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The Effects of Paraquat Exposure on Serial Reaction Time Performance in Rats (Rattus norvegicus) and Neuroprotection by Water-Soluble COQ10

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ABSTRACT

Nissen and Bullemer (1987) developed the serial reaction time (SRT) task to measure attention in humans. The SRT task in rats is typically modeled after studies with humans and uses repeated or random sequences to test anticipatory reactions. In the current study, paraquat (PQ)-induced Parkinson's disease (PD) model in Long-Evans hooded rats was used to examine the rats' sequential learning. A water-soluble formulation of coenzyme Q_{10} (WS- CoQ_{10}) was used as a therapeutic agent. Rats were induced with Parkinson's disease via the administration of paraquat. The aim of this study was to study the neuroprotective effects of CoQ_{10} using the SRT task to measure sequence performance in rats. The results indicated that the rats were much faster in responding to fixed sequences compared to random sequences. However, this study did not find significant results to indicate that exposure of paraquat with or without a neuroprotective agent, WS-CoQ_{10} affected serial reaction performance. The implications of these findings are discussed with suggestions for further research with this task.

DEDICATION

Dedicated to my grandparents.

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CHAPTER I

INTRODUCTION

One of the common motor disorders affecting between 80,000 to 100,000 Canadians is Parkinson's disease (PD), which results in the loss of dopamine-producing brain cells present in the substantia nigra pars compacta region of the brain. Much of the information regarding the etiology and pathogenesis of PD has been obtained from clinical, postmortem and epidemiological studies. Currently, the goals of therapy in PD are to maintain function and quality of life by treating its symptoms while trying to avoid or minimize any drug-induced complications. To date, levodopa is the most effective treatment for symptom relief in PD. The aim of all dopaminergic strategies is to restore dopamine transmission in the striatum which is accomplished by stimulating postsynaptic receptors (directly with dopamine agonists), increasing dopamine precursor availability (levodopa), blocking the metabolism of levodopa in the brain, and blocking the catabolism of dopamine at the presynaptic terminal. Treatment however, does not reverse the morphologic changes and fails to arrest the progression of the disease. As the disease progresses, drug therapies tend to become less effective and symptoms become more difficult to manage. Thus the priority must be to move beyond symptom control and develop neuroprotective therapies. Unfortunately, no such therapy is currently available, leaving this area open for further research.

To make a model of neurodegeneration functionally relevant, a behavioural component is necessary. Along with various motor gaiting and balancing tasks, the serial reaction time task (SRT) may prove to be one of the most important assessment tools as it combines motor function skills with attentional and other cognitive capacities. The SRT

task consists of a sequence of a connected series of events that engages processes supporting the temporal organization of behaviour, the formation of high-order associations, and the prediction of future events (Chafee & Ashe, 2007; Keele et al., 2004). Moreover this test is easy to administer and manipulated to assess a patient's attention and other cognitive abilities as a function of the type and severity of his/her neurodegenerative disorder. Despite such advantages, many inconsistent findings have been reported from studies employing this test on human patients with PD. The use of different samples of patient populations at uncontrolled different stages of the disease have made comparisons between studies for replication of effects difficult to obtain. Therefore developing animal models has become imperative to (a) better understand of the pathogenesis of PD and for (b) preclinical testing of possible neuroprotective therapeutics to treat PD. Moreover, in animal models, it may be easier to control the degeneration of dopamine neurons with dose-specific exposure to neurotoxins like paraquat. Rodents are a common choice of animal used to study PD, they have a short life span and comparative brain structures with humans. In the neurological sciences, rodents have proved to be an important tool for the study of neural development, diseases, neurodegeneration, addiction, and general principles of cognitive behaviour. Given that rodents are a common choice of animal model to investigate neurodegeneration, we sought to use an SRT preparation derived from Eckart et al. (2010), who reported specific effects on SRT performance from lesions to dopamine areas of the rat's brain.

Recent studies suggest that PD may arise from a combination of genetic susceptibility and exposure to environmental toxins (McCormack et al., 2002). Several environmental risk factors such as metals and herbicides have been linked to the greater

incidence and progression of PD (Dinis-Oliveira et al., 2006). Among these factors, the herbicide paraquat (PQ) shows a clear neurotoxicity in the central nervous system (CNS). Sub-lethal doses of this toxin have been shown to have targeted effects on dopamine neurons in the substantia nigra. PQ was chosen as the toxin to induce Parkinsonism in the current study because it is an environmental toxin associated with increased incidence of PD in areas where it has been used in agriculture (Liou et al. 1997) and also because it's chemical structure is similar to that of the synthetic narcotic MPTP (1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine) known to have neuro-degenerative effects in the substantia nigra pars compacta (SNc) (Norris et al. 2007). Consideration of PQ as a candidate neurotoxicant requires that systemic delivery not only produces dopamine (DA) neuron loss in the substantia niagra (SN) but also exhibits the resultant behavioural effects reflecting dopamine depletion.

CoQ_{10} is known to be a powerful antioxidant that reduces oxidative stress in in-vitro preparations (Beal, 2003b). This in-vitro neuroprotectant has recently been shown to have an in-vivo prophylactic effect in rats exposed to PQ and therefore might also have a post-exposure therapeutic effect. This possibility could lead to important clinical applications for preventing further loss of dopamine producing neurons in human PD patients. Therefore the major goals of the current study are (a) to examine signal sequence factors that affect rats' SRT performance, (b) to determine how such performance is affected by rats' exposure to PQ, and (c) to determine whether post-PQ exposure treatment with CoQ10 can ameliorate any SRT behavioral changes. We note that this experiment is part of a larger preclinical study on the therapeutic effects of CoQ10 conducted in the University of Windsor's biochemistry department by Facecchia

et al. who are carrying out the biochemical assays on the brain tissue of these animals following our laboratory's behavioural assessments.

In the next chapter, we discuss in further detail rats' serial pattern learning, PD and animal models of this disorder, and mechanisms of neuroprotection by CoQ10.

CHAPTER II

REVIEW OF LITERATURE

Parkinson's Disease

Epidemiology

Parkinson's disease (PD) is one of the most common degenerative disorders of the nervous system. Occurring in about 1% of people over the age of 65, its peak age of onset is in the 60s (range is 35 to 85 years) and the course of the illness ranges between 10 and 25 years (Kasper et al., 2005). Familial clusters of autosomal dominant and recessive forms of PD comprise approximately 5% of cases (Cory-Slechta et al., 2005). These cases are characterized by an earlier age of onset (typically before age 50) and a longer course than the more typical sporadic PD.

Clinical Features

An accurate diagnosis of PD can usually be made (with some confidence) in patients who present with rest tremor, rigidity and bradykinesia. Tremor is particularly important because it is found in 85% of patients with PD (Kasper et al., 2005). A unilateral and gradual onset of symptoms further supports the diagnosis. Masked faces, decreased eye blinking, stooped posture and decreased arm swing are some of the early symptoms of this disease. The most disabling feature of PD is bradykinesia, which interferes with all aspects of daily living. Fine motor control is also impaired as noted by a decrease in manual dexterity and handwriting (micrographia). Rest tremor typically appears unilaterally, first distally, involving the digits and wrist, where it may have a "pill rolling" character. Tremor usually spreads proximally, ipsilaterally and occasionally to the leg before crossing to the other side after a year or more after the onset of the disease.

Tremor may later appear in the lips, tongue and jaw. Rigidity is felt as a uniform resistance to passive movement about a joint throughout the full range of motion, giving rise to a characteristic "plastic" quality. Short lived and regular interruptions of resistance during passive movement may give rise to a "cogwheeling" sensation.

Gait disturbance with shuffling and short steps is a prominent feature of PD. Festinating gait is a classic Parkinsonian sign and results from the combination of flexed posture and loss of postural reflexes which in turn cause the patient to accelerate in an effort to "catch up" with the body's center of gravity. Freezing of gait, a feature of more advanced PD, commonly occurs at the onset of locomotion (start hesitation), when attempting to change direction or turn around, and upon entering a narrow space. Postural instability is one of the most disabling features of advanced PD, contributing to falls and injuries and leading to major morbidity and mortality in this population.

Non motor aspects of PD include depression, anxiety, cognitive impairment, sleep disturbances, sensory abnormalities and pain, loss of smell (anosmia), and disturbances of autonomic function. Together they may contribute as much to the burden of the disease as the more obvious motor abnormalities.

Pathology

On gross pathologic exam, the typical macroscopic findings in a PD brain are pallor of the substantia nigra and locus ceruleus due to degeneration of dopamine-producing neurons in the substantia nigra pars compacta (SNc), a region within the midbrain (Corti et al., 2005). As mentioned previously, PD exhibits four classic symptoms: a resting tremor, muscular rigidity or stiffness, an inability to initiate movement, and generalized slowness of movement and postural instability. These

symptoms become fully developed after approximately 80% of the dopaminergic neurons in the SN are already lost, resulting in reduced synthesis and release of DA from the SNc region (Schober, 2004). Pharmacologic restoration of dopamine transmission is thus the basis for symptomatic drug treatment of PD.

PD is assumed to be a multicentric disease. It is also believed that an association exists between the nigrostriatal degeneration and degenerative processes elsewhere in the central and peripheral nervous systems. Three possible mechanisms are hypothesized: damage to the SNc and other regions occurs simultaneously; the disease primarily begins in the SNc, which influences the involvement of other areas; or the involvement of the SNc occurs later in the disease (Lang & Lozano, 1998). Associated with this neuronal loss is the presence of large eosinophilic inclusions called "Lewy bodies," which are found in the cytoplasm of the surviving neurons in the SN region. Lewy bodies are single or multiple, intracytoplasmic, eosinophilic round to elongated inclusions that often have a dense core surrounded by a pale halo (Cotran et al., 1999). They are abnormal aggregates made up of a series of proteins including neurofilaments, -synuclein fibrils, ubiquitin, parkin, and proteasomal elements and are a hallmark of genetic diseases such as PD (Schapira, 1999).

Basal Ganglia

Control of movement is highly varied in humans, from manipulating objects as light as a needle to swinging objects as heavy as a baseball bat to drive a ball across a field. Parkinson's disease (PD) is a disorder of "controlling" movements (Kolb, 2001). The brain areas that allow us to adjust the force of our movements include the basal ganglia (BG), which are a group of nuclei situated at the base of the forebrain and are

connected with the cerebral cortex, thalamus and other brain areas. The BG has two subdivisions: (i) the rostral subdivision, containing the striatum (putamen and caudate nucleus), pas externa (GPe), and pars internal (GPi) segments of the globus pallidus; and (ii) the caudal subdivision, containing the subthalamic nucleus (STN) and the substantia nigra (SN), which encompasses the SN pars compacta (SNc), SN pars reticulate (SNr) and the SN pars lateralis (SNI) (Obaso et al., 2008) (Figure 1). The BG are associated with a variety of functions, including voluntary motor control, procedural learning relating to routing behaviours, or "habits", eye movements and most importantly, cognitive and emotional functions. In particular, the motor circuit has two entry points into the BG, the striatum and the STN, and an output, the GPi, which connects to the cortex via the motor thalamus (Obaso et al., 2008). The signals for intentional movements are initiated in the cerebral motor cortex and eventually reach the brain stem. From the brain stem the signals are transmitted to the muscles. Before this they reach the muscles, centers such as the cerebellum and the basal ganglia pose their influence on these signals. Both of these centers exert their influence on the final motor signals via the thalamus (Groenewegen, 2003). One theory holds that an inhibitory pathway and an excitatory pathway affect the activity of the motor cortex: (Alexander & Crutcher, 1990). Both these pathways converge on an area of the basal ganglia called the internal path of the globus pallidus (GPi). The GPi in turn, projects into the ventral thalamic nucleus and the thalamus projects to the motor cortex. The thalamic projections determine the size or force of a movement that the cortex produces and is influenced by the GPi. The GPi acts metaphorically like the volume dial on a radio because it controls the output thereby dictating whether a movement will be strong or weak. The direct projections to the GPi

are via the striatum and STN. Neurons in this pathway are the D1 dopamine receptor subtype and they provide a direct inhibitory effect on the GPi/SNr (Figure 2A). The indirect projections to the GPi are via the GPe. Neurons in this pathway are the D2 dopamine receptor subtype, which cause the excitation of GPi/SNr (Figure 2B). The GPe regulates the motor output of the BG (Obaso, 2008). The output from the basal ganglia is influenced by the opposing effects of the direct and indirect pathways. If the activity in the indirect pathway dominates, the thalamus shuts down, and the cortex is unable to produce movement. If direct-pathway activity dominates, the thalamus can become overactive thereby amplifying the movements (Kolb, 2006). The DA deficiency that precedes PD leads to a cascade of functional changes in basal ganglia circuitry. Dopamine modifies striatal input and neuronal striatal activity, modulated GPe and GPi, and STN activity. The loss of DA neurons in PD disrupts the corticostriatal balance, which increases activity in the indirect circuit and ultimately reduces the activity in the direct circuit (Obaso, 2008). Therefore, PD is characterized by increased neuronal activity in the STN, GPi and SNr regions, leading to an inhibition of motor nuclei, which regulate the body's ability to execute smooth and controlled movements.

Dopamine Pathways

Dopamine (DA) is a catecholamine neurotransmitter present in all animals and readily available in the substantia nigra (SN). Dopamine has many functions in the brain; among them are vital roles in cognition, voluntary movement, motivation, punishment and reward. Dopamine is produced in several brain areas, including the SN and the ventral tegmental area (VTA) (Purves et al., 2007). Dopamine biosynthesis in the body begins with hydroxylation of the amino acid L-tyrosine to L-DOPA via the enzyme

tyrosine hydroxylase and then by decarboxylation of L-DOPA by dopa decarboxylase (Figure 3). In some neurons, DA is further processed into epinephrine and norepinephrine by dopamine beta-hydroxylase (Purves et al., 2007). In neurons, DA is packaged after synthesis into vesicles, which are then released into the synapse in response to a presynaptic action potential. In most areas of the brain, including the striatum and BG, DA is inactivated by reuptake via the dopamine transporter (DAT) (Dziedzicka-Wasylewska, 2004), then is broken down by monoamine oxidase (MAOA and MAOB).

Dopamine neurons are mostly present in the VTA of the midbrain, the SNc and the arcuate nucleus of the hypothalamus. This forms the neurotransmitter system, composed of axon projections to large areas of the brain which are divided into four major pathways: mesocortical, mesolimbic, nigrostriatal, and tuberoinfundibular pathways (Hung et al., 1995). The mesocortical pathway connects the VTA to the frontal lobe of the prefrontal cortex. Neurons with somas in the VTA project axons into the prefrontal cortex. This pathway is important for normal cognitive functions in the dorsolateral prefrontal cortex (part of the frontal lobe) and is involved in motivation, emotion, and aspects of learning and memory. The mesolimbic pathway carries DA from the VTA to the nucleus accumbens via the amygdala and hippocampus. The somas of the projecting neurons are in the VTA. This is widely known as the “reward” pathway. The tuberoinfundibular pathway runs from the hypothalamus to the pituitary gland. This pathway controls the secretion of prolactin from the anterior pituitary gland. Of particular importance is the nigrostriatal pathway, which runs from the SN to the neostriatum. Somas in the SN project axons into the caudate nucleus and putamen. This pathway is involved in the production of movement and belongs to a system called the basal ganglia

motor loop. The degeneration of the nigrostriatal dopaminergic pathway consequently results in a substantial striatal dopamine reduction, ultimately manifesting as symptoms of PD (Dinis-Oliveria et al., 2006).

