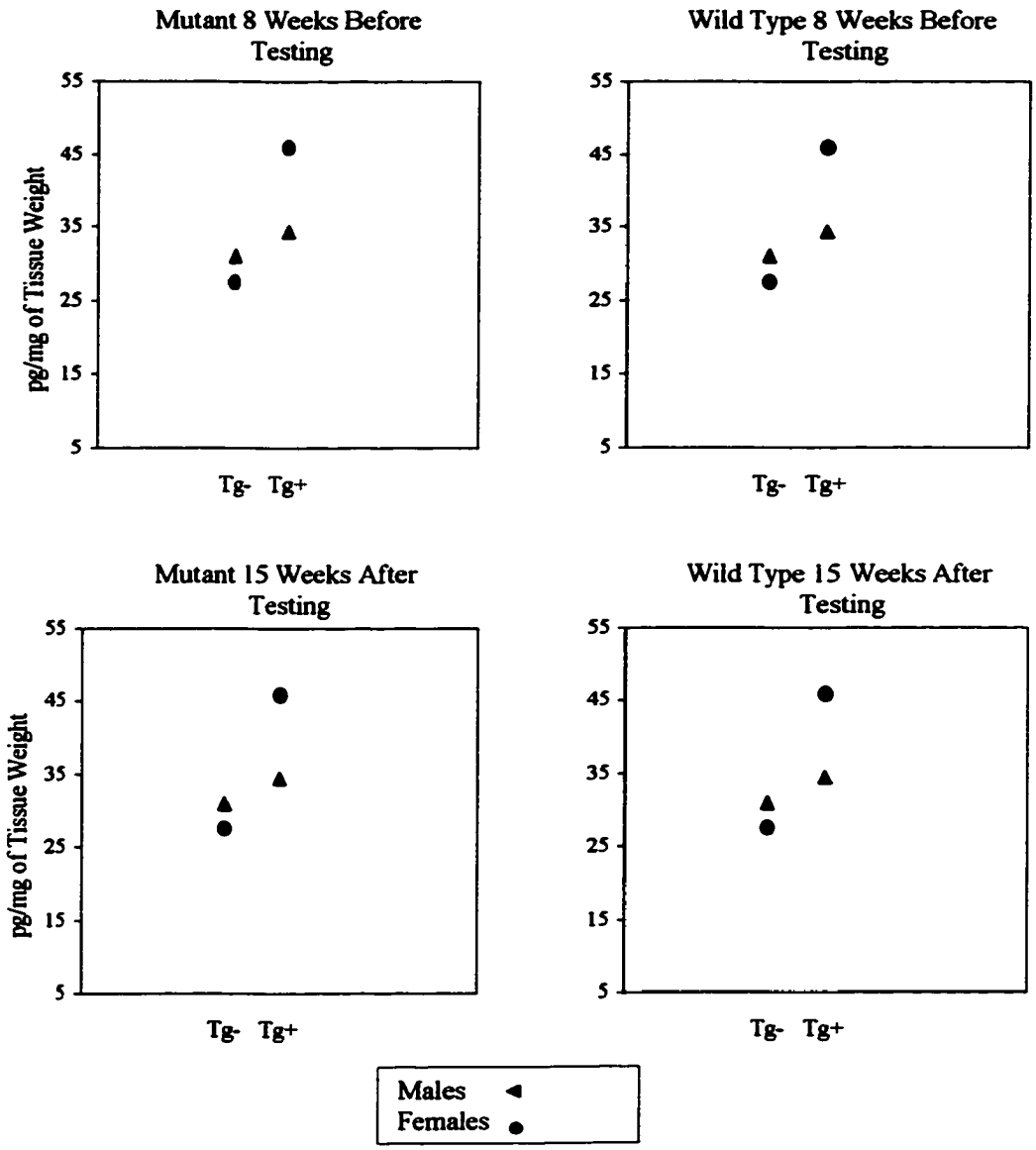
Both DOPAC and HVA, metabolites of DA are significantly elevated in MSOD1 mice at eight weeks in the frontal cortex in interactions of transgenic line and age ($p<0.01$, Figure 10a, $p<0.001$, Figure 12, respectively). Another two-way interaction results in significantly elevated concentrations of DOPAC in the frontal cortex of Tg+ female mice ($p<0.05$, Figure 10b). In the hippocampus, DOPAC and HVA are significantly elevated in both transgenic lines at eight weeks of age when compared with mice evaluated at eight weeks of age ($p<0.001$, Figure 11; $p<0.1$, Figure 13, respectively).

Figure 10a
Fitted Concentrations of 3,4-dihydroxyphenylacetic acid (DOPAC) in the Frontal Cortex of MSOD1 and WT SOD1 Mice



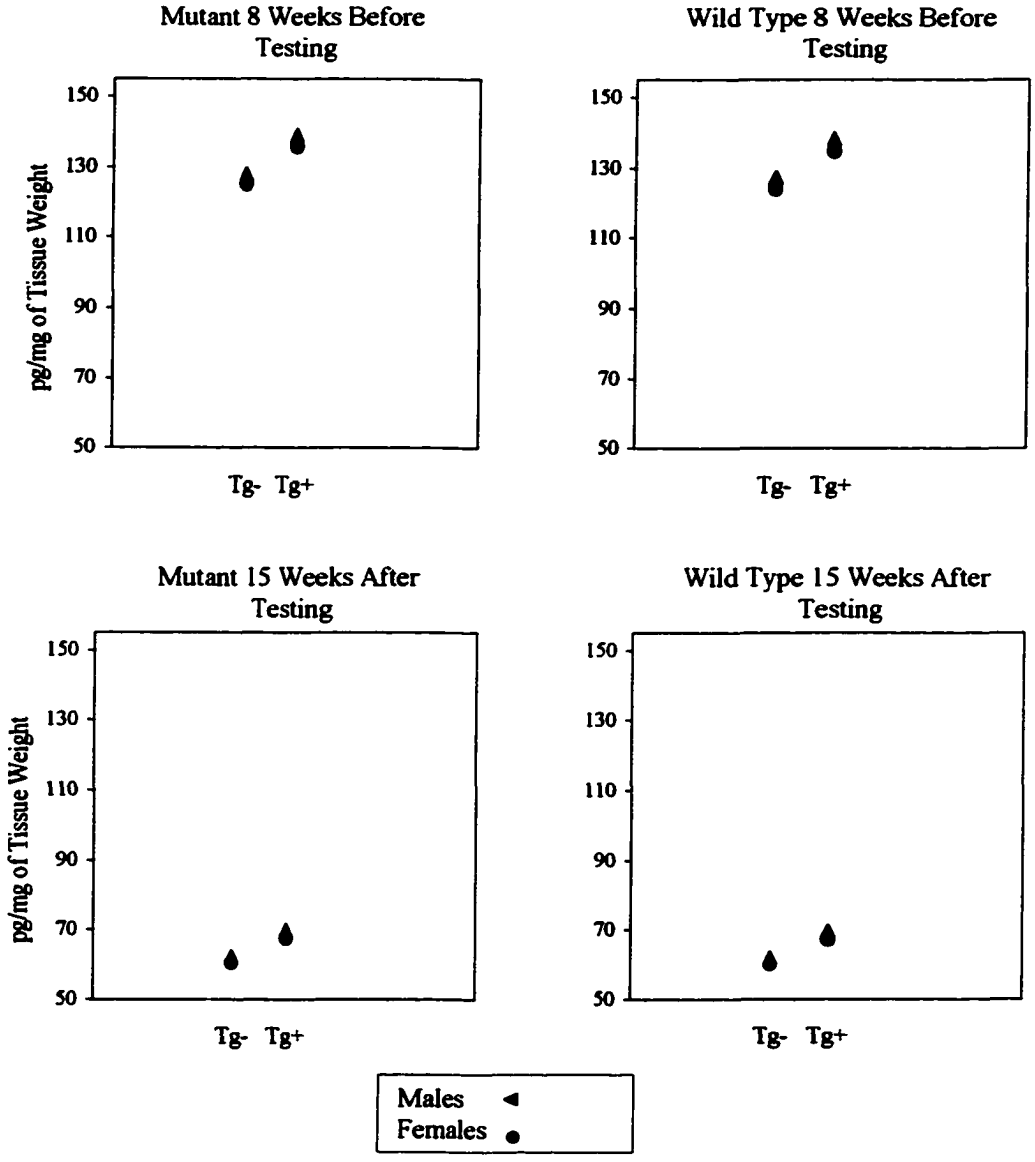
Significantly elevated concentrations of DOPAC are present in the frontal cortex of MSOD1 mice at 8 weeks of age the result of an interaction of transgenic line and age ($p<0.01$).

Figure 10b
Fitted Concentrations of 3,4-dihydroxyphenylacetic acid (DOPAC) in the Frontal Cortex of MSOD1 and WT SOD1 Mice



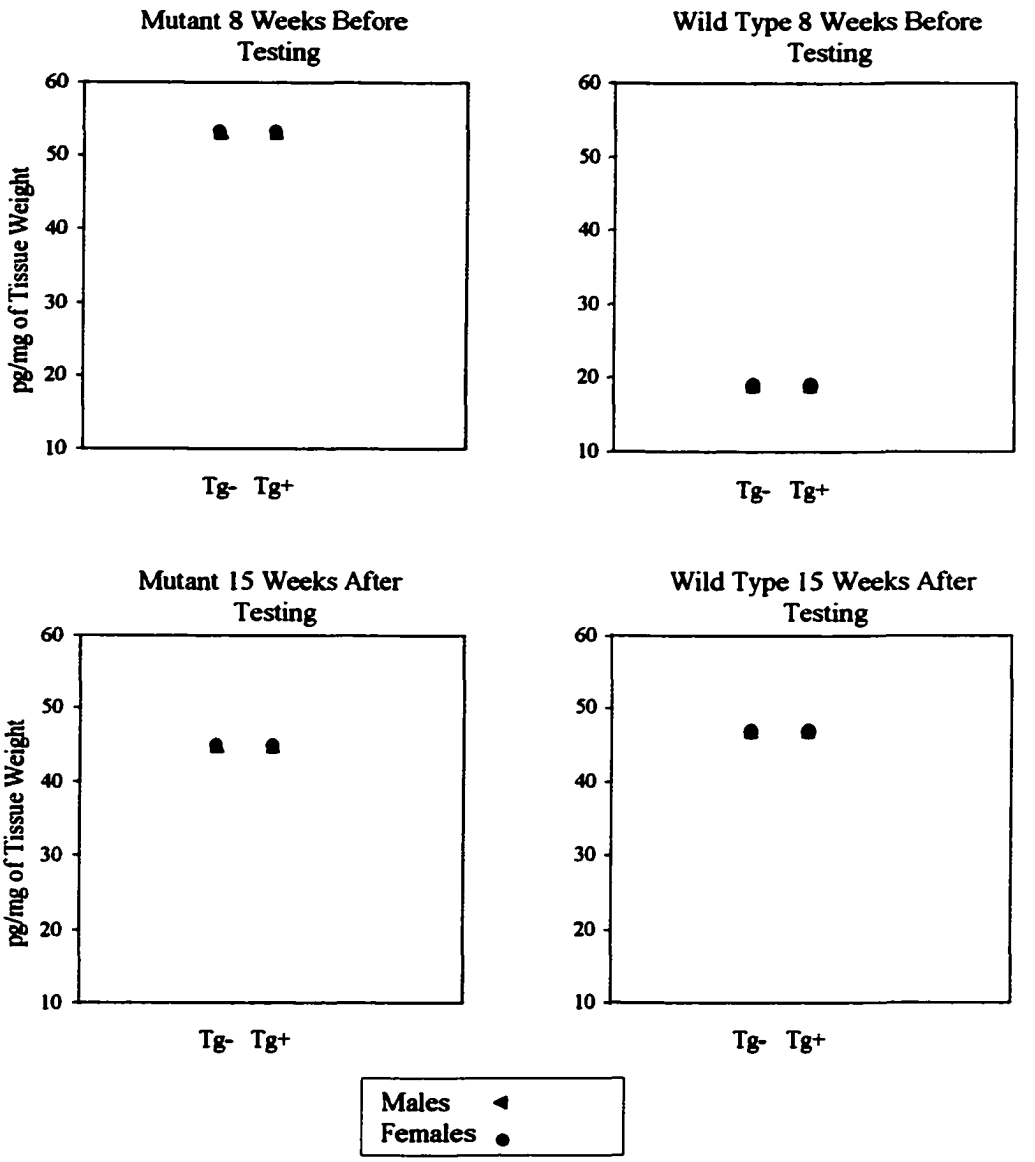
An interaction of transgene and gender results in significantly elevated concentrations of DOPAC in the frontal cortex of the Tg+ female mice ($p<0.05$).

Figure 11
Fitted Concentrations of 3,4-dihydroxyphenylacetic acid (DOPAC) in the Hippocampus of MSOD1 and WT SOD1 Mice



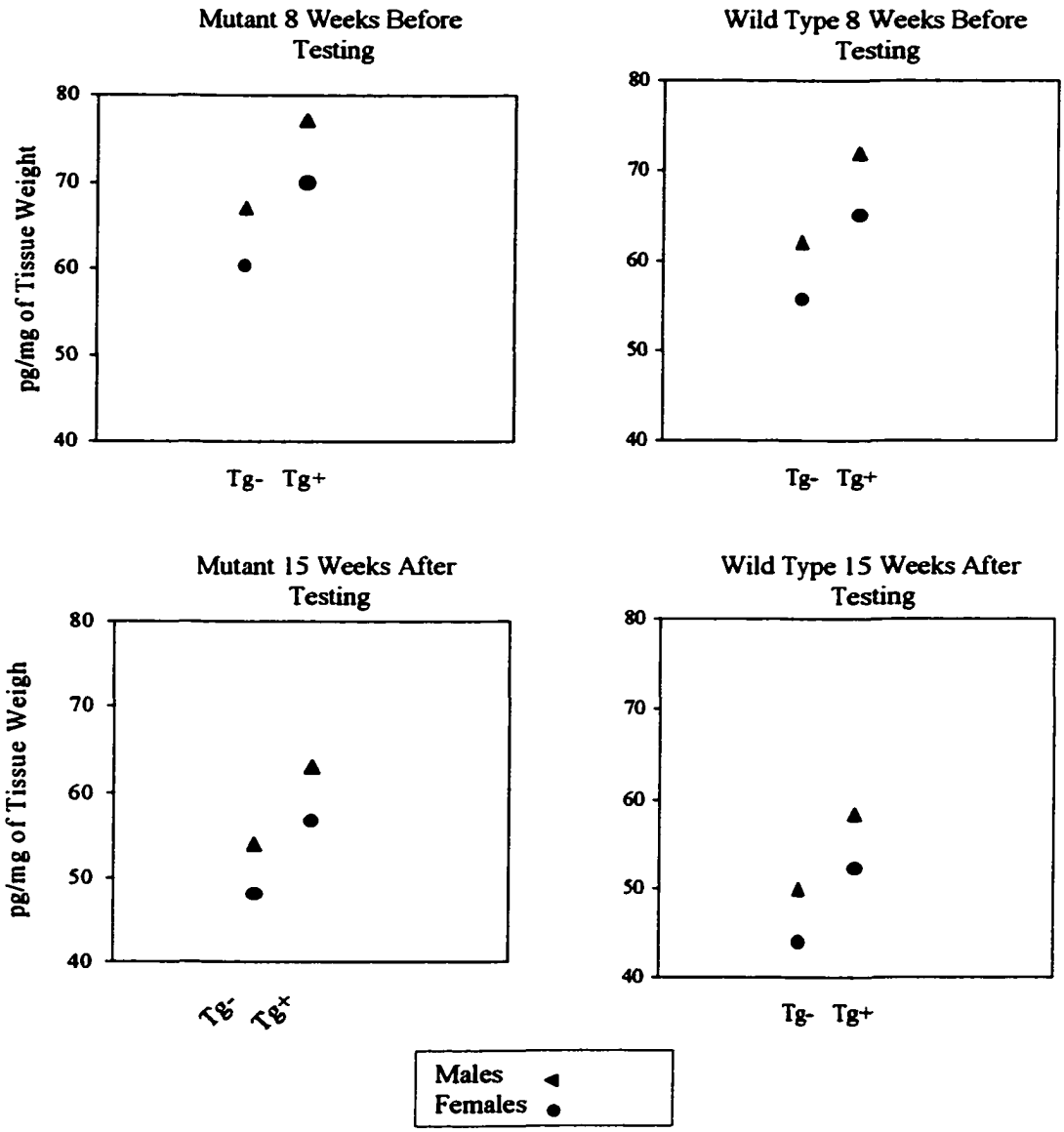
Both transgenic lines of mice at eight weeks of age show significantly elevated concentrations of DOPAC in the hippocampus when compared with mice at 15 weeks of age ($p<0.001$).

Figure 12
Fitted Concentrations of Homovanillic acid (HVA) in the Frontal Cortex of MSOD1 and WT SOD1 Mice



An interaction of transgenic line and age results in significantly elevated concentrations of of HVA in MSOD1 mice at eight weeks of age ($p<0.001$).

Figure 13
Fitted Concentrations of Homovanillic acid (HVA) in the Hippocampus of MSOD1 and WT SOD1 Mice

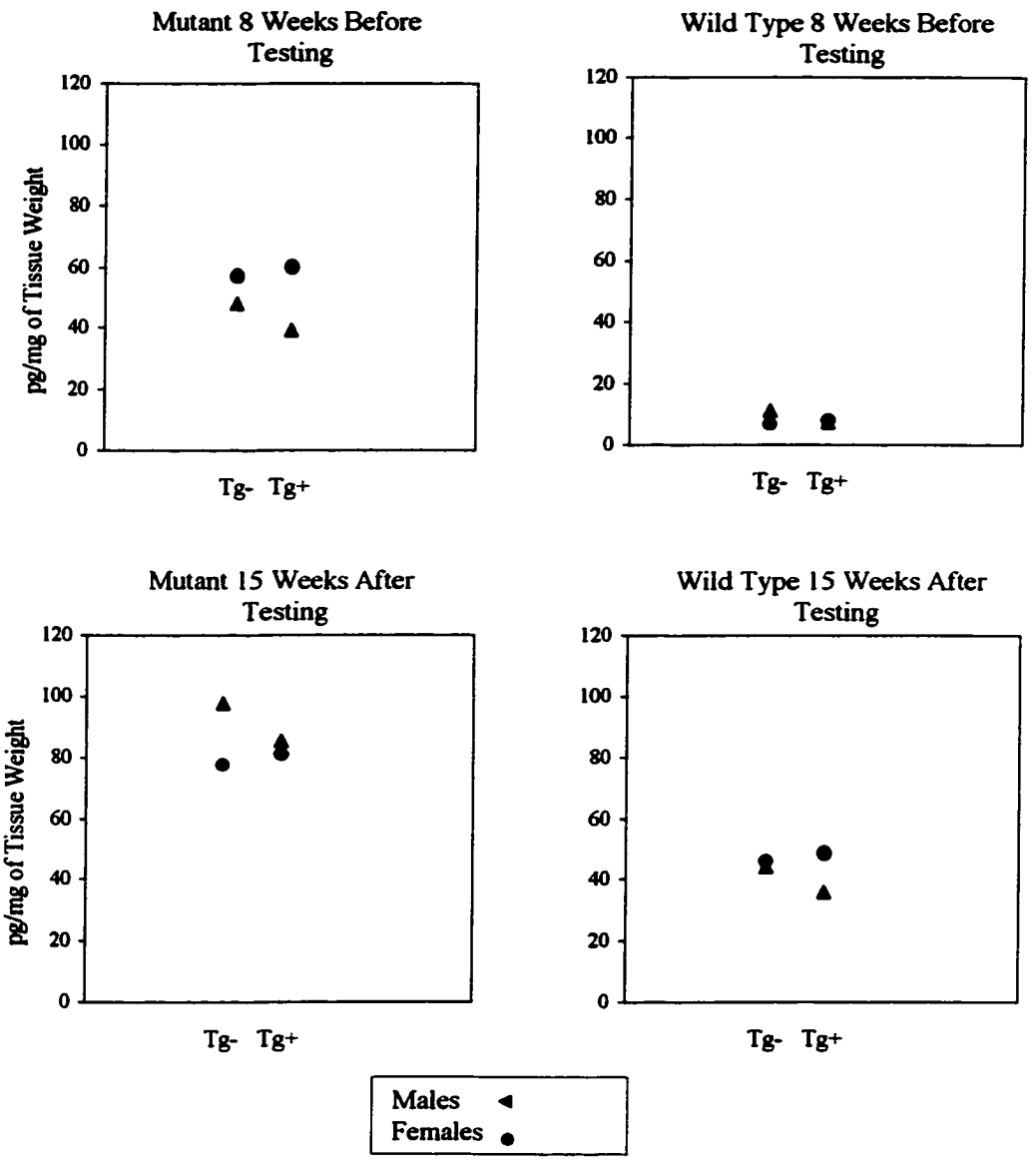


Mice evaluated at 8 weeks of age showed significantly elevated concentrations of HVA in the hippocampus when compared with mice at 15 weeks of age ($p<0.1$).

Norepinephrine. A three-way interaction involving transgenic line, gender and age shows significantly elevated levels of the adrenergic neurotransmitter, NE in the frontal cortex of MSOD1 males and WT SOD1 females at 15 weeks of age. Interestingly, the pattern reverses with MSOD1 female mice and male WT SOD1 mice showing slightly higher levels of NE in the frontal cortex at eight weeks of age ($p<0.01$, Figure 14).

