THE PERCENTILE IRT SCHEDULE: HIGH RATE BEHAVIOR AS A TOOL FOR EXAMINING THE TOXIC MOTOR EFFECTS OF METHYLMERCURY EXPOSURE

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Wendy Dawn Donlin

Certificate of Approval:

_____	_____
James Johnston Professor Psychology	M. Christopher Newland, Chair Alumni Professor Psychology
_____	_____
Christopher Correia Assistant Professor Psychology	James McCoy Associate Professor Psychology

_____

Stephen L. McFarland
Acting Dean
Graduate School

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Wendy Dawn Donlin

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THE PERCENTILE IRT SCHEDULE: HIGH RATE BEHAVIOR AS A TOOL FOR EXAMINING THE TOXIC MOTOR EFFECTS OF METHYLMERCURY EXPOSURE

Wendy Dawn Donlin

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(B.A., West Virginia University, 1996)

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Methylmercury exposure has become a topic of interest in both the toxicology literature and among the general media within the last decade. Concern about methylmercury's toxicity has grown with the discovery that fish-consuming individuals can be exposed to the toxicant at varying levels. One dietary constituent thought to prevent or counteract the toxicity of methylmercury is selenium, which is also available through fish consumption. The scientific community is trying to develop a balanced approach to fish consumption, by designating which fish are safest, and the amount of that fish which would be unlikely to cause any noticeable physical or behavioral deficits.

Previously, it was found that prenatal methylmercury exposure resulted in deficits in high rate responding reinforced under a differential reinforcement of high-rates 9:4 (DRH

9:4) schedule. The differences may have been due to motor deficits or differential reinforcer rates. The current study, using a percentile schedule instead of a DRH, was designed to reduce the influence of reinforcer rate on behavior. The percentile IRT 10:0.5 schedule reinforces an interresponse time (IRT) if it is shorter than the median of the previous ten. The schedule produces high response rates, and minimizes direct effects of reinforcement rate. In experiment 1, behavior of 8 rats under the percentile IRT 10:5 schedule was characterized using a log survivor plot analysis. Bouts of responses were separated by periods of disengagement. Overall response rates ranged from 50 to 150 responses per minute. These overall rate differences were most closely correlated with within-bout response rates and bout lengths, with a smaller contribution from bout-initiation rates. Inter-correlations among these measures were low, indicating that they contribute independently to overall response rates.

In experiment 2, forty eight rats were used in a 2x3 design to examine the interaction of methylmercury and selenium on behavior. Rats were chronically exposed to 0, 0.5 or 5 ppm of methylmercury through drinking water daily. Rats consumed diets which contained either high (0.05 PPM) or low (0.5 PPM) levels of selenium. At 56 weeks of age, (247 days on methylmercury and 267 days on selenium), animals were placed on a percentile IRT 10:5 schedule of reinforcement. Methylmercury exposure resulted in slower behavioral acquisition, lower steady state response rates, and longer post-reinforcement pause durations. Selenium deficiency resulted in slower behavioral acquisition and lower steady state response rates. High selenium levels were associated with decreasing bout initiation rates over time. There were no statistically significant interactions between methylmercury and selenium.

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CHAPTER 1: METHYLMERCURY TOXICITY IN HUMANS AND ANIMALS: A LITERATURE REVIEW

Mercury is an element present in our environment, contributed by both natural and anthropogenic sources. The natural form of mercury is mercuric sulfide, or cinnabar, mostly present in soil. Through the degassing of soil and water, mercury is released to the atmosphere. Once in the atmosphere, mercury can convert amongst its three valence states, Hg^0, Hg^{1+} and Hg^{2+}. Through interaction with other chemicals and/or microorganisms, mercury can remain a metallic ion, or be transformed into both organic and inorganic compounds. The different forms of mercury can then be reabsorbed into soil and water. In addition to this natural process of mercury transformation, vapors can be released into the atmosphere through the burning of fossil fuels. Between 25% (estimated by World Health Organization) and 75% (estimated by EPA) of estimated emissions are due to fossil fuel burning (National Research Council, 2000).

Humans and other animal species come into contact with the various forms of mercury through these environmental sources: water, air and soil. However, the primary source of dangerous exposure to mercury compounds is through the consumption of contaminated fish. In water, elemental mercury can be converted to the organic forms of methylmercury and dimethylmercury through bioconversion by microorganisms. Small fish consume these microorganisms, bigger fish consume the smaller fish, and there is an ultimate bioaccumulation of dangerous methylmercury through the food chain. Large

predatory fish such as shark, swordfish and whale contain the highest levels of methylmercury (National Research Council, 2000). Other methods of exposure to mercury pose less threat, but that are still of concern are through inhalation of mercury vapors, and through dental amalgams.

Methylmercury is distributed throughout the body when an organism has been exposed. It can be inhaled, absorbed through skin, or consumed. Food exposure poses the greatest risk, with approximately 95% methylmercury absorbed from the gastrointestinal tract. MeHg is lipophillic, and is primarily transported into cells and across the blood brain barrier through cysteine active transport. The half-life of is around 70-80 days, but is slower for brain and liver elimination.

Epidemic Exposures

Two significant epidemics of exposure to methylmercury have been recorded in recent times. Marine exposure in Minamata, Japan, and contaminated grain exposure in Iraq have resulted in many instances of mercury poisoning in those areas. Although tragic, these incidences contribute to our understanding of mercury poisoning.

Minamata, Japan. In the early 1950's, doctors in the Minamata area were seeing patients that had mysterious maladies that they were unable to accurately diagnose. The disease was named Minamata disease in May 1956, because there was no knowledge of the causes of the illness. Nearly half of the original 34 diagnosed cases resulted in death within 3 months. Children were exhibiting symptoms that resembled those of encephalitis. Infants were being born with cerebral palsy at an alarming rate. High incidences of miscarriages and stillbirths were occurring, as well as death soon after birth. Both adults and children were showing other symptoms such as: constriction of

visual field, sensory disturbances, ataxia, dysarthria, auditory disturbances, gait disturbances, tremor, chorea, athetosis, contracture, tendon reflex, pathologic reflexes, hemiplegia, hypersalivation, sweating, focal cramps, pain in limbs and mental disturbances. Between 1955 and 1958, 29.1 % of children were being born with varying degrees of mental retardation. Up to 17.5% of secondary school students were exhibiting motor dysfunction in the form of general clumsiness and poor dexterity (Harada 1995).