Etiology

The etiology behind almost 95% of the cases of Parkinson's disease is unknown (Corti et al., 2005). The genetic inheritance in the origin of PD has been debated for more than a century. A study by Tanner et al. (1999) initiated a large twin study of PD by using the World War II twin's registry and the National Academy of Sciences registry. They studied concordance rates in both monozygotic and dizygotic twin pairs and used 19,842 white males. These studies suggested that heredity plays an important role in cases with age of onset <50 years and a less important role in older patients. Four genes have been clearly linked to familial forms of PD. PARK 1 and PARK 5 lead to an autosomal dominant form of PD with typical features such as early age of onset and rapid progression of symptoms. PARK 1 encodes α-synuclein, leading to its abnormal aggregation. PARK 2 and PARK 7 lead to autosomal recessive disorders, also with atypical features, including juvenile forms of Parkinsonism (Kasper et al., 2005). PARK 2 encodes parkin, an E3 ubiquitin protein ligase. Mutations in parkin appear to be the major cause of autosomal recessive PD. The identification of these and other mutations is proving invaluable in refining the correlation between genotypes and phenotypes, in generating animal models to study pathogenesis, and in identifying target pathways for possible therapeutic intervention.

Models to Study Parkinson's disease

Numerous concerns exist in studies involving patients with Parkinson's disease. Several studies have not succeeded in ruling out the possibility that the oxidative stress indices found in PD brains are anything other than the nonspecific expression of dying neurons. The postmortem tissue from end-stage PD is difficult to access due to ethical issues, and when these tissues were obtained, the studied samples lacked dopaminergic neurons. Another issue is that the majority of patients being used for postmortem studies are patients that have used a battery of drugs, such as L-DOPA, which, like dopamine, can readily auto-oxidize, giving rise to reactive oxygen species (ROS) (Parkinson's study group, 2004). This however does not imply that chronic use of this drug will induce PD, but does raise the concern that many observations from studies in humans may reflect artifacts from the treatments received by PD patients before their death. For these reasons, animal models of PD may be desirable in that they can provide controls against such confounds as well as provide pre-clinical tests of therapeutic interventions. Since PD does not spontaneously develop in animals, neurotoxic agents have had to be used to induce the characteristic functional changes associated with PD. Some of the important toxin-induced models are discussed in the following section of this paper.

MPTP Model

Several causative factors have been found to induce Parkinsonism similar to that of idiopathic PD, including repeated head trauma, neuroleptic drugs, and manganese toxicity (Adler, 1999). In particular, the toxicant MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine), which was accidently discovered in the 1980s, resulted in the development of Parkinsonism symptoms in a small group of drug addicts (Langston et

al., 1983). These individuals injected themselves with the synthetic narcotic. The postmortem analysis of these addicts revealed a selective loss of neurons and Lewy body–like inclusions in the substantia nigra. These findings suggested that compounds similar to MPTP in structure or biological activity might be the primary cause of sporadic PD (Smeyne, 2005). By itself, MPTP is not toxic, but after reacting with monomine oxidase and converting to its toxic metabolite MPP+, it is believed to cause cell death by interfering with mitochondrial respiration (Costello et al., 2009) because it concentrates in mitochondria and inhibits complex-I of the electron transport chain.

Several studies with MPTP have been conducted in nonhuman primates, rodents and cats although a majority of studies have been performed on rodents such as mice (Terzioglu, 2008). These studies showed that in various animals, MPTP induces damage to the dopaminergic neurons in the nigrostriatal pathway identical to that seen in PD (Bove et al., 2005). Various regimes and doses of MPTP were used to study PD (Betarbet, 2002). Chronic administration of MPTP induces about 50–60% loss of dopaminergic neurons in the substantia nigra. Susceptibility to this compound varies in different species. In the MPTP mouse model of PD, time-course experiments suggest that oxidative stress is an early event that may directly kill some of the dopaminergic neurons (Beal, 2001). Systemic MPTP administration in mice induces PD-like symptoms, including bradykinesia, rigidity and posture anomalies.

Pesticide and Herbicide Induced PD Models

Although the exact cause of PD remains unclear, numerous environmental risk factors have been identified in the modulation of the disease onset and/or its progression (De Monte, 2001, 2003; Di Monte et al., 2002). Several environmental agents are known

to cause nigrostriatal damage and may contribute to PD, namely, aluminum (Altschuler, 1999); solvents such as methanol (Davis & Adair, 1999), and carbon monoxide (Klawans et al., 1982). Gorell et al. (1998) assessed exposure to pesticides, farming, well-water use, and rural living as risk factors for PD. They enrolled 144 patients with PD and 464 control subjects from metropolitan Detroit. They found a significant association of occupational exposure to herbicides, insecticides and farming in general with PD. It has been hypothesized that many chemicals used in agriculture are capable of selectively targeting dopaminergic neurons, thereby accelerating the development of PD. Brown et al. (2006) conducted a literature search of 10 major studies and found a consistent association between exposure to pesticides and an increased risk of developing PD. Particular classes of pesticides found to be associated with PD include herbicides, in particular paraquat, and insecticides. Duration of exposure of more than 10 years was found to be a risk factor in developing PD.

Paraquat Model of PD

Paraquat (PQ), or 1, 1'- Dimethyl-4, 4'-bipyridinium, is a herbicide commonly used in many developing countries. This is a hydrophilic-charged molecule and therefore does not diffuse across the blood–brain barrier (BBB) (Denis-Oliveira et al., 2006) but rather through the neutral amino acid transporters. This toxicant is quick acting and kills plant tissue on contact. Paraquat is an agent known to induce Parkinsonian symptoms in humans and animals (Langston, 1983). Ingesting large dosages of paraquat causes liver, lung, heart, and kidney failure within several days to several weeks and may cause death. Moreover, studies have revealed a strong correlation between the amount of exposure to non-lethal dosages of paraquat and Parkinson's disease (Liou et al., 1997; Morano et al.,

1994). Liou et al. (1997) explored environmental risk factors for PD in Taiwan. They recruited 120 patients with PD and 240 control participants in this study. All participants underwent structured interviews and open-ended questionnaires to obtain histories of their exposure to environmental factors, including place of residence, source of drinking water and environmental and occupational exposures to various agricultural chemicals. The authors suggested that there was an increased risk of PD associated with a history of living in rural environments, farming, and the use of herbicides and pesticides, specifically the agricultural use of paraquat (PQ). Moreover, the risk of PD was greater among participants who had used paraquat than in those who had used other types of herbicides and pesticides. It is important to note that the molecular structure of PQ is similar to that of MPP+ (Figure 5). Recent mammalian and yeast-cell experiments suggest that mitochondria actively take up PQ across their membranes where complex-I reduce it to the paraquat radical action that subsequently produced mitochondria-damaging superoxide (Cocheme et al., 2008).

Studies of PQ toxicity have recently focused on its CNS effects. Unlike the exposure to high levels of PQ that mainly produce pulmonary toxicity, chronic low levels resulting from prolonged exposure to nonpneumotoxic doses may produce damage to the basal ganglia. Toxic damage of the brain has been observed in patients who died from PQ poisoning (Grant et al., 1980; Hughes, 1988). Autopsy findings in cases of acute PQ poisoning showed cerebral damage with edema and neural death in the SN region. However, in these studies, the possibility of whether the observed tissue changes occurred either postmortem or as a consequence of anoxia due to respiratory dysfunction could not be ruled out (Grant et al., 1980).

Mechanism of Paraquat Toxicity

One mechanism by the cellular toxicity of PQ is essentially due to its redox cycle which leads to the production of reactive oxygen species (ROS) (Castello et al., 2007). Paraquat is reduced, mainly by NADPH-cytochrome P-450 reductase (Clejan & Cederbaum, 1989). NADPH-cytochrome c reductase (Fernandez et al., 1995) and the mitochondrial complex 1, also known as NADH:ubiquinone oxidoreductase (Fukushima et al., 1993; Yamada & Fukushima, 1993) combine to form a PQ monocation free radical (PQ+). It is generally accepted that PQ uses cellular diaphoresis, which are a class of enzymes that transfer electrons from NAD (P) H to small molecules, such as PQ (Day et al., 1999). The PQ monocation free radical is then rapidly reoxidized in the presence of oxygen, generating the superoxide radical (O_{2-}) (Bush et al., 1998). This then sets off a cascade of reactions leading to the generation of other ROS, mainly hydrogen peroxide (H_2O_2), hydroxyl radical (HO), and the consequent adverse cellular effects. Indeed, hydroxyl radicals (Bush et al., 1998) have been implicated in the initiation of membrane damage by lipid peroxidation during the exposure to PQ in vitro (Busch et al., 1998) and in vivo (Burk et al., 1980). These results were confirmed by McCarthy et al (2004) who investigated the mechanism of cell death of differentiated in vitro human neuroblastoma cells. They found that exposure to PQ produced cell death by causing oxidative stress and subsequent mitochondrial dysfunction (McCarthy et al., 2004).

Paraquat has been found to selectively kill nigrostriatal dopaminergic neurons in in-vivo animal models (Suntres, 2002). This study revealed that paraquat induced cell death by causing oxidative stress. The details behind this interaction are still not clearly understood. Somayajulu et al (2009) concluded that PQ administration into rat's causes

the loss of DA neurons, specifically in the SNc. Results showed that with the doses of 10 mg/kg of PQ, damage was caused specifically to the DA neurons in the SNc without producing any decrease in cells in other brain areas.

Paraquat-Maneb Model

Parkinson's disease has been reported to occur at higher rates among farmers and in rural populations, leading to the hypothesis that agricultural pesticides, such as rotenone, maneb (MB), as well as paraquat (PQ), might be causal agents. Additionally, data from epidemiological studies point to an association between increased PD risk and specific environments. Costello et al. (2008) investigated whether exposure to maneb (fungicide) and paraquat (herbicide) alone and in combination increased the risk of incidents of PD among residents of the Central Valley of California, an area well known for its intensive agriculture and potential for pesticide exposure. They conducted extensive interviews with 377 patients with PD and 755 eligible controls. The researchers collected information on pesticide use as far back as 1974. The authors found that during the period between 1974 and 1999 that agricultural application of both maneb and paraquat within 500 meters of residences greatly increased the risk of developing PD. Exposure to both pesticides during the earlier years studied also doubled the risk for older cases. Association was strong for "younger-onset" patients (≤60 years), who would have been children, teenagers, and/or young adults during the exposure period. Among those who were exposed in the earlier years, risk was increased more than two times with exposure to just one of the pesticides and more than four times with exposure to both pesticides. Consistent with some theories regarding the progression of PD pathology (Braak et al., 2003), these data suggest that the critical window of exposure to toxicants

may be years before the onset of motor symptoms, which ultimately leads to a positive diagnosis. Human data are insufficient to support this claim for any specific pesticide, largely because of challenges in exposure assessment. Given the public health implications concerning risk factors for the development of PD, the study of the environmental factors involved in the etiology of PD has gained the renewed interest of the scientific and medical community.

Experimental evidence of the neurotoxicity of such organic compounds has been demonstrated in mice and rats. Studies have shown exposure to PQ alone or in combination with MB results in loss of DA neurons in SNc and reduction in animals' general activity. Norris et al. (2007) found that exposure to a combination of the fungicide maneb and the herbicide paraquat in mice led to increased substantia nigra neuronal pathology. In that study, mutant human α-synuclein transgenic mice (M83) were treated with the pesticides paraquat and maneb, either alone or in combination. The researchers found that chronic treatment of M83 mice with both pesticides drastically increased neuronal α-synuclein pathology throughout the CNS, including the hippocampus, cerebellum, and sensory and auditory cortices. This study supports the notion that environmental factors causing nitrative damage are closely linked to mechanisms underlying the onset of Parkinson's- like neurodegeneration. Thiruchelvam et al. (2003) tested whether exposing mice to different herbicides and/or pesticides would produce Parkinsonism features. The authors used young adult mice of different age groups (6 weeks, 5 months, or 18 months old). Subjects were exposed to the herbicide paraquat, fungicide maneb, or paraquat and maneb. The results showed that paraquat and maneb induced reductions in locomotor activity and motor coordination. The 18-month-

old mice were most affected and exhibited failure to recover 24 hours posttreatment. Progressive reductions in dopamine metabolites and dopamine turnover were exhibited, and numbers of nigrostriatal dopaminergic neurons were reduced in all age groups following exposure. Collectively, these data demonstrate enhanced sensitivity of the ageing nigrostriatal dopamine pathway to these pesticides, particularly the paraquat+ maneb group, resulting in irreversible and progressive neurotoxicity. Cicchetti et al. (2005) studied the effects of PQ and MB on dopaminergic (DA) neuron-glia, both in vitro and in vivo cultures in young adult rats. In vitro, PQ led to a loss of DA compared to non-DA neurons and microglial activation in a dose-dependent manner. The addition of MB had no further effect nor did it lead to microglial activation when used alone. In vivo, 2-month-old rats were subjected to intraperitoneal injections (IP) of placebo, PQ alone, or PQ in combination with MB, twice a week for 4 weeks, and then were sacrificed the day following the last injection. The results showed a significant loss of nigral DA neurons in both treatment groups. Microglial activation was seen in the substantia nigra of rats subjected to PQ with or without MB. The authors further conducted behavioural-speed and mobility testing, which measured the degree of hunchback position; speed testing consisted of determining the speed at which animals conducted daily grooming, moving, and exploring the cage; and mobility testing consisted of placing the animal on a trellis at a 45-degree angle and assessing the degree of akinesia or bradykinesia. The behavioural analyses showed a mixed pattern of motor impairments, which may have been due to early effects of DA neuronal loss and/or systemic effects associated with MB exposure in addition to PQ. This study concluded that exposure to PQ with or without MB induced

neurodegeneration, which is thought to occur via an early inflammatory response in young adult animals.

Molecular Mechanisms Contributing to PD

In Parkinson's disease, dopaminergic cells die due to a combination of factors including excitotoxicity, mitochondrial dysfunction, and oxidative stress. However, the exact mechanism of neurotoxicity is not yet fully known. A current available research in this area will be discussed below.

Excitotoxicity

One of the mechanisms of neurodegeneration in several neurological diseases (Dugan & Choi, 1999) is by the excitotoxicity induced by N-methyl-D-aspartate (NMDA) receptor over activation. Excitotoxins like NMDA and kainic acid which bind to these receptors as well as high levels of glutamate, can cause excitotoxicity by allowing high levels of calcium (Ca^{2+}) ions to enter the cell. Glutamate is an excitatory neurotransmitter implicated in the development of the brain and synaptic plasticity (Sandu et al., 2003). In vivo studies on the mechanisms of PQ-induced toxicity in the striatum indicate that PQ stimulates glutamate efflux, initiating excitotoxicity mediated by reactive nitrogen species (RNS). Increasing evidence shows that the excitotoxic injury plays a critical role in the progressive degeneration of DA neurons in PD (Beal, 1998). PQ induces continuous dopamine overflow and a consequent reduction of dopamine synthesis (Dinis-Oliveira et al., 2006). However, no direct evidence has been found to indicate excitotoxicity in PD.