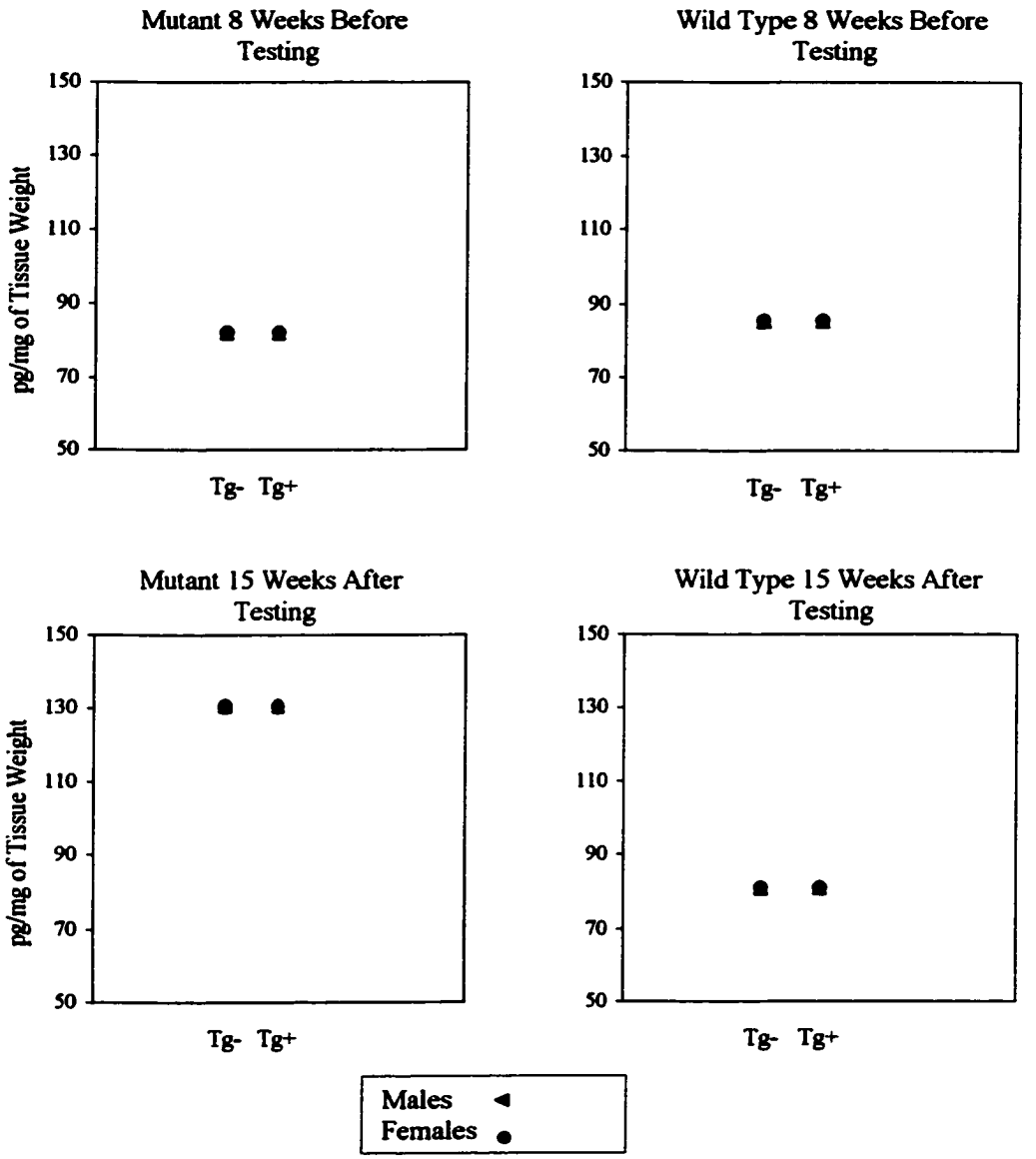
Findings from the analysis of concentrations of NE in the hippocampus reveal two two-way interactions involving transgenic line, genotype and age. An interaction of transgenic line and age results in significantly elevated levels of NE in the hippocampus of SOD1 mice at 15 weeks of age ($p<0.001$, Figure 15a). The interaction of transgenic line and Tg is shown in elevations of NE in the hippocampus of MSOD1 Tg- mice and WT SOD1 Tg+ mice ($p<0.001$, Figure 15b).

Figure 14
Fitted Concentrations of Norepinephrine (NE) in the Frontal Cortex of MSOD1 and WT SOD1 Mice



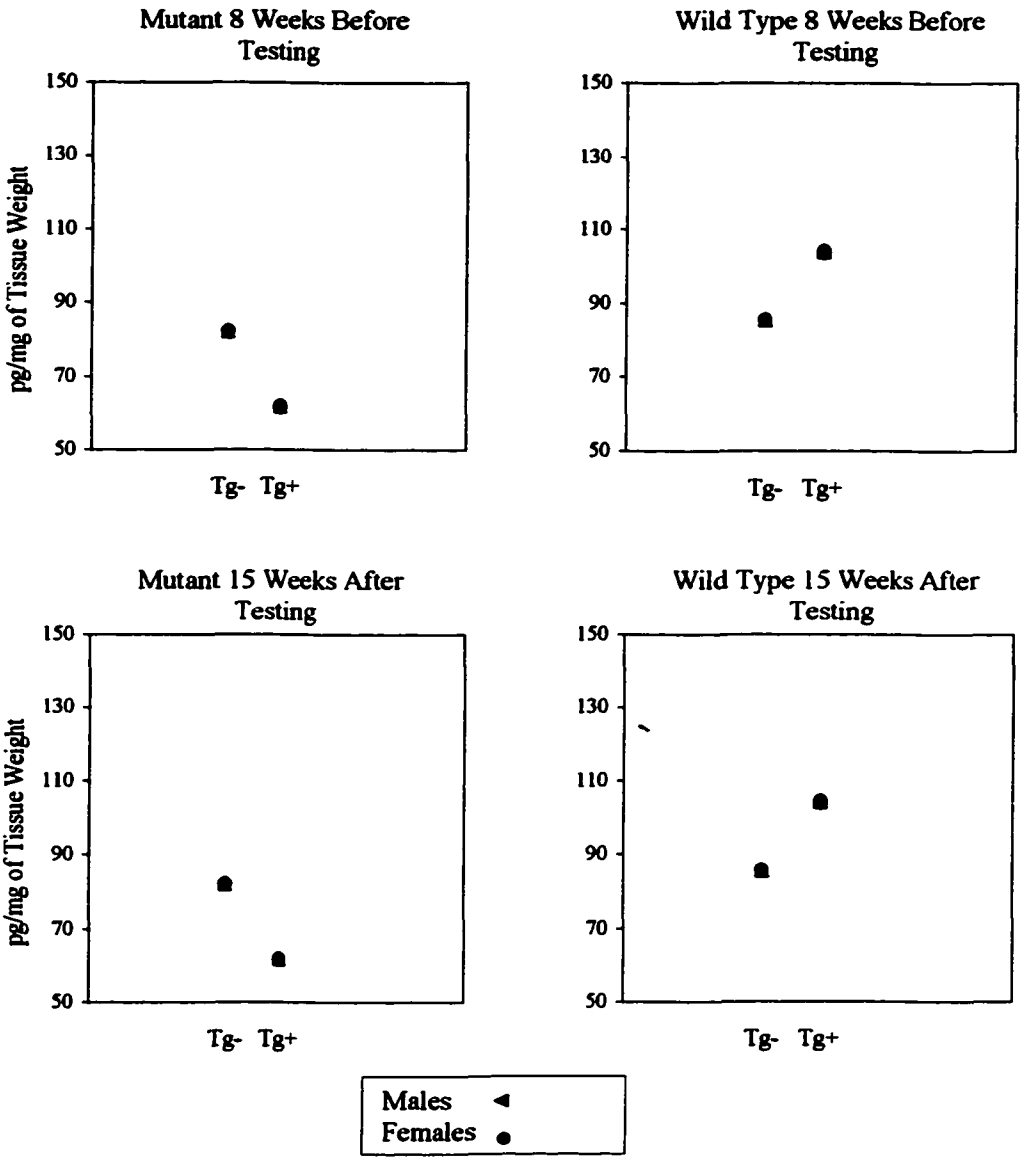
Significantly elevated concentrations of NE are shown MSOD1 males and WT SOD1 females at 15 weeks and MSOD1 females and WT SOD1 males at 8 weeks in a three way interaction of transgenic line, gender and age ($p<0.01$).

Figure 15a
Fitted Concentrations of Norepinephrine (NE) in the Hippocampus of MSOD1 and WT SOD1 Mice



Fitted concentrations of NE from the hippocampus of SOD1 mice at 15 weeks reflect a significant interaction of transgenic line and age ($p<0.001$).

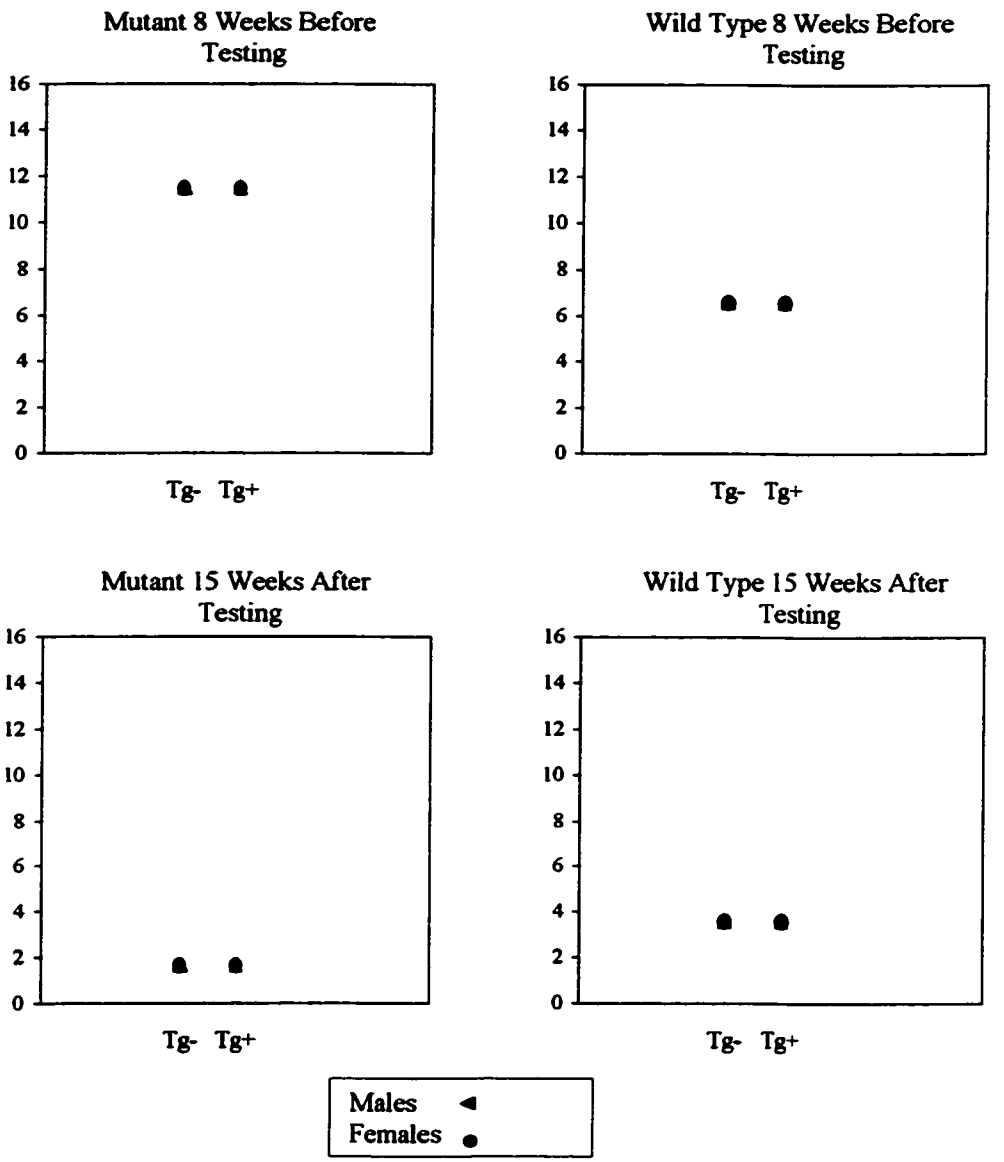
Figure 15b
Fitted Concentrations of Norepinephrine (NE) in the Hippocampus of MSOD1 and WT SOD1 Mice



Significantly elevated concentrations of NEpresent in Tg- SOD1 and Tg+ WT SOD1 mice reflects an interaction of transgenic line and genotype ($p<0.001$).

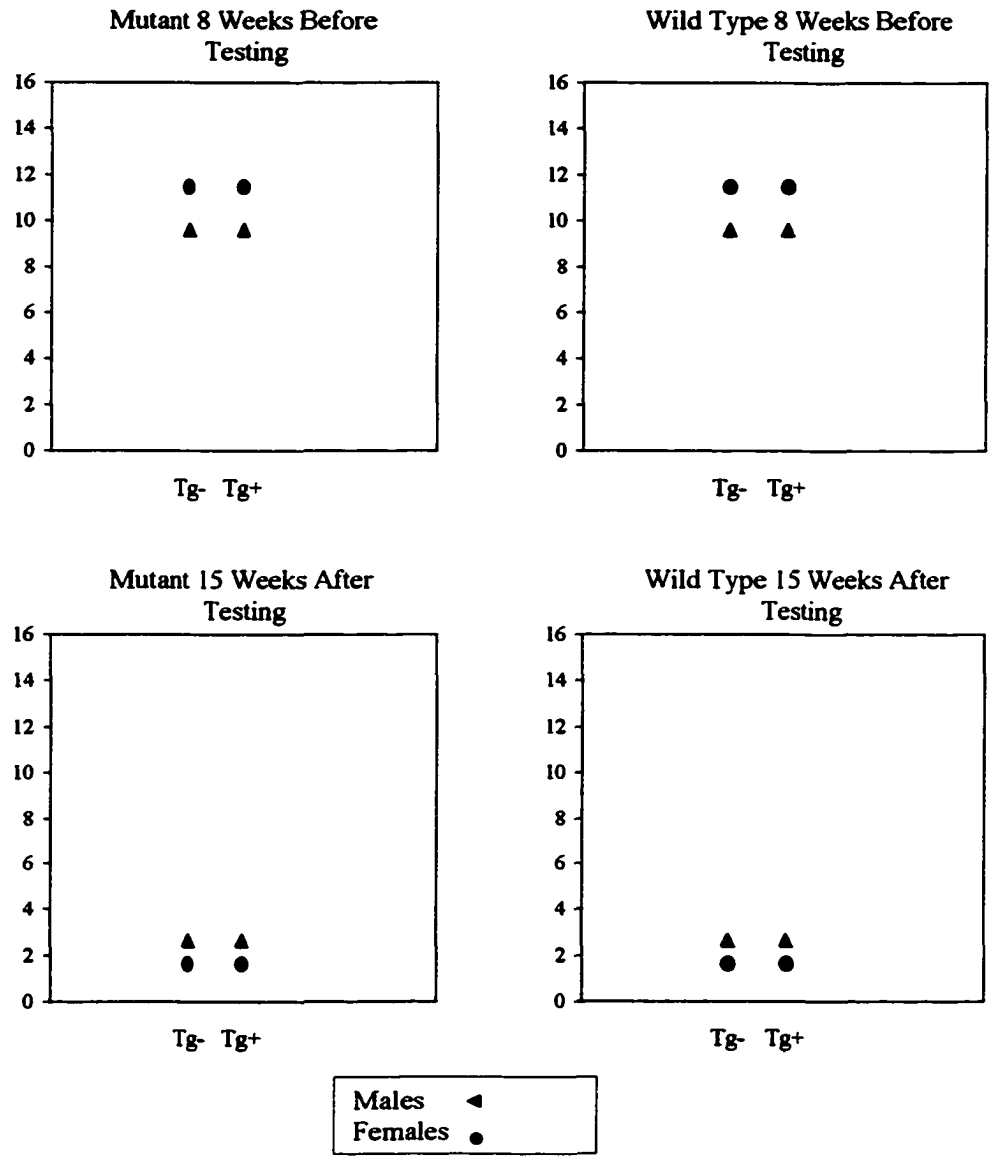
Dopamine Turnover. Turnover of DA in the frontal cortex is identified by examining the ratio of HVA/DA relative to concentration levels of DA. In the frontal cortex a two-way interaction involving transgenic line and age shows a significantly higher ratio of HVA/DA in both transgenic lines at eight weeks of age ($p<0.001$, Figure 16a). The lower levels of DA at eight weeks of age suggest that DA is metabolized more slowly rate in younger mice. Interestingly, an interaction of gender and age results in significantly low ratios in male mice at 15 weeks of age ($p<0.1$, Figure 16b) and high ratios in female mice at eight weeks of age. The lower ratios of HVA/DA in the SOD1 mice and the corresponding higher levels of DA may reflect a higher rate of DA turnover in the frontal cortex in SOD1 mice at 15 weeks of age. However, the ratio is HVA/DA in the hippocampus is significantly high in MSOD1 mice than in WT SOD1 mice ($p<0.001$, Figure 17) and significantly high in both MSOD1 and WT SOD1 mice at eight weeks of age ($p<0.001$, Figure 17). Levels of DA are highest in MSOD1 mice at 15 weeks of age. The high level of DA in the hippocampus and the low ratio again suggests that DA is turned over rapidly in older mice and in this case most specifically, MSOD1 mice.

Figure 16a
Fitted ratios of HVA/DA in the Frontal Cortex of MSOD1 and WT SOD1 Mice



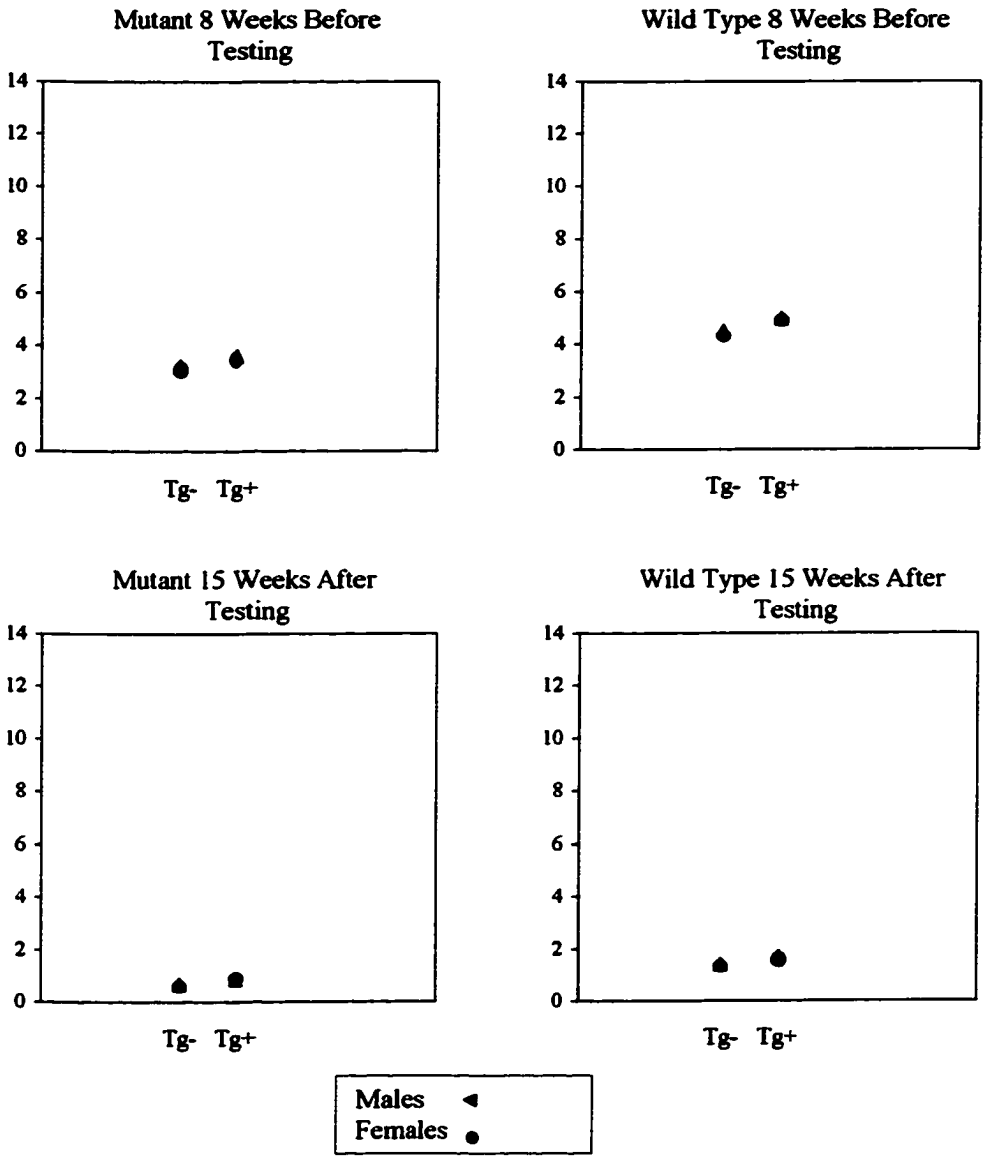
A significant interaction between transgenic line and age is shown in significantly higher fitted ratios of HVA/DA in MSOD1 and WT SOD1 mice at eight weeks of age ($p<0.001$).

Figure 16b
Fitted Ratios of HVA/DA in the Frontal Cortex of MSOD1 and WT SOD1 Mice



The elevated fitted ratios of HVA/DA in female mice at 8 weeks of and male mice at 15 weeks ($p<0.1$) are the result of a significant interaction of gender and age.

Figure 17
Fitted Ratios of HVA/DA in the Hippocampus of MSOD1 and WT SOD1 Mice



MSOD1 mice show significantly elevated fitted ratios when compared with WT SOD1 mice ($p<0.001$) and both transgenic lines reflect significantly elevated ratios of HVA/DA ($p<0.001$) at 8 weeks of age.

Serotonin and 5-HIAA. Neurochemical findings regarding the indolamine 5-HT quantified from both the frontal cortex and hippocampus reflects two-way interactions involving transgenic line, age, gender and genotype (as shown in Tables 8 and 9). A two-way interaction of transgenic line and age demonstrates significantly elevated levels of 5-HT in MSOD1 mice at 15 weeks of age ($p<0.001$, see Figure 18a).

While another interaction involving transgenic line and gender shows significantly elevated levels of 5-HT in MSOD1 females and WT SOD1 males ($p<0.05$, see Figure 18b). Also in the frontal cortex, the metabolite of 5-HT, 5-HIAA is shown at significantly elevated levels in MSOD1 mice at eight weeks of age and WT SOD1 mice at 15 weeks of age ($p<0.001$, Figure 20) the result of a two-way interaction involving transgenic line and age.

In the hippocampus, a two-way interaction of transgenic line and age results in significantly higher levels of 5-HT in MSOD1 mice at 15 weeks of age ($p<0.001$, see Figure 19a). Significantly elevated levels of 5-HT are observed in MSOD1 and WT SOD1 female mice at 15 weeks of age in an interaction involving gender and age ($p<0.1$, Figure 19b). Finally, an interaction of transgenic line and genotype produces significantly elevated levels of 5-HT in the hippocampus of WT SOD1 Tg+ mice ($p<0.1$, see Figure 19c). Finally, an interaction between transgenic line and age demonstrates that 5-HIAA is significantly elevated in WT SOD1 mice at eight weeks of age and MSOD1 at 15 weeks of age ($p<0.001$, Figure 21).