Concurrently, with the unusually high incidences of these human disturbances, animals in the region began exhibiting disturbing behavior as well. Fish floated belly up in the water, or rotated continuously, shellfish decomposed, and birds fell from the sky while flying. The most revealing species turned out to be cats, which were convulsing, salivating excessively, were unable to walk straight, and eventually collapsed dead. In 1959, based on a proposal by the Minimata Disease Research group, feeding studies were conducted on cats and it was concluded that the water in the area was contaminated with methylmercury. There was up to 1 mg/kg of MeHg in the tissue of the cats examined that had been eating seafood from the local waters. Based on this finding, fishing in the region was banned. There was also a movement to study how to decontaminate the water, and wide-ranging studies of individuals in the area (Harada 1995).

Mercury samples obtained from waters near the sludge plant revealed levels as high as 2010 ppm. Several species of marine life were also tested, revealing a wide range of mercury levels: Hormomya nutabilis from 11.4 to 39 ppm, oysters at 5.61 ppm, crabs at 35.7 ppm, and scindena schelenglii at 14.9 ppm. Further, autopsies in humans revealed levels of 22 to 70.5 ppm in the liver, 2.6 to 24.8 ppm in the brain, and 21.2 to 140 ppm in

kidneys. Hair samples from humans indicated a range of 2.46 to 705 ppm up to 5 years after original contamination (Harada, 1995)

Many mild cases of methylmercury poisoning were initially overlooked, especially if the symptoms were atypical. Much later, it was discovered that symptoms lie on a continuum, with delayed onset, and exacerbation with aging. In addition to the typical neurological problems, the disease was found to be co-morbid with several physical complications, such as: hypertension, cerebral arteriosclerosis, hepatopathy, gastric disorders, immune disorders, thyroid disorders and liver disorders (Harada, 1995). Humans that show early signs of methylmercury toxicity, the "early onset type" of minimata disease, have improved over time after exposure to methylmercury has ended. Those individuals with delayed onset of symptoms, the chronic minimata disease patients, show progressively worsening symptoms after exposure has ceased (Castoldi, Coccini and Manzo, 2003). The minimum amount of mercury exposure that would produce acute Minimata disease was calculated to approximately .417 mg/kg in adults, or equivalent to 50 ppm in hair samples (NRC, 2000).

Iraq. Another major incident of methylmercury exposure occurred in rural Iraq in 1971-1972. Iraqi farmers received grain treated with mercury (as a fungicide) from the United States. The grain was intended for planting, but the starving farmers and their families instead made bread from the grain. A total of 50,000 individuals were exposed directly to the methylmercury, 6530 patients were admitted to the hospital, and 459 died. Most patients first complained of parathesias, although they also exhibited ataxia and dysarthria at extreme levels. Children prenatally exposed to methylmercury in Iraq showed deficits in reaching developmental milestones. Dose-dependent relationships

were often found, with levels of Hg in maternal hair as low as 10 ppm resulting in adverse fetal effects (Myers, Davidson, Cox, Shamalaye, Cernichiari, and Clarkson, 2000).

Human Studies

The risk of being exposed to methylmercury in the general population is highest in populations that consume large amounts of predatory fish. Fish intake can vary with geographic location, culture, and socioeconomic status. Island and coastal populations, therefore, are the most at risk for dangerous levels of methylmercury exposure. Large scale studies have taken place in two of these populations: The Faroe Islands, and the Seychelles.

The Faroe Islands. Located between Iceland and Norway, the 42,000 inhabitants of the Faroe Islands primarily consume a marine diet, which includes fish, pilot whale meat and blubber. Inhabitants with the highest mercury concentration biomarkers ate more whale meat dinners than others. Whale blubber consumption was predictive of organochlorine concentrations. Omega-3 fatty acid levels correlated with fish intake. Neurological optimality scores, obtained in babies 2 weeks old, indicated a negative association between mercury biomarker levels, with no correlation or interaction with organochlorine or omega-3 fatty acid (Steurwald, Weihe, Jorgensen, MEng, Kristian, Brock, Heinzow, Birger, Budtz-Jorgensen, and Grandjean, 2000).

The Seychelles. The Seychelles Development Study (SCDS) is a longitudinal study of pre and postnatal methylmercury exposure. Children born between February 1989 and February 1990 on the Island of Mahe were evaluated at 6.5, 19, 29 and 66 months of age. Cognitive, perceptual, motor and language functioning were assessed. No adverse effects

were found with either prenatal or postnatal exposure to methylmercury through a fish diet (Davidson, Myers, Cox, Axtell, Shamlaye, Sloane-Reeves, Cernichiari, Needham, Choi, Wang, Berlin and Clarkson, 1998; Axtell, Cox, Myers, Davidson, Choi, Cernichiari, Sloane-Reeves, Shamlaye, and Clarkson, 2000). Paradoxically, benefits for males on the Woodcock-Johnson and the Bender Gestalt test were found (Davidson, Kose, Myers, Cox, Clarkson, and Shamlaye, 2001).

Summary of Human Exposure. Epidemic high-level exposures and low-level chronic exposures to methylmercury have affected resulted in detrimental effects in both adults and developing children. Discrepancies between exposure dose and adverse effects exist, which is most likely due to the time-course of exposure, and possibly to other factors such as nutritional intake. Because several factors contribute to the dose of methylmercury which may produce adverse effects, it is important to account for the most sensitive conditions and populations which may be exposed.

Reference Dose

One important goal of examining data associated with human exposure to any toxicant is to determine the level of exposure should not be harmful, which is called a no-observed adverse effect level, or a NOAEL. Determination of this level can be calculated through direct observations of human exposures, or through animal experimentation and extrapolation. Usually, a lowest observed-adverse effect level (LOAEL) is designated by the accumulation of experimental and/or general exposure data. Based on factors of uncertainty, this level is extrapolated to a level that is assumed to produce no adverse effect, the NOAEL. From the NOAEL, a reference dose (RfD) can be calculated. Considerations for extrapolation include interspecies variability, intraspecies variability,

conversion from acute to chronic exposure, or when a LOAEL is available instead of a NOAEL (National Research Council, 1992; EPA, 1997). In determining the RfD for methylmercury, the data from the Iraqi exposures, as well as data examining a population of New Zealanders was used. Detrimental effects were observed in the Iraqis children at a level corresponding to 11 ppm of Hg in maternal hair. Using calculations that take into account bioavailability, absorption factors, blood levels, and body levels of methylmercury, a daily dietary intake of methylmercury can be calculated which would result in this level of mercury in hair. This calculation indicates that 1.1 ug/kg/day of Hg is the maximum exposure is unlikely to cause harm. This level, however, is divided by an uncertainty factor of 10 to account for interspecies variability (EPA, 1997). Further support for this reference dose comes from the inverse relationship noted between maternal hair levels of Hg and IQ in offspring in a New Zealand population (Gearhart, Clewell, Crump, Shimp and Silvers, 1995; Kjellstrom, Kennedy and Wallis, 1989). Maternal hair Hg levels ranged from 5 to 15 ppm in this population, which leads to a range of 0.8 to 2.5 ug/kg/day as a reference dose, with an uncertainty factor applied for interindividual variability. This further extrapolation results in a range of 0.08 to .25 ug/kg/day. The EPA currently supports a .1 ug/kg/day RfD of Hg (EPA, 1997).