Mitochondrial Dysfunction in PD

Many lines of research point to possible mitochondrial dysfunction in PD. Impaired electron transport hampers adenosine triphosphate production and leads to the diversion of electrons from their normal electron transport recipients resulting in further formation of damaging free radicals (Cassarino et al., 1999). The initial hypothesis that mitochondrial complex-I deficiency may be involved in the etiology of PD came from the findings that the mitochondrial complex-I inhibitor MPTP causes a clinical syndrome indistinguishable from PD and selective dopaminergic cell loss in the substantia nigra (SN) (Langston et al., 1983). Subsequently, complex-I activity was assessed and found to be significantly reduced in platelet mitochondria in patients with PD compared with controls (Hass et al., 1993). Complex-I activity was also reduced in the SN region but not in other regions of the brain (Schapira et al., 1990 a, b).

Several studies suggest that mitochondrial complex-I of the electron transport chain (ETC) is responsible for reducing PQ into its radical, which inhibits complex-I activity, and finally leading to mitochondrial dysfunction (Dinis-Oliveira et al., 2006). Castello et al. (2007) investigated the implications of the mitochondria on exposure to PQ. Their results suggested that at least in the rat brain, mitochondria are a principal site for PQ_2+H_2O_2 production and that this production requires the presence of respiratory substrates. This study found complex-III of the mitochondrial ETC as the main site for reactive oxygen species (ROS) production.

Oxidative Stress in PD

Oxidative stress has been hypothesized to be one of the central mechanisms linked to both the initiation and the progression of PD. Oxidative stress refers to the

undue oxidation of biomolecules leading to cellular damage caused by ROS. The brain depends mainly on energy produced from the mitochondria and almost 95% of the molecular oxygen that is inhaled is metabolized by the mitochondrial electron transport chain. ROS may be formed during a number of cellular processes including mitochondrial oxidative respiration and dopamine metabolism. Mitochondria are the major source of ROS at the cellular level. Free radicals produce oxidative damage by reacting with DNA, lipids and proteins (Betarbet et al., 2002).Various neurodegenerative disorders and syndromes are associated with oxidative stress (Behl, 2002). At several sites along the ETC are sites of "electron leak" (Arnaiz et al., 1999). These electrons may combine with molecular oxygen and thereby form ROS, such as superoxide (O_2-) and hydrogen peroxide (H_2O_2) (Halliwell, 1987). The mitochondrial electron transport chain produces ROS at complex-I and complex-III. It is suggested that increase in ROS is a consequence of the impairment of the mitochondrial respiratory chain (Fluery et al., 2002). H_2O_2 is not a free radical but can penetrate cell membranes, making it very toxic to the cell (Rhee, 1999). Several studies emphasize the contribution of oxidative stress in nigral cell death in PD. This has been demonstrated by postmortem studies in patients with PD as well as toxin-induced PD models (Dickson, 2007; Jenner, 2003). The evidence of increased oxidative damage includes increased levels of lipid peroxidation, DNA damage, and protein oxidation observed in the SN region in patients with PD (Zhang et al., 1999). Furthermore, oxidative stress is linked to other cellular processes such as cell death, excitotoxicity and mitochondrial dysfunction; therefore it is not easy to establish whether oxidative stress is the primary initiating agent or a product of these events (Jenner, 2003).

Advances in Therapeutic Approaches to PD

Much progress has been made in the treatment of PD as a result of advances in experimental therapeutics. Many promising therapies are available for PD, and currently symptomatic treatments are available focusing on treating the symptoms of the disease. These options include dopamine replacement strategies, nondopaminergic therapies, surgical approaches and very few neuroprotective therapies that are under research. Pharmacological treatment has two main objectives: (1) to increase the activity of remaining dopamine synapses and; (2) to suppress the activity in structures that show heightened activity in the absence of adequate dopamine action. Levodopa (L-DOPA), which is directed toward comfort and support, is the most potent drug for controlling PD symptoms. L-DOPA is converted into dopamine in the brain, enhancing effective dopamine transmission. Long-term (> 5 years) use of this drug is associated with significant complications, such as a "wearing off" effect, L-DOPA-induced dyskinesias, and other motor complications, such as fluctuations and dyskinesias and other complications (Stern, 2004; Jankovic, 2005). Further, L-DOPA is shown to be toxic to dopamine neurons and can produce harmful ROS by oxidative metabolism of dopamine, which in turn may hasten the rate of progression of the disease (Olanow, 2008).

Another extreme form of therapy available is surgery which involves destruction of certain parts of the brain (thalamus, the globus pallidus, and the subthalamic nucleus) or insertion of electrodes into these areas for electrical stimulation is available for patients with PD (Savitt, 2006). This option is usually given to patients with moderate to severe PD and/or to patients who are not able to tolerate medications available for this disease.

Neuroprotective approaches have shown to be very promising in slowing the progression of the disease and limit the extent of neuronal cell loss in PD. The use of neurotrophic factors such as neurturin (NTN) (Kordower et al., 2006) and the glial cell line–derived neurotrophic factor (GDNF) (Gash et al., 1998), have been reported to enhance the survival of midbrain dopaminergic neurons. Bioenergetic compounds such as creatine, riboflavin, and CoQ_{10} are also shown to be neuroprotective agents for PD in both animal models and humans (Beal, 2003b). These studies are still under research and have not shown any efficacy in slowing down neurodegerative disorders such as PD.

CoQ10 as a neuroprotective agent.

The etiology of several neurodegenerative disorders is thought to involve impaired mitochondrial function and oxidative stress, which are evident from studies with animal models, studies of mitochondria from patients, and analysis of genetic defects. Coenzyme Q_{10} (also known as CoQ_{10}; 2, 3, dimethoxy-5 methyl-6-decaprenyl benzoquinone; ubiquinone) is a naturally occurring compound that participates in electron transfer in the mitochondrial oxidative respiratory chain for complexes-I and complexes-II of the electron transport chain. CoQ_{10} can accept one electron and be converted to an intermediate semi-ubiquinone, which can then accept one electron to be converted to its reduced form called ubiquinol (Figure 4). When reduced to ubiquinol, it is a powerful antioxidant that prevents oxidative damage from free radicals, including oxidation of lipids within the mitochondrial membranes, and protects DNA and proteins from free radicals (Ernster, 1995; Geromel et al., 2002; Matthews et al., 1998). Oxidative stress burden in the midbrain is usually high under normal conditions due to generation of reactive metabolites of DA and is further elevated during aging, especially in patients

with PD (Brown et al., 2006; Ernster, 1995). Schults et al. (1997) measured levels of CoQ_{10} in mitochondria from subjects who have PD. CoQ_{10} levels were found to be significantly lower in patients with PD compared to age-matched controls. These findings support the idea that increasing CoQ_{10} levels could be of therapeutic benefit.

Clinical trials, including an open-label phase trial of CoQ_{10} in patients with PD, have found good CoQ_{10} absorption and tolerability (Beal, 2003b). A recent study by Storch et al. (2007) conducted a randomized clinical trial of a 300 mg dose of CoQ_{10} in 131 patients with PD who did not have changes in motor functions and were on stable treatment for their condition. Those assigned to the treatment group took 100 mg of CoQ_{10} three times daily for three months, followed by a two-month "washout" period. The researchers assessed Parkinson's disease symptoms before treatment began, each month during treatment, and again after the washout period. The compound was well tolerated and the occurrences of adverse effects like viral infection, diarrhea and hearing loss were equal between the two groups. This study demonstrated an increase in blood levels of CoQ_{10} in the treatment group, from an average of 0.99 mg/L to an average of 4.46 mg/L after three months. However, this study failed to show improvement of Parkinson's disease symptoms as it did not meet its primary or secondary end points, which were changes on scales that measured Parkinson's disease symptoms and their effects on physical and mental functioning. The pharmaceutical applications seem to suffer from the lack of solubility and low bioviability, both of which are needed to achieve therapeutic effects.

Although CoQ_{10} is classified as a lipophilic compound, its degree of solubility in lipids is limited, and it is practically insoluble in aqueous solutions. Recently, a water-

soluble formulation of CoQ_{10} (WS- CoQ_{10}) was prepared by Drs. M. Sikorska and H. Browhy-Borowski of the national Research Council of Canada using a patented protocol. In 2006, Bhagava and Chopra reviewed available research data on the absorption, metabolism, and pharmacokinetics of CoQ_{10}. They found that in many cases, the soluble form of CoQ_{10} shows enhanced bioviability in its uptake in cells; moreover, its beneficial effects on cardiovascular and neurodegenerative diseases, and its antioxidant properties were demonstrated (Bhagava & Chopra, 2006).

Human neuroblastoma cells pre-treated with WS- CoQ_{10} have been shown to prevent against PQ-induced neuronal cell death (McCarthy et al., 2004). Sandu et al. (2003) exposed neurons to 10 μg/ml of WS- CoQ_{10} for three days. The investigators found that this treatment resulted in an increase of cellular ATP levels, with an increase of CoQ_{10} in cellular mitochondria membranes and cell membranes. In addition, they found that WS- CoQ_{10} can prevent against neuronal cell death caused by glutamate excitotoxicity (Sandu et al., 2003). Somayajulu-Nitu (2009) reported that prophylactic application of a water-soluble formulation of CoQ_{10} could effectively offset the effects of the environmental neurotoxin paraquat. They used a model of paraquat-induced dopaminergic neurodegeneration in adult rats that received three weekly intraperitoneal injections of the herbicide paraquat. They found increased levels of oxidative stress markers and a loss of approximately 65% of dopamine neurons in the substantia nigra region. In addition, the researchers found that rats receiving a water-soluble formulation of CoQ_{10} in their drinking water prior to and during the paraquat treatment neither developed neurodegeneration nor reduced performance on their behavioural assessment (rotorod) than the control paraquat-untreated rats. Given the research discussed on the

potential benefits of WS- CoQ_{10} and protection against neurodegeneration, the current study is aimed at further evaluating the efficacy of WS- CoQ_{10} in rats induced with Parkinson's disease.

Serial Reaction Time

Sequential learning entails learning to organize sequences of behaviour so as to anticipate events occurring in a consistent sequential order. Stewart Hulse and colleagues initially studied such learning in rats by systematically varying amount of food at the end of a long runway over trials and examining rats' changes in running speeds over these trials as food quantities consistently changed. Results from a series of studies suggested that rats learn to anticipate patterned sequences of events or "serial patterns". Hulse and Dorsky (1977) presented rats with a simple monotonic pattern sequence of 14-7-3-1-0 food pellets in Experiment 1 which they considered promoted a single "less than" rule to describe the relationships of all successive pairs of quantities. Other rats received nonmonotonic pattern sequence 14-1-3-7-0 food pellets which would generate a more complex combinations of "less than," "greater than", and "equal" rules to describe the patterns. Rats learned to anticipate (run more slowly down the non-rewarded runway) more easily with the simple monotonic than nonmonotonic pattern. In Experiment 2, some rats experienced a weaker monotonic pattern 14-5-5-1-0 pellets that was intermediate between the monotonic and nonmonotonic pattern sequences given to other rats. These animals learned to adjust their running speeds less easily to weaker than stronger monotonic pattern. Hulse and Dorsky (1977) concluded that rats are sensitive to pattern structure and can learn the rules that described the structure. In a later experiment,

they also showed that rats could generalize a rule from one set of patterns to a different pattern (Hulse and Dorsky, 1979).

Capaldi and Molina (1979) challenged the theory that rats learned rules by maintaining that patterned performance could be accounted by rats acquiring simple stimulus associations and generalizations in pattern learning. According to this notion, food quantities earlier in a sequence serve as cues for the later food quantities. Fountain and his research team provided clearer evidence that rats learn a simultaneous rule from sequential patterns rather than simple successive associations. For example, Fountain et al., (2006) trained rats in an octagonal operant chamber equipped with a retractable lever mounted on each wall designated 1-8 in a clockwise fashion. In their typical procedure, all levers are presented at the beginning of each trial allowing the rat to press any of the 8 levers. If the correct lever is chosen, the rat receives brain-stimulation reward (BSR) via implanted hypothalamic electrodes. If an incorrect lever is chosen, all levers except the correct lever are withdrawn from the box and the rat must make a correct choice to receive BSR before continuing to the next trial. Rats were required to learn two patterns of successive lever choices, a "perfect" pattern (123-234-345-456-567-678-781-812) and one containing a "violation" pattern (123-234-345-456-567-678-781-818). They were both identical, but the violation pattern contained a single element at the end of the pattern that violated the simple structure. Rats were given 24-element response patterns which can be described as composed of either 3-element "chunks" that share a common base "rule" within chunks, usually "+1" rule. In the violation pattern, there is an exception to the rule for the last element. Now, the rule is "+2", which would be a correct response. The authors found that rats learn to use combinations of multiple pattern elements to respond

properly in a trial and anticipate the violated element. We note that this task requires considerable training over several sessions before rats show any reasonable accuracy in lever choices. Although Fountain has successfully used this as a behavioural assessment to examine neurological function of NMDA receptors, a specific type of glutamate receptor which plays a critical role in hippocampal learning (Fountain et al. 2000) we do not consider this preparation logistically suitable for our goals. Rather, in reviewing the literature on serial pattern learning we discovered a far easier-to-learn serial reaction time task initially given to humans and later shown to be similarly easy to acquire by animals.

The main reasons behind studying sequential learning by using the serial reaction time (SRT) task are: (1) sequencing of information and actions is a fundamental human ability; (2) sequence learning is an easily studied example of skill acquisition (i.e., reaction time and error rates are reliable measures for determining improvement in sequential learning; and (3) sequence learning may be a complex form of implicit learning.

Nissen and Bullemer (1987) developed a simple paradigm that now forms the basis of much of the research on sequence learning. In this task, human participants sit in front of the computer screen and are shown an asterisk which appears in one of four locations across the monitor. Participants are instructed to react to the presentation of the stimulus by pressing one of the four keys positioned below the stimulus. A correct response makes the stimulus disappear and another asterisk later appears in one of the other three positions. Location of response follows a pattern whereby the stimuli follow a 10-trial sequence which is then repeated. The beginning and end of the sequence are not marked. Each participant typically receives 10 repetitions of the sequence (100 trials),

which makes up a block, and typically a total of four blocks are presented. At the end of the fourth block, a fifth block is presented in which the position of the stimuli is randomly determined. The dependent variable is reaction time to respond to each stimulus (RT), and learning of the sequence is inferred from a reduction in RT over the four learning blocks followed by an increase in RT on the fifth block containing a random sequence. The RT provides a measure of participants' growing expertise in performing not only the sequence but also in learning the visuomotor association or mapping between the position of the visual cue and the required response. This difference in RT between pattern and random trials indicates the extent of learning to fixed pattern. Despite the performance difference, some participants are unable to express declarative knowledge of the nature of the fixed and random sequences. Consequently, their learning is considered to be implicit.