Table 8

Parameter Slope Estimates (and approximate p-values) from the OLS Regression from the Frontal Cortex on Transgene, Gender and Behavioral Testing for MSOD1 and WT SOD1 Mice

	OUTCOME		
Predictors	**Sq Rt 5-HT**	**Sq Rt 5-HIAA**	**Sq Rt 5-HIAA/5-HT**
Intercept	3.7238***	12.1144***	3.3538***
Mutant SOD1 (MSOD1)	0.7648~	3.1359***	-0.5388*
Positive for Transgene (Tg+)	-0.517*	-0.7593*	0.1249
Male (M)	0.2104	-0.3245	-0.2346
Mice 15 Weeks after Behavioral Testing (B)	7.3689***	-0.4357	-2.075***
MSOD1*B	4.4852***	-4.518***	-0.4152~
MSOD1*M	1.0674*		-0.7226**
R^2 between	0.8512	0.4054	0.7188

~ p < .10
* p < .05
** p < .01
*** p < .001

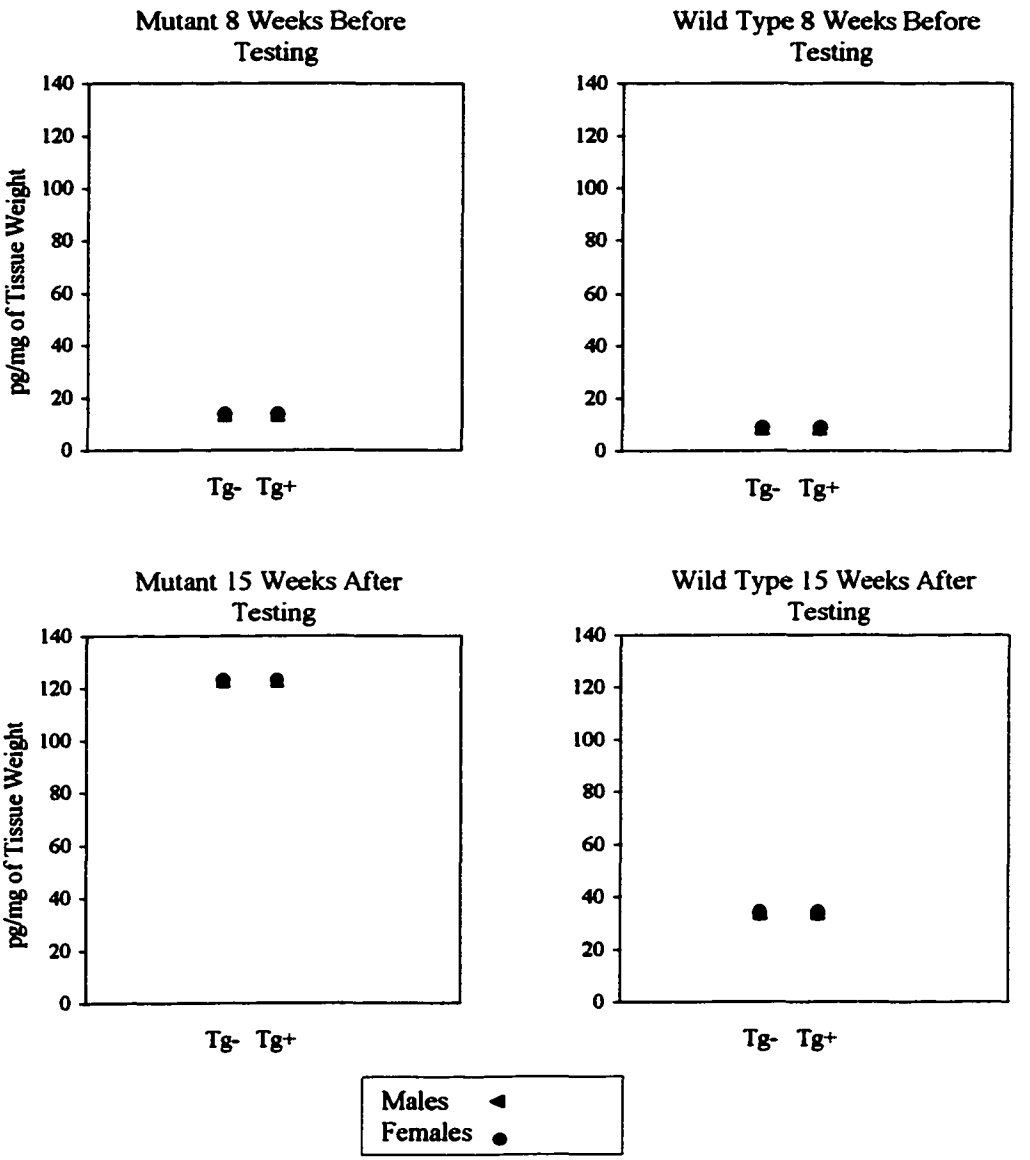
Table 9

Parameter Slope Estimates (and approximate p-values) from the OLS Regression of Monoamines from the Hippocampus on Transgene, Gender and Behavioral Testing for MSOD1 and WT SOD1 Mice

	OUTCOME		
Predictors	**Sq Rt 5-HT**	**Sq Rt 5-HIAA**	**Sq Rt 5-HIAA/5-HT**
Intercept	8.5144***	10.5905***	1.2531***
Mutant SOD1 (MSOD1)	0.5859	-1.9051***	-0.2278***
Positive for Transgene (Tg+)	-0.0520	0.0641	0.0302
Male (M)	-0.0543	-0.1062	0.0193
Mice 15 Weeks after Behavioral Testing (B)	6.9424***	2.0771***	-0.4045***
*MSOD1*B*	5.0642***	2.7046***	-0.1793**
*M*B*	-1.4227~		
*MSOD1*Tg+*	-1.4002~		0.1419*
R^2 *between*	0.6289	0.1902	0.5143

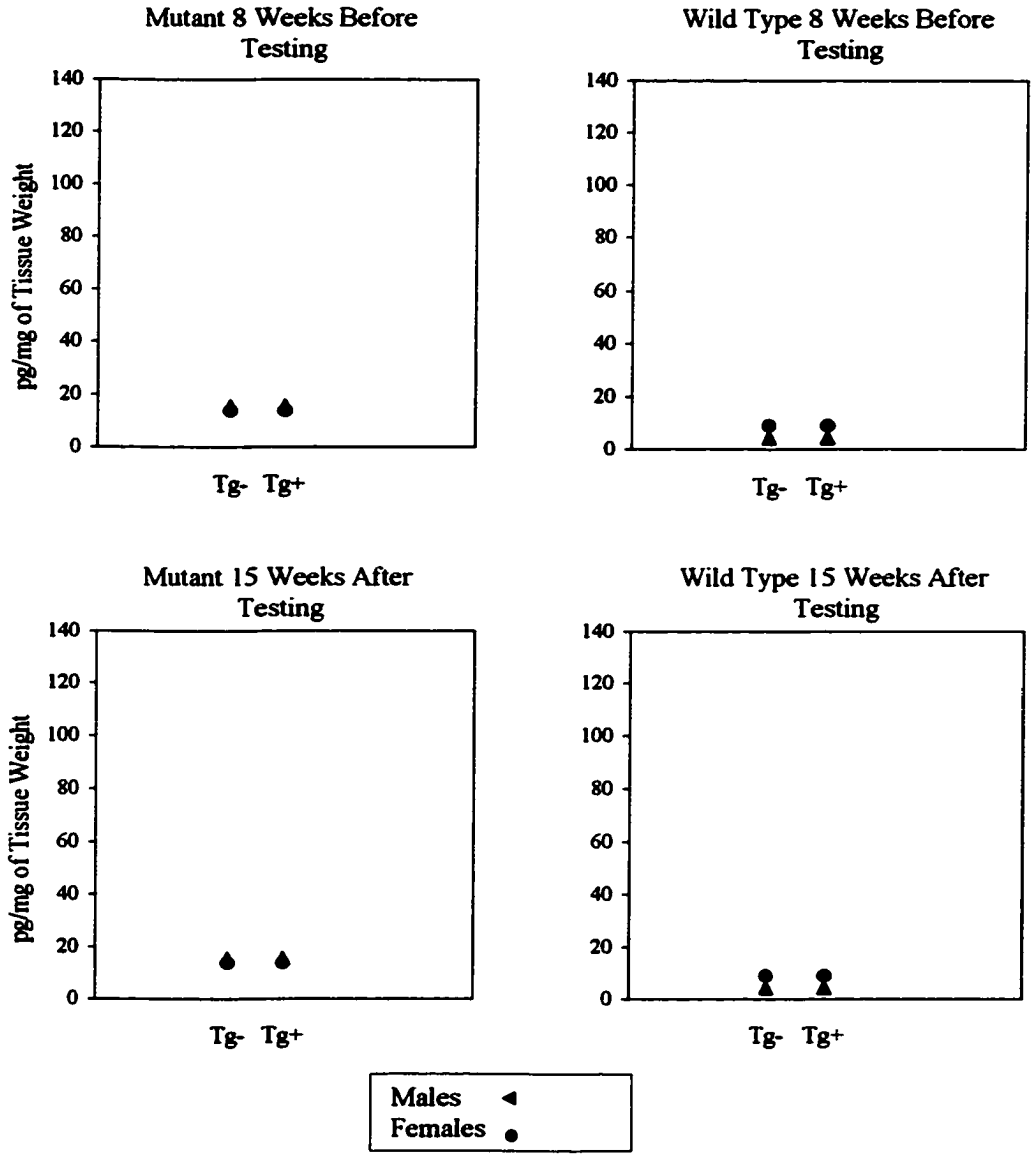
~ p < .10
* p < .05
** p < .01
*** p < .001

Figure 18a
Fitted Concentrations of Serotonin (5HT) in the Frontal Cortex of MSOD1 and WT SOD1 Mice



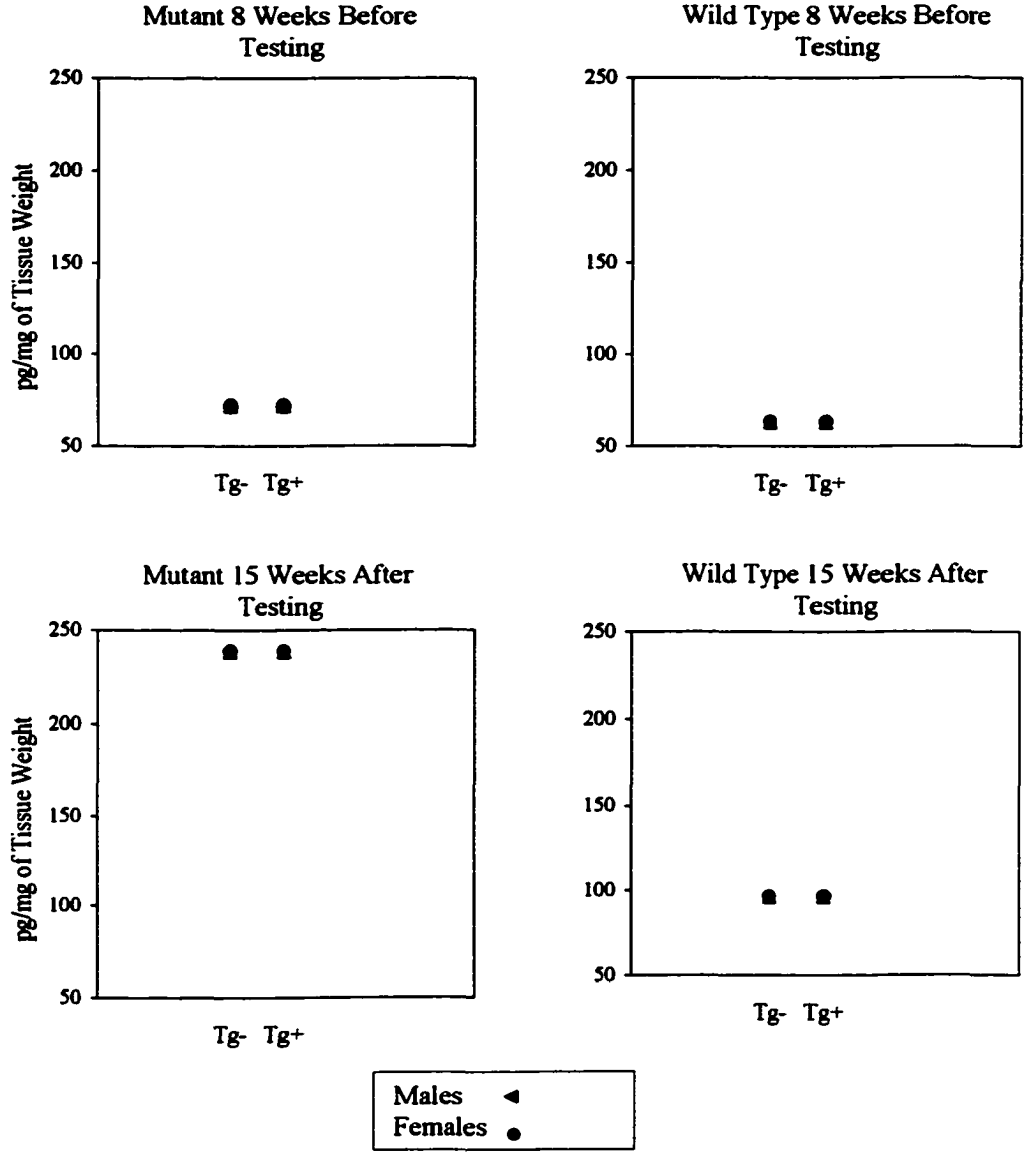
Significantly elevated levels of 5-HT are shown in MSOD1 mice at 15 weeks of age the result of an interaction of transgenic line and age ($p<0.001$).

Figure 18b
Fitted Concentrations of Serotonin (5HT) in the Frontal Cortex of MSOD1 and WT SOD1 Mice



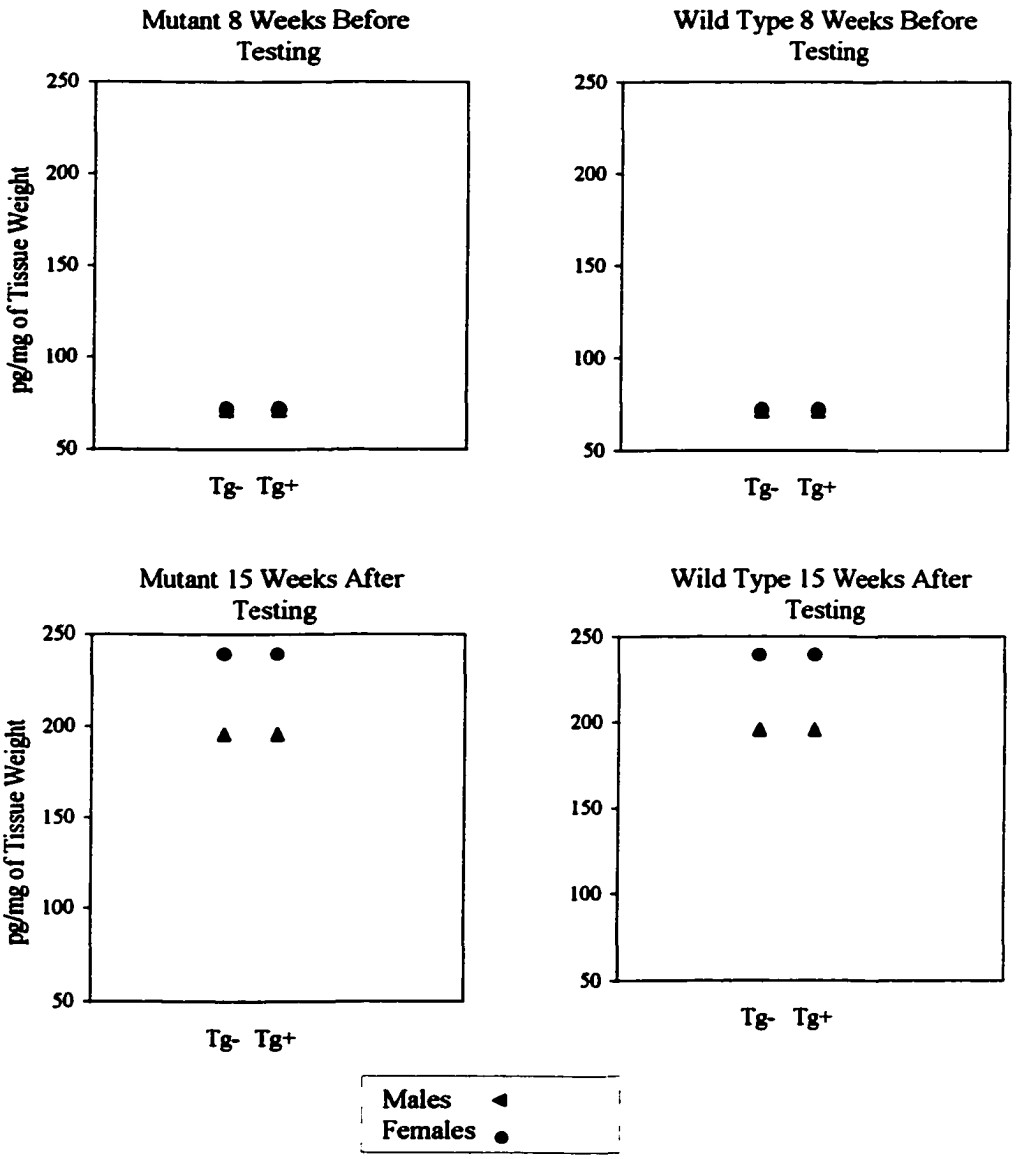
Significantly elevated levels of 5-HT are shown in the frontal cortex of MSOD1 female and WT SOD1 male mice reflecting a significant interaction of transgenic line and gender ($p<0.05$).

Figure 19a
Fitted Concentrations of Serotonin (5HT) in the Hippocampus of MSOD1 and WT SOD1 Mice



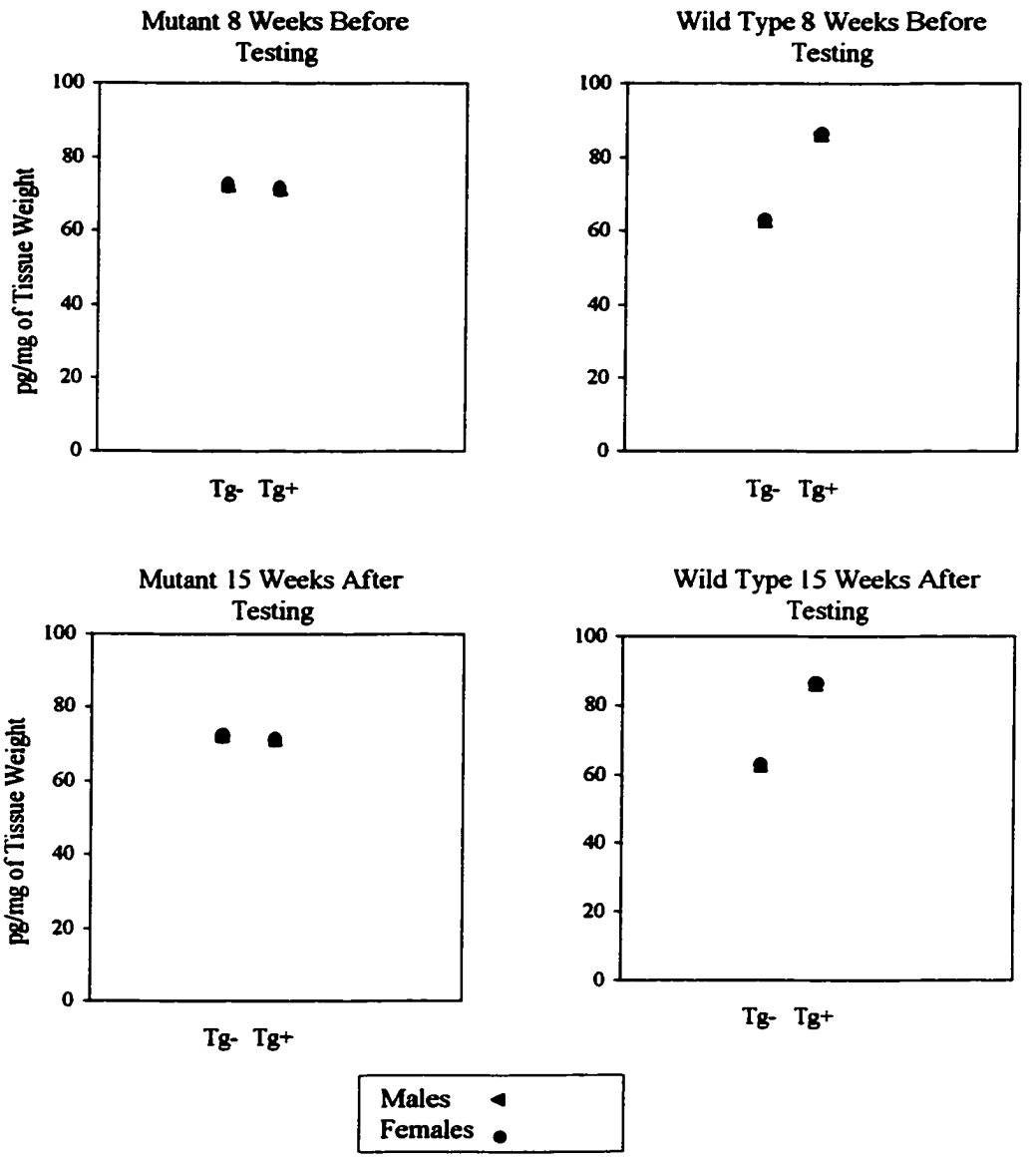
An interaction of transgenic line and age results in significantly higher levels of 5-HT in SOD1 mice at 15 weeks of age ($p<0.001$).