Neurotoxicity

The behavioral effects found in humans are undoubtedly due to alterations of the nervous system by methylmercury. In adults exposed to methylmercury, specific types of damage occur: there is a loss of neurons in the visual cortex, a disappearance of granule cells in the cerebellum, and degeneration of axons and myelin disruption in the sensory branch of the peripheral nerve (Castoldi, Coccini, and Manzo, 2003).

The developing, prenatally exposed brain shows more diffuse damage, including: degeneration and fewer nerve cells in every lobe of the cortex, and reduction in purkinje cells and granule cells in the cerebellum (Castoldi, 2001). Oligodendroglia and microglia flourish beyond normal levels, and unusual astrocytes are found in the white matter (glial proliferation (gliosis) is considered a sign of damage in most cases). Similar structural abnormalities are found in humans, primates and other mammals exposed to methylmercury (Burbacher, 1990). In rats exposed to a very high dose of methylmercury (10 mg/kg/day), large motor neurons of the spinal cord and glial cells exhibited accumulation of mercury after only 15 days of exposure which eventually resulted in a loss of these neurons (Su, Wakabayashi, Kakita, Ikuta, and Takahashi, 1998).

The neurotoxic effects of methylmercury are exerted through several different types of mechanisms. For both prenatal and postnatal exposure, methylmercury can induce apoptosis and necrosis of cells, can impair mitochondrial activity, depolymerize and inhibit cerebral microtubules, disrupt calcium signaling, and increase oxidative stress. The glutamate, cholinergic and dopaminergic neurotransmitter systems are also altered by methylmercury toxicity (Castoldi, 2001).

Animal Studies

Two types of methylmercury exposure are of concern for humans: prenatal exposure, and postnatal exposure. Because similar molecular and behavioral abnormalities have been found in humans, primates and rodents in respect to methylmercury exposure, using an animal model of methylmercury exposure is ethical, economical and useful (Burbacher, 1990).

The focus of much of the animal literature is pre-natal or developmental exposure to methylmercury. Prenatal exposure occurs when a pregnant dam is exposed to methylmercury and the offspring are the organisms of interest. Figure 1 summarizes the findings of this literature (from Newland and Paletz, 2000). Along the X-axis, a dose of mercury is indicated in µg/kg/day. The dose of mercury represented is the lowest observed-adverse-effect level, the LOAEL. This dose represents the point at which the first signs of detrimental effects were observed in each respective study. Open circles represent rodents, and filled circles represent primates. Note that several behavioral domains are affected by prenatal methylmercury exposure, but that some are not evident until very high doses are administered. The points on the lower left side of the graph are indicative of the measures that are most sensitive to methylmercury toxicity. In general, primates are more sensitive the low doses of Hg, but rats showed detriments on a DRH schedule of food reinforcement at a dose as low as 10 µg/kg/day Hg (Bornhausen, Musch, and Greim, 1980). Newland and Paletz (2000) argued that this enhanced sensitivity is due to at least three reasons. First, rat blood is very high in sulfur-containing hemoglobin that binds mercury. A higher daily dose is required to overcome this binding and achieve brain levels associated with neurotoxicity. Second, with some exceptions, the investigators using rodents usually use cruder behavioral measures than those investigating nonhuman primates. Third, chronic, low-level dosing regimens were used in primate studies while rodent studies typically (not always) involved the administration of a fairly high daily dose over the course of a few days, preventing the effects of chronic low-level exposure from appearing. Thus, the sensitivity may lie in the experimental preparation or blood, but not the brain of the species examined. Monkeys

(Macaca Fascicularis) showed detriments in responding under a fixed interval schedule at doses of 10 µg/kg/day (Rice, 1992; Gilbert, Rice and Burbacher, 1996). More global measure of functioning, like gait, startle, and tremor were evidenced only at much higher doses above 1000 µg/kg/day Hg. This divergence seems to be emphasizing the sensitivity of operant tests to chemical assault. Although these results are interesting in that they reflect methylmercury exposure, they do not necessarily correspond with the effects of adult exposure to methylmercury because the subjects were all exposed prenatally. Adult-exposed animals look and behave differently than prenatally-exposed organisms.

Chronic developmental and adult exposure in primates. Fewer studies examine the behavioral effects of methylmercury when the animal is only exposed after birth. The most thorough research program examining these effects is a series of studies conducted by Rice and colleagues at the Canadian Health Protection Branch. A cohort of macaque monkeys were raised, with developmental exposure to 50 µg/kg/day of methylmercury a day (orally) from birth to 7 years of age. Blood levels of mercury were approximately 0.7 ppm at steady state. These monkeys underwent a series of behavioral tests starting at 3 years of age, and they continued until 20 years of age (Rice, 1996).

Testing examining spatial-visual function was conducted in these animals exposed to methylmercury as well as a control group of macaques (Rice and Gilbert, 1982). The monkeys were restrained in a chair in a light-tight room. Two oscilloscopes were placed 114 cm from their eyes. During a trial, one oscilloscope presented a vertical sine wave grating, while the other showed a blank field with the same luminance. Spatial frequency of the grating was varied, using thick versus narrow bars. Monkeys pressed a button to

indicate which scope presented the grating. Correct responses were reinforced with apple juice. Three of five treated monkeys showed impairments in discrimination; two were impaired at high spatial frequencies, while one was impaired at both middle and high frequencies. When luminance was low, all treated monkeys showed impairments. Control monkeys performed well at all frequencies and luminance. No general health or global behavioral effects were noted in any of the treated monkeys.

At five years of age, vision was tested using a similar experimental design, except that the stimuli presented through the oscilloscopes were either a flickering or a steady light (Rice and Gilbert, 1990). Monkeys pushed a button to indicate which stimulus was flickering for juice reinforcement. Treated and control monkeys showed no discrimination differences at high luminance, but surprisingly, treated monkeys discriminated flickering lights better than controls at low luminance. The authors suggested that during development, the visual system may remodel itself to compensate for damage to the parvocellular system, resulting in the expansion of the magnocellular system. The parvocellular system transmits information about color and fine detail from the eye to the primary visual cortex. The magnocellular system transmits information important in the perception of form, depth, movement and luminance from the eye to the primary visual cortex (Carlson, 1998).