Nissen and Bullemer (1987) also gave the SRT task to patients with amnesia. They found that amnesic patients show these learning patterns despite their lack of awareness of the sequence. Willingham (1989) showed that reaction times can be improved with repeated sequences even if the subject was not aware of this learning. This study used an SRT task similar to that used by Nissen and Bullemer (1987) in which they investigated procedural learning in normal subjects. Locurto et al. (2010) examined the acquisition of sequence information by cotton-top tamarins (*Saguinus oedipus*). The authors wanted to know what subjects learn in the absence of explicit reinforcement for correct responses. Two experiments were conducted using an implicit chaining procedure. Visual stimuli were presented serially on a touch screen. Subjects were required to touch one stimulus to advance to the next stimulus. These stimuli were

presented in a fixed pattern, but learning the pattern was not necessary for reinforcement. In experiment 1, five different visual stimuli were presented in the same order on each trial, each occurring at any one of the six positions. In experiment 2, the same visual element was presented serially in the same five locations on each trial, which allowed a behavioural pattern to be correlated with the visual pattern. In addition, two new tests, a Wild-Card test and a Running-Start test were used to assess what was learned in this procedure. Results indicated that tamarins acquired more information about the sequential nature of the stimuli than by the rules of reinforcement. The authors concluded that even without explicit reinforcement for correct responding, tamarins can learn several features of patterned information, including the sequence position of elements in a series and the perceptual characteristics of those elements.

Domenger and Schwarting (2007) developed a rat version of the human SRT task. They trained rats to nose-poke one of the four locations (illuminated nose-poke hole) to obtain a reward under sequential conditions. The authors investigated the effects of violating a single stimulus position in the series to determine whether the rats still attended to the actual stimulus order when confronted with sequence violations. Random conditions showed slower reaction times and lower response accuracy compared to fixed sequential conditions. In-depth analysis of the incorrect responses showed that most rats directed their errors to the position where the stimulus would have normally appeared.

In the standard serial reaction time task, individuals might learn either the perceptual or motor sequence separately or in some combination. Although the SRT task is often viewed as a motor learning task, it is not clear that learning is taking place solely in the motor domain. The motor theory of implicit sequence learning focuses on

responding to each stimulus. This model suggests that individuals learn a sequence of manual response movements and this learning is tied to motor-related output (Goschke et al., 2001). Goschke et al. (2001) modified the common SRT tasks as follows. In their task, four letters (A, B, C, and D) were presented horizontally in discrete locations and each letter corresponded to one of the four response keys on the keyboard. In contrast to previous SRT tasks, where each stimulus would appear one at a time, all letters were presented visually on every trial. Following the visual stimuli, one of the four letters was spoken and heard through headphones. Participants were instructed to respond by pressing the key directly below the letter they heard. Unlike previous SRT tasks, location of stimuli on the computer screen changed with each trial. The change of location altered the associated motor response from one trial to the next. Results showed that normal, healthy participants demonstrated implicit based sequence learning in the absence of spatio-motor sequencing. The serial pattern could be learned as a sequence of motor responses with participants learning the correct sequence of response buttons (Robertson, 2007). Alternatively, the serial pattern could be learned perceptually, that is, as a sequence of visual cue positions. Over time, participants would learn to predict the position of each visual cue and so would quickly respond to the appearance of the visual cue. Dennis et al. (2006) investigated people's ability to learn perceptual sequences in the absence of motor sequencing, using an SRT task similar to the one used in the Goschke et al. (2001) study. Dennis et al.'s (2006) study concluded that both younger and older adults can learn simple first- and second-order sequences in the absence of a spatiomotor sequence (i.e., sequence learning can be supported by purely perceptual learning, but this only occurs when the SRT task has been modified to remove the motor sequence). With

no motor sequence, individuals are forced to learn perceptually. In contrast, when both perceptual and motor sequences are available, then learning occurs in both domains. Work done by Willingham et al. (1999) tested the hypothesis that implicit sequence learning could be supported by purely perceptual information. They concluded that sequence learning is not purely perceptual learning. Thus, sequential learning can have both motor and perceptual learning.

While performing the SRT task, individuals acquire the skill of producing the sequence, which is shown by the reduction of reaction time over trials for fixed-pattern sequences. In these cases, individuals can also acquire an ability to declaratively describe the sequence. After performing the SRT task, an individual might be able to verbally describe some or all of the sequences contained within the task. Thus, the SRT task is not exclusively a motor learning task; it includes an important declarative component and is demonstrated by the ability to verbally describe some or all of the sequences within the SRT task. Brown et al. (2007) disrupted sequence learning in individuals by having participants learn a word list immediately after sequence learning. After 12 hours of being tested with SRT and learning a word list, the participants were retested on the SRT and word list and then were administered a free recall test of the sequence. The results showed that learning a word list immediately after acquiring the SRT disrupted the declarative representation of the sequence, thereby reducing participants' ability to describe the sequence.

SRT and Brain Processes

Motor skill learning is supported by a circuit that includes motor cortical and subcortical areas such as the striatum and cerebellum (Robertson, 2007). Keele et al.

(2004) analyzed several studies and theories behind the brain processes of the SRT task and found that a similar pattern of areas are engaged during learning in the SRT task. The prefrontal cortex, striatum and cerebellum all make contributions during this task. Several studies report that the medial temporal lobes (MTL) are involved in SRT only for the acquisition of high-order sequence learning but not low-order sequence learning. In second-order sequences, predicting the next event requires knowing the two immediately preceding events (Curran et al., 2007; Schendan et al., 2003). Procedural memory has been associated with subcortical structures of the basal ganglia (Eichenbaum et al., 2001). Further, Positron Emission Tomography (PET) studies of SRT learning have shown that activation changes within the basal ganglia are associated with learning and the parietal and temporal cortex (Grafton et al., 1995; Haseltine et al., 1997). Under implicit conditions, activation of areas is commonly associated with motor control, including motor cortex and subcortical areas in the basal ganglia (Curran, 1998).

Over the past 20 years many studies using SRT have been performed on primates, healthy human subjects, and patients with brain damage (i.e., Huntington's disease [HD], Alzheimer's disease [AD], and Parkinson's disease [PD]). These studies attempted to detail possible brain mechanisms involved in sequential learning. Knopman and Nissen (1991) studied procedural learning in patients with HD using a four-choice SRT task. Participants were given a total of five blocks; the first four blocks were of a fixed pattern of stimuli and the last block consisted of random pattern of stimuli. Results showed that although there was a reduction in reaction time in the fixed sequences compared to the random sequences, there was an increased reaction time compared to the healthy controls for reaction time for both types of sequences. More importantly, the individual analysis

of patients with HD showed that these patients failed to exhibit a sequence-specific learning. Knopman (1991) later studied procedural learning in patients with Alzheimer's disease using the same SRT task. Results showed that both patients with AD and normal controls had the same reaction times across the sessions; however, all patients with AD lacked the awareness of these sequences.

A number of studies have examined the performance of patients with Parkinson's disease using the SRT task. Interest in this group arises from evidence that suggests that the basal ganglia, which are compromised in this group of patients, may play a critical role in procedural learning. Ferraro et al. (1993) examined implicit memory performance using the SRT task in four groups of subjects: (1) healthy and aged individuals; (2) non-demented individuals with Parkinson's disease; (3) individuals with mild Alzheimer's disease; and (4) individuals with mild cognitive impairment. The SRT task involved four blocks of a repeated pattern of a 10-item key press sequence that tapped general skill development along with a fifth block of a non-repeated sequence that emphasized the impact of switching from a learned set of associations. The researchers found that patients with non-demented PD demonstrated less sequence learning than controls in the SRT task. Siegert et al. (2006) conducted a meta-analysis of sequential learning in patients with PD and found impaired implicit learning in this population. However, it was pointed out that these studies were not methodologically consistent. For example, several studies lacked the inclusion of random blocks after sequential conditions and did not control for the severity of the disease when recruiting participants. Despite these problems, researchers in animal behavior and cognition have begun to use SRT tasks to

examine effects of interference with dopamine production on such sequence learning and performance in rats.

Eckart et al (2010) investigated the effects of bilateral neostriatal dopamine lesions induced by 6-hydroxydopamine on sequential learning in rats. This study used the operant rat version of the human four-choice SRT. The rats were tested on an interference test. That is, the rats were presented with a stimulus that switched between a sequential and a pseudo-random order every 5 minutes and a violation test in which one sequence item was skipped. The lesion group received bilateral intracranial injections of 6-OHDA.HCl into the striatum, with two injection sites per hemisphere before the start of training, which resulted in subtotal dopamine depletions (35–56%) in the medial neostriatum. This study also included operated and non-operated control groups. Results from this study revealed that the lesioned group showed less response accuracy and slower RT than the control groups. During the alternating phases of sequential and random stimulus presentations, RT and accuracy of the control group were better during sequential sequences when compared to random sequences. In the lesion group, only a moderate advantage in accuracy was observed. In the violation test, the control group showed an expected increase in RT on the violated positions. In the lesion group, RT did not increase, which suggested less automation of sequential behaviour. This study concluded that neostriatal dopamine plays an important role in acquiring or maintaining sequential behaviour. The current thesis further examines the effects of destruction of dopaminergic brain area on rats' SRT performance and on the potential to use of this task as a diagnostic tool in assessing the neuro-protective effects of post-insult treatment with a water soluable formaulation of CoQ10. The neuro-protected group should show no

change in their RT or errors compared to the placebo group in their post-injection behavioural assessments. The non-protected group should show increased RT and more errors compared to the protected group and the placebo group.

Objectives

1. To develop a rat SRT preparation for investigating performance as a function of sequence structure
2. To study the neuroprotective effects of water-soluble coenzyme Q_{10} (WS- CoQ_{10}) by evaluating the behavioural effects on the SRT using the paraquat-induced sporadic Parkinson's disease model in Long-Evans hooded rats

CHAPTER III

DESIGN AND METHODOLOGY

Materials

Experimental animals.

Twenty-five male Long-Evans hooded rats, purchased from Charles River Breeding Farms, St. Constant, Quebec, were used in this study. Only twenty-four animals were used for data analysis, one animal was dropped out of the study. When the rats arrived at the facility, they were randomly split into two groups: one that would receive injections of PQ (10 mg/kg) and the other that would receive injections of saline. These two groups were then further divided into groups based on their water regimen: whether they received WS- CoQ_{10} (50 µg/mL) in their drinking water (H_2O) (Figure 5). On average, the rate of water consumption for a rat is 10 ml per 100 g of body weight each day. An average rat weighing 500 g would therefore consume 5 mg of either WS- CoQ_{10} or H_2O. The rats were approximately 2 months old and weighed an average of over 300 g at the beginning of the study. There were three to four rats per group cage. After each experimental session, they were fed 20–25 g of food (Purina Rodent Chow) and its specific liquid for 2 hours in individual holding cages only before being returned to their large-group holding cage in the colony room. This regimen was necessary for long-term maintenance of the rats' weight and prevented dominance hierarchies based on the degree of neurodegeneration between the paraquat-injected animals and the saline-injected animals. This regimen prevented the possibility that the saline-injected rats and/or the neuroprotected rats might dominate other rats and gain access to liquid and food. The rats' specific liquid was freely available in group and holding cages. This regimen

maintained rats at approximately 90% of their free-feeding weights over the course of the experimental period. Towards the end of the study, the rats weighed between 400 g and 500 g. Rats in either their individual cages or group cages had access to drinking solutions (regular water or with water supplemented with WS- CoQ_{10}). Bottles that contained WS- CoQ_{10} were wrapped in aluminum foil to prevent degradation of the compound and were refilled every day. Illumination in the colony room was maintained on a reversed 12:12 hour dark/light cycle, and experimental sessions always began within 3 hours before the beginning of the dark cycle to ensure that these nocturnal animals were awake and active during their SRT sessions. The colony room was maintained at temperatures of 20°C–24°C. All animal care, procedures, and treatments were approved by the University of Windsor's Animal Care Committee and were in accordance with the Canadian Council for Animal Care Guidelines.

Water-soluble formulation of CoQ_{10} (WS- CoQ_{10}).

Water soluble CoQ_{10} (WS- CoQ_{10}) was obtained from the National Research Council. The WS- CoQ_{10} was produced through a patented protocol (U.S. patent #6 045 826), which contained a 2:1 (w/w) ratio of oxidized and reduced forms of CoQ_{10}, respectively. CoQ_{10} was contained within a water-soluble "cage" composed of polyethylene glycol and α-tocopherol (vitamin E). Stock solutions of WS- CoQ_{10} (50 mg of CoQ_{10} and 150 mg of PTS per mL in phosphate buffered saline) were diluted with regular drinking water to a final concentration of 50 µg WS- CoQ_{10} per mL.

SRT apparatus

Three 48.3×22.9×27.9 cm standard operant chambers (Figure 5) were placed in separate sound-attenuated chambers. In each chamber, five light-equipped nose-poke

holes, each covered by a translucent plastic disk and were arranged horizontally. The pellet receptacle was placed on the opposite side of the nose-poke holes, and a house light and speaker were attached directly above. The pellet receptacle was attached to a dispenser, which delivered sugar pellets. Infrared devices detected entries into the nose-poke holes and the food receptacle. The whole system was controlled and monitored by a program created by the Tech-Support Centre, University of Windsor with LabVIEW software.

Behavioural Assessment

Phase 1- Shaping

The rats were trained on the SRT apparatus and were required to respond to a discriminative visual stimulus which in this case, was the illumination of middle of five nose-poke translucent keys. Rats were required to poke the key before it was darkened to receive a food pellet before being trained on a sequence of lit keys before each reinforcement. Following this initial training, rats were trained to respond to a series of illuminated holes before being reinforced; the final level in this experiment was on a fixed-ratio schedule of 5 (FR5). During the first 3 weeks of shaping, the rats were trained daily for 10–30 min per day. Initially, one hole was illuminated for 20,000 ms and poking at this hole was reinforced on a continuous reinforcement schedule (FR1). For each illuminated hole that the rat pokes, the rat would get reinforcement. When the rat learned to respond at this signal, it was shaped to respond to an FR2, FR3, and finally an FR5 schedule. A typical trial (FR5) was initiated by a house light which lasted for 5,000 ms. Immediately after the house light went off, one of five nose-poke keys was illuminated for 20,000 ms. The subject terminated the key by poking the illuminated key. The inter-

signal interval was 0 ms, at which point the next key was lit for 20,000 ms. After all five keys had been poked, the tone (75 dB noise) would turn on for 1000 ms, indicating that the reinforcement was ready. The subject would then poke the food receptacle with its nose to obtain a reward. The next trial was initiated after the subject obtained reinforcement.