Figure 19b
Fitted Concentrations of Serotonin (5HT) in the Hippocampus of MSOD1 and WT SOD1 Mice



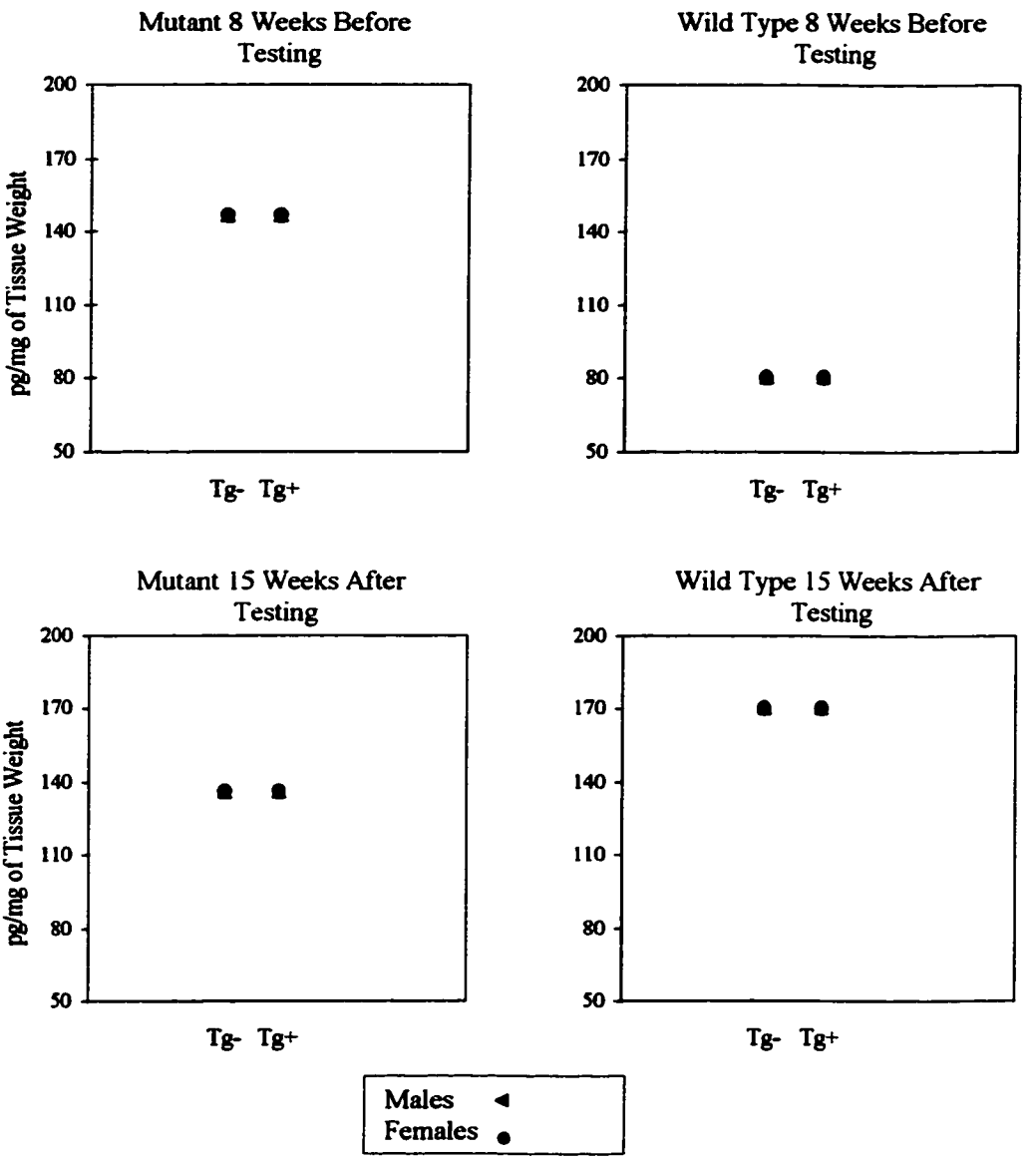
An interaction of gender and age in MSOD1 and WT SOD1 female mice at 15 weeks of age results in significantly high concentrations of 5-HT ($p<0.05$).

Figure 19c
Fitted Concentrations of Serotonin (5HT) in the Hippocampus of MSOD1 and WT SOD1 Mice



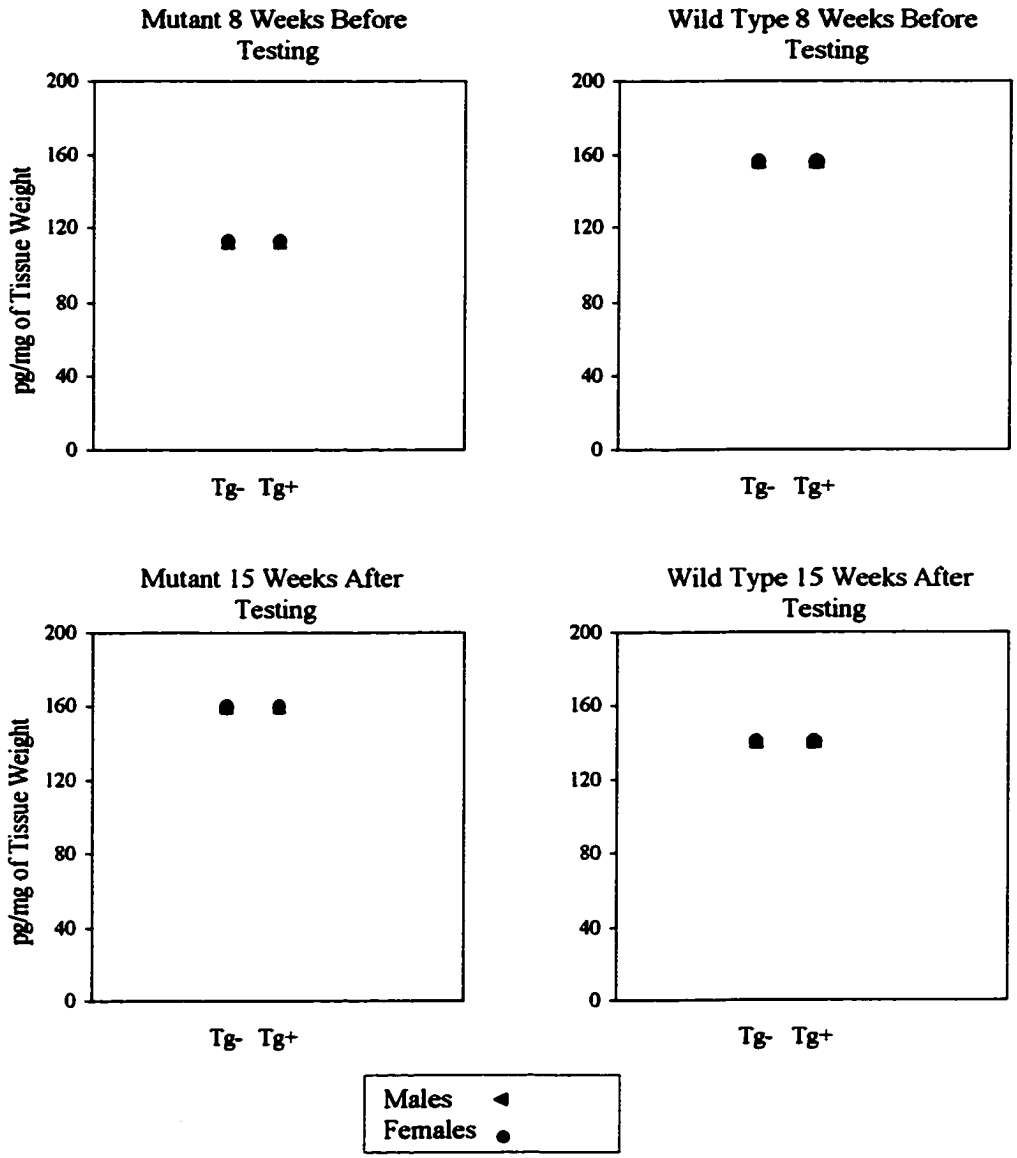
An interaction of transgenic line and genotype results in WT SOD1 Tg+ mice demonstrating significantly elevated levels of 5-HT ($p<0.05$).

Figure 20
Fitted Concentrations of 5-hydroxyindoleacetic acid (5-HIAA) in the Frontal Cortex of MSOD1 and WT SOD1 Mice



An interaction between transgenic line and age results in significantly elevated concentrations of 5-HIAA in MSOD1 mice at 8 weeks and WT SOD1 mice at 15 weeks of age ($p<0.001$).

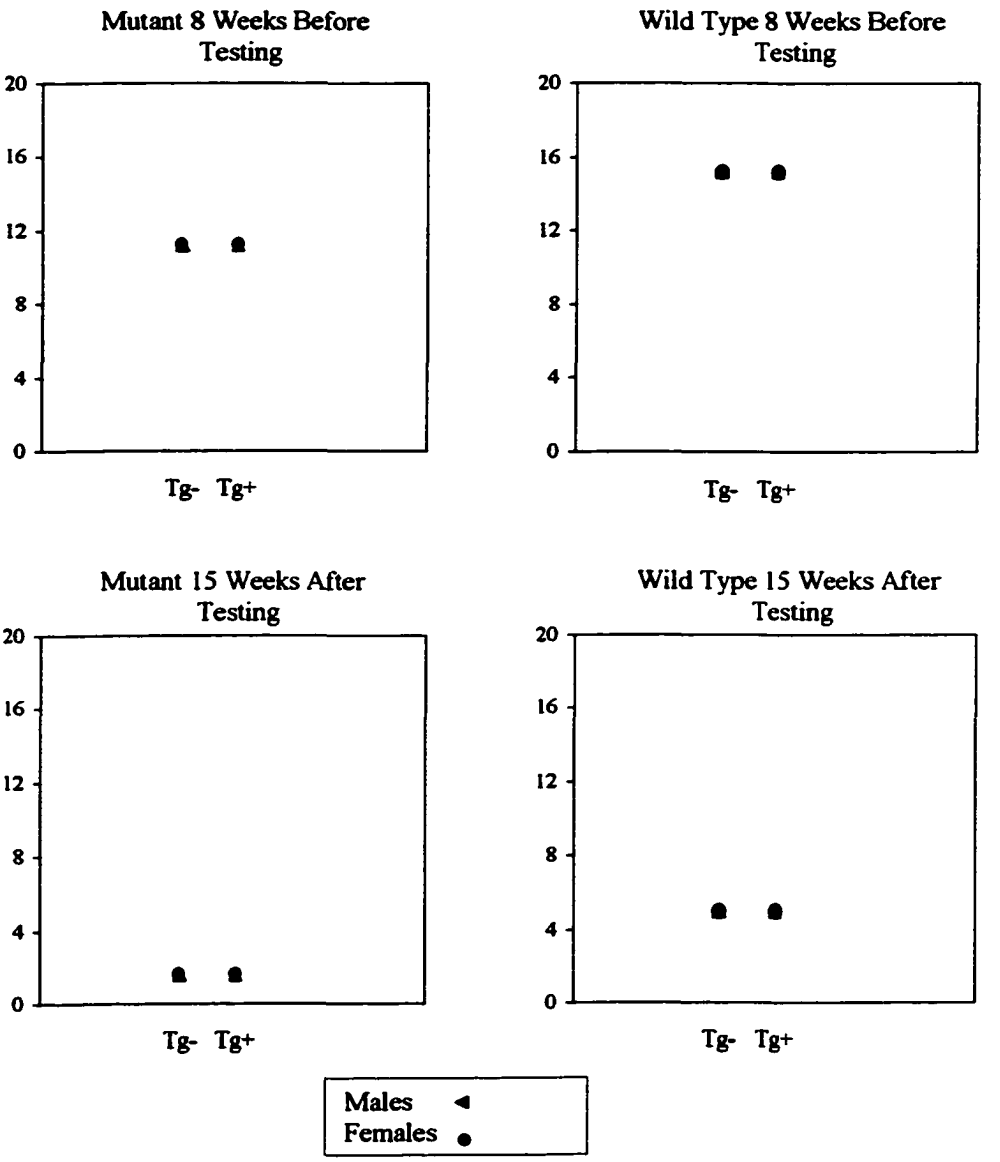
Figure 21
Fitted Concentrations of 5-hydroxyindoleacetic acid (5-HIAA) in the Hippocampus of MSOD1 and WT SOD1 Mice



Significantly elevated concentrations of 5-HIAA are present in the WT SOD1 mice at 8 weeks and MSOD1 mice at 15 weeks of age ($p<0.001$) demonstrating an interaction between transgenic line and age.

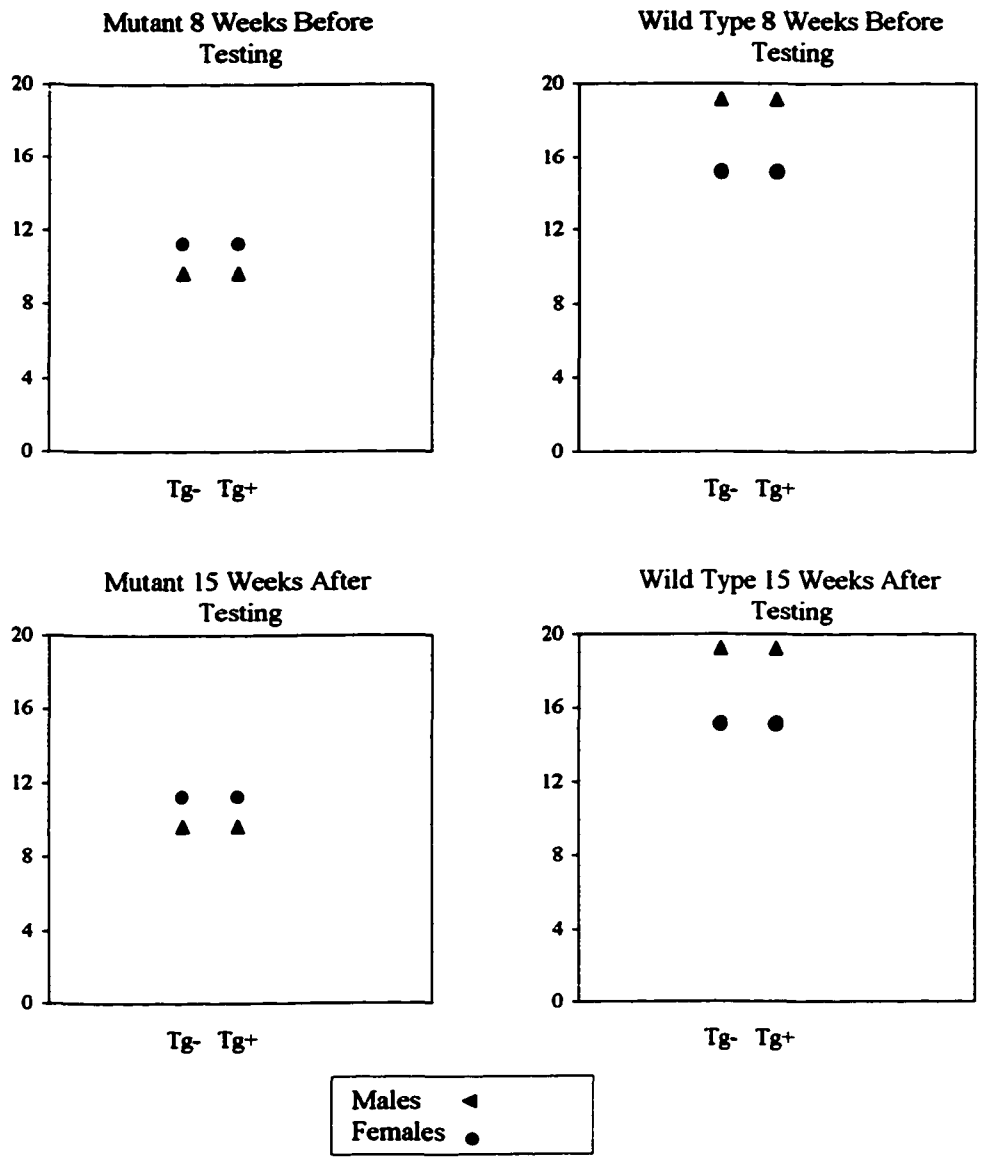
Serotonin Turnover. 5-HT turnover is determined by examining the ratio of 5-HT/5-HIAA. In the frontal cortex, the 5-HT ratio is significantly higher in MSOD1 and WT SOD1 mice at eight weeks of age reflecting an interaction of transgenic line and age ($p<0.1$, see Figure 22a). Another interaction of 5-HT/5-HIAA that involves transgenic line and gender shows significantly elevated ratios in male WT SOD1 mice ($p<0.01$, Figure 22b). The high levels of 5-HT and low ratios in MSOD1 mice at 15 weeks of age suggest rapid 5-HT turnover in the frontal cortex. Furthermore, the interaction of transgenic line and gender proposes that turnover may be more rapid in the male MSOD1 and female WT SOD1 mice. Interestingly, in the hippocampus the 5-HT/5-HIAA ratio is significantly elevated in WT SOD1 mice at eight weeks of age demonstrating an interaction between transgenic line and age ($p<0.01$, please see Figure 23a). An interaction between transgenic line and transgene results in the WT SOD1 Tg- mice showing a significantly elevated ratio of 5-HT/5-HIAA ($p<0.05$, Figure 23b). In the hippocampus, ratios for both eight and 15 week old mice are low. Levels of 5-HT are high at 15 weeks of age and highest in MSOD1 mice. This neurochemical finding suggests that 5-HT turnover at 15 weeks of age and particularly in the MSOD1 mice is rapid in the hippocampus.

Figure 22a
Fitted Ratios of 5-HIAA/5-HT in the Frontal Cortex of MSOD1 and WT SOD1 Mice



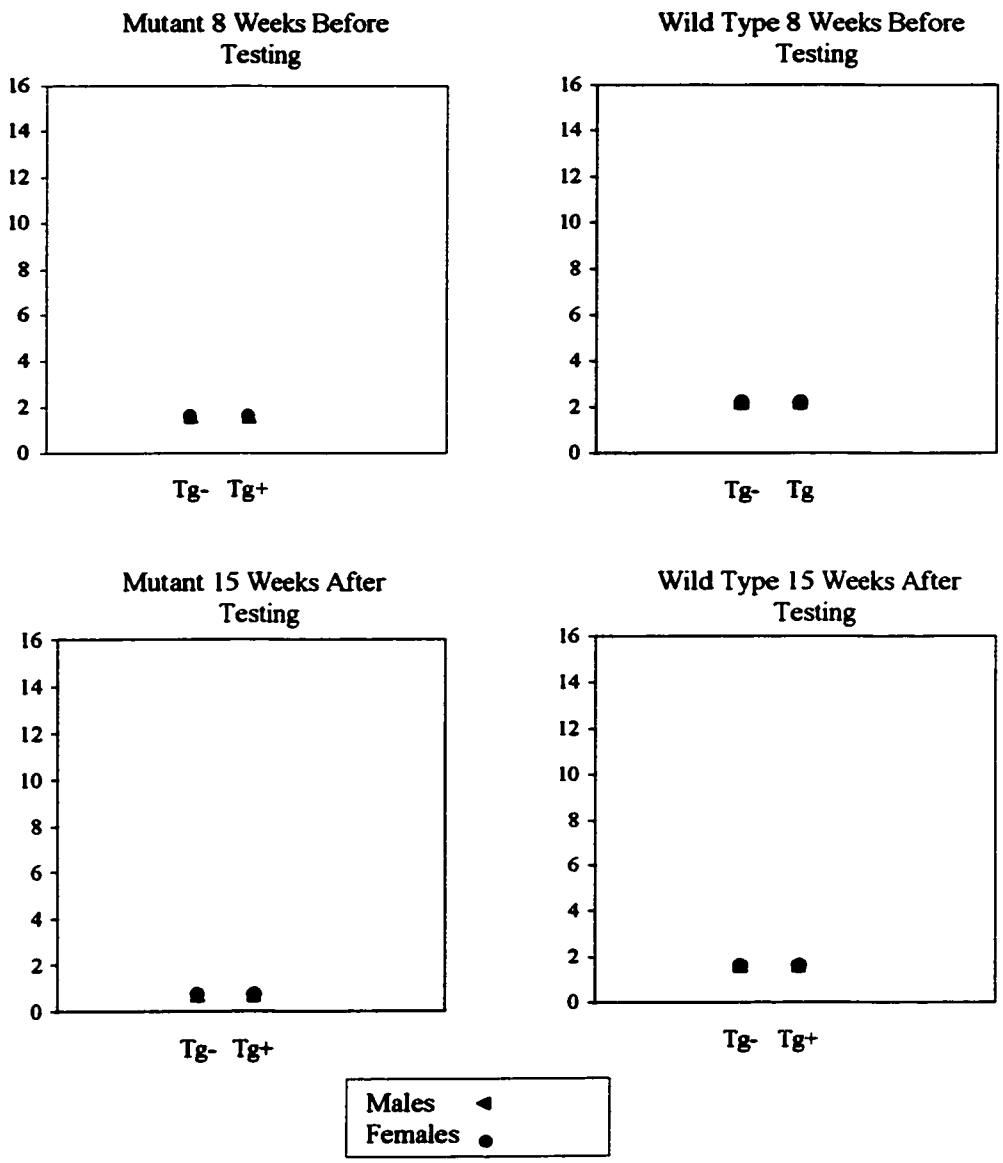
Significantly higher ratios of 5-HT/5-HIAA are present in the MSOD1 and WT SOD1 mice at 15 weeks demonstrating an interaction of transgenic line and age ($p<0.1$).