Daily oral doses of methylmercury ceased in this cohort of monkeys at 7 years of age. General health and behavior of these animals was followed after dosing to examine delayed effects of methylmercury and interaction of age. At 13, treated monkeys were observed to be clumsy and hesitant. When required to pick up raisins out of compartments, treated monkeys were slow and less accurate in retrieval. Monkeys failed

to respond to pin pricks and touch on hands, feet and tail, and they were observed to slip on bar in their cages while climbing. These general problems were not noted at younger ages (Rice, 1989).

At age 14, auditory function was assessed. Monkeys were taught to hold onto a bar until a tone was detected through a pair of headphones (Rice, 1992). Upon releasing the bar when a tone was present, juice was presented. Frequency and amplitude of tones were varied. All monkeys performed well at low frequencies, but 3 of 5 monkeys were impaired at high frequencies. One monkey performed poorly at all but low frequencies.

Sensitivity to vibration was tested at age 18 by reinforcing the release of touching a small stimulus once vibration was detected. Four of five monkeys showed detection impairments, with thresholds being higher for several frequencies and amplitudes (Rice and Gilbert, 1995).

Information processing speed was unaffected in treated monkeys at 20 years of age (Rice, 1998). Monkeys were required to hold on to a metal bar until a light appeared on a series of keys located in front of the monkey. Upon presentation of the light, the monkey received a reinforcer for releasing the bar, and pushing a corresponding key. The treated monkeys responded as accurately and as quickly as control monkeys, which indicates that gross motor movement in the arms, and central processing speed were not affected by methylmercury exposure.

Visual function was reassessed in these monkeys at 20 years of age as well (Rice and Hayward, 1999). Two of five monkeys showed a constriction of visual field, which none of the control animals exhibited. The previously described spatial frequency test revealed that all animals had a decrease in sensitivity, with the methylmercury treated animals

showing greater declines. Retesting of temporal visual function, using flickering lights, revealed a general decrease in sensitivity to low frequency presentations, but no differences between groups.

This series of studies characterizes several domains of sensory and behavioral changes due to methylmercury exposure. Deficits were noted in seeing high spatial frequencies early in development, with increasing deficits with age. Temporal visual discrimination was superior in young animals dosed with methylmercury over controls, which diminished over time. High frequency auditory testing performance was impaired in treated monkeys, with all monkeys performing well at low frequencies. Fine motor skills were diminished even several years after methylmercury dosing ceased, with gross motor deficits evident as well. Rice (1996) pointed out that the presence of deficits in one domain did not predict the presence of deficits in different domains. For example, monkeys that had visual decrements did not necessarily have motor or auditory decrements. Visual deficits were exhibited earlier in life, whereas motor deficits were not observed until higher cumulative doses of methylmercury were administered, which necessarily occurred at a later age.

Motor function and methylmercury. Chronic exposure to methylmercury can result in a significant reduction of granule cells in the granule layer of the cerebellum (Castoldi, 2001). The function of the cerebellum is to regulate movements by comparing afferent sensory data to the dispatched efferent motor data. If there are discrepancies between the motor signals and the feedback of the sensory signals, the cerebellum modifies the motor plan to adjust accordingly. The cerebellum receives input from both the central nervous system, and the periphery. It sends information via the deep nuclei

and vestibular nuclei to the motor regions of the cerebral cortex and brainstem (Kandel, Schwartz, and Jessell, 1995). Figure 2 illustrates the complex interactions between the cerebellum and other parts of the brain in both planning and executing movements. Because the cerebellum does not directly control motor movements, impairment, or even complete disruption of the cerebellum will not disrupt sensory perception or muscle strength, but instead would result in problems of coordination, balance and muscle tone (Kandel, Scwartz and Jessell, 1991).

The cerebellar cortex, part of the spinocerebellum, has three distinct layers: the molecular (outermost) layer, the Purkinje cell layer, and the granular layer (innermost). Within these layers, purkinje cells extend axons into underlying white matter, acting as the only output from the cerebellar cortex. These purkinje cells are excited indirectly by afferent input from brain stem nuclei. The information from the brain stem nuclei is passed from afferent mossy fibers to granule cells, which in turn excite purkinje cells (Kandel, Schwartz, and Jessell, 1995). Reductions in granule cell volume, therefore, could influence the coordination of movements, muscle tone and balance. Tasks that require repetitions of extensions and flexions should be sensitive to the toxicity of methylmercury due to these neurochemical alterations. Deficits in motor control should be evident using tests that require a large motor component, and could be observable using simple measures of gait and posture.

Motor neurotoxicity in animals. Motor function has been shown to be disrupted by methylmercury exposure in humans and primates. The cerebellar damage associated with exposure points towards specific types of deficits that should be expected in the

motor system in other species as well. Both avians and rodents have been used to examine the effects of adult methylmercury exposure on motor functioning.

Evans, Garman and Laties (1982), exposed adult pigeons to 5 mg/kg/week, 7.5 mg/kg/week or 10 mg/kg/week of methylmercury through intubation until overt behavioral signs of toxicity were noted. The birds were tested and observed on several tasks of general motor functioning. The first evidence of toxicity was revealed on a feeding test. After 24 hours of food deprivation, the birds were presented with 40 grains of food in a clear glass dish. The time to consume all grain, accuracy, and rate were all measured. Over 4 to 5 weeks of exposure, rates of pecking declined for the two highest doses of methylmercury. One pigeon declined to 70% accuracy after 57 days of exposure. After 8 weeks of exposure, the highest methylmercury exposed group of pigeons began exhibiting overt signs of motor dysfunction, through examination of flying and walking. They became clumsy, some had a high-stepping gait, and eventually, the wings would remain in a constant flexed position. The lower mercury groups showed similar deficits, but after dose-dependent longer exposure times (up to 10 weeks for the lowest exposure group). Dose-dependant blood and brain levels of mercury were found as well: 25 ppm for the 10 mg/kg/week group, 20 ppm for the 7.5 mg/kg/week group and 12 ppm for the 5 mg/kg/day group after 30 days of exposure.

Wakabayashi, Kakita, Sakamoto, Su, Iwanaga, and Ikuta (1995) sought to characterize deficits associated with methylmercury exposure at different developmental stages in rats. Rats 2 days old, 15 days old, or 60 days old were dosed with 10 mg/kg/day Hg for 10 days orally. In newborns, no change in appearance or body weight was noted. In the young rats, motor disturbances, such as unsteadiness, gait disturbances and hindlimb

cross were noted 14 days after dosing. Three of 24 rats had died by the 14th day. Widespread neuronal damage was noted in the cerebral neocortex, neostriatum, red nucleus and brainstem as early as the 11th day after dosing. No alterations were noted in the cerebellum. The adult rats showed different patterns of deficits and brain alterations. Weight loss began after day 6, with hindlimb cross evident on the 13th day. One sixth of the animals died by the 17th day. Molecular analysis revealed changes in the granule cells of the cerebellum at day 13, while myelin breakdown was noted in the spinal sensory system at day 11.