Phase 2- Pre-injection

Subjects were then tested using the ABA sequences. The sequences were of two types: fixed (A) or random (B). In fixed sequences, one of five nose-poke locations (Figure 6) was presented in a fixed order with no repetition (e.g. 31452, 31452 and 31452). In the random condition, the five nose-poke locations were presented randomly with no repetition among the sequences (e.g.13452, 41325, 51234 and 12354). The random patterns for each subject were computer generated. Subjects were trained one session per day for 15 days. Each session consisted of 300 nose pokes with every fifth correct nose poke reinforced. Of the 300 correct nose pokes, the first 100 were of fixed sequence (A), followed by a random sequence for 100 nose pokes (B), and finishing off with the same fixed sequence of 100 nose pokes (A). For these sequences, the house light was on for 5,000 ms and the inter-signal-interval was 1,000 ms. These parameters were chosen in these experiments because they were used in previous studies using the SRT. The signal light was illuminated for 3,000 ms. The rats' latency, or reaction time, to press a lit key was measured in milliseconds. If the rats failed to poke a lit key, the signal would turn off and reappear after 1,500 ms. This sequence continued until the animal poked the lit key. If the animal poked an unlit key while the other key was lit, the lit key would be terminated and would turn on after a 1,500 ms delay. Poking an unlit key was

labeled an incorrect choice while failure to respond was designated an omission..During this phase, all rats received H_2O (tap water) in their individual holding and group home cages.

Phase 3- Post-Injection

Injection regimes.

Figures 7 and 8 describe the group allocation and the time line for this phase respectively. Previous studies, such as the one conducted by Somayajulu et al. (2009), conducted several experiments to induce selective damage to DA neurons in rats' SNc. For the first experiment, rats were given three intraperitoneal injections of PQ (10 mg/kg) to induce selective damage to DA in the SN in mice, used similar protocol to (McCormack et al., 2002). In the second experiment, rats were given eight intraperitoneal injections of a combination of PQ (10 mg/kg) and the fungicide MB (30 mg/kg), same regimens as per (Thiruchelvam et al., 2000a; Thiruchelvam et al., 2000b). These treatment regimens were fatal to a large number of rats during injections and were discontinued. Since the PQ and MB regime was not tolerated by the rats, Somayajulu (2009) decided to use eight injections of 10 mg/kg PQ alone twice a week for four weeks. This regime was toxic to the rats, and the experiment was discontinued. In the final experiment, rats were given five injections of PQ to yield greater neuronal loss than in the three-injection regime. In all of the above experiments, rats were divided into six groups. These groups included saline water, saline placebo, saline WS- CoQ_{10} PQ-water, PQ-placebo, and PQ-WS- CoQ_{10}. The rats were fed drinking water supplemented with their treatments after 2 weeks of arrival to the facility. In her pilot research, Somayajulu found greater neuronal loss when the rats were injected five times compared to three times.

Since the five-injection regime yielded greater neuronal loss, the current experiment used this schedule.

In the current study, subjects were randomly divided into three groups (Figure 7), with the following number of subjects in each: (i) paraquat and WS- CoQ_{10} group (n=12), (ii) paraquat and H_2O group (n=6), and (iii) saline and H_2O group (n=7). This experiment was designed to study only the therapeutic effect of WS- CoQ_{10}. The number of subjects for each group was determined by the research team from the biochemistry department at the University of Windsor that was also responsible for injection and post-mortem protocols (Facecchia et al., 2011, in progress). After the collection of 15 days of baseline data (pre-injection phase), the rats were injected with either 10 mg/kg intraperitoneal paraquat injections or saline injections every five days for a total of five injections. During the time of injections, subjects were fed tap water (H_2O), and were not run on the SRT task. Immediately after their fifth injection, the 12 rats that received paraquat were fed WS- CoQ_{10} to investigate its therapeutic effects in halting any post-injection PQ-induced neurodegeneration. The remaining rats were fed with H_2O. After three days of the last injection, the subjects were run through the SRT protocol for another 15 days. Each subject was given the same sequence as previously indicated in the preinjection phase. After conducting the postinjection behavioural studies, the rats were dissected, and tissues were collected for histochemical analyses as part of Katie Facecchia's master's thesis at the University of Windsor. We note that one rat in the PQ+WS-CoQ_{10} group failed to acquire the SRT task during baseline training was not further tested on this task during the remainder of the study. Thus the PQ+WS-CoQ_{10} group was reduced to 11 rats so that SRT performance was assessed and analyzed on 24 animals.

Statistical Analysis

Individual response types and reaction times were analyzed. The following response types were used: (a) correct nose-pokes (responses to illuminated holes), (b) incorrect nose-pokes (responses to non-illuminated holes), and (c) omissions (no response during a lit key). The reaction time (ms) elapsed between the onset of the stimulus and the nose-poke of each correct response was normalized to reduce variation. This was done by dividing 1,000 by the reaction time for each rat to each signal. These reciprocal RT values were then averaged over blocks of three days for each signal at each block of fixed and random sequences and then reconverted into normalized mean RT scores by dividing the mean reciprocal RT by 1000 of each rat. The various two-way and three-way Analyses of Variance were conducted on rats' normalized mean RTs as described in the results section. Each rat's total number of omissions and total number of incorrect choices over all pre-and post-injection sessions and over each block of three sessions within each pre- and post-injection phase for fixed and random sequences were also analyzed by two- and three-way ANOVAs respectively. All statistical analyses were calculated with IBM SPSS (version 19) software. For this and all other analyses a significance level used was $p < .05$.

CHAPTER IV

ANALYSIS OF RESULTS

Reaction Time

The means of each rat's normalized reaction time (RT) for each session's signal position in from each within-session block of sequences (1st fixed, 2nd random, 3rd fixed) were calculated in three ways. 1.) To determine RT as a function of signal position, we calculated their mean signal RTs for each position for each of the three within-session blocks of sequences collapsed over all sessions in each phase. We separately analyzed rats' pre-injection and post-injection data by separate three way ANOVAs (3 groups x 3 sequence blocks x 5 signal positions). 2.) To determine any changes in SRT performance over training in the pre-and post-injection phases, we calculated each rat's mean normalized signal RT for each within-block of sequences (collapsed over signal positions) for each successive block of three sessions (5 blocks). We conducted a separate three-way ANOVA (3 groups x three sequences x five blocks) for each phase to determine whether patterns of post-injection SRT differed from those found in the pre-injection phase. 3.) Finally we calculated each rats mean normalized signal RT for each within-session block of sequences collapsed over signal position and sessions that allowed us to directly statistically compare data from pre-and and post-injection phases as a function of groups and sequence type. We carried out a three-way ANOVA (3 groups x 3 sequences x 2 injection phases).

1.) Signal RT as a function of Signal Position and Sequence. Figure 9 summarizes data describing these functions during the pre-injection phase. These data were collapsed over groups because as expected no significant effects for this factor were

uncovered. A similar summary of the post-injection data are also not shown for reasons already discussed. As seen in this figure and supported by significant main effect for sequence, F (2, 42) = 25.453, $p < 0.001$, rats developed longer RTs during the middle within-session blocks of random sequences than during either of the within-session blocks of fixed sequences. Rats also reduced their RTs over signal positions within each within-sessions block of sequences but demonstrated greater declines during the fixed than random sequence blocks. These observations were confirmed by a significant main effect for signal position, F (4, 84) = 21.365, $p < 0.001$, and a significant interaction between sequence and signal position, F (8, 168) = 12.214, $p < 0.001$.

2.) Signal RT as a function of sequence type and successive 3-session blocks. Figures 10A and 10B summarize mean signal RTs for each sequence condition for each group over successive three-session blocks during the pre- and post-injection phases respectively. During the pre-injection phase, as seen in Figure 10 A and confirmed by a main effect for blocks of sessions, F (4, 84) = 4.380, $p = 0.003$, rats decreased their RTs over blocks during each type of session. Their decline within the random sequences appeared somewhat less steep but this observation was not statically supported by any interaction between blocks and sequence type, F (8, 168) =1.067, $p = 0.388$. A significant main effect for sequence type, F (2, 42) = 18.560, $p < 001$, not surprisingly replicated that seen in the initial analysis. As seen in Figure 10B, rats no longer displayed any further declines in signal RTs over post-injection blocks of sessions but continued to display greater RTs during random than fixed sequences as confirmed by a significant main effect for sequence type, F (2, 42) = 22.189, $p < 001$. No significant interaction between block and sequence was obtained, F (1, 16) = 1.561, $p = 0.140$. Of

particular importance is that fact no obvious overall differences between groups were observed during this phase nor did group significantly interact with sequence, F (4, 42) = 0.531, p = 0.714.

3.) Pre-injection vs post-injection RT comparisons as functions of group and sequence type. Figure 11 shows the overall mean RT for each group during each phase as a function of sequence type. We note that no apparent or significant effects for groups were obtained for this analysis, F (16, 168) = 0.927, p = 0.540. The only apparent effect was that rats displayed generally lower RTs in the post-injection phase but this observation was not supported by main effect for phase, F (4, 42) = 0.587, p = 0.674.

Number of Errors

Error rates were either omissions or incorrect nose pokes (to an unlit key in the presence of a lit key). The means of each rat's error rates for each type of error for each within-session block of sequences (1st fixed, 2nd random, 3rd fixed) were calculated. We calculated mean number of incorrect choices and mean number of omissions for each of the three within-session blocks of sequences collapsed over all sessions in each phase. We separately analyzed rats' pre-injection and post-injection data by separate three way ANOVAs (3 groups x 3 sequence blocks x error type). This was done for both omissions and incorrect choices separately. To determine any changes in SRT performance over training in the pre-and post-injection phases, we calculated each rat's mean errors for each type for each within-block of sequences for each successive block of three sessions (5 blocks). We conducted a separate three-way ANOVA (3 groups x three sequences x five blocks) for each phase to determine whether patterns of post-injection SRT differed from those found in the pre-injection phase.

There were significantly more omissions compared to incorrect choices, as shown in Figure 12: F (2, 18) = 17.843, p = 0.00. As shown in Figure 13A, when analyzing omissions in more detail in the pre-injection phase, the 15 sessions were broken down to three-session blocks. ANOVA was conducted for 3 (groups) by 3 (sequence conditions) by 5 (three-session blocks). Results were not significant for blocks: F (4, 84) = 0.690, p = 0.601. The same analysis was conducted for post-injection data, as shown in Figure 13B. Similarly, results were again not significant for blocks: F (16, 168) = 1.226, p = 0.253; however, sequence was found to be significant: F (2, 42) = 4.367, p = 0.019. An in-depth analysis of the sequence types revealed that the rats made significantly fewer omissions during their first fixed sequence than during the random sequence or the final fixed sequence, Fs (2, 21) = 8.891; 6.969, p = 0.007; 0.015. Differences in omissions between the random and third fixed sequence were not significant, F (2, 21) = 1. 032, p = 0.321. As shown in Figure 14, when directly comparing omissions between the pre-injection and post-injection phases, no significant interactions among sequence type, group, and blocks: F (4, 42) = 1.359, p = 0.264 occurred but a main effect for sequence type was significant: F (2, 42) = 4.170, p = 0.022.

When looking at three-session blocks (acquisition data for incorrect responses for preinjection, as shown in Figure 15A), results were not significant for sequence type and groups: F (16, 168) = 1.594, p = 0.075. Sequence type was significant: F (2, 42) = 12.450, p = 0.000. The same analysis was performed for post-injection data, as shown in Figure 15B. No significant results were obtained for blocks: F (4, 84) = 0.817, p = 0.518. Sequence type however was found to be significant: F (2, 42) = 17.907, p = 0.000. An in-depth analysis of sequences for postinjection data revealed a significant effect for the

random sequence and overall significant decline in the mean number of incorrect choices over blocks: F (4, 84) = 2.846, p = 0.029. In addition, a separate analysis within each block of sequences revealed a significant effect for group in the first fixed sequence: F (2, 42) = 6.826, p = 0.005. There were more errors with the paraquat and water group compared to the other groups (saline group and paraquat and CoQ10 group).

When comparing omissions between the two phases, pre injection and post injection, as shown in Figure 16, there were no significant interactions among sequence type, group, and blocks: F (16, 168) = 0.919, p = 0.549. Again, sequence type was found to be significant: F (2, 42) = 16.871, p = 0.00. There were more errors for the random sequences versus the fixed sequences. These tests may not have sufficient power to detect group differences.

Histochemistry

Histochemical assays from Phase 3 are not yet available from Facecchia's master's thesis (2011) at the University of Windsor biochemistry department.

CHAPTER V

DISCUSSION

Although considerable evidence has shown that the development of PD is a result of a gene–environment interaction, exposure to environmental toxins has been a major focus in current research (Di Monte, 2003). In particular paraquat (PQ), a herbicide, has been linked to the incidence and progression of PD (Castello, 2007). The current study was designed in part to assess a PQ-induced model of PD in Long-Evans hooded rats and to evaluate the neuroprotective properties of water-soluble coenzyme Q_{10} using the serial reaction time task. Reaction time and error (omissions and incorrect choices) were measured as a function of sequence structure.

In the SRT task, when the location of stimuli followed a repeated fixed sequence, rats' reaction times decreased dramatically. The improvement of reaction time over trials in the same session was due to learning of the specific sequence. However, the same improvement occurred for the random sequences. In the random condition, the individual stimulus–response relations do not allow prediction of subsequent ones, but in the fixed condition, stimulus-response relations are highly predictive. Thus, it can be assumed that enhanced performance under sequential conditions reflects the establishment of a higher-order motor plan (Hoffmann & Koch, 1998). Domenger and Schwarting (2007) interfered with these cognitive mechanisms through violations of well-trained sequences by replacing only one specific position in the FR schedule. They found that the major effect of this violation was an increase in reaction times at the violated position. These findings suggested that the SRT was an automated task but still required some attention to the ongoing stimuli. This method of introducing violations might be of importance when

using this task in neurodegenerative disorders. It might show strong effects between the disorder of interest and the placebo group.

Important methodological differences exist between human and nonhuman preparations. In SRT task experiments with humans, the experimenter can use verbal instructions to communicate to the subjects, encouraging them to perform the task as quickly and accurately as possible. This cannot be done with nonhuman subjects where the experimenter must communicate the objectives nonverbally. With the development of a simple paradigm of studying SRT, variations in methodology make comparisons among studies difficult. For example, simple parametric manipulations, such as varying the response to stimulus interval, can influence the amount of learning (Willingham et al., 1997) (i.e., if this interval was too long, these animals do not express learning). Evidence suggests that multiple forms of representations exist even within implicit learning (Seger, 1998). A study by Heindel et al. (1989) demonstrated a double dissociation between patients with Huntington's disease and patients with Alzheimer's disease in implicit motor learning and implicit perceptual learning. Heindel and colleagues suggested that deficits in the motor learning task were correlated with the amount of dementia in these patients, which suggests that implicit learning can affect multiple levels and different neural structures.

For phase 3, when comparing the different groups, (a) paraquat and WS- CoQ_{10}, (b) paraquat and H_2O, and (c) saline and H_2O, there were no RT differences among groups . There are several possibilities why this might have occurred. One possibility is that not enough paraquat reached the CNS to kill enough dopaminergic neurons in the SN region. The second possibility could be that five weeks was not enough time to cause cell

death in these rats. Lastly, the therapeutic effects of the neuroprotectant, WS- $CoQ_{10,}$ may not have been observed due to the short period of administration to the rats.