Figure 22b
Fitted Ratios of 5-HIAA/5-HT in the Frontal Cortex of MSOD1 and WT SOD1 Mice



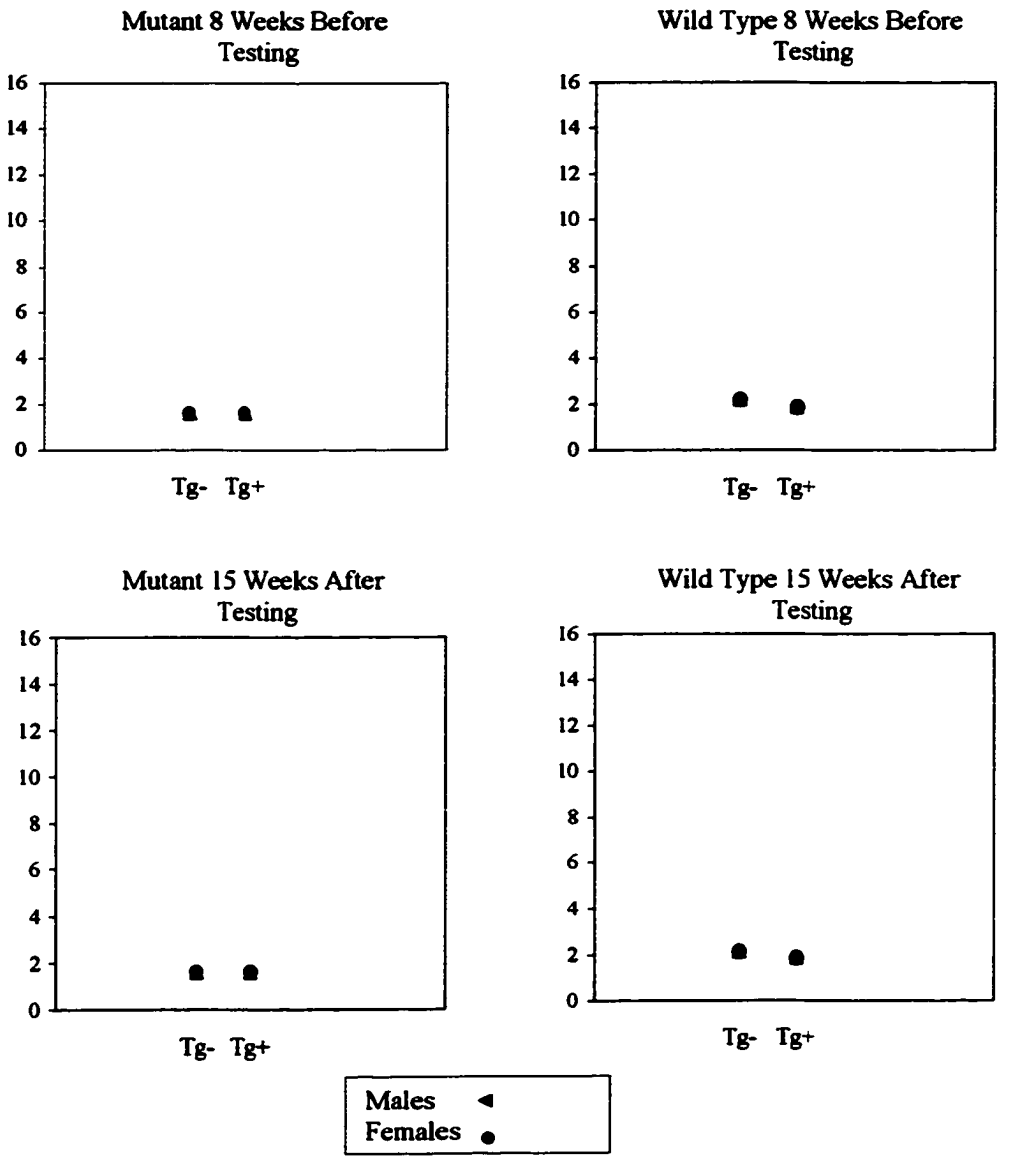
An interaction of transgenic line and gender shows significantly elevated ratios of 5-HIAA/5-HT in MSOD1 males and WT SOD1 females ($p<0.01$).

Figure 23a
Fitted Ratios of 5-HIAA/5-HT in the Hippocampus of MSOD1 and WT SOD1 Mice



An interaction between transgenic line and age results in significantly higher ratios of 5-HIAA/5-HT in WT SOD1 mice at 8 weeks of age ($p<0.1$).

Figure 23b
Fitted Ratios of 5-HIAA/5-HT in the Hippocampus of MSOD1 and WT SOD1 Mice



Significantly higher ratios of /5-HIAA/5-HT are present in WT SOD1 Tg- mice the result of an interaction of transgenic line and the presence of the transgene ($p<0.05$).

Summary of Hypotheses Three and Four

Levels of glutamate are significantly low in the frontal cortex of the MSOD1 mice when compared with the WT SOD1 mice. However, when comparing age groups of both transgenic lines, glutamate was significantly high in mice at fifteen weeks of age.

Monoamines, DA and NE are significantly elevated in MSOD1 mice in both the frontal cortex and hippocampus. DA is significantly high in MSOD1 mice in the frontal cortex. A two-way interaction involving transgenic line and age results in significantly elevated levels of DA in the hippocampus of MSOD1 mice at 15 weeks of age. With respect to NE, a three-way interactions result in elevated levels of NE in MSOD1 male mice and WT SOD1 female mice at fifteen weeks and MSOD1 females and WT SOD1 males at eight weeks of age. Interactions of transgenic line, gender, age and transgene show significantly high levels of 5-HT in both the frontal cortex and hippocampus in MSOD1 mice at 15 weeks of age with females more often than males reflecting the higher levels.

Chapter V

CONCLUSIONS AND STUDY LIMITATIONS

Overview

The final Chapter is organized into five sections: 1) Behavior, 2) Amino Acids, 3) Monoamines, 4) Limitations, and 5) Conclusions.

Behavior

The primary goal of this study was to determine if one of the SOD1 mutations associated with FALS affects cognition before the presentation of the first symptom or onset of motor neuron disease. A secondary aim of the study was to determine if there was an association of amino acids and monoamines from brain regions involved in cognition and memory function. In order to control for the effect of the SOD1 mutation on cognition, two transgenic lines mice were evaluated with the RAM, a hippocampal dependent memory task: MSOD1 Tg+ and WT SOD1 Tg+ and their Tg- littermates. MSOD1 mice express the largest amount of mutant SOD1 and carry at least eighteen copies of the MSOD1 gene. MSOD1 mice develop motor neuron loss and symptomatic features comparable to human FALS, including paralysis and die within five to six months. The severity of the disease corresponds to the number of copies of the mutated gene. WT SOD1 mice carry the normal human SOD1 gene. WT SOD1 mice harbor at least eight copies of the normal human SOD1 gene and do not develop symptoms of FALS.

This study showed that there was very little difference between the two transgenic lines. There were significant differences however, between the younger MSOD1 and older MSOD1 mice, between the males and females and between the transgenic and non-transgenic mice. For example, YMSOD1 mice made their first error later than OMSOD1 mice, chose more correct arms in the first eight choices on the RAM, and recorded fewer errors. Similarly, MSOD1 and WT SOD1 males made their first error later than female counterparts, chose more correct choices in the first eight choices on the RAM and made fewer errors. Interestingly, mice carrying the SOD1 transgene recorded more errors on the RAM when compared with their Tg- littermates. Therefore, over-expression of either the mutant or wild-type SOD1 gene had a deleterious effect on the performance of the mice on the RAM. Finally, while YMSOD1 mice required more time to learn the RAM than the OMSOD1 and WT SOD1 mice, performance of YMSOD1 mice with practice dramatically improved over the study period.

What can these behavioral findings tell us about the effect of mutant SOD1 on cognition and parallels with FALS in humans and specifically concerns about decision-making in patients diagnosed with ALS? Both MSOD1 and WT SOD1 transgenic mice may be models of accelerated aging and disease processes exacerbated by the presence of SOD1. Luine's laboratory has already observed differences in attention, spatial memory and monoaminergic systems between young and aging rodents (1990; 1994). Effective completion of the RAM requires two types of memory: spatial memory that utilizes the hippocampus; and, temporal memory that relies on the frontal lobe. Therefore, the cognitive impairments that we have observed on the RAM may be indicative of both hippocampal and frontal lobe dysfunction as seen in ageing systems, but specifically,

dysfunction of the frontal lobe. With the restrictions posed by using an animal model, we are, of course, limited in applying findings to human ALS. However, when we consider that attention and task acquisition underlie the basic decision-making process, we could speculate that parallels could be drawn between outcomes on the RAM and difficulties experienced with protracted decision-making. While one cannot exclude the effect of excitotoxic activity generated by the mutant SOD1, the differences in these mice may be relegated to SOD1, its role in the developing system affected by ALS or FALS, and its interaction with the neural processes controlling frontal lobe functioning.

One aspect of this study is to consider why the Tg- mice never performed at levels comparable with other strains of mice. For example, Bernstein et al. (1985), utilizing a highly controlled water deprivation paradigm, tested mice on the RAM three times a day, with 90 minutes between each trial for a period of thirty-six days. Both groups of older and younger mice performed at approximately the same level. When mice were first exposed to the RAM, they initially chose 5.3 correct choices out of the first eight, but after 20 blocks of trials their performance improved to approximately 7 choices out of the first eight. This level of performance in mice differs from the current study. The performance of the MSOD1 and WT SDO1 mice improved over time increasing to approximately 5.8 to 6.0 correct choices out of eight and average performance for all groups of mice ranged between 5.5-5.8 correct choices out of eight. MSOD1 and the WT SOD1 mice never performed at this level and initial performance suggests that the mice may have had difficulty with task acquisition and the trajectories of the mice showed steady albeit depressed progression and improvement. Alternatively, the strain of mice

used in this study may have inherently different RAM performance compared to other mice.

What do we make of the differences between the male and female mice with respect to task duration? Other reports in the rodent literature state that males perform cognitively better than females in tasks involving spatial memory (Luine, Beck, Bowman, & Kneavel, 2001). It may be that this decrement in motor activity observed in the males in this study reflects early motor impairment. In human ALS, there is a higher incidence of the disease in males; however, the ratio evens after women experience menopause. Gurney et al. (1994) originally reported that MSOD1 mice develop signs of the disease such as tremor in one or more limbs at approximately 91 $\pm$ 14 days. Paralysis occurs between the fourth and fifth month. Progression of muscle impairment was described in a study assessing the effects of lysine acetylsalicylate on MSOD1 mice. Investigators taught mice to grip a small loaded grid weighing 10, 20, 30 or 40 grams and then timed the mice in their ability to maintain their grasp on the grid. Investigators found that while motor function in MSOD1 mice did not differ from controls at seven weeks; significant differences were found at 15 weeks of age. Specifically, changes in muscle strength were noted at approximately 12 to 14 weeks of age, but depended upon the weight of the grid. When the grid was heavier, performance of the mice was worse. Performance improved in the mice when the lighter grids were used (Barneoud & Curet, 1999). Study limitations were that motor decrements were only examined in male mice. Investigators did not examine differences between genders.

Gender differences in motor behavior were identified in evaluating the effects of riluzole (2-amino-6-trifluromethoxy-benzothiazole) on MSOD1 mice. MSOD1 mice

were trained on a running wheel located in their cages. After approximately one week of practice, mice ran 7 to 10 km per night and female mice ran significantly longer than male mice (10.7 ± 0.4 versus 7.4 ± 0.3 km). The length of the stride of the mice was similar, although males weighed more than females. The increased activity of the female mice did not alter disease onset or survival (Gurney, Fleck, Himes, & Hall, 1998). Therefore, as mice in the present study were evaluated from 42 to 109 days of age, it is possible that motor performance of the male mice was affected by muscle weakness. However, if this were the case, comparable task duration would not be found in the male MSOD1 Tg- mice who completed the task at approximately the same rate as the MSOD1 Tg+ males.

Over-expression of SOD1 may affect both Tg+ and Tg- mice. Morphological changes have been found in mice that overexpress the WT SOD1 gene. Avraham, Schickler, Sapoznikov, Yarom & Groner (1988) identified muscle impairment in two to four month old transgenic mice carrying elevated levels of WT SOD1. Pathological features such as flattened and degenerated nerve terminals, vacuoles, neurofilaments and other structures were present in the neuromuscular junctions of tongues. The area around the end plates contained increased mitochondria, membranous degenerate structures and convoluted nuclei. Schwann cells also showed abnormalities. Interestingly, similar neuropathological features developed in the Tg- littermates of these mice at one year of age. Therefore, investigators suggest that the elevated levels of SOD1 may provoke these neuropathological abnormalities and accelerate aging similar to processes inherent in DS.

Examining how SOD1 affects individuals with DS may facilitate our understanding SOD1 and its effect in FALS. SOD1 is elevated in individuals diagnosed

with DS and progressive cognitive impairment is a hallmark of DS. Biological markers of SOD1 in DS have not been widely correlated in non-demented or demented individuals with DS although some markers have been observed in transgenic mice (Epstein et al. 1987). However, one study examined activity of SOD1, as well as glutathione peroxidase (GSHPx) and catalase (CAT) in erythrocytes of non-demented adults with DS ranging in age from 21-51 years. Erythrocyte SOD1 and GSHPx activities were significantly elevated in DS adults relative to controls: mean SOD1 activity was 50% greater. Two neuropsychological measures indicating mild dementia were also significantly correlated with erythrocyte GSHPx activity, but not with CAT or SOD1 activity. However, investigators emphasize that levels of SOD1 and GSHPx are highly variable and findings may be misleading. GSHPx's role in oxidative stress is compensatory and protective. Although neuropsychological impairment was not found in these DS adults, they were found to be impaired in another study. Brugge examined these same DS adults with other neuropsychological tests and found that they demonstrated significant cognitive impairment when compared with IQ and age matched non-DS controls (as cited in Brugge, Nichols, Saitoh & Trauner, 1999). Therefore, findings relative to SOD1 are still being ascertained; the role relationship between SOD1 and cognition is unclear. The present study of cognitive behavior in MSOD1 and WT SOD1 mice supports the notion that SOD1 activity is involved with cognitive performance and over-expression of wild-type or mutant SOD1 specifically impaired performance on the RAM. The mutation associated with SOD1 may have a deleterious effect, but additional studies need to be pursued.

Amino Acids.

Glutamate, the most prevalent excitatory transmitter in the central nervous system, facilitates neuronal death by increasing intracellular calcium and is released by neurons during apoptosis. Elevated levels of glutamate have been found in the spinal cords of patients diagnosed with ALS (Rothstein, 1995). Glutamate's potential involvement with the production of free radicals might lead one to speculate that increased levels of glutamate may be present in other regions of the affected degenerating nervous system. Additionally, glutamate is also involved with cognitive behavior. The amino acid acts on two receptors, one of which is the N-methyl-D-aspartic acid (NMDA) receptor, which is associated with learning. Therefore, it is surprising to find that levels of glutamate are significantly lower in MSOD1 mice when compared with WT SOD1 mice. Although glutamate may not have a primary role in the degeneration of neurons in ALS or FALS, these findings may support the notion that glutamate levels change as the result of secondary processes in the degenerating physiological system.

However, although levels of glutamate were significantly low in MSOD1 mice when compared to WT SOD1 mice, elevations of glutamate were significantly higher in all older mice when compared with younger mice. The older mice were exposed to behavioral testing, RAM and weeks of food restriction. Despite adaptations in the protocol aimed toward reducing stress, these factors may have increased stress in the mice. Moghaddam found that stress was associated with increased levels of glutamate (as cited in Luine, Martinez, Villegas, Magarinos, & McEwen, 1996) and the associated experience of stress may explain the difference in findings relative to age.

In cognitive behavior, glutamate is responsible for transmitting LTP, a hippocampal NMDA receptor dependent task. LTP has not been tested in the brains of MSOD1 mice but it has been tested in slice cultures of brains from extra-cellular over-expressor SOD1 mice. Results have varied and have been dependent upon the paradigms used for LTP (Levin et al. 1998; Gahtan, Auerbach, Groner & Segal, 1998; Thiels et al, 2000). Therefore, impaired performance of OMSOD1 mice relative to YMSOD1 mice may be a reflection of glutamate availability and the corresponding response of the NMDA receptor. As the load of the cognitive task increases or the paradigm shifts to one that is unfamiliar to the mice, demand for glutamate may be increased due to increased LTP activity. MSOD1 mice may require increased supplies of glutamate because of increased demands on the physiological system provoked by the SOD1 mutation. Lower levels of glutamate that were found in the MSOD1 mice could be attributed to deficiency of the synthetic pathway supplying glutamate caused by stress and depletion of the neurotransmitter. This supply and demand cycle may underlie the argument for attentional deficits in learning acquisition. For example, the YMSOD1 mice required a longer duration to learn the RAM when compared with the OMSOD1 and WT SOD1 mice. However, once the younger mice learned the task, their duration shortened rapidly.

This enhanced performance that corresponds to a shift in cognitive load may also be present in the suspected decision-making difficulty in patients diagnosed with ALS. For example, if we consider the association of SOD1, enhanced LTP and corresponding variations in learning paradigms, the decision making process in patients diagnosed with ALS may occur in the follow manner: as information regarding technological options are presented to the patient, it is as though each option presents a paradigm that must be

learned. In order for the individual with ALS to feel comfortable with the decision that they are making, the paradigm must be presented over and over again until the learning paradigm is no longer unfamiliar but familiar. Habituation occurs and with increased exposure to the paradigm the patient may be more able to make a decision. Alternatively, if the patient is presented with the information once or rapidly the patient is limited in his or her ability to accommodate the paradigm. This finding may suggest that alternative complementary paradigms of educations may enhance information acquisition in patients diagnosed with ALS.

Another amino acid, GABA was significantly low in MSOD1 mice. GABA has also been found to be low in the prefrontal cortex of patients diagnosed with ALS. Lloyd, Richardson, Brooks, Al-Chalabi & Leight (2000) used PET scan and flumazenil, a benzodiazepine GABA marker, to examine cerebral dysfunction in patients diagnosed with ALS. When ALS patients were compared to normal controls, ALS patients showed significantly decreased flumazenil in the prefrontal cortex, parietal cortex, visual association cortex and left motor/premotor cortex. Therefore, lack of adequate levels of GABA may have contributed to the impaired cognitive performance of the MSOD1 mice, and particularly the impaired motor performance of the male MSOD1 mice as demonstrated in increased task duration. GABA is the one of the major inhibitory neurotransmitters of the central nervous system although it may sometimes promote excitation. For example, Durkin (1992) found that GABA provided synaptic input to the hippocampal circuit, but could not determine if the effect was excitatory or inhibitory as both findings were shown. If GABA was acting in a excitatory manner, the neurotransmitter may have compensated for deficits induced by the mutated SOD1.

Alternatively, the elevated levels of GABA may have inhibited performance of the WT SOD1 mice by exaggerating their cognitive deficits. When Menses injected GABA into the prefrontal cortex, learning deficits were exaggerated in aged rats (as cited in Haroutunian & Santucci, 1999).

Monoamines

Dopamine. The MSOD1 mice showed significantly elevated levels of DA in the frontal cortex and the hippocampus when compared with WT SOD1 mice. Metabolites of DA are also elevated: DOPAC and HVA were significantly increased in both the frontal cortex and hippocampus of the MSOD1 mice when compared with WT SOD1 mice.

There are four major dopaminergic tracts. Three of these pathways originate in the substantia nigra: the nigrostriatal pathway affects movement, the mesolimbic and mesocortical affect emotion and motivation, and the fourth tract originates in the hypothalamus and projects to the pituitary gland and affects the secretion of hormones (Schwartz, 2000). Of these four systems, the two systems whose cell bodies originate in the ventral tegmental area may influence this studies' findings of DA: the mesolimbic system utilizes neurons that project to the limbic system; and, the mesocorticol system utilizes neurons that project to the neocortex. While the hippocampus is involved with spatial orientation and memory, the prefrontal cortex directs temporal organization such as planning, attention, motivation and social behavior.

Therefore, the dopaminergic system is involved with frontal lobe function and working memory. Patients with neurodegenerative diseases such as Parkinson's disease and reduced DA have difficulty with working memory and prefrontal lobe function (Frick

& Sweeney, 1999). If patients diagnosed with ALS are suspected to have difficulty with prefrontal lobe functioning and working memory, why is DA elevated in MSOD1 mice?

Weinberger (1987) examining the physiological processes involved with schizophrenia proposed that the mesolimbic and mesocortical systems operate in tandem. Investigations of the role of DA in the positive and negative symptoms associated with schizophrenia suggested that increased activity of the mesocortical pathway inhibits the mesolimbic pathway by feedback inhibition. When these pathways are imbalanced they facilitate the negative and positive symptoms associated with schizophreniza. To investigation the inter-relationship of the two systems Pycock (1980) lesioned the mesocortical pathway and found that activity was enhanced in the mesolimbic pathway.

In this study, DA levels in the frontal cortex in the MSOD1 mice were significantly elevated and all mice showed elevated levels at 15 weeks of age. However, an interaction of transgenic line and age induced elevated DA levels in the hippocampus of MSOD1 mice at 15 weeks of age. This finding suggests that the dopaminergic pathways may be functioning in a compensatory manner similar to the interaction described in schizophrenia. The inter-relationship of the two pathways results in an increased supply of DA to both the frontal cortex and hippocampus. Stimulation of the frontal cortex, enhanced attention to task and motor activity promoted elevated levels of DA in the MSOD1 mice. Perhaps because of the enhanced effect of the mutant SOD1 gene on their physiological functioning, MSOD1 mice work harder to complete the task. The inhibitory feedback interaction between the two pathways appears to be diminished. MSOD1 mice may have lost this compensatory feedback ability. Thus, DA levels are high in both the frontal cortex and the hippocampus. The high level of DA in the

hippocampus and the low FVA/DA ratio suggests that DA is turned over rapidly in these mice.

Stimulation may also have been provided by the RAM, which served as a novel stressor for the MSOD1 mice comparable to the role of food deprivation in another study involving cognitive performance (Beck and Luine, 1999). We now know that the release of DA and its subsequent interaction with D2 receptor is involved with a pleasure-reward circuit. When DA is released, feelings of well-being are increased and stress is reduced (Blum et al. 2000; Comings & Blum, 2000). Dysfunction in receptivity, for example, compromise D2 receptors, promoted craving of alternate substances such as, cocaine, nicotine, etc., in order to promote the release of DA. The SOD1 mice demonstrated high variability of performance on the RAM and incidences of stereotypy were frequent. The highly variable prolonged trials may have prompted enhanced DA release, and thus, served as a form of self-stimulation for the mice. Release of DA may also have been affected by these changes in the cortical regulation of the subcortical DA system. The increased DA and changes in the hippocampus influences the processing of information from the prefrontal cortex (Grace, 2000) thus, provoking high variability and stereotypy in performance. Again, while there are restrictions in applying findings from an animal model, this variability of behavior may be evident as in protracted difficulty in decision-making in patients diagnosed with ALS.

Norepinephrine. NE is significantly elevated in a number of complex interactions in both the frontal cortex and hippocampus of the MSOD1 and WT SOD1 mice. Neurons that produce NE are located in the locus ceruleus and project throughout the cortex, cerebellum, and spinal cord (Schwartz, 2000). Gresch, Sved, Zigmond & Finlay (1994)

assessed how stress affected catecholamine release in the medial prefrontal cortex of rats as well as other brain regions. Stressors were presented within two paradigms: continuous (coldness) and novel (tail shock). When the novel stressor was presented after the continuous stressor of coldness, increased NE efflux was shown in the medial prefrontal cortex. The responsiveness of the frontal cortex to continuous and novel stressors in the rodent is noteworthy. The complexity of the interactions of NE in both the frontal cortex and hippocampus may further support the suggestion that the RAM served as novel stressor to the MSOD1 and WT SOD1 mice encouraging the expression of a circulatory loop of stimulation, pleasure and reward.