Su, Wakabayashi, Kakita, Ikuta and Takahashi (1998) exposed adult rats to 10 mg/kg/day of methylmercury over 10 days as well, with an equimolar amount of l-cysteine. l-cysteine is thought to be important in transfer of methylmercury across the blood brain barrier. Weight loss and fur roughness began 6 days after initial exposure. By the 11th day, an accumulation of mercury was noted in the large motor neurons of the spinal cord. Hind limb cross was first noted 13 days after exposure. Degenerated myelin fibers, as well as other changes in the spinal gray matter were noted at day 14. By the 16th day, mercury was accumulating in glial cells, and the gluteus muscle of the hind limbs was exhibiting neurogenic atrophy. Flaccid and atrophic limbs were evident at day 18, in the 20 of the 34 animals who had survived.

The cumulative dose of 100 mg/kg over a short period of time is very high, and leads to quick impairments of the motor system in these two studies. Impairments and accumulation of mercury in the nervous system was usually evident before any overt motor deficits were observed. These levels are representative more of epidemic acute exposures rather than long term chronic exposure.

Yasutake, Nakano, Miyamoto and Eto (1997) exposed rats to much lower doses, 0, 1 or 5 ppm of Hg in chow. No hindlimb cross or other obvious motor deficits were evident at 2 years of age in any group, but all animals (including controls) exhibited the hindlimb cross at 2.5 years of age. Fifty percent of animals were dead in the high group at 32 months of age, but it took 34 months for the low and control groups to reach the same mortality rate. Gross motor measures were not a sensitive to these low level chronic exposure arrangements.

Summary of methylmercury effects on motor behavior. Motor deficits are a primary concern when an organism has been exposed to methylmercury either as adults or prenatally. Humans, primates, rodents and birds have shown dose dependent deficits in motor function when naturally or experimentally exposed. Adult organisms, whether human, primate or rodent, have shown reductions in granular cells of the cerebellum, as well as demyelination of sensory neurons of the spinal cord. The effects of low-level chronic exposure to methylmercury on motor function has not been satisfactorily studied to this point. A rodent model of this type of exposure, with a challenging behavioral motor component, could be informative about the effects that humans may be subject to through exposure to methylmercury by consuming fish. This model could help to discover reasons that different fish-eating populations of humans exhibit different levels of motor, cognitive and sensory deficits. Selenium intake has been proposed to be one variable which may affect the toxicity of methylmercury. Selenium reacts with methylmercury in the body, altering systemic, neurological and behavioral measures.

Selenium. Selenium is an element that is naturally found in rock, soil, water, air and food. Usually, it exists as part of a substance, not in its elemental form. Animals are

exposed to selenium primarily through food intake, although levels up to 50 ppb can be found in US water supplies (USDHHS, 2003.) Both deficiencies and excesses in selenium intake can lead to adverse effects. The FDA's recommended daily allowance (RDA) for both men and women is 55 µg/day, or approximately 0.8 µg/kg/day (Rayman, 2000.) The highest intake level should not exceed 5.7 µg/kg/day (USDHHS, 2003.)

Selenium is essential to normal bodily functioning for several reasons. Thirty-five selenoproteins have been identified, and the different proteins can function as antioxidants, act as catalysts for the production of hormones, and maintain membrane integrity. Selenium has also been shown to have a direct relationship with immune system functioning, prevent some forms of cancer and cardiovascular disease, as well as alleviating symptoms of arthritis, pancreatitis, and asthma (Rayman, 2000.)

Excessive selenium intake is associated with health problems as well. An isolated high dose of selenium can lead to nausea, vomiting and diarrhea, tachycardia, and myocardial degeneration. Chronic exposure to high levels of selenium can produce a condition called selenosis, which is characterized by hair loss, nail frailty, and neurological motor problems (USDHHS, 2003.) This condition is rare, and has been found in populations that inhabit areas where high selenium content has been found in the soil, such as certain areas of China.

In the early 1970s, it was discovered that selenium taken concurrently with mercury compounds resulted in a less severe toxic effect of mercury. Ganther, Goudie, Sunde and Kopecky (1972) reported evidence that consumption of mercury from tuna containing selenium was less toxic than other instances of methylmercury poisoning. This may be because mercury and selenium for a complex, bis(methylmercuric) selenide (BMS), when

co administered (Imura and Naganuma, 1991.) This binding results in varying distributions of both selenium and mercury concentrations throughout the body, as compared to controls. Selenium concentrations are lower in most organs, except the liver, where there are elevated concentrations relative to conditions in which mercury exposure did not occur. (Komsta-Szumska, Reuhl, and Miller , 1983.) Mercury concentrations in brain (Prohaska and Ganther, 1977), blood, liver and testes (Whanger, 1992) are elevated when selenium and methylmercury are co-administered relative to mercury-only administration. It has been proposed that the molar ratio of selenium molecules to mercury molecules is the variable that is most important to the interactions of selenium and methylmercury.

Selenium also seems to ameliorate the behavioral effects of methylmercury. It reduces weight loss that is a function of age and methylmercury exposure, as well as delaying the onset of signs of neurotoxicity (Iwata, Okamoto and Ohsawa, 1973.) Watanabe, Yin, Kasanuma and Satoh (1999) examined the behavior of mice exposed prenatally to 0, 5 or two doses of 3 mg/kg Hg and a diet either sufficient (0.4 µg Se/kg) or deficient (<0.02 µg Se/kg) in selenium. The mice exposed to high doses of methylmercury showed selenium-dose-dependant deficiencies in the righting reflex, walking ability, and open-field locomotion, as well as increased thermal preference. These differences were less pronounced with aging (from post-natal day 4 to 14.)

The research, therefore, indicates that there is a chemical reaction between selenium and methylmercury, which leads to a BMS complex. This complex results in different distributions of both elements throughout the body, which ultimately results in effects on brain, cellular, renal and cardiovascular functioning. Selenium supplementation

ameliorates the prenatal effects of methylmercury on early motor function in rodents. However, interactions have not been examined for chronic low level exposure to methylmercury and selenium.

Behavioral Testing

Behavior has been an excellent endpoint in the study of the toxicity of various chemicals and metals. Behavior "represents the net sensory, motor and integrative outputs of the central, peripheral and autonomic nervous systems" (Kulig and Jaspers, 1999). Toxicants can therefore detrimentally affect sensory systems, motor systems and cognitive abilities. With over 65,000 chemicals being used in the environment, discovering the behavioral tests that are most sensitive to chemical assault is an important task.