Parkinson's disease (e.g., tremor, impaired facility of movement, rigidity, and loss of postural reflexes) allows for the possibility that patients with PD are capable of sequence learning but are simply unable to demonstrate this learning through a decrease in reaction time over trials (Westwater et al., 1998). One concern of using the motor version of the SRT task is that the performance of PD patients may be seriously compromised by the motor difficulties that characterize this disease. This study examined the performance of patients with PD (n=13) and healthy controls (n=11), matched for verbal fluency on a verbal version of the SRT task, where the standard button-pressing response was replaced by a spoken response. They found that the PD group demonstrated less sequence learning than the controls, independent of age and severity of illness.

Evaluation of the PQ-Induced PD Model and Susceptibly of the SN

Five injections of PQ (10 mg/kg) or placebo was injected into the rats. No fatalities were reported. An important aspect to consider in the effectiveness of the five-injection regime was to determine whether the amount of PQ administered to the rats was enough to selectively target and cause neuronal cell death in the substantia nigra while leaving other brain regions and organs unharmed. Unfortunately, due to technical difficulties, these staining results were not obtained (Facecchia, 2011, pending thesis). However, previous research found that PQ selectively targets dopaminergic neurons in the substantia nigra by exerting its toxic effects through the production of ROS (Dinis-Oliveira et al., 2006). Several authors have suggested that DA neurons are susceptible to the oxidative damage caused by toxins due to pre-existing vulnerabilities in the neurons.

Dopaminergic neurons are already under considerable oxidative stress because the metabolisation of dopamine involving MAO produces hydrogen species (H_2O_2) as a by-product (Dinis-Oliveira et al., 2006). Furthermore, H_2O_2 is converted by Fenton reactions to produce highly toxic hydroxyl radicals in the presence of the high levels of iron usually present in the SN (Youdmin et al., 1989). Postmortem analysis of PD patients has confirmed the presence of high levels of iron in the substantia nigra (Dexter et al., 1989). It is evident that PQ-induced redox cycling within the SN may exceed the oxidative defences of the DA neurons, thus proving detrimental for the neurons.

Although rodent models are advantageous because subjects are easily accessible, a varied susceptibility exists between species in their response to neurotoxins. For example, mice are more susceptible to MPTP than rats. Furthermore, age, gender, and body weight also play important roles in determining the sensitivity of an animal to neurotoxins (Przedborski & Vila, 2001). Differences have also been found among different strains of rats. For example, Lewis rats require two-fold higher dosing of oxidopamine than Fischer or Sprague-Dawley rats. Interestingly, when rotenone is administered in Lewis rats, less variability and more consistency are exhibited compared to Sprague-Dawley rats (Betarbet et al., 2002). Evidence of neuroprotection in rodents does not guarantee similar results in humans (Emborg, 2004). The advantage of using an animal model is to obtain a phylogenetic perspective about the odds of success when translating a therapy to humans. Obviously these models must continue to be refined in order to improve techniques for evaluating neuroprotective compounds.

WS- CoQ_{10}**as a Therapeutic Agent in Rats**

Coenzyme Q10 is a hydrophobic and is localized in the inner mitochondrial membrane. Previous studies have used oil formulations of CoQ_{10}. Since CoQ_{10} is very hydrophobic, cells cannot absorb it easily. Recently, water-soluble CoQ_{10} containing both the oxidized and reduced forms was formulated and prepared by Drs. M. Sikorska and H. Browhy-Borowski. Water formulations of CoQ_{10} are readily absorbed by the cells when added to tissue culture media, making it possible to study the mechanism by which CoQ_{10} offers protection against oxidative stress. An increase in CoQ_{10} content in the mitochondrial membranes and cell membranes has been observed in cells pre-treated with CoQ_{10} (Sandhu et al., 2003). WS- CoQ_{10} may be exerting its neuroprotective effects by acting as an antioxidant and reducing the amount of harmful reactive oxygen species by scavenging free radicals (Beal et al., 2003). In vivo studies have shown the ability of the enzyme to prevent the reduction of ATP levels. Clinical trials have evaluated oil-soluble CoQ_{10} in PD patients and found a fair amount of absorption and tolerance of CoQ_{10} but cautioned that very high doses were needed to achieve any positive effects (Shults et al., 2004). However, no efficacy trials have proved that CoQ_{10} stabilizes symptoms of PD with this other formulation. WS- CoQ_{10} is an improved formulation, as it is a stable water-soluble complex that can be administered orally. Sikorska et al. (2003) found elevated plasma levels of WS- CoQ_{10} in rats fed with this compound.

One of the major objectives of this study was to assess the neuroprotection provided by WS- CoQ_{10} as a therapeutic agent in rats. Data from Facecchia (2011, pending thesis) have yet to be obtained.

Susceptibility of Rats to PQ Toxicity and Protection by WS- CoQ_{10}

In the current study, the susceptibility of paraquat toxicity in rats are being assessed by another colleague participating in this project. To data these results have not been obtained. However, in previous studies, Saint-Pierre and colleagues (2006) found a significant loss of DA neurons observed in the SNc after 6 weeks of only two toxin injections (30 mg/kg) in 6-month-old rats. Shultz and colleagues (1999) have attributed the greater effectiveness of CoQ_{10} in aged animals as a result of the decline in brain levels of CoQ_{10} with age. The decline of CoQ_{10} levels can be attributed to reduced synthesis of age-dependent increase in lipid peroxidation (Beyer et al., 1985).

Challenges Associated in Finding a Neuroprotective Therapy for PD

There are limitations to current models used for neuroprotection. Cell cultures are used in a variety of research. They are especially important when drugs are first tested to determine whether they can protect cells from a variety of toxins. Cell culture models do not provide insight into how a drug will behave in an organism. To evaluate the capacity of drugs to protect DA neurons from toxic insults, its side effects and treatment-associated complications in vivo models are indispensable.

One of the challenges in developing neuroprotective strategies is that the exact causative factors of PD remain unclear. Most cases of PD are sporadic and of unknown etiology. Knowing the precise mechanism of PD would then allow the identification of probable targets for the development of neuroprotective agents (Olanow et al., 2008). Which, if any, of the proposed mechanisms in PD is primary and is responsible for cell death remains uncertain. As discussed earlier, many mechanisms cause cell death in PD. Studies have shown that patients carrying the same gene mutation and who are family

members may exhibit different clinical symptoms and different pathology (Olanow et al., 2008), implying that a combination of various neuroprotectants acting simultaneously on different mechanisms might be required to achieve neuroprotection.

Future Work

In future studies, the present test might be useful to study and distinguish brain mechanisms critical for skill and attention. Electrophysiological and brain imaging studies have shown that sequential learning and performance are correlated with a number of changes in neuronal activity, including basal ganglia structures, cerebellum, and various cortical areas, especially within the motor cortex (Keele et al., 203; Saing-Cyr 2003). The use of violations in humans yield activations in the basal ganglia as shown in the fMRI (Huettel et al., 2002). A role of the basal ganglia has also been specifically demonstrated with lesion studies in rodent models using the SRT (Keel et al., 2003). The use of this SRT task in Parkinson's disease may be of value since impaired sequential learning reflects the loss of dopamine function within the basal ganglia.

Due to the lack of histochemical results, we cannot conclude that there was any neuroprotection by CoQ_{10} or if paraquat did indeed cause a loss of dopamine neurons in these rats. Once a standard protocol has been developed to count the cells in the brain tissue of rats, it would be beneficial to run this test again to see whether there was any neuroprotection by CoQ_{10} using the SRT.

Therapy for PD begins after diagnosis, by which time a large number of DA neurons have already been lost. For a candidate to be considered as a good therapeutic/restorative agent, it should be able to arrest the further loss of neurons and thereby prevent the progression of this disease. Further experiments will be needed to

confirm our studies aimed at evaluating the potential effects of WS- CoQ_{10} as a therapeutic intervention in PD. Since PD is a slowly progressive disease, it would be important to have a longer-term follow-up of these rats in the SRT task. This follow-up may be needed to identify clinically meaningful benefits to the administration of WS- CoQ_{10}.

WS- CoQ_{10} is now being developed to cross the blood–brain barrier (BBB), an evaluation of the amount of WS- CoQ_{10} that diffuses across the BBB and furthermore, the amount taken up by the mitochondria would be helpful findings. More research is required in this area. The next steps would be to determine if this compound is safe for human consumption, as this would be a potential candidate for clinical trials.

Conclusion

It is of value to have an animal model of a human implicit memory task for which there is already reasonable evidence in humans that it reflects a motor skill. In the current study, rats were more efficient on 5CSRT under fixed rather than random sequences for RTs and incorrect choices. This effect occurs for RTs as a function of signal position and also when collapsed over positions.

While we did not demonstrate significant RT differences between the neuroprotected group and the non-neuroprotected group, it remains an open question as to whether this represents a true absence of learning differences between the groups or rather a problem of sensitivity of our testing methods. Repeating this study with probe sequences containing single violations might produce more reliable effects as a function of PQ-induced neurodegeneration similar to that reported by Eckart et al (2010). The fact that our PQ+H2O group did produce more incorrect choices during the first third or each

session than the other two groups is interesting and needs to be replicated in further research. The questions we need to determine are whether such a difference resulted from the inclusion of random sequences mid-way through the session or if it merely reflects poorer performance at the beginning of a session. The fact that this effect emerged over blocks suggests that neuro-degeneration of domapine areas may have continued to progress in these animals but was halted by post-injection treatment with WS-CoQ10.

FIGURES

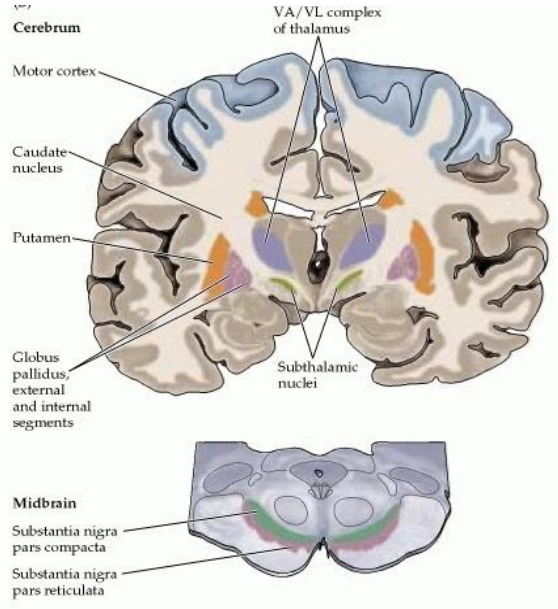

Figure 1. Motor components of the human basal ganglia (Purves 2001). Coronal section through the brain showing anatomical locations of structures involved in the basal ganglia pathway. Most of these structures are in the telencephalon, although the substantia nigra is in the midbrain and the thalamic and subthalamic nuclei are in the diencephalon.

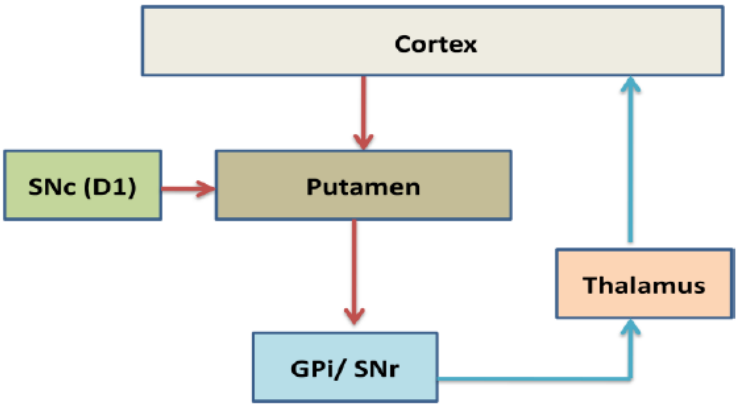

Figure 2A. The basal ganglia circuitry: the direct pathway (Purves 2001).

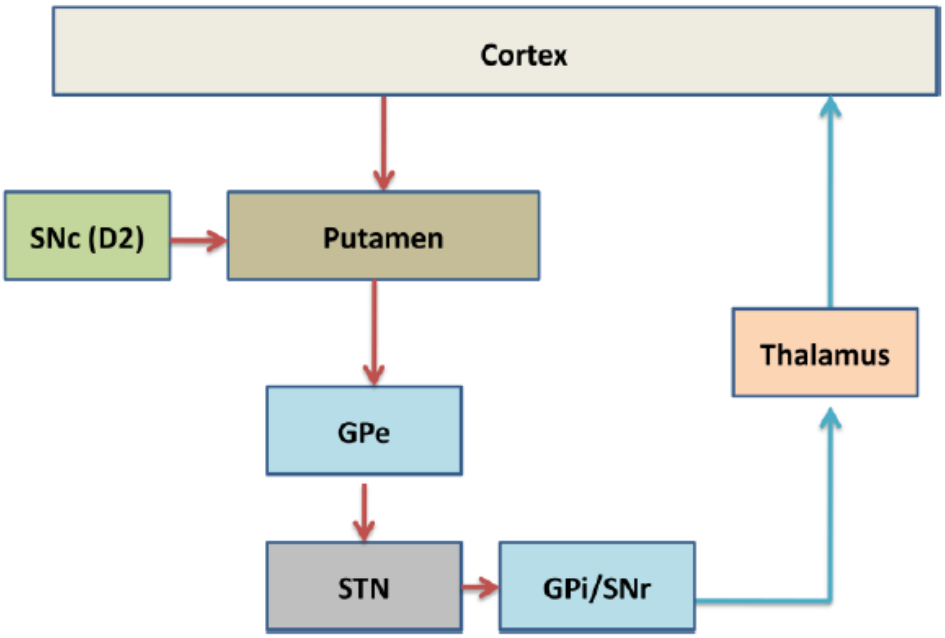

Figure 2B. The basal ganglia circuitry: the indirect pathway (Purves 2001).

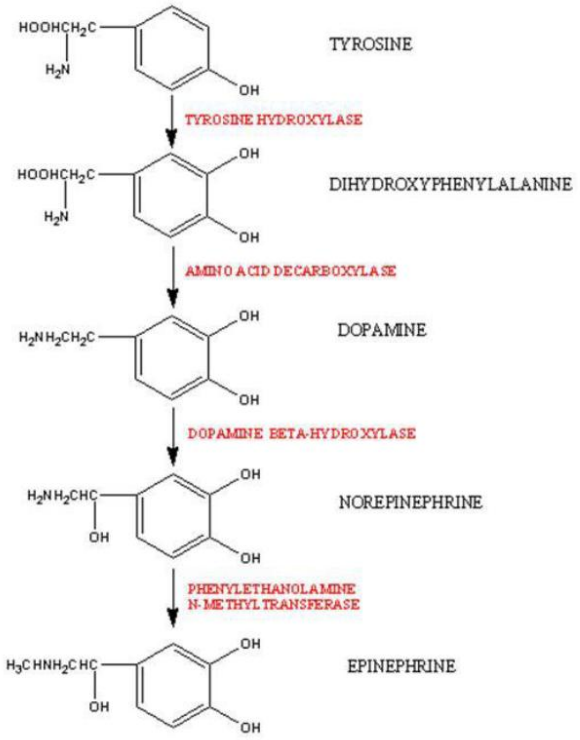

Figure 3. Catecholamine synthesis. Dopamine functions as both a neurotransmitter and a precursor for other catecholamines (Wurtman 1980).