The complexity and variability of NE and other monoamines in the frontal cortex may be regulated directly by expression of SOD1. The intricacies of this relationship may be observed in another study examining the role of SOD1 and monoaminergic activity. In that study, CD-1 mice were administered 1-methyl-4-phenyl-1,2,3,6-tetrahydrophyridine (MPTP) analog 1-methyl-4-(2'-aminophenyl)-1,2,3,6-tetrahydrophyridine (2'-NH2-MPTP) and decreases of 5-HT and NE were found in the cortex and hippocampus, while striatal levels of DA were unchanged. However, when homozygous SOD mice with 5-fold SOD brain activity were administered the same agent 5-HT and NE levels were unaffected; and, when heterozygous SOD mice (showing a 3-fold increase of SOD) were administered 2'-NH2-MPTP 5-HT and NE were decreased moderately. Decreasing levels of SOD activity corresponded with decreasing availability of 5-HT uptake sites (Andrews, Ladenheim, Epstein, Cadet & Murphy, 1996). SOD1 appears to be neuroprotective when considering the effects of this neurotoxic lesioning agent.

Serotonin. Complex interactions between the effects of transgenic line, presence of transgene, gender and age resulted primarily in significantly elevated 5-HT in the frontal cortex and hippocampus primarily in the older MSOD1 mice. Neurons that produce 5-HT are found in the raphe nuclei of the brain stem and affect cognitive function especially attention and project throughout the brain and spinal cord (Schwartz, 2000). Interactions of transgenic line and age resulted in significantly increased levels of 5-HT in MSOD1 mice at 15 weeks in both the frontal cortex and hippocampus. WTSOD1 female mice were affected by an interaction involving gender and age: WT SOD1 mice, along with the MSOD1 mice, at 15 weeks of age, showed significantly increased levels of 5-HT in the hippocampus. These elevated levels of 5-HT may have contributed to impaired performance of the WT SOD1. This inhibitory effect of 5-HT on cognitive behavior has been noted. For example, Luine, Villegas, Martinez & McEwen, (1990) found that increased levels of 5-HT are present in the aging system and furthermore, that 5-HT demonstrates an inhibitory effect on cognition and learning. In that study, investigators found that the drug tianeptine, which affects 5-HT reuptake, blocked the effects of stress-induced atrophy of CA3 dendrites and attenuated stress-dependent impairments in spatial memory. Therefore, in the present study the increased levels of 5-HT in the MSOD1 mice at 15 weeks of age may have inhibited performance.

In another study, the corresponding and variable roles of both 5-HT and DA in cognitive performance were demonstrated when levels of monoamines were measured in rats after they were taught a five choice serial reaction time task. Puumala & Sirvio (1998) used multivariate regression analysis with a stepwise method to show that metabolites of 5-HT from the left frontal cortex and metabolites of DA from the right

frontal cortex accounted for 49% of variability in attention between subjects. Findings relative to 5-HT and choice accuracy were negatively correlated and findings pertaining to DA and choice accuracy were positively correlated. Furthermore, when 5-HT utilization was measured in the right frontal cortex positive correlations were associated with 24% of the variability of impulsivity between animals, as shown in the proportion of premature hole responses. Similar to the stereotypy and variability, these findings relative to impulsivity may further support our understanding the difficulty in decision making that may be observed in patients diagnosed with ALS.

Limitations

Limitations of this study were the variable nature of the SOD1 transgenic, a response-reward paradigm that would accommodate this variability (as well as the fragility of the transgenic), and the lack of comparison of SOD1 transgenic mice with another line of over-expressing SOD1 mice and normal control mice. Another limitation was posed by the examination of whole tissue using HPLC as opposed to methods of intracellular analysis. Therefore, neurochemical findings in this study were relegated to concentration release, as opposed to indications of system failure.

Conclusions

Although mutations of SOD1 do not appear to dramatically effect cognition as indicated by the performance of MSOD1 mice on the RAM, SOD1 does appear to be related to the functioning of the frontal cortex and modulates pathways involving amino acids and monoamines. Difficulties lie in determining if the effect of SOD1 is primary or

secondary. The inhibitory and excitatory nature of amino acids and monoamines in SOD1 mice influence the performance of the mice and the behavior of the mice influences the levels and expressions of the amino acids and monoamines. It is difficult to determine if the expressions noted in this study were pure baseline expressions of neurochemical activity or the result of an artifact of the transgenic system. For example, the transgene is incorporated into the genome in a random tandem fashion. The incorporation of the transgene may have occurred near one of the amino acid or monoamine pathways, thus, inhibiting or exacerbating neurochemical release. Another transgenic line producing SOD1 would facilitate the investigation of this potential artifact.

ALS and FALS are diseases that have many forms of expression. Some investigators state that ALS is not one disease but many. However, FALS and its associated mutation of SOD1 is probably one of the purer forms of the disease process, which is why its mechanisms are important. Understanding the mechanisms involved with FALS may lead to greater understanding of ALS. Synaptic plasticity and the compensatory measures of the neuronal and monoaminergic system have been identified as factors that either impair or enhance cognitive performance in this study. Therefore, these are factors that one would wish to consider in monitoring the progression of ALS in individuals and may account for the considerable heterogeneity observed in ALS and FALS. The potentially positive effect of novelty and pleasure in the environment may have facilitated this synaptic plasticity in the rodent. Specifically, that SOD1 or its subsequent modifications to associated systems may enhance the reward-response circuit controlled by GABA, 5-HT and DA. Thus, stimuli in the environment assume proportions of novel enhancement and potential sources of self-stimulation. This finding

too may underlie the emotional heterogeneity also observed in patients diagnosed with ALS and FALS. Some patients diagnosed with ALS respond to stressors in their environment with greater resilience. This novelty pleasure circuit provided by the monoaminergic system and enhanced by the protective features of SOD1 may enhance resilience to the degenerating features of the progressive disease.

Accordingly, SOD1 protects areas of the brain potentially impacted by mutant cytotoxic activity. In this study, we see that YMSOD1 mice are superior in performance to OMSOD1 and WT SOD1 mice. Therefore, SOD1's protective influence may override or compensate for the toxic influences of the mutant protein. Alternatively, factors influenced by the SOD1 cascade downstream may provide this protection until onset of symptoms. Degeneration begins and other factors initiating other biological cascades in disease pathology override all protective mechanisms. Biological substrates or lesions developed in ontogeny are activated and similar to Weinberger's (1987) theory of the development of schizophrenia in young adults the lesion developed early in development interacts with normal maturation later in life. When we consider the cognitive performance of the three groups of mice over the evaluation period, we see performance measured and repressed when compared with other strains of mice. There is improvement in the mice, but it is not dramatic. A striking feature in the cognitive performance of all of the mice on the RAM was that the trajectories for each behavioral outcome were essentially parallel. Although the trajectories may have been close to one another depending upon the gender, the trajectories of each genotype reflected their own course. A Tg+ mouse was clearly different from a Tg-.This finding may suggest that the pathological effect of both mutated and over-expressing SOD1 begins early, in

development, and further, that demonstrable signs of pathology may not be present until later in the aging process. Although we cannot undermine the possibility that mutated SOD1 influences cognitive behavior on the RAM, the excitotoxicity of the mutation appears to have no more effect than the developmental phenomenon that produced the differences in SOD1 in the evolving system in the first place.

APPENDIX I

INITIAL PRELIMINARY FITTED GLS REGRESSION MODEL FOR FIRST ERROR

```
xtreg er YMSOD1 WT SOD1 DP Tg+ M YMSDP WTSDP TDP MDP YMST WTST YMSM
WTSM TM YMTM WTTM YMTDP WTTDP YMMDP WTMDP TMDP YMTMDP WTTMDP, i(id)

Random-effects GLS regression                   Number of obs      =      1649
Group variable (i) : id                         Number of groups   =        99
R-sq:  within  = 0.0282                         Obs per group: min =        11
       between = 0.1420                                        avg =      16.7
       overall = 0.0384                                        max =        27
Random effects u_i ~ Gaussian                   Wald chi2(23)      =     61.97
corr(u_i, X)       = 0 (assumed)                Prob > chi2        =    0.0000

------------------------------------------------------------------------------
          er |      Coef.   Std. Err.      z    P>|z|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
      YMSOD1 |  -.0020033    .335687    -0.01   0.995    -.6599378    .6559312
      WTSOD1 |  -.0932786   .3307787    -0.28   0.778    -.7415928    .5550357
          DP |  -.0051365    .007257    -0.71   0.479    -.0193599     .009087
           T |   .3959589    .329318     1.20   0.229    -.2494926     1.04141
           M |  -.0812328    .287115    -0.28   0.777    -.6439678    .4815021
       YMSDP |   .0189073   .0129742     1.46   0.145    -.0065216    .0443363
       WTSDP |   .0158976   .0110652     1.44   0.151    -.0057898    .0375851
         TDP |  -.0047965   .0107512    -0.45   0.655    -.0258685    .0162754
         MDP |   .0156524   .0095346     1.64   0.101    -.0030352    .0343399
        YMST |  -.6908238   .4905222    -1.41   0.159     -1.65223    .2705821
        WTST |  -.5744695   .4992973    -1.15   0.250    -1.553074    .4041353
        YMSM |  -.0176672   .4651369    -0.04   0.970    -.9293189    .8939844
        WTSM |  -.0566241   .4382519    -0.13   0.897     -.915582    .8023338
          TM |  -.6107098   .4378132    -1.39   0.163    -1.468808    .2473883
        YMTM |   1.130177   .6849287     1.65   0.099    -.2122589    2.472612
        WTTM |   1.341621   .6399834     2.10   0.036     .0872765    2.595965
       YMTDP |   .0164004   .0191402     0.86   0.392    -.0211137    .0539145
       WTTDP |   .0101715   .0164181     0.62   0.536    -.0220073    .0423503
       YMMDP |   .0025776   .0180647     0.14   0.887    -.0328286    .0379839
       WTMDP |  -.0009271   .0146579    -0.06   0.950    -.0296561    .0278019
        TMDP |   .0077888   .0141815     0.55   0.583    -.0200065    .0355841
      YMTMDP |  -.0310983   .0264786    -1.17   0.240    -.0829955    .0207989
      WTTMDP |  -.0433058   .0210129    -2.06   0.039    -.0844902   -.0021213
       cons  |   4.744001   .2156626    22.00   0.000      4.32131    5.166692
-------------+----------------------------------------------------------------
     sigma_u |  .15628845
     sigma_e |  1.5843396
         rho |  .00963721   (fraction of variance due to u_i)
------------------------------------------------------------------------------
```

FITTED GLS REGRESSION MODEL FOR FIRST ERROR

Hypothesis Test

test YMTMDP WTTMDP

(1) YMTMDP = 0.0
(2) WTTMDP = 0.0

chi2(2) = 4.46
Prob > chi2 = 0.1076

FITTED GLS REGRESSION MODEL FOR FIRST ERROR

```
xtreg er YMSOD1 WT SOD1 DP Tg+ M YMSDP WTSDP TDP MDP YMST WTST YMSM
WTSM TM YMTM WTTM YMTDP WTTDP YMMDP WTMDP TMDP, i(id)

Random-effects GLS regression                   Number of obs      =      1649
Group variable (i) : id                         Number of groups   =        99
R-sq:  within  = 0.0256                         Obs per group: min =        11
       between = 0.1383                                        avg =      16.7
       overall = 0.0357                                        max =        27
Random effects u_i ~ Gaussian                   Wald chi2(21)      =     56.83
corr(u_i, X)       = 0 (assumed)                Prob > chi2        =    0.0000

------------------------------------------------------------------------------
          er |      Coef.   Std. Err.      z    P>|z|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
      YMSOD1 |  -.1705756    .312436    -0.55   0.585    -.7829389    .4417877
      WTSOD1 |  -.3565477   .3076687    -1.16   0.247    -.9595673    .2464719
          DP |  -.0108167   .0067101    -1.61   0.107    -.0239682    .0023347
           T |   .1074221   .3005775     0.36   0.721     -.481699    .6965433
           M |   -.301551   .2689676    -1.12   0.262    -.8287178    .2256157
       YMSDP |   .0267601   .0113081     2.37   0.018     .0045967    .0489235
       WTSDP |   .0278644   .0094054     2.96   0.003     .0094301    .0462986
         TDP |   .0076859   .0088778     0.87   0.387    -.0097142    .0250861
         MDP |   .0254771   .0082559     3.09   0.002     .0092959    .0416584
        YMST |  -.3136522   .4079951    -0.77   0.442    -1.113308    .4860035
        WTST |    .021408   .4103601     0.05   0.958     -.782883    .8256991
        YMSM |   .2887219    .395895     0.73   0.466     -.487218    1.064662
        WTSM |   .4052096    .380064     1.07   0.286    -.3397022    1.150121
          TM |  -.1031417   .3661807    -0.28   0.778    -.8208426    .6145593
        YMTM |   .4419067   .4253262     1.04   0.299    -.3917173    1.275531
        WTTM |   .3576776   .4339314     0.82   0.410    -.4928122    1.208167
       YMTDP |  -.0007439   .0132145    -0.06   0.955    -.0266439    .0251561
       WTTDP |  -.0161278   .0102422    -1.57   0.115    -.0362021    .0039464
       YMMDP |  -.0116676   .0132065    -0.88   0.377    -.0375519    .0142167
       WTMDP |  -.0218829   .0104984    -2.08   0.037    -.0424593   -.0013064
        TMDP |  -.0139778   .0094777    -1.47   0.140    -.0325537    .0045981
       _cons |   4.869522   .2085925    23.34   0.000     4.460689    5.278356
-------------+----------------------------------------------------------------
     sigma_u |  .17529561
     sigma_e |  1.5854752
         rho |  .01207665   (fraction of variance due to u_i)
------------------------------------------------------------------------------
```

FITTED GLS REGRESSION MODEL FOR FIRST ERROR

Hypothesis Test

```
. test YMTM WTTM YMTDP WTTDP YMMDP WTMDP TMDP

 ( 1)  YMTM   = 0.0
 ( 2)  WTTM   = 0.0
 ( 3)  YMTDP  = 0.0
 ( 4)  WTTDP  = 0.0
 ( 5)  YMMDP  = 0.0
 ( 6)  WTMDP  = 0.0
 ( 7)  TMDP   = 0.0
```

FITTED GLS REGRESSION MODEL FOR FIRST ERROR

```
xtreg er YMSOD1 WT SOD1 DP Tg+ M YMSDP WTSDP TDP MDP YMST WTST YMSM
WTSM TM, i(id)

Random-effects GLS regression                   Number of obs      =      1649
Group variable (i) : id                         Number of groups   =        99
R-sq:  within  = 0.0186                         Obs per group: min =        11
       between = 0.1275                                        avg =      16.7
       overall = 0.0283                                        max =        27
Random effects u_i ~ Gaussian                   Wald chi2(14)      =     45.09
corr(u_i, X)       = 0 (assumed)                Prob > chi2        =    0.0000

------------------------------------------------------------------------------
          er |      Coef.   Std. Err.      z    P>|z|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
      YMSOD1 |  -.1244747   .2221951    -0.56   0.575    -.5599692    .3110197
      WTSOD1 |   .0465331    .220877     0.21   0.833    -.3863778     .479444
          DP |    .002805   .0048232     0.58   0.561    -.0066483    .0122584
           T |   .3001848   .2031474     1.48   0.139    -.0979769    .6983464
           M |  -.0253258   .1949811    -0.13   0.897    -.4074818    .3568302
       YMSDP |   .0199603   .0066046     3.02   0.003     .0070155    .0329051
       WTSDP |   .0054502   .0050913     1.07   0.284    -.0045286    .0154289
         TDP |  -.0077355   .0046373    -1.67   0.095    -.0168244    .0013535
         MDP |   .0076792   .0047224     1.63   0.104    -.0015766    .0169349
        YMST |   -.124928   .2071517    -0.60   0.546    -.5309378    .2810817
        WTST |  -.1316725   .2079055    -0.63   0.527    -.5391598    .2758148
        YMSM |   .2401559   .2072719     1.16   0.247    -.1660895    .6464013
        WTSM |   .0759937   .2129053     0.36   0.721    -.3412931    .4932805
          TM |  -.1406233   .1729179    -0.81   0.416    -.4795361    .1982894
       _cons |   4.637023   .1702683    27.23   0.000     4.303303    4.970743
-------------+----------------------------------------------------------------
     sigma_u |  .15095624
     sigma_e |  1.5885822
         rho |  .00894909   (fraction of variance due to u_i)
------------------------------------------------------------------------------
```

FITTED GLS REGRESSION MODEL FOR FIRST ERROR

Hypothesis Test

```
. test YMSDP WTSDP TDP MDP YMST WTST YMSM WTSM TM

 ( 1)  YMSDP= 0.0
 ( 2)  WTSDP= 0.0
 ( 3)  TDP  = 0.0
 ( 4)  MDP  = 0.0
 ( 5)  YMST = 0.0
 ( 6)  WTST = 0.0
 ( 7)  YMSM = 0.0
 ( 8)  WTSM = 0.0
 ( 9)  TM   = 0.0

           chi2(  9) =   15.71
         Prob > chi2 =    0.0732
```

FINAL FITTED GLS REGRESSION MODEL FOR FIRST ERROR

```
xtreg er  YMSOD1 WT SOD1 DP Tg+ M, i(id)

Random-effects GLS regression                   Number of obs      =      1649
Group variable (i) : id                         Number of groups   =        99
R-sq:  within  = 0.0098                         Obs per group: min =         1
       between = 0.1111                                        avg =      16.7
       overall = 0.0189                                        max =        27
Random effects u_i ~ Gaussian                   Wald chi2(5)       =     29.86
corr(u_i, X)       = 0 (assumed)                Prob > chi2        =    0.0000

------------------------------------------------------------------------------
          er |      Coef.   Std. Err.      z    P>|z|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
      TMSOD1 |    .359979   .1013748     3.55   0.000     .1612882    .5586699
      WTSOD1 |     .14341   .1016262     1.41   0.158    -.0557736    .3425936
          DP |   .0090688   .0023059     3.93   0.000     .0045493    .0135882
           T |  -.0358939   .0833515    -0.43   0.667    -.1992598    .1274721
           M |   .1777416   .0845994     2.10   0.036     .0119298    .3435534
       _cons |   4.510075   .1052469    42.85   0.000     4.303795    4.716355
-------------+----------------------------------------------------------------
     sigma_u |  .12781158
     sigma_e |  1.5936409
         rho |  .00639109   (fraction of variance due to u_i)
------------------------------------------------------------------------------
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PRELIMINARY INITIAL FITTED OLS REGRESSION MODEL FOR SQUARE ROOT OF NOREPINEPHRINE

```
reg sqne WT SOD1 Tg+ M B WTB TB MB WTT WTM TM WTTM WTTB WTMB TMB WTTMB

      Source |       SS       df       MS      Number of obs =     138
-------------+------------------------------   F( 15,   122) =   24.55
       Model |  728.513258    15  48.5675505   Prob > F      =  0.0000
    Residual |  241.387147   122  1.97858318   R-squared     =  0.7511
-------------+------------------------------   Adj R-squared =  0.7205
       Total |  969.900405   137    7.079565   Root MSE      =  1.4066

------------------------------------------------------------------------------
        sqne |      Coef.   Std. Err.      t    P>|t|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
      WTSOD1 |  -4.054421    .952287    -4.26   0.000    -5.939569   -2.169274
         Tg+ |   .4648254   .7033106     0.66   0.510    -.9274482    1.857099
           M |  -.6471384   .6420318    -1.01   0.315    -1.918104    .6238277
           B |   1.203071    .683495     1.76   0.081    -.1499759    2.556117
         WTB |   2.216912    1.18716     1.87   0.064    -.1331889    4.567013
          TB |   .0616755   .9807198     0.06   0.950    -1.879757    2.003108
          MB |   2.419214   .9010653     2.68   0.008     .6354653    4.202963
         WTT |  -1.395397   1.198678    -1.16   0.247    -3.768299    .9775056
         WTM |   .3847731   1.136475     0.34   0.736    -1.864992    2.634539
          TM |  -.7741166   .9377475    -0.83   0.411    -2.630481    1.082248
        WTTM |   1.474475   1.490391     0.99   0.324    -1.475903    4.424854
        WTTB |   1.055598    1.58632     0.67   0.507    -2.084681    4.195878
        WTMB |   -2.55682   1.502198    -1.70   0.091    -5.530571    .4169308
         TMB |  -1.502538   1.313113    -1.14   0.255    -4.101976      1.0969
       WTTMB |   .3254882    2.02816     0.16   0.873    -3.689457    4.340434
        cons |   7.436668   .4973157    14.95   0.000     6.452182    8.421154
------------------------------------------------------------------------------
```

Hypothesis Test

```
. test  WTTM WTTB WTMB TMB WTTMB

 ( 1)  WTTM  =  0.0
 ( 2)  WTTB  =  0.0
 ( 3)  WTMB  =  0.0
 ( 4)  TMB   =  0.0
 ( 5)  WTTMB = 0.0

       F(  5,   122) =    2.52
            Prob > F =    0.0331
```

FITTED OLS REGRESSION MODEL FOR SQUARE ROOT OF NOREPINEPHRINE

```
reg sqne WT SOD1 Tg+ M B WTB TB MB WTT WTM TM WTTM WTTB WTMB TMB

      Source |       SS       df       MS      Number of obs =     138
-------------+------------------------------   F( 14,   123) =   26.51
       Model |  728.462299    14  52.0330213   Prob > F      =  0.0000
    Residual |  241.438106   123  1.96291143   R-squared     =  0.7511
-------------+------------------------------   Adj R-squared =  0.7227
       Total |  969.900405   137    7.079565   Root MSE      =   1.401

------------------------------------------------------------------------------
        sqne |      Coef.   Std. Err.      t    P>|t|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
      WTSOD1 |  -3.982664   .8374523    -4.76   0.000    -5.640349   -2.324979
         Tg+ |   .5039658   .6570519     0.77   0.445    -.7966281     1.80456
           M |  -.6145214   .6065973    -1.01   0.313    -1.815243    .5862007
           B |   1.240037   .6409595     1.93   0.055    -.0287034    2.508777
         WTB |   2.105393   .9587167     2.20   0.030     .2076722    4.003114
          TB |  -.0144308   .8550332    -0.02   0.987    -1.706917    1.678055
          MB |   2.354968   .8040514     2.93   0.004     .7633979    3.946539
         WTT |   -1.50909   .9630871    -1.57   0.120    -3.415462    .3972817
         WTM |   .2825732   .9375586     0.30   0.764    -1.573266    2.138413
          TM |  -.8436995   .8281921    -1.02   0.310    -2.483055     .795656
        WTTM |    1.65024   1.006818     1.64   0.104    -.3426946    3.643175
        WTTB |   1.254717   .9845034     1.27   0.205    -.6940468    3.203481
        WTMB |   -2.37826   1.005275    -2.37   0.020    -4.368139   -.3883798
         TMB |    -1.3661   .9967732    -1.37   0.173    -3.339152    .6069514
       _cons |   7.417098     .48022    15.45   0.000     6.466532    8.367664
------------------------------------------------------------------------------
```

Hypothesis Test