Motor functioning is a domain of behavior that is subject to toxic effects of many chemicals. Currently, there are basic functional batteries of behavioral tests that are used to detect motor deficits, but often these tests are not as sensitive as appropriate operant experiments. Due to the stability of behavior afforded by operant arrangements, much more subtle changes in behavior can be detected. Behavioral changes can be detected between different animals, and also when two instances of one animal's behavior are compared.

The most common arrangement for tests of operant behavior involve lever pressing for reinforcers by rats in a standard operant chamber. Much is known about the behavior engendered by reinforcement schedules under these conditions. Deviation from expected behavior on these schedules was the first evidence that operant behavior would be sensitive to chemical assault. Behavior under different types of reinforcement schedules

reflects functioning of various sensory and motor systems, as well learning and experience with the schedule. To some degree, all of the schedules rely on motor ability, discrimination ability, and reinforcement mechanisms. Some schedules, however, emphasize some components over others. Animals can be taught "to respond rapidly or slowly, to exert great or small amounts of force, to hold down a lever for a specified amount of time, to pause for a specified time before responding, or to emit a specific number of responses before doing something else" and "to respond differently to various physical dimensions of their environment" (Laties, 1978). Among other possibilities, dimensions of motor function can be assessed when high rates of behavior are produced.

High-rate behavior. Many reinforcement schedules tend to produce high rates of behavior by manipulating response contingencies. Ratio and interval schedules can produce these steady high rates by delivering reinforcers after number of responses or after a short interval has elapsed, respectively. These particular schedules do not *require* that response rates be high, it only adventitiously reinforces higher rates of behavior. For example, in a variable ratio 30 (VR 30), an average of 30 responses must be emitted to receive reinforcement, and the faster that those responses occur, the higher the reinforcement rate will be. For interval schedules, there is a similar increase in reinforcement rates with low rates of responding, but the relationship at higher levels of responding level off to some maximum reinforcement rate programmed by the schedule (2 reinforcers/ min for a VI 30 second). Catania and Reynolds (1968) described this relationship as a negatively accelerating relationship between response rate and reinforcement rate. Hernnstein (1970) describes this phenomenon using a hyperbolic function, graphing response rate as a function of reinforcement rate. The asymptote of

the hyperbola represents the maximum reinforcement rate, where increasing response rate has no effect on reinforcement. Catania and Reynolds (1968) also point out that longer IRTs may be differentially reinforced in interval schedules, since the longer the subject waits to respond, the more likely a reinforcer will be available. Galbicka and Platt (1986) describe this phenomenon as "the conditional probability of reinforcement increasing monotonically as a function of IRT duration under VI schedules.

Schedules such as differential reinforcement of high rates of behavior, or DRH schedules, as well as IRT < t schedules, set criteria of reinforcement specifically on high rates of responding. DRH schedules designate a number of responses that must occur in a set amount of time. A DRH 9:4 means that 9 responses must occur in four seconds. IRT < t schedules place contingencies specifically on interresponse times. An IRT < 1 s schedule will deliver reinforcement if a response occurs after an interresponse time of less than 1 second. Both arrangements tend to generate high rates of responding (Lattal, 1991). Like ratio schedules, reinforcement rates are higher when response rates are higher.

Methylmercury and high-rate behavior. A DRH 9:4 schedule has previously been shown to be sensitive to the effects of prenatal methylmercury exposure. Newland and Rasmussen (2000) implemented a Multiple DRH 9:4 EXT schedule of food reinforcement in offspring of female rats exposed to 0, 0.5 or 6.4 ppm of Hg as methylmercury during gestation. Rats began operant testing at 120 days old and continued until the oldest survivors were 900 days of age. Baseline behavior was established, then the age at which reinforcement rates fell to below 50% of baseline rate were calculated. There was a dose-related decline in age to the 50% of baseline mark.

Rats at higher methylmercury doses reached the 50% mark before those at the lower dose, while controls maintained constant reinforcement rates across the experiment. Response rates declined over the course of the experiment as well. The response rates most likely reflected greater increases in longer (non-criterion) IRTs, but the shorter (criterion) IRTs remained intact.

The decline in response rates found in this study could be due to different factors: declining motor ability, a decrease in reinforcement rate, or a combination of the two. If rats who have been exposed to mercury have more pronounced declines in motor ability, then response rates could decline. This decline in responding would result in lower reinforcement rates, which could in turn lower responding, resulting in a downwardly spiraling interaction of responding and reinforcement.

Percentile-IRT schedules. Generating high-rates of behavior is a useful way of studying motor functioning, and its decline across aging. It may be the case that both sensitivity to reinforcement and motor changes across the lifespan can result in lower or less efficient responding. In order to minimize the effect of reinforcement rate on behavior in schedules that generate high-rates of behavior, a percentile IRT schedule can be used. Modifying the percentile arrangements described by Kuch and Platt (1976) and Galbicka and Platt (1986), short interresponse times can be specifically targeted, while reinforcement rates are held constant both for one subject and across subjects.

Kuch and Platt (1976) developed a schedule arrangement which could separate isolate these effects holding reinforcement rate constant, but differentially reinforcing different IRT classes. Pigeons either received reinforcement for emitting long IRTs or short IRTs, based on their own baseline IRT distribution. Reinforcement rate remained constant

throughout the sessions. Behavior tracked the contingency in place, with either IRTs getting longer or shorter. Therefore, IRTs were differentially reinforced without the effect of reinforcement rate altering responding.

When long IRTs were followed by food, the mean IRT rate reached asymptotic levels of about 10 seconds. Response rates increased with increasing reinforcement rate. When short IRTs were followed by food, reinforced IRTs averaged between 0.1 and 0.2 seconds. These IRT values greatly differed from baseline IRT values, which clustered around 1.0 seconds.

Galbicka and Platt (1986) manipulated the degree to which reinforcement depended on IRT duration. Pigeons received food reinforcers for key pecking. The reinforcement contingency between IRT duration were systematically manipulated by using a phi coefficient calculation. Increasing reinforcement rate generally increased response rate when IRT contingencies were weak. By increasing the contingency between reinforcement and long interresponse times, lower response rates and longer IRT durations were produced. With weaker IRT contingencies, where reinforcement was independent of IRT length, response rates tended to increase with increasing reinforcement rates. It was concluded that IRT contingencies account for a majority of the control of responding in VI schedules, with minimal effects of reinforcement rates.