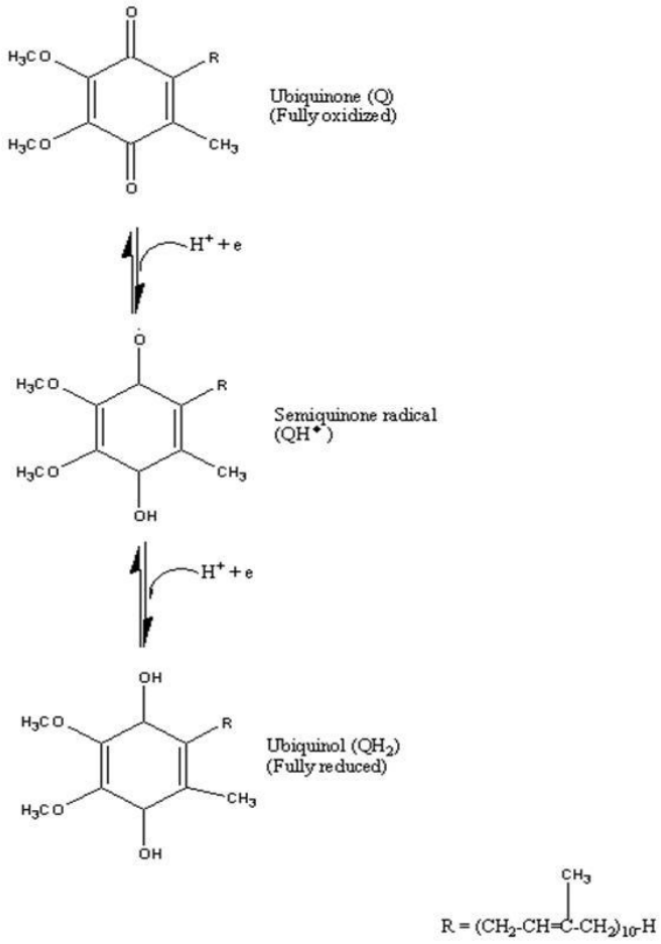

Figure 4. The oxidized and reduced forms of CoQ10 (Chew 2004).

Figure 5. Serial reaction time task operant chamber.

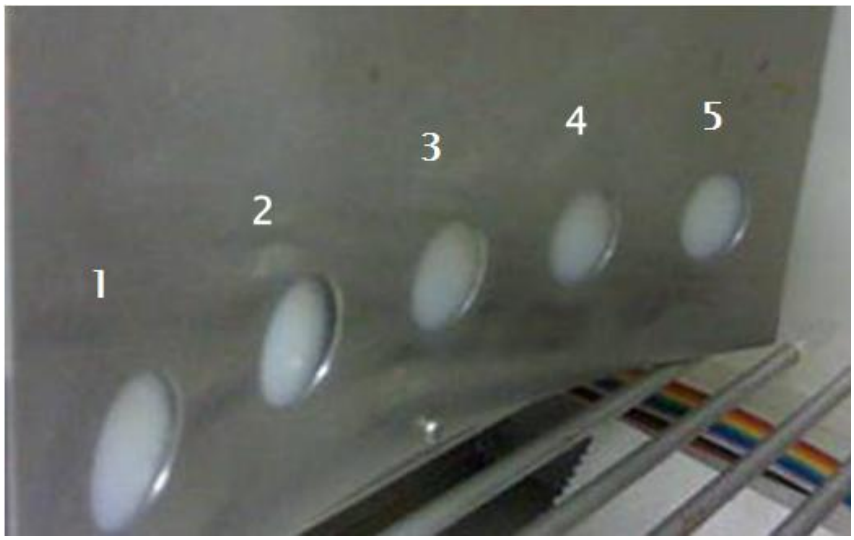

Figure 6. Nose Poke Key Locations

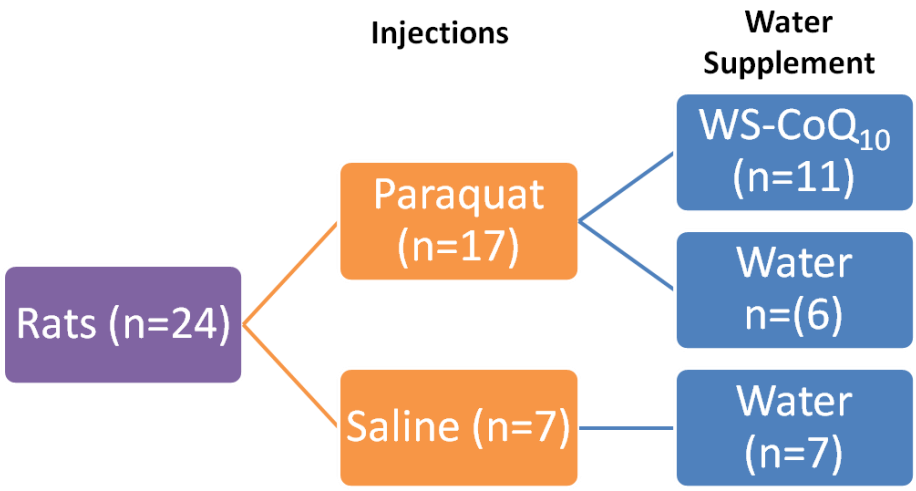

Figure 7. Rat groupings based on the injection regime and water supplementation.

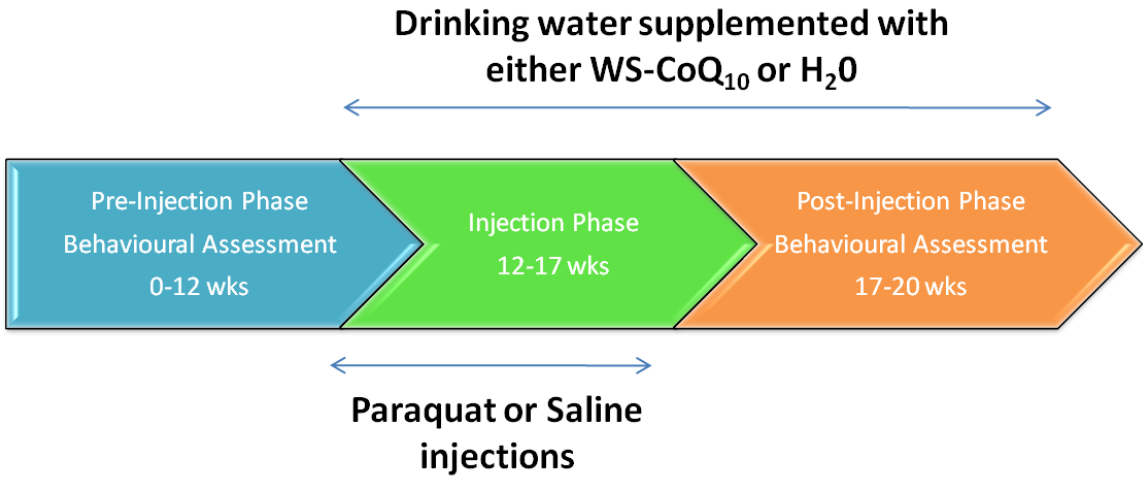

Figure 8. Schematic outline for the three phases.

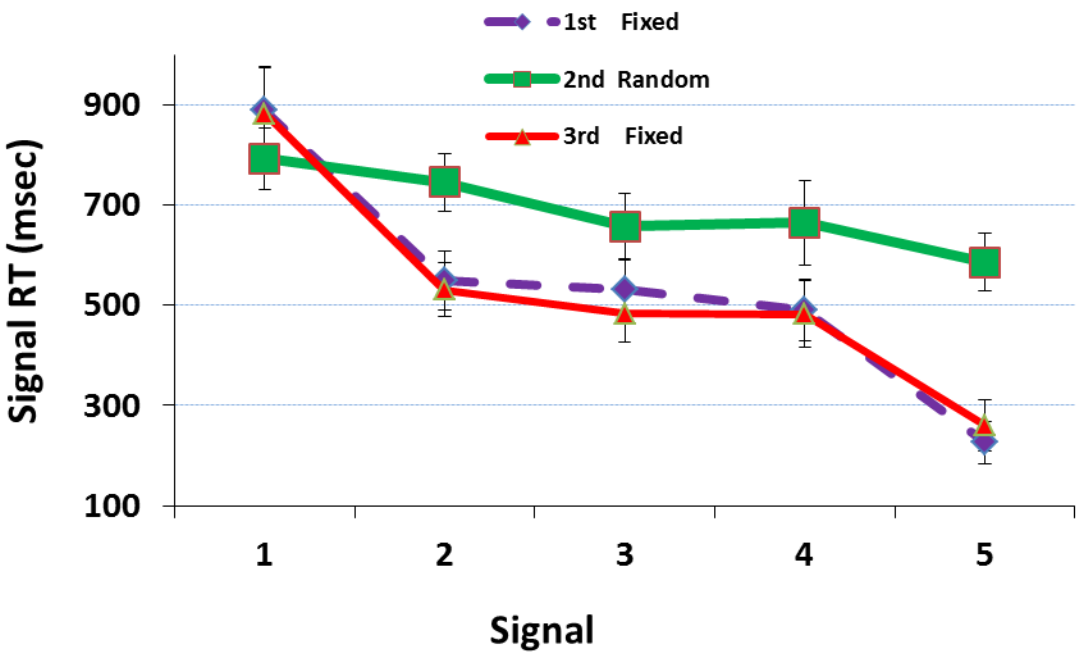

Figure 9. Normalized average RTs plotted as a function of sequence for each signal position over pre-injection trials.

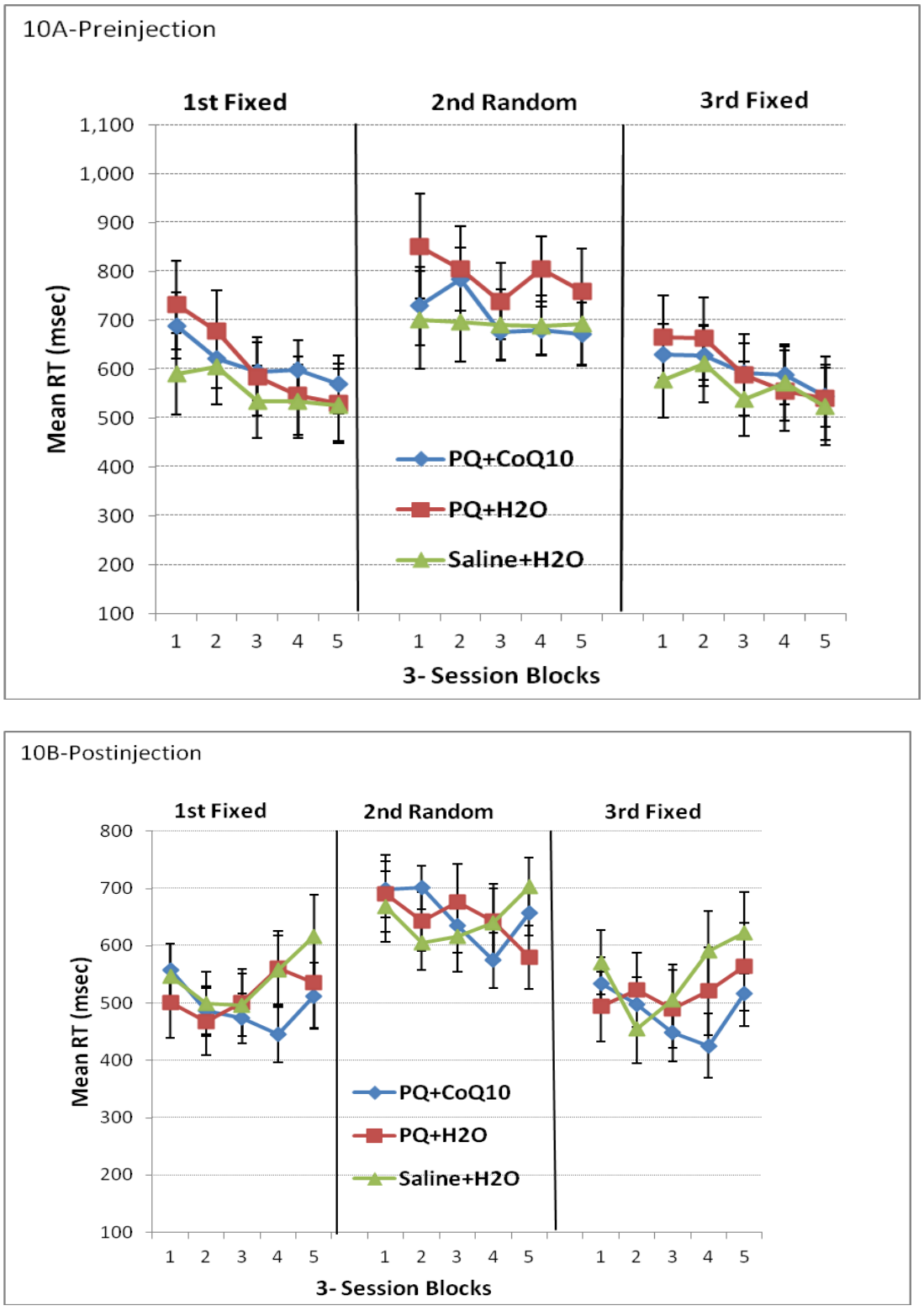

Figure 10. Rats' acquisition curves for normalized average RTs plotted as a function of sequence for 3-session blocks.

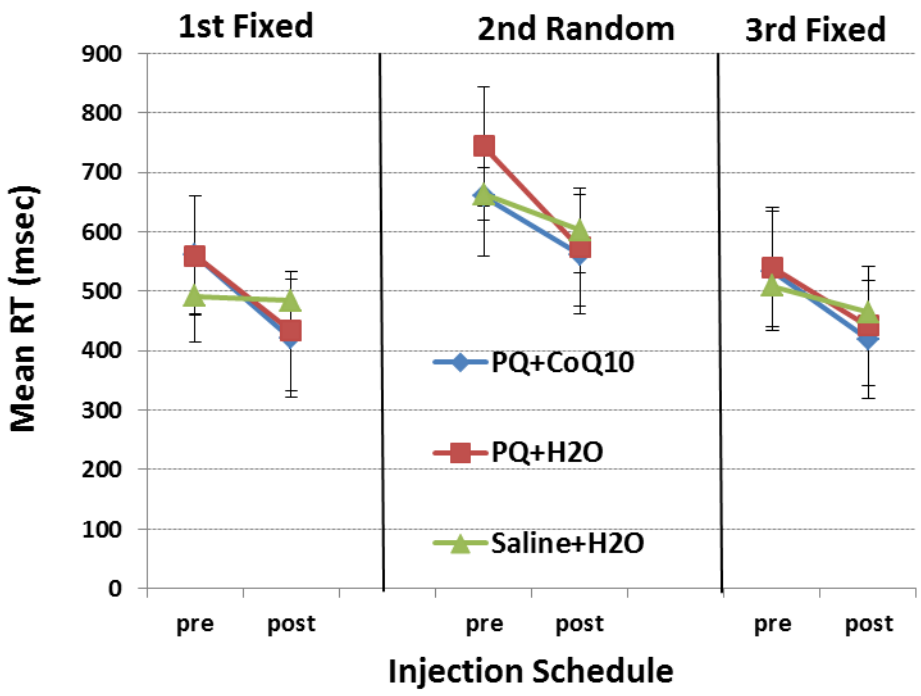

Figure 11. Normalized average RTs plotted for both pre-injection and post-injection

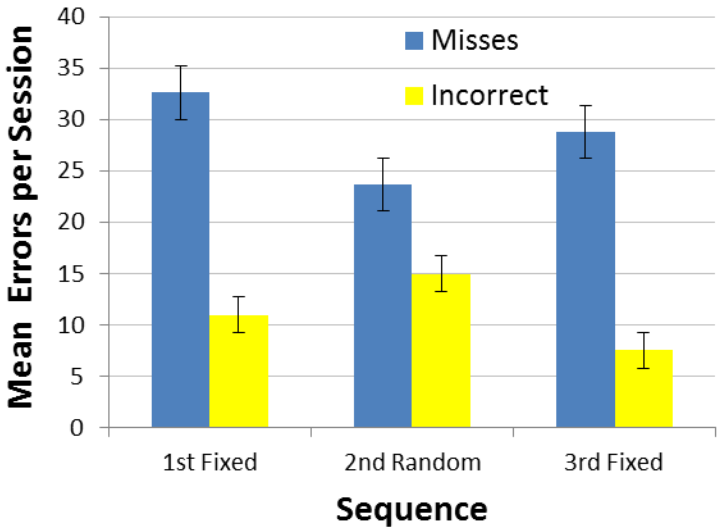

Figure 12. Overall error data for both misses and incorrect choices as a function of sequence.