```
. test  WTTM WTTB WTMB TMB

 ( 1)  WTTM = 0.0
 ( 2)  WTTB = 0.0
 ( 3)  WTMB = 0.0
 ( 4)  TMB  = 0.0

       F(  4,   123) =    3.17
            Prob > F =    0.0162
```

FITTED OLS REGRESSION MODEL FOR SQUARE ROOT OF NOREPINEPHRINE

```
reg sqne WT SOD1 Tg+ M B WTB TB MB WTT WTM TM WTTM WTMB TMB

      Source |       SS       df       MS      Number of obs =     138
-------------+------------------------------   F( 13,   124) =   28.28
       Model |  725.274007    13  55.7903082   Prob > F      =  0.0000
    Residual |  244.626398   124  1.97279353   R-squared     =  0.7478
-------------+------------------------------   Adj R-squared =  0.7213
       Total |  969.900405   137    7.079565   Root MSE      =  1.4046

------------------------------------------------------------------------------
        sqne |      Coef.   Std. Err.      t    P>|t|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
      WTSOD1 |  -4.434832   .7604917    -5.83   0.000    -5.940058   -2.929606
         Tg+ |   .2573287   .6294851     0.41   0.683    -.9885985    1.503256
           M |  -.6082986   .6081026    -1.00   0.319    -1.811904    .5953067
           B |   1.007102    .615893     1.64   0.105    -.2119231    2.226126
         WTB |   2.808113   .7862639     3.57   0.001     1.251877    4.364349
          TB |   .4651413   .7697101     0.60   0.547    -1.058331    1.988613
          MB |   2.317044   .8055207     2.88   0.005     .7226934    3.911395
         WTT |  -.7926681   .7839772    -1.01   0.314    -2.344379    .7590423
         WTM |   .4324782   .9324896     0.46   0.644     -1.41318    2.278136
          TM |  -.8993255   .8291205    -1.08   0.280    -2.540387    .7417362
        WTTM |   1.550121   1.006272     1.54   0.126    -.4415738    3.541815
        WTMB |  -2.415328    1.00738    -2.40   0.018    -4.409215   -.4214404
         TMB |  -1.246642   .9948512    -1.25   0.213    -3.215731    .7224474
       _cons |   7.540416   .4715532    15.99   0.000      6.60708    8.473752
------------------------------------------------------------------------------
```

FINAL FITTED OLS REGRESSION MODEL FOR SQUARE ROOT OF NOREPINEPHRINE