Methylmercury and a percentile-IRT schedule. Behavior has previously been shown to be sensitive to mercury exposure when food reinforcement is provided according to a DRH 9:4 schedule (Newland and Rasmussen, 2000). As previously stated, there is multiple control of behavior by the rate contingency and the reinforcement rate under this schedule. By modifying the procedure to reinforce short IRTs according to the

animals own performance criterion, reinforcement rate can be held constant both across an experiment for a single animal and across subjects. By removing indirect effects of reinforcement rates on responding, the direct effects of neurotoxicant exposure may become more visible. This focuses on the motor ability of the rat to emit lever presses at a high rate and its sensitivity to the consequences of behavior.

Feedback and conditioned reinforcement. As previously described, motor execution and feedback is disrupted by methylmercury exposure, primarily through cell loss in the granular layer of the cerebellum, and a loss of large motor neurons in the spinal cord. This loss of feedback during any motor task could result in less refined movements, as well as a possible loss in the emission of the behavior. Providing external feedback, which indicates successful emission of a behavior, could act as a "behavioral prosthesis" which could lessen the effects of methylmercury exposure.

Consequences are key to the shaping and maintenance of behavior, especially operant behavior, which comprises what is commonly considered "voluntary" activities. Operant behavior is described by the three-term contingency:

$$S^d: R \rightarrow S^{R+}$$

The first element, S^d, is the discriminative stimulus. This can signify a particular stimulus or environment in which a response is likely to be reinforced, or when it is not likely to be reinforced. In other words, the discriminative stimulus "occasions" certain behaviors that are likely to be reinforced. The R in the model represents the response, or behavior.

As a consequence of this behavior occurring, the S^{R+} is presented (or in the case of negative reinforcement, the S^{R-} is taken away). The S^{R+} or the S^{R-} represent the

reinforcer that maintains behavior. When these consequences are presented, the response is more likely to occur in the future. Reinforcing qualities are not "possessed" by a stimulus. We assess their reinforcing value by their effect on behavior. If the stimulus is presented (or removed) as a consequence of a certain behavior, which in turn makes the behavior more likely to occur in the future, the stimulus is designated a reinforcer. Circumstances can be arranged to make a stimulus more (or less) likely to be a reinforcer, and are called "establishing operations." An example of an establishing operation is food depriving an animal to ensure that food will serve as a reliable reinforcer (Michael, 1993). Some stimuli reliably serve as reinforcers, which are classified as "primary reinforcers." These include food, water, ambient temperature, and sex. All of these stimuli or events are associated with survival of the species, and their reinforcing effectiveness is usually only lessened through satiation.

Feedback Schedules

The reinforcement contingency "drives" behavior. Behavior, however, can vary along many dimensions, and a deficient animal may have trouble discriminating the relevant dimensions to meet the criterion of a response. In an operant chamber, the completion of an electrical circuit with a lever is counted as a response. Many variations of behavior emitted by the rat in an operant chamber could result in a recorded response: the rat may bump the lever, press it with a paw, poke it with its nose, fall backwards on to it, with several other possibilities without doubt. All of these responses collectively are referred to as the "response class." Even if the subject seems to complete the circuit in a similar way each time a response is recorded (for example, it always presses a lever with its left

paw), there is an inherent variability in responding. Different forces or durations may occur, but would be counted the same way as each other response.

Schedules can be arranged to bring these dimensions of responding under the control of reinforcement contingencies. Reinforcement criteria can be set for particular rates, durations, forces and patterns of operant responding. For example, differential reinforcement of high rates (DRH) and differential reinforcement of low rates (DRL) specify reinforcement criteria for particular response rates. Schedules such as these usually bring behavior under control of the reinforcement, by modifying the targeted dimension of behavior. Acquisition and maintenance of these types of behavior can be altered by the presence of feedback stimuli. The animal receives sensory feedback when a certain behavior is performed and reinforcement follows. This feedback is essential to shaping and maintaining behavior. External feedback stimuli can also supplement the sensory feedback that the animal receives. These stimuli, which are presented contingent upon behavior, indicate the "effectiveness" of that response. In most experimental preparations, feedback can be a tone or a light contingent on the correct emission of a behavior. (A primary reinforcer delivery also acts as feedback for correct behavior as well). This feedback has been found to have effects on acquisition of the behavior during shaping procedures, and to increase response strength of steady state operant behavior (eg. Armus and Mikesell, 1990; Williams and Dunn, 1991;) Feedback has been shown to affect the dimensions of force (Slifkin and Brener, 1998; DiLollo, Ensminger and Notterman, 1965; Fowler and Notterman, 1974), IRT distribution (Hake and Azrin, 1969, Staddon, 1969), topography (Critchfield and Lattal, 1993) and variability (Machado, 1989). The presence of contingent, informative feedback in these studies has resulted in

steady state behavior being acquired more quickly, less behavioral variability at steady state, and less perturbation by various interventions. In other words, informative feedback facilitates "quicker" learning and "stronger" behavior. The feedback is thought to serve the functions of both discriminative stimuli and conditioned reinforcers.

Conditioned reinforcement. Stimuli that are typically neutral can be paired with reinforcing stimuli, to produce "conditioned reinforcers." Second order schedules, including chained, brief stimulus, and tandem schedules are well suited to characterizing the discriminative-stimulus and reinforcing properties of previously neutral stimuli. Second order schedules refer to arrangements in which behavior within a series of components is treated as a unit of behavior, and the completion of which results in delivery of a reinforcer. In second-order schedules, consequences can be the flash of a light rather than a primary reinforcer, and these consequences have powerful effects on behavior. Events that occur as a consequence of behavior can also function as discriminative stimuli. Discriminative control of these stimuli can be assessed through examination of response rate (Lattal, 1991). Based on results of these arrangements, there are many theories on how and why these stimuli come to acquire the properties similar to that of the primary reinforcer.

Brief stimulus schedules. Findley and Brady (1965) found that responding in a chimpanzee increased with presentation of a stimulus change every 400 responses in an FR 4000 reinforcement schedule. This arrangement, now known as a brief-stimulus schedule, is the simplest of the second-order schedules. When a designated formal unit of behavior is completed, a stimulus changes for a very short amount of time. With this stimulus change, a new component starts. This pattern continues until an ultimate

reinforcement delivery. The brief-stimulus is called "paired" if it is also presented just prior to food delivery and "unpaired" if it is not presented just prior to food delivery (Marr, 1979).

Marr (1979) summarizes findings pertaining to conditioned reinforcement using brief stimulus schedules. He concludes that stimuli that become conditioned reinforcers are apparent if there is a rate enhancement when a stimulus is presented compared with when it is not presented, and if behavior during components resembles behavior that is typically obtained when a primary reinforcer is delivered. However, these results do not imply that a stimulus is absolutely a conditioned reinforcer. For example, Stubbs (1971) found that even the presentation of brief stimuli, unpaired with food reinforcement, increased response rates. Results like these require the dual functionality of stimuli to be considered: as both conditioned reinforcers and discriminative stimuli.