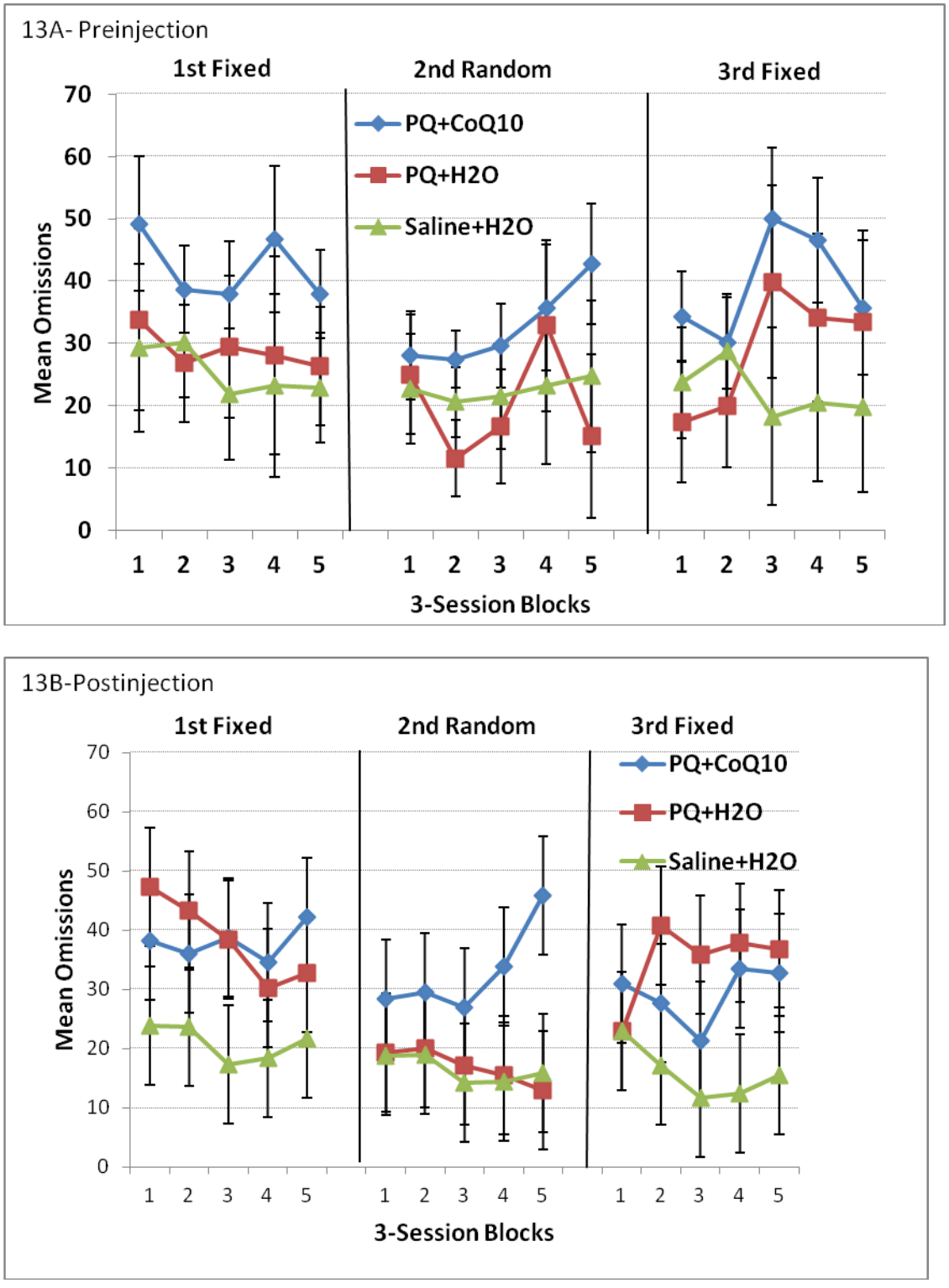

Figure 13. Rats' acquisition curves for mean number of omissions as a function of sequence structure over 3-session blocks.

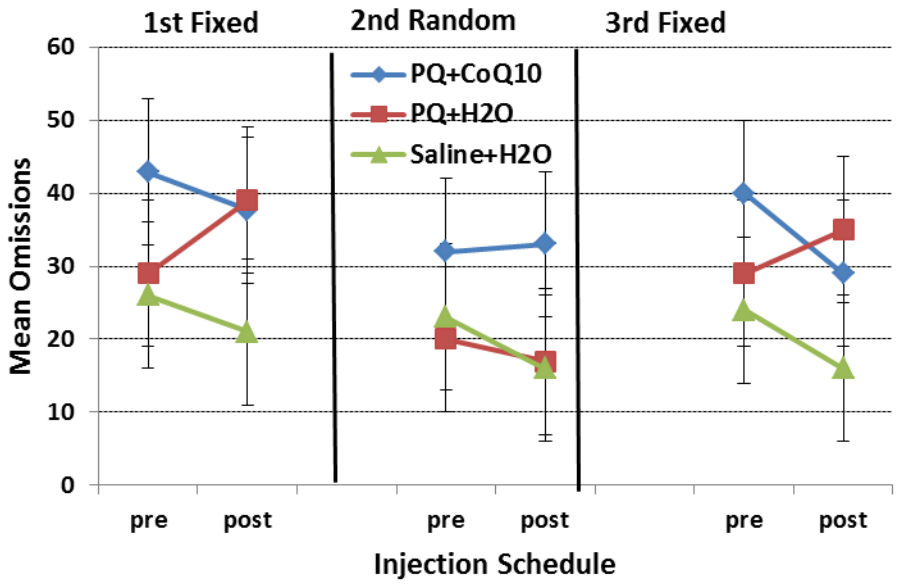

Figure 14. Mean number of omissions for both pre and post-injection as a function of sequence

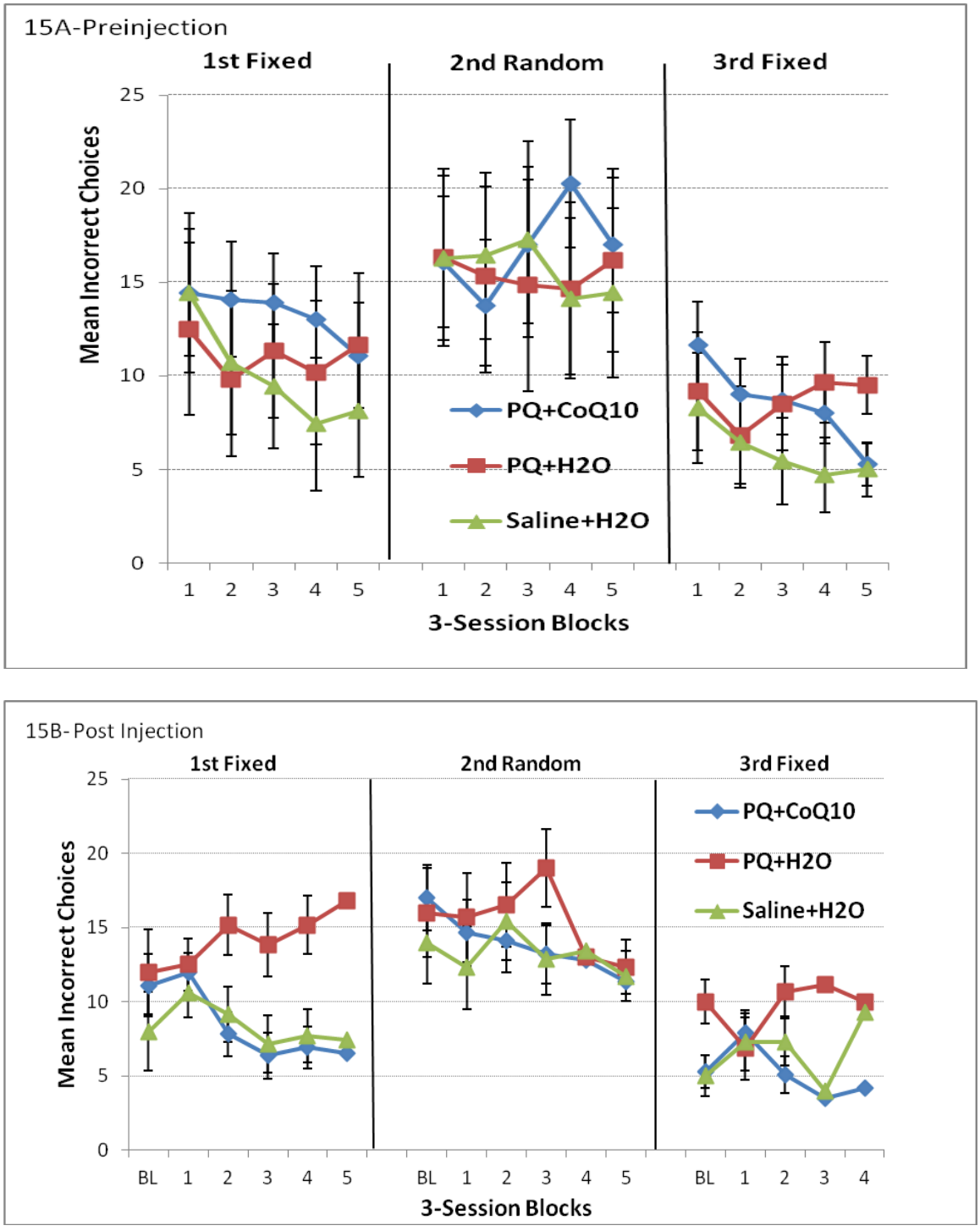

Figure 15. Rats' acquisition curves for mean number of incorrect choices as a function of sequence structure over 3-session blocks.

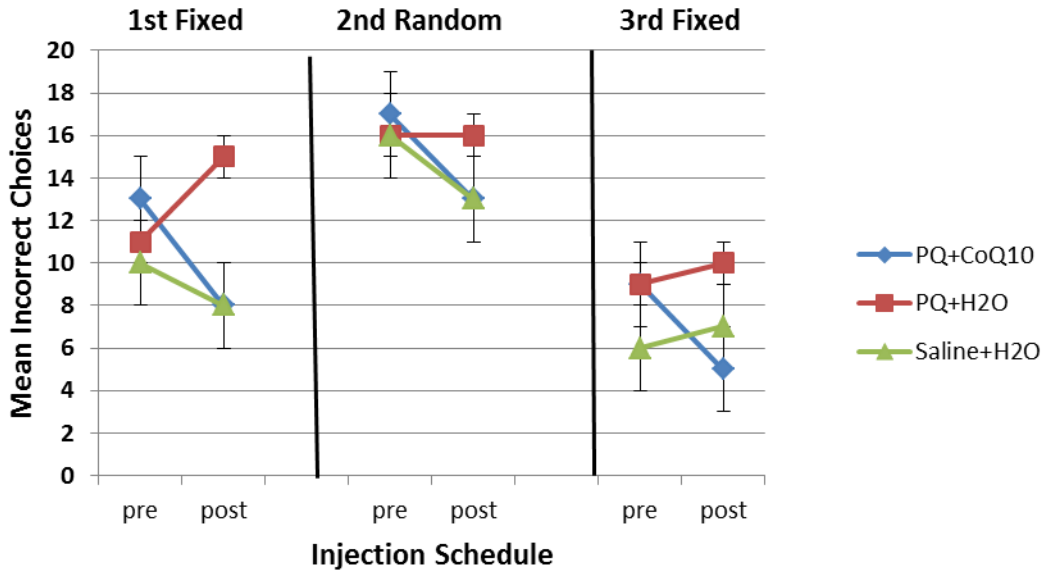

Figure 16. Mean number of incorrect choices for both pre and post-injection as a function of sequence.

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Caregiver Burden scales/Resource Utilization scales:
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Presentations Given:

Parameswaran, V., Gallant, S., Cohen, J., (03 March2011). *Factors Affecting Rats' 5-Choice Serial Reaction Time Performance*. Oral presentation presented at the 18th Annual International Conference on Comparative Cognition, Melbourne Beach, FL, March 2-4 2011.

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Parameswaran, V., Cohen, J., & Matei, A., (2006, April). *What do rats remember in a working-memory object recognition task?* Oral presentation presented at the 19th Annual Tri-State Plus Conference on Animal Learning and Behavior Indiana University Purdue University, Indianapolis, IN, April 7-8.

Pandey, S., Somayajulu, M., Vergel de Dios, J., Matei, A., Parameswaran, V., Cohen, J., Sandhu, J., Borowy- Borowski, H., & Sikorska, M. (2006, March). *Paraquat Induces Oxidative Stress, Neuronal Loss in SN Region and Parkinsonism in Rats: Neuro-Protection and Amerlioration of Symptoms by Water-Soluble COQ10.* Platform presentation presented at the 45th Society of Toxicology Conference, San Diego, CA.
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Parameswaran, V., and Cohen, J. (2005, November). *Rat's Working Memory for Objects Based on Configuration and Location Stability.* Oral presentation presented at the 46th Annual Psychonomic Society Conference, Toronto, ON.

Parameswaran, V., Cohen J., & Matei, A. (2005, May). *Rats' Object Recognition Working Memory in a Foraging Task.* Oral presentation presented at the 18th Annual Tri-State Plus Conference on Animal Learning and Behavior University of Windsor, Windsor, ON, May 13-14.

Published Articles

Cohen, J., Han, X., Matei, A., Parameswaran, V., Zuniga, R., Hlynka, M. (2010). Rats' visual-spatial working memory: New object choice accuracy as a function of a number of objects in the study array. *Learning and Motivation*, 41, 125-140.

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