```
reg sqne WT SOD1 Tg+ M B WTB TB MB WTT WTM TM WTTM WTMB
```

Source	SS	df	MS		
Model	722.17624	12	60.1813533	Number of obs	= 138
Residual	247.724165	125	1.98179332	F(12, 125)	= 30.37
Total	969.900405	137	7.079565	Prob > F	= 0.0000
				R-squared	= 0.7446
				Adj R-squared	= 0.7201
				Root MSE	= 1.4078

sqne	Coef.	Std. Err.	t	P>|t|	[95% Conf.	Interval]
WTSOD1	-4.559529	.7556708	-6.03	0.000	-6.055095	-3.063963
Tg+	.6411104	.5512091	1.16	0.247	-.4498009	1.732022
M	-.3081955	.560231	-0.55	0.583	-1.416962	.800571
B	1.369562	.5449825	2.51	0.013	.290974	2.44815
WTB	2.89792	.7847747	3.69	0.000	1.344754	4.451087
TB	-.2811008	.4887685	-0.58	0.566	-1.248434	.6862327
MB	1.728322	.6558128	2.64	0.009	.4303871	3.026257
WTT	-.7241818	.7838517	-0.92	0.357	-2.275522	.8271579
WTM	.5807785	.9270564	0.63	0.532	-1.253981	2.415538
TM	-1.535602	.6569441	-2.34	0.021	-2.835776	-.2354286
WTTM	1.494464	1.007582	1.48	0.141	-.4996651	3.488594
WTMB	-2.580167	1.00103	-2.58	0.011	-4.561329	-.599004
_cons	7.348525	.4470123	16.44	0.000	6.463833	8.233218

References

Abrahams, S., Goldstein, L. H., Lloyd, C.M., Brooks, D. J., & Leigh, P. N. (1995). Cognitive deficits in non-demented amyotrophic lateral sclerosis patients: a neuropsychological investigation. Journal of the Neurological Sciences, 129 (Suppl.), 54-55.

Abrahams, S., Goldstein, L. H., Kew, J .J .M., Brooks, D.J., Lloyd, C. .M., Frith, C. D., & Leigh, P. N. (1996). Frontal lobe dysfunction in amyotrophic lateral sclerosis a PET study. Brain, 119, 2105-2120.

Abrahams, S., Goldstein, L. H., Al-Chalabi, A., Pickering, A., Morris, R. G., Passingham, R. E., Brooks, D. J., & Leigh, P. N. (1997). Relation between cognitive dysfunction and pseudobulbar palsy in amyotrophic lateral sclerosis. Journal of Neurology, Neurosurgery, and Psychiatry, 62, 464-472.

Al-Chalabi, A., & Leigh, P. N. (2000). Recent advances in amyotrophic lateral sclerosis. Current Opinion in Neurology, 13, 397-405.

Alexianu, M. E., Ho, B.-K, Mohamed, H., La Bella, V., Glenn Smith, E., & Appel, S. (1994). The role of calcium-binding proteins in selective motorneuron vulnerability in amyotrophic lateral sclerosis. Annals of Neurology, 36, 846-858.

Ammassari-Teule, M., Fagioli, S., Maritati, M., Populin, R., & Pavone, F. (1990). Chronic administration of phosphatidylserine during ontogeny enhances subject environment interactions and radial maze performance in C57BL/6 mice. Physiology & Behavior, 47, 755-760.

Ammassari-Teule, M., Hoffman, H-J., & Rossi-Arnaud, C. (1993). Learning in inbred mice: strain-specific abilities across three radial maze problems. Behavior Genetics, 23, 405-412.

Ammassari-Teule, M., Fagioli, S., & Rossi-Arnaud, C. (1994). Radial maze performance and open-field behaviors in aged C57BL/6 mice: further evidence for preserved cognitive abilities during senescence. Physiology & Behavior, 55, 341-345.

Andrews, A. M., Ladenheim, B., Epstein, C. J., Cadet, J. L., & Murphy, D. L. (1996, Dec). Transgenic mice with high levels of superoxide dismutase activity are protected from the neurotoxic effects of 2'-NH2-MPTP on serotonergic and noradrenergic nerve terminals. Molecular Pharmacology, 50, 1511-1519.

Appel, S. H., Smith, R. G., Alexiano, M. F., Engelhardt, J. I., & Stefani, E. (1995). Autoimmunity as an etiological factor in sporadic amyotrophic lateral sclerosis. Advances in Neurology, 68, 47-57.

Appel, S. H., & Smith, G. (2001). The pathogenesis of amyotrophic lateral sclerosis. In M. P. Mattson (Ed.), Pathogenesis of Neurodegenerative Disorders (pp. 149-171). Totowa, NJ: Humana Press Inc.

Avraham, K. B., Schickler, M., Sapoznikov, D., Yarom, R., & Groner, Y. (1988). Down's syndrome: abnormal neuromuscular junction in tongue of transgenic mice with elevated levels of human Cu/Zn-Superoxide dismutase. Cell, 54, 823-829.

Azar, B. (2000, February). Wanted: behavioral researchers for a penchant for genetics. Monitor on Psychology, 36-39.

Bach, M. E., Hawkins, R. D., Osman, M.., Kandel, E. R., & Mayford, M. (1995). Impairment of spatial but not contextual memory in CaMKII mutant mice with a selective loss of hippocampal LTP in the range of 0 frequency. Cell, 81, 905-915.

Barneoud, P. & Curet, O., (1999). Beneficial effects of lysine acetylsalicylate, a soluable salt of aspirin, on motor performance in a transgenic model of amyotrophic lateral sclerosis. Experimental Neurology, 155, 243-251.

Beck, K. D., & Luine, V.N. (1999). Food deprivation modulates chronic stress effects on object recognition in male rats: role monoamines and amino acids. Brain Research, 830, 56-71.

Becker, J. T., Walker, J. A., & Olton, D. S. (1980). Neuroanatomical bases of spatial memory. Brain Research, 200, 307-320.

Beckman, J.S., Carson, M., Smith, C.D., & Koppenol, W. H. (1993). ALS, SOD and peroxynitrate (letter). Nature, 364, 584.

Belsh, J. M. (1996). Definition of terms, classification, and diagnostic criteria of ALS. In J. M. S. Belsh, P.L. (Ed.), Amyotrophic Lateral Sclerosis: Diagnosis and Management for the Clinician (pp. 25-45). Armonk, NY: Futura Publishing Company.

Bernard, N. D., & Kaufman, S.R. (1997, February). Animal Research is Wasteful and Misleading. Scientific American, 80-82.

Bernstein, D., Olton, D. S., Ingram, D. K., Waller, S. B., Reynolds, M. A., & London, E. D. (1984). Radial maze performance in young and aged mice: neurochemical correlates. Pharmacology Biochemistry & Behavior, 22, 301-307.

Blum, K., Braverman, E. R., Holder, J. M., Lubar, J. F., Monastra, V. J., Miller, D., Lubar, J. O., Chen, T. J., & Comings, D. E. (2000). Reward deficiency syndrome: a

biogenetic model for the diagnosis and treatment of impulsive, addictive, and compulsive behaviors. Journal Psychoactive Drugs, 32, Suppli:i-iv, 1-112.

Boyar, J. I., & Decker, E. (1985). Amyotrophic lateral sclerosis: an unusual case report. The International Journal of Clinical Neuropsychology, 7, 137-140.

Brooks, G. R. (1998). El Escorial Revisted: Revised Criteria for the Diagnosis of Amyotrophic Lateral Sclerosis. http://www.wfnals.org/Articles/elescorial 1998introduction.htm.

Brown, R. H. (1995). Amyotrophic lateral sclerosis. Insights from genetics. Archives Neurology, 54, 1246-1250.

Brownell, B., Oppenheimer, D. R., & Hughes, J. T. (1970). The central nervous system in motor neurone disease. Journal of Neurology Neurosurgery and Psychiatry, 33, 338-357.

Brugge, K., Nichols, S., Saitoh, T., & Trauner, D. (1999). Correlations of glutathione peroxidase activity with memory impairment in adults with Down syndrome. Biological Psychiatry, 46, 1682-1689.

Carpenter, S. (1968). Proximal axonal enlargement in motor neuron disease. Neurology, 18, 842-851.

Caselli, R. J., & Boeve, B. F. (1999). The Degenerative Dementias. In C. G. P. Goetz, E.J. (Ed.), Textbook of Clinical Neurology (pp. 629-654). Philadelphia: W.B. Saunders Company.

Cavalleri, F., & DeRenzi, E. (1994). Amyotrophic lateral sclerosis with dementia. Acta Neurologica Scandinavica, 89, 391-394.

Chance, P. F., Rabin, B. A., Ryan, S. G., Ding, Y., Scavina, M., Crain, B., Griffin, J. W., & Cornblath, D. R. (1998). Linkage of the gene for an autosomal dominate form of juvenile amyotrophic lateral sclerosis to chromosome 9q34. American Journal Human Genetics, 62, 633-640.

Chiu, A. Y., Zhai, :P., Dal Canto, M. C., Peters, T. M., Kwon, Y. W., Prattis, S. M., & Gurney, M. E. (1995). Age-dependent penetrance of disease in a transgenic mouse model of familial amyotrophic lateral sclerosis. Molecular and Cellular Neuroscience, 6, 349-362.

Cleveland, D. W., (1999). From Charcot to SOD1: mechanisms of selective motor neuron death in ALS. Neuron, 24, 515-520.

Collard, J., Cote, F. & Julien, J. (1995). Defective axonal transport in a transgenic mouse model of amyotrophic lateral sclerosis. Nature, 375, 61-65.

Comings, D. E., & Blum, K. (2000). Reward deficiency syndrome: genetic aspects of behavioral disorders. Progress Brain Research, 126, 325-341.

Conn, P. M., & Parker, J. (1998, November 20). Animal rights: reaching the public. Science, 282, 1417.

Crawley, J. N. & Paylor, R. (1997). A proposed test battery and constellations of specific behavioral paradigms to investigate the behavioral phenotypes of transgenic and knockout mice. Hormones and Behavior, 31, 197-211.

Crawley, J. N., Belknap, J. K., Collins, A., Crabbe, J. C., Frankel, W., Henderson, N., Hitzemann, R. J., Maxson, S. C., Miner, L. L., Silva, A. J., Wehner, J. M., Wynshaw-Boris, A., & Paylor, R. (1997). Behavioral phenotypes of inbred mouse strains:

implications and recommendations for molecular studies. Psychopharmacology, 132, 107-124.

Cudkowicz, M. E., Mc Kenna-Yasek, D., Sapp, P. E., Chin, W., Geller, B., Hayden, D. L., Schoenfeld, D. A., Hosler, B. A., Horvitz, H. R., & Brown, R. H. (1997). Epidemiology of mutations in superoxide dismutase in amyotrophic lateral sclerosis. Annals Neurology, 41, 210-221.

Dal Canto, M. C. & Gurney, M. E. (1995). Neuropathological changes in two lines of mice carrying a transgene for mutant human Cu,Zn SOD, and in mice overexpressing wild type human SOD: a model of familial amyotrophic lateral sclerosis (FALS). Brain Research, 676, 25-40.

David, A. S., & Gillham, R. A. (1986). Neuropsychological study of motor neuron disease. Psychosomatics, 27, 441-445.

Deng, H-X., Hentati, A., Tainer, J. A., Iqbal, Z., Cayabyab, A., Hung, W-Y., Getzoff, E. D., Hu, P., Herzfeldt, B., Roos, R. P., Warner, C., Deng, G., Soriano, E., Smyth, C., Parge, H. E., Ahmed, A., Roses, A. D., Hallewell, R. A., Pericak-Vance, M. A., and Siddique, T. (1993). Amyotrophic lateral sclerosis and structural defects in Cu/Zn superoxide dismutase. Science, 261, 1047-1051.

Donnelley, S. (1989, March/April). Speculative philosophy, the troubled middle, and the ethics of animal experimentation. Hastings Center Report, 15-21.

Drachman, D. B., Fishman, P. S., Rothstein, J.D., Motomura, M., Lang, B., Vincent, A., & Mellits, E. D. (1995). Amyotrophic lateral sclerosis an autoimmune disease? Advances in Neurology, 68, 59-72.

Durkin, T. P. (1992). GABAergic mediation of indirect transsynaptic control over basal and spatial memory testing-induced activation of septo-hippocampal cholinergic activity in mice. Behavioral Brain Research, 50, 155-165.

Epstein, C. J., Avraham, K. B., Lovett, M., Smith, S., Elroy-Stein, O., Rotman, G., Bry, C., & Groner,Y. (1987). Transgenic mice with increased Cu/Zn-superoxide dismutase activity: Animal model of dosage effects in Down syndrome. Proceedings National Academy of Science, 84, 8044-8048.

Estevez, A. G., Crow, J.P., Sampson, J.B., Reiter, C., Zhuang, Y., Richardson, G.J., Tarpey, M.M., Barbeito, L., & Beckman, J.S. (1999, December 24). Induction of nitric oxide-dependent apoptosis in motor neurons by zinc-deficient superoxide dismutase. Science, 286, 2498-2500.

Facchinetti, F., Dawson, V. L., & Dawson, T. M. (1998). Free radicals as mediators of neuronal injury. Cellular and Molecular Neurobiology, 18, 667-682.

Ferrer, I., Tunon, T., Serrano, M. T., Casas, R., Alcantara, Zujar, M. J., & Rivera, R. M. (1993). Journal Neurology, Neurosurgery and Psychiatry, 56, 257-261.

Flood, D. G., Reaume, A. G., Gruner, J. A., Hoffman, E. K., Hirsch, J. D., Lin, Y., Dorfman, K. S., & Scott, R. W. (1999). Hindlimb motor neurons require Cu/Zn superoxide dismutase for maintenance of neuromuscular junctions. American Journal of Pathology, 155, 66-672.

Frick, K. M. & Berger-Sweeney, J. (1999). Neurochemical systems involved in learning and memory. In D. S. Charney, E. J. Nestler & B. S. Bunney (Eds.). Neurobiology of Mental Illness (pp. 685-692.

Gahtan, E., Auerbach, J. M., Groner, Y., Segal, M. (1998). Reversible impairment of long-term potentiation in transgenic Cu/Zn-SOD mice. European Journal of Neuroscience, 10, 538-544.

Gonatas, N. K., Steiber, A., Mourelatos, Z., Chen, Y., Gonatas, J. O., Appel, S. H., Hays, A. P., Hickey, W. F., & Hauw, J. J. (1992). Fragmentation of the Golgi apparatus of motor neurons in amyotrophic lateral sclerosis. American Journal Pathology, 140, 731-737.

Grace, A. A. (2000, March). Gating of information flow within the limbic system and the pathophysiology of schizophrenia. Brain Research Review, 31(2-3), 330-341.

Green, S. L., & Tolwani, R.J. (1999). Animal models for motor neuron disease. Laboratory Animal Science, 49, 480-487.

Gresch, P. J., Sved, A. F., Zigmond, M. J., & Finaly, J. M. (1994). Stress-induced sensitization of dopamine and norephinephrine efflux in medial prefrontal cortex of the rat. Journal of Neurochemistry, 63, 575-583.

Gurney, M. E., Pu, H., Chiu, A. Y., Dal Canto, M. C., Polchow, C. Y., Alexander, D. D., Caliendo, J., Hentati, A., Kwon, Y. W., Deng, H. X., Chen, W., Zhai, P., Sufit, R. L., & Siddique, T. (1994, June 17). Motor neuron degeneration in mice expressing a human Cn,Zn superoxide dismutase mutation. Science, 264, 1772-1775.

Gurney, M. E., Cutting, F. B., Zhai, P., Doble, A., Taylor, C. P., Andrus, P. K., & Hall, E. D. (1996). Benefit of Vitamin E, Riluzole, and Gabapentin in a transgenic model of familial amyotrophic lateral sclerosis. Annals of Neurology, 39, 147-157.

Gurney, M. E. (1997). Transgenic animal models of familial amyotrophic lateral sclerosis. Journal of Neurology, 244 (Sppl. 2), S15-S20.

Gurney, M. E., Liu, R., Althaus, J. S., Hall, E. D., & Becker, D. A. (1998a). Mutant CuZn superoxide dismutase in motor neuron disease. Journal of Inherited Metabolic Disorders, 21, 587-987.

Gurney, M. E., Fleck, T. J., Himes, C. S., & Hall, E. D. (1998b). Riluzole preserves motor function in a transgenic model of familial amyotrophic lateral sclerosis. Neurology, 50, 62-72.

Haroutunian, V., & Santucci, A. C. (1999). Pharmacological animal models of dementia. In D. S. Charney, E. J. Nestler, & B. S. Bunney.(Eds.). Neurobiology of Mental Illness (pp. 669-678). New York: Oxford University Press.

Hartikainen, P., Elkala, E. L., Soininen, H., & Riekkinen, Sr. (1993). Cognitive and memory deficits in untreated Parkinson's disease and amyotrophic lateral sclerosis patients: a comparative study. Journal Neural Transmission, 6 (P-D Sect), 127-137.

Hentati, A., Bejaoui, K., Pericak-Vance, M. A., Hentati, F., Speer, M. C., Hung, W. Y., Figlewicz, D. A., Haines, J., Rimmler, J., Ben Hamida, C. B., Hamida, M. B., Brown, R. H., Jr., & Siddique, T. (1994). Linkage of recessive familial amyotrophic lateral sclerosis to chromosome 2q33-q35. Nature Genetics, 7, 425-8.

Hentati, A., Ouahchi, K., Pericak-Vance, M.A., Nijhawan, D., Ahmad, A., Yang, Y., Rimmler, J., Hung, W., Schlotter, B., Ahmed, A., Ben Hamida, M., Hentati, F., & Siddique, T. (1998). Linkage of a commoner form of recessive amyotrophic lateral sclerosis to chromosome 15q15-q22 markers. Neurogenetics, 2, 55-60.

Hirano, A., Kurland, L. T., Krooth, R. S., & Lessel, S. (1961). Parkinsonism-dementia complex, an endemic disease on the island of Guam. I. Clinical features. Brain, 84, 642-661.

Hirano, A., Armugasamy, N., & Zimmerman, H. M. (1967). Amyotrophic lateral sclerosis. A comparison of Guam and classical cases. Archives Neurology, 16, 357-363.

Hirano, A., Kurland, L. T., & Sayre, G. P. (1967). Familial amyotrophic lateral sclerosis. A subgroup characterized by posterior and spinocerebellar tract involvement and hyaline inclusions in the anterior horn cells. Archives Neurology, 16, 232-243.

Hirano, A. (1996). Neuropathology of familial amyotrophic lateral sclerosis. In I. Nakano & A. Hirano (Eds.) Amyotrophic Lateral Sclerosis: Progress and Perspectives in Basic Research and Clinical Application (pp. 102-108). New York: Elsevier Science B.V.

Hirano, A. (1996). Neuropathology of ALS: a review. Neurology, 47(Supp2), S63-S66.

Holden, C. (2000, November 24). Researchers pained by effort to define distress precisely. Science, 290, 1474-1475.

Hong, S., Brooks, B. R., Hung, W. Y., Siddique, N. A., & Rimmler, J. (1998). X-linked dominant locus for late-onset familial amyotrophic lateral sclerosis. Society Neuroscience Abstract, 24, 478.

Horoupian, D. S., Thal, L., Katzman, R., Terry, R. D., Davies, P., Hirano, A., DeTeresa, R., Fuld, P. A., Petito, C., Blass, J., & Ellis, J. M. (1984). Dementia and motor neuron disease: morphometric, biochemical, and golgi studies. Annals Neurology, 16, 305-313.

Hosler, B. A., Siddique, T., Sapp, P. C., Sailor, W., Huang, M. C., Hossain, A., Daube, J. R., Nance, M., Fan, C., Kaplan, J., Hung, W., McKenna-Yasek, D., Haines, J. L., Pericak-Vance, M. A., Horvitz, H. R., & Brown, R. H. (2000). Linkage of familial

amyotrophic lateral sclerosis with frontotemporal dementia to chromosome 9q21-q22. Journal American Medical Association, 284, 1664-1669.

Hsiao, K., Chapman, P., Nilsen, S., Eckman, C., Harigaya, Y., Younkin, S., Yang, F., & Cole, G. (1996). Correlative memory deficits, A Elevation, and Amyloid Plaques in Transgenic Mice. Science, 274, 99-102.

Hudson, A. J. (1981). Amyotrophic lateral sclerosis and its association with dementia, parkinsonism and other neurological disorders: a review. Brain, 104, 217-247.

Hyde, L. A., Hoplight, B. J., Denenberg, V. H. (1998). Water version of the radial-arm maze: learning in three inbred strains of mice. Brain Research, 785, 236-244.

Hyde, L. A., & Denenberg, V. H. (1999). BXSB mice can learn complex visual pattern discriminations. Physiology & Behavior, 66, 437-439.

Iannelo, R. C., & Kola, I. (2001). Oxidative stress in Down syndrome. In M. P. Mattson (Ed.) Pathogenesis of Neurodegenerative Disorders (pp. 139-148). Totowa, NJ: Humana Press, Inc.

Iwasaki, Y., Kinoshita, M., Ikeda, K., Takamiya, K., & Shiojima, T. (1990). Neuropsychological dysfunctions in amyotrophic lateral sclerosis: relation to motor disabilities. International Journal Neuroscience, 54, 191-195.

Jiang, F., DeSilva, S., & Turnbull, J. (2000). Beneficial effect of ginseng root in SOD-1 (G93A) transgenic mice. Journal of the Neurological Sciences, 180, 52-54.

Kato, T., Hirano, A., & Donnenfeld, H. (1987). A Golgi study of the large anterior horn cells of the lumbar cords in normal spinal cords and in amyotrophic lateral sclerosis. Acta Neuropatholigica, 75, 34-40.

Kew, J. J .M., Goldstein, L. H., Leigh, P. N., Abrahams, S., Cosgrave, N., Passingham, R. E., Frackowiak, R. S .J., & Brooks, D. J. (1993). The relationship between abnormalities of cognitive function and cerebral activation in amyotrophic lateral sclerosis. Brain, 116, 1399-1423.

Klivenyi, P., Ferrante, R. J., Matthews, R. T., Bogdanov, M. B., Klein, A. M., Andreassen, O. A., Mueller, G., Wermer, M., Kaddurah-Daouk, R., & Beal, M. F., (1999). Neuroprotective effects of creative in a transgenic animal model of amyotrophic lateral sclerosis. Nature Medicine, 5, 347-350.

Kneavel, M., Gogos, J., Karayiorgou, K., & Luine, V. N., (2000). Interaction of COMT gene deletion and environment on cognition. Society of Neuroscience, Abstract.

Kostic, V., Jackson-Lewis, V., de Bilbao, F., Dubois-Dauphin, M., & Przedborski, S. (1997a, July 25). Bcl-2: prolonging life in a transgenic mouse model of familial amyotrophic lateral sclerosis. Science, 277, 559-562.

Kostic, V., Gurney, M. E., Deng, H-X., Siddique, T., Epstein, C. J., and Przedborski, S. (1997b). Midbrain dopaminergic neuronal degeneration in a transgenic mouse model of familial amyotrophic lateral sclerosis. Annals of Neurology, 41, 497-504.

Kurland, L. T., & Mulder, D. W. (1955). Epidemiologic investigations of amyotrophic lateral sclerosis. 2. Familial aggregations indicative of dominant inheritance. Neurology, 5, 182-196.

Lauerman, J. F. (1999, January-February). Animal research. Harvard Magazine, 49-57.

Levin, E. D., Brady, T. C., Crapo Hochrein, E., Oury, T. D., Jonsson, L. M., Marklund, S. L., and Crapo, J. D. (1998). Molecular manipulations of extracellular superoxide dismutase: functional importance for learning. Behavior Genetics, 28, 381-390.

Levy, A., Kluge, P. B., & Elsmore, T. F. (1983). Radial arm maze performance of mice: acquisition and atropine effects. Behavioral and Neural Biology, 39, 229-240.

Li, M., Ona, V. O., Guegan, C., Chen, M., Jackson-Lewis, V., Andrews, L. J., Olszewski, A. J., Stieg, P. E., Lee, J-P., Przedborksi, S., & Friedlander, R. M. (2000, April 14). Functional role of caspase-1 and caspase-3 in an ALS transgenic mouse model. Science, 288, 335-339.

Lin, C. G., Bristol, L.A., Jin, L., Dykes-Hoberg, M., Crawford, T., Clawson, L., & Rothstein, J.D. (1998). Aberrant RNA Processing in neurodegenerative disease: the cause for absent EAAT2, a glutamate transporter, in amyotrophic lateral sclerosis. Neuron, 20, 589-602.

Lloyd, C. M., Richardson, M. P., Brooks, D. J., Al-Chalabi, A., & Leight, P. N. (2000, Nov). Extramotor involvement in ALS: PET studies with the GABA (A) ligand [(11)C] flumazenil. Brain, 123 (PT 11), 2289-2296.

Ludolph, A. C., Langen, K. J., Herzog, R. M., Kempter, B., Kuwert, T., Bottger, I. G. & Feinendegen, L. (1992). Frontal lobe function in amyotrophic lateral sclerosis: a neuropsychologic and positron emission tomography study. Acta Neurologica Scandinavica, 85, 81-89. .

Luine, V. N., Bowling, D., & Hearns, M. (1990). Spatial memory deficits in aged rats: contributions of monoaminergic systems. Brain Research, 537, 271-278.

Luine, V. N., Spencer, R. L., & McEwen, B. S. (1993). Effects of chronic corticosterone ingestion on spatial memory performance and hippocampal serotonergic function. Brain Research, 616, 65-70.

Luine, V. N., & Rodriguez, M. (1994). Effects estradiol on radial arm maze performance of young and aged rats. Behavioral and Neural Biology, 62, 230-236.

Luine, V., Villegas, M., Martinez, C., & McEwen, B. S. (1994). Repeated stress causes reversible impairments of spatial memory performance. Brain Research, 639, 167-170.

Luine, V. N. (1994). Steroid Hormone Influences on Spatial Memory. Hormonal Restructuring of the Adult Brain. Annals of the New York Academy of Sciences, 743, 201-211.

Luine, V. N. (1997). Steroid hormone modulation of hippocampal dependent spatial memory. Stress, 2, 21-36.

Luine, V. N., Richards, S. T., Wu, V. Y., & Beck, K. D. (1998). Estradiol enhances learning and memory in a spatial memory task and effects levels of monoaminergic neurotransmitters. Hormones and Behavior, 34, 149-162.

Luine, V., Beck, K., Bowan, R., & Kneavel, M., (2001). Sex differences in chronic stress effects on cognitive function and brain neurochemistry, in press.

Lynch, T., Sano, M., Marder, K. S., Bell, K. L., Foster, N. L., Defendini, R. F., Sima, A. A. F., Keohane, C., Nygaard, T. G., Fahn, S., Mayeux, R., Rowland, L. P., & Wilhelmsen, K. C. (1994). Clinical characteristics of a family with chromosome 17-linked disinhibition-dementia-parkinsonism-amyotrophy complex. Neurology, 44, 1878-1884.

Majoor-Krakauer, D., Ottman, R., Johnson, W. G., & Rowland, L. P. (1994). Familial aggregation of amyotrophic lateral sclerosis, dementia, and Parkinson's disease: evidence of shared genetic susceptibility. Neurology, 44, 1872-1877.

Malakoff, D. (2000, April 14). The rise of the mouse, biomedicine's model mammal. Science, 288, 248-252.

Malakoff, D. (2000, October 6). Researchers fight plan to regulate mice, birds. Science, 290, 23.

Massman, P. J., Sims, J., Cooke, N., Haverkamp, L. J., Appel, V., & Appel, S. H. (1996). Prevalence and correlates of neuropsychological deficits in amyotrophic lateral sclerosis. Journal of Neurology, Neurosurgery, and Psychiatry, 61, 450-455.

Matsumoto, S., Goto, M., Kusaka, H. Imai, T., Murakami, N., Hashizume, Y., Okazaki, H., & Hirano, A. (1993). Ubiquitin-positive inclusion in anterior horn cells in subgroups of motor neuron disease: a comparative study of adult-onset amyotrophic lateral sclerosis, juvenile amyotrophic lateral sclerosis and Werdnig-Hoffman disease. Journal Neurological Science, 115, 208-213.

Mitsuyama, Y., (1984). Presenile dementia with motor neuron disease in Japan: clinico-pathological review of 26 cases. Journal of Neurology, Neurosurgery and Psychiatry, 47, 953-959.

Mitsuyama, Y., (2000). Dementia with motor neuron disease. Neuropathology, 20, S79-S81.

Mizusawa, H., Matsumoto, S., Yen, S-H. Hirano, A., Rojas-Corona, R. R., & Donnenfeld, H. (1989). Focal accumulations of phosphorylated neurofilaments within

anterior horn cells in familial amyotrophic lateral sclerosis. Acta Neuropathologica, 79, 37-43.

Morrison, B. M., & Morrison, J. H. (1999). Amyotrophic lateral sclerosis associated with mutations in superoxide dismutase: a putative mechanism of degeneration. Brain Research Reviews, 29, 121-135.

Mukerjee, M. (1997, February). Trends in animal research. Scientific American, 86-93.

Muller, M., Vieregge, P., Reusche, E., & Ogomori, K., (1993). Amyotrophic lateral sclerosis and frontal lobe dementia in alzheimer's disease. European Neurology, 33, 320-324.

Murphy, P. L., Albert, S. M., Weber, C. M., Del Bene, M., & Rowland, L. P. (2000). Impact of spirituality and religiousness on outcomes in patients with ALS. Neurology, 55, 1581-1584.

Nakano, I. (1993). Temporal lobe lesions in amyotrophic lateral sclerosis with or without dementia: a neuropathological study. Neuropathology, 13, 215-227.

Nelson, L.M. (1996). Epidemiology of ALS. Clinical Neuroscience, 3, 327-331.

Obrink, K. J. & Rehbider, C. (2000). Animal definition: a necessity for the validity of animal experiments. Laboratory Animals, 34, 121-130.

Offen, D., Halevi, S., Orion, D., Mosberg, R., Stern-Goldberg, H., Melamed, E., & Atlas, D. (1998). Antibodies from ALS patients inhibit dopamine release mediated by L-type calcium channels. Neurology, 51, 1100-1103.

Odetti, P., Angelini, G., Dapino, D., Zaccheo, D., Garibaldi, S., Dagna-Bricarelli, F., Piombo, G., Perry, G., Smith, M., Traverso, N., & Tabaton, M. (1998). Early

Glycoxidation Damage in Brains from Down's Syndrome. Biochemical and Biophysical Research Communications, 243, 849-851.

Olton, D. S., & Samuelson, R. J. (1976). Remembrance of places passed: spatial memory in rats. Journal of Experimental Psychology, 2, 97-116.

Olton, D. S., Walker, J. A., & Gage, F. H. (1978). Hippocampal connections and spatial discrimination. Brain Research, 137, 295-308.

Olton, D. S., & Feustle, W. A. (1981). Hippocampal function required for nonspatial working memory. Experimental Brain Research, 41, 380-389.

Puumala, T., & Sirvio, J. (1998, Mar). Changes in activities of dopamine and serotonin systems in the frontal cortex underlie poor choice accuracy and impulsivity of rats in an attention task. Neuroscience, 83, 489-499.

Poole, T. (1996). Happy animals make good science. Laboratory Animals, 31, 116-124.

Price, D. L., Sisodia, S.S., & Borchelt, D.R. (1998, November 6). Genetic neurodegenerative diseases: the human illness and transgenic models. Science, 282, 1079-1083.

Przedborski, S., Donaldson, D. M., Murphy, P. L., Hirsch, O., Lange, D., Naini, A.B., McKenna-Yasek, D., & Brown, R.H. (1996). Blood superoxide dismutase, catalase and glutathione peroxidase activities in familial and sporadic amyotrophic lateral sclerosis. Neurodegeneration, 5, 57-64.

Przedborski, S., Dhawan, V., Donaldson, D. M., Murphy, P. L., McKenna-Yasek, D., Mandel, F. S., Brown, R. H., & Eidelberg, D. (1996). Nigrostriatal dopaminergic

function in familial amyotrophic lateral sclerosis patients with and without copper/zinc superoxide dismutase mutations. Neurology, 47, 1546-1551.

Pycock, C. J., Kerwin, R. W., Carter, C. J. (1980). Effect of lesion of cortical dopamine terminals on subcortical dopamine receptors in rats. Nature, 286, 74-77.

Reaume, A. G., Elliott, J. L., Hoffman, E. K., Kowall, N. W., Ferrante, R. J., Siwek, D. F., Wilcox, H. M., Flood, D. G., Beal, M. F., Brown, R. H., Jr., Scott, R. W., & Snider, W. D., (1996). Motor neurons in Cu/Zn superoxide dismutase-deficient mice develop normally but exhibit enhanced cell death after axonal injury. Nature Genetics, 13, 43-47.

Reeves, R. H., Irving, N. G., Moran, T. H., Wohn, A., Kitt, C., Sisodia, S. S., Schmidt, C., Bronson, R. T., & Davisson, M. T. (1995). A mouse model for Down syndrome exhibits learning and behavior deficits. Nature Genetics, 11, 177-183.

Reinstein, D. K., DeBoissiere, T., Robinson, N., & Wurtman, R. J. (1983). Radial maze performance in three strains of mice: role of the fimbria/fornix. Brain Research, 263, 172-176.

Rosen, D. R., Siddique, T., Patterson, D., Figlewicz, D.A., Sapp, P., Hentati, A., Donaldson, D., Goto, J., O'Regan, J. P. Deng, H.-X, Rahmani, Z., Krizus, A., McKenna-Yasek, D., Cayabyab, A., Gaston, S. M., Berger, R., Tanzi, R. E., Haperin, J. J., Herzfeldt, B., Van den B. (1993). Mutations in Cu/Zn superoxide dismutase are associated with familial amyotrophic lateral sclerosis. Nature, 362, 59-62.

Rothstein, J. D. (1995). Excitotoxic mechanisms in the pathogenesis of amyotrophic lateral sclerosis. Advances Neurology, 68, 7-20.

Rothstein, J. D., Van Kammen, M., Levey, A. I., Martin, L. J., & Kuncl, R. W. (1995). Selective loss of glial glutamate transporter GLT-1 in amyotrophic lateral sclerosis. Annals Neurology, 38, 73-84.

Rowan, A.N. (1997, February). The benefits and ethics of animal research. Scientific American, 79.

Rowland, L. P., Lange, D. J., & Murphy, P. L. (1997). Amyotrophic Lateral Sclerosis. In Johnson, R. T., & Griffen, J.W. Current Therapy in Neurologic Disease, Fifth Edition (pp. 307-311). St. Louis: Mosby.

Rowland, L. P. (1998). Diagnosis of amyotrophic lateral sclerosis. Journal of Neurological Sciences, 160 (Suppl. 1), S6-S24.

Rowland, L. P. (2000). Six important themes in amyotrophic lateral sclerosis (ALS) research, 1999. Journal of Neurological Sciences, 180, 2-6.

Rowland, L. P. and Shneider, N. A. (2001). Amyotrophic Lateral Sclerosis. New England Journal of Medicine, 344 (22), 1688-1700.

Sago, H., Carlson, E. J., Smith, D. J., Kilbridge, J., Rubin, E. M., Mobley, W. C., Epstein, C. J., & Huang, T-T. (1998). Ts1Cje,a partial trisomy 16 mouse model for Down syndrome, exhibits learning and behavioral abnormalities. Proceedings National Academy of Science, 95, 6256-6261.

Schwartz, J. H. (2000). Neurotransmitters. In E. R. Kandel, J. H. Schwartz & T. M. Jessel (Eds.), Principles of Neural Science (4th ed., pp. 280-297). New York: McGraw Hill.

Shaw, P. J. (1999). Calcium, glutamate, and amyotrophic lateral sclerosis: more evidence but no certainties. Annals of Neurology, 46(6), 803-805.

Sherwin, C. M. (1997). Observations on the prevalence of nest-building in non-breeding TO strain mice and their use of two nesting materials. Laboratory Animal, 31, 125-132.

Shibata, N., Hirano, A., Kobayashi, M., Siddique, T., Deng, H. X., Hung, W. Y., Kato, Y., & Asayama, K. (1996). Intense superoxide dismutase-1 immunoreactivity in intracytoplamic hyaline inclusions of familial amyotrophic lateral sclerosis with posterior column involvement. Journal Neuropathology Experimental Neurology, 55, 481-490.

Siddique, N., Sufit, R., & Siddique, T. (1999). Degenerative Motor, Sensory, and Autonomic Disorders. In C. G. P. Goetz, E. J. (Ed.), Textbook of Clinical Neurology (pp. 695-717). Philadelphia: W.B. Saunders Company.

Siddique, T., & Deng, H. X. (1996). Genetics of amyotrophic lateral sclerosis. Human Molecular Genetics, 5, 1465-1470.

Silberberg, R., Jarrett, S. R., & Silberberg, M. (1961). Life span of mice fed enriched or restricted diets during grWT h. American Journal of Physiology, 200, 332-334.

Sommer, B., Kohler, M., Sprengel, R., Seeburg, P. H. (1991). RNA editing in brain controls a determinant of ion flow in glutamate-gated channels. Cell, 67, 11-9.

StataCorp, (2001). Stata Statistical Software: Release 7.0 [Computer Software and Manual]. College Station, TX: Stata Corporation.

Strong, M. J., Grace, G. M., Orange, J. B., Leeper, H. A., Menon, R. S., & Aere, C. (1999). A prospective study in cognitive impairment in ALS. Neurology, 53, 1665-1670.

Tanaka, M., Kondo, S., Hirai, S., Sun, X., Yamagishi, T., & Okamoto, K., (1993). Journal of Neurological Sciences, 120, 22-28.

Tannenbaum, J. R., & Rowan, A.N. (1985). Rethinking the morality of animal research. Hastings Center Report, October, 32-43.

Thiels, E., Urban, N. N., Gonzalez-Burgos, G. R., Kanterewics, B. I., Barrionuevo, G., Chu, C. T., Oury, T. D., & Klann, E. (2000). Impairment of long-term potentiation and associative memory in mice that overexpress extracellular superoxide dismutase. Journal of Neuroscience, 20(20), 7631-7639.

Thomas, C. L. (Ed.). (1989). Taber's Cyclopedic Medical Dictionary, 16th Edition. Philadelphia: F. A. Davis Company.

Toumane, A., Durkin, T., Marighetto, D., Galey, D., & Jaffard, R. (1988). Differential hippocampal and cortical cholinergic activation during the acquisition, retention, reversal and extinction of a spatial discrimination in an 8-arm radial maze by mice. Behavioral Brain Research, 30, 225-234.

Trull, F. L. (1999, November 19). Biomedical attacks [Letter to the Editor]. Science, 286, 1477.

Tu, P-H., Gurney, M. E., Julien, J-P., Lee, V. M-Y., & Trojanowski, J. Q. (1997). Oxidative stress, mutant SOD1, and neurofilament pathology in transgenic mouse models of human motor neuron disease. Laboratory Investigation, 76, 441-456.

Umahara, T., Hirano, A., Shibata, N., & Yen, S. H. (1994). Demonstration of neurofibrillary tangles and neuropil thread-like structures in spinal cord white matter in parkinsonism-dementia complex on Guam and in Guamanian amyotrophic lateral sclerosis. Acta Neuropathologica, 88, 180-184.

Van de Weerd, H. A., Van Loo, P. L. P., Van Zutphen, L. F. M., Koolhaas, J. M., & Baumans, V. (1997). Preferences for nesting material as environmental enrichment for laboratory mice. Laboratory Animals, 31, 133-143.

Vogel, G. (2000, April 14). The mouse house as a recruiting tool. Science, 288, 254-255.

Warita, H., Hayashi, T., Murakami, T., Manabe, Y., & Abe, K. (2001). Oxidative damage to mitochondrial DNA in spinal motorneurons of transgenic ALS mice. Molecular Brain Research, 89, 147-152.

Weinberger, D. R. (1987). Implications of normal brain development for the pathogenesis of schizophrenia. Archives General Psychiatry, 44, 660-669.

Whishaw, I. Q., & Tomie, J-A. (1996). Of mice and mazes: similarities between mice and rats on dry land but not water mazes. Physiological Behavior, 60, 1191-1196.

Wightman, G., Anderson, V. E. R., Martin, J., Swash, M., Anderton, B. H., Neary, D., Mann, D., Luthert, P., and Leigh, P. N. (1992). Hippocampal and neocortical ubiquitin-immunoreactive inclusions in amyotrophic lateral sclerosis. Neuroscience Letters, 139, 269-274.

Wickelgren, I. (1997, May 2). Estrogen stakes claim to cognition. Science, 276, 675-678.

Williams, R. S., & Wagner, P. D. (2000). Transgenic animals in integrative biology: approaches and interpretations of outcome. Journal Applied Physiology, 88, 1119-1126.

Williamson, T. L., Brujin, L. I, Zhu, Q., Anderson, K. L., Anderson, S. D., Julien, J. P., & Cleveland, D. W., (1988). Absence of neurofilaments reduces the selective

vulnerability of motor neurons and slows disease caused by familial amyotrophic lateral sclerosis-linked superoxide dismutase 1 mutant. Proceedings National Academy Science, USA, 95, 2631-2636.

Williamson, T. L., & Cleveland, D. W., (1999). Slowing of axonal transport is a very early event in the toxicity of ALS linked SOD1 mutants to motor neurons. National Neuroscience, 2, 50-56.

Wilson, C. M., Grace, G. M., Munoz, D. G., He, B. P., & Strong, M. J. (2001). Cognitive impairment in sporadic ALS: a pathologic continuum underlying a multisystem disorder. Neurology, 57, 651-657.

Wilson, I. A., Puolivali, J., Heikkinen, T., & Riekkinen, P. (1999). Estrogen and NMDA receptor antagonism: effects upon reference and working memory. European Journal of Pharmacology, 381, 93-99.

Wong, P. C., Price, D. L., & Subramaniam, J., (2001). Experimental genetics as a tool for understanding pathogenesis of ALS. In M. P. Mattson (Ed.) Pathogenesis of Neurodegenerative disorders (pp. 173-190). Totowa, NJ: Humana Press Inc.

Wong, P. C., Waggoner, D., Subramaniam, J. R., Tessarollo, L., Bartnikas, T. B., Culotta, V. C., Price, D. L., Rothstein, J., & Gitlin, J. D., (2000). Copper chaperone for superoxide dismutase is essential to activate mammalian Cu/Zn superoxide dismutase. Proceedings National Academy of Science, 97, 2886-2891.

World Federation of Neurology El Escorial Criteria for Diagnosis of ALS. Journal of Neurological Sciences, 124 (suppl), 96-107.