Further, the administration of drugs can differentially alter behavior maintained by brief stimulus schedules. Marr (1970) found that chlorpromazine disrupted behavior more when no brief stimulus was delivered after the completion of components in an FR 20 (FI 1 min) than when a brief stimulus was presented after each component.

Summary of contingent-stimuli roles in behavior. Contingent presentations of stimuli can therefore modify both the structure and strength of operant responding. These effects can be contributed to discriminative, feedback and reinforcing properties that the stimuli come to acquire. The functions are not mutually exclusive, and therefore any combination of functions can be allocated to contingently presented stimuli. If the stimulus is often paired with reinforcement, then the stimulus itself may become a reinforcer that maintains behavior. Feedback can make correct emissions of behavior

more likely, while minimizing the emission of extraneous behavior. It may act as a behavioral prosthesis in animals with deficiencies. With contingent discriminative stimuli, behavior often reflects the proximity to availability of reinforcement, and the schedule of reinforcement in place.

Analysis of Operant Behavior: Response Strength and Components of Response Rate

Response rate, the traditional measure of response "strength" is a molar variable. If each response were exactly equal, then rate would probably be enough to analyze the effects of independent variables on behavior. However, different determinants may affect the various dimensions of response rate.

Behavior may usefully be examined as periods of disengagement and periods of engagement, or bouts. A response that terminates a period of disengagement could be considered an initiation response. After initiation, several responses may occur in a row without any disengagement, and this can be considered a bout of behavior. Relevant dimensions of bouts include the length of the bout and the rate of within-bout responding, which may be independently influenced by different environmental manipulations.

Shull, Gaynor and Grimes (2001) propose that independent variables may be grouped according to their effects on these components of responding. They hypothesized that motivation or incentive variables affect bout initiation rates, while more molecular schedule type variables would influence bout lengths and within bout responding. I hypothesize that motor deficits such as those found with methylmercury exposure would likely affect bout length or within bout response rates.

A key problem with executing this component analysis of responding arises when trying to define "engagement" and "disengagement." Figure 3 (from Shull et al, 2001).

illustrates the ambiguity in the classifications of responses. Each hatch on the line represents one response, across a period of 30 seconds. Many of the responses are easily identifiable as initiations or bouts, but in the section labeled "a", it is difficult to determine whether or not the animal was disengaging from a bout or if the rate within a bout just slowed for a period. The figure illustrates that interresponse times are important in defining periods of disengagement and in defining bouts. One approach is to simply specify an IRT, for example 1 second, which would differentiate bouts from disengagement, which is the IRT cutoff method (Shull et al, 2001). The problem with this method is that the IRT distributions probably would overlap, resulting in the misclassification of an unidentifiable number of responses. It also dichotomizes IRTs rather than using the important information found in IRT distributions.

Shull, Gaynor and Grimes (2001) sought to resolve this misclassification dilemma by examining IRT distributions to determine bout initiation rates and bout lengths. In their analysis, all IRTs in a session were collected and sorted in sequential order, beginning with the shortest. IRTs are then plotted on a "log survivor plot", which shows the proportion of IRTs (on the Y-axis) "surviving" or lasting longer than t seconds (on the X-axis). The Markov chain in figure 4 illustrates the probabilistic interpretation of two-mode responding, diagramming engagement versus disengagement.

As previously explained, responding is viewed as periods of disengagement that can be followed by a single response or a series of responses. These probabilities are more easily conceived of as particular rates and lengths associated with responding. A bout initiation rate can be ascertained from the probability of initiating a bout after a period of elapsed time. Once a response has occurred, there can be another response that follows

in a short period of time, or the organism can disengage in the particular behavior. A within bout response rate can be found based on the probability of a response within a small amount of time. Average bout lengths can be calculated based on the probabilities of continuing or ending a bout.

Shull and colleagues developed a computer simulation that would generate different patterns of responding, varying both bout initiation rates and bout lengths based on assigned probabilities of engaging and disengaging in responding. A within bout IRT of .22 seconds was held constant. The simulation was carried out in real time, with 40 alternating 50-second components (the equivalent of a multiple schedule).

Figure 6 shows log survivor plots produced with the computer simulation. Elapsed time (t) in seconds is graphed along the X-axis, which represents the IRT length. The proportion of responses with IRTs greater than t is graphed on the Y-axis. Note the "broken stick" appearance; the initial decline in the slope is very steep, and a second portion declines more slowly. This phenomenon occurs because of two different types of responding. The first, steep, portion represents the within-bout responding characterized by short IRTs, while the second shallower portion represents pausing in responding.

A line was fitted to data falling between 1 second and 12 seconds using equation 1:

$$\mathbf{y = a\ exp^{bt}} \qquad \mathbf{(1)}$$

y = proportion of response times > t

a = proportion of responses that are bout initiations

b= visit initiations per second

t = elapsed time in seconds

The tables under the graphs present the response dimension values that are derived from the formula (except for total responses per minute, which was obtained by dividing total responses by time in the session). Extrapolating the line back to the y intercept will give the proportion of responses that are bout initiations (a); the inverse of this number will give the number of responses per bout (1/a). Visit initiations per minute is obtained by multiplying (b) by 60 seconds.

Simulation 1, represented in the left graph, illustrates a pattern of responding in which responses per bout is held constant. The initial portions of the lines are equal, yielding approximately a value of 2.9 responses/bout. The visit initiation rate was 8.9 initiations per minute for the shallower line (A), and 22.2 initiations per minute for the steeper line (B).

Simulation 2, shown in the right graph of figure 6, reflects a different pattern of IRTs by altering the number of responses per bout while holding initiations per minute and within bout IRT constant. In line B, there is a much steeper initial decline, representing more responses per bout than in line A, with values of 8 resp/bout and 2.9 resp/bout respectively. The second portions of the two lines are parallel, indicating equivalent initiation rates of approximately 8.9 initiations per minute.

Although total response rates are very similar for both lines labeled B in figure 5, the curves on the log survivor plots indicate very different patterns of responding. It becomes apparent through this analysis that similar changes in overall rate can be due to very different changes in patterns of behavior. Shull et al (2001) concluded that the log survivor plots offer a way to examine the components of response rate. To show that components are influenced by different variables, Shull et al (2001) manipulated

motivational variables (reinforcement rates, amount of reinforcement, and percentage of food pellets that are response contingent) and schedule definitions (by adding a tandem response requirement to an interval schedule). The motivational variables and schedule definitions were varied separately in four experimental conditions. In each condition, nose poking in 7 rats was reinforced with food pellets according to the designated schedule (1: mult VI 1 min / VI 4 min; 2: mult VI 2 min with 1 pellet / VI 2 min with 4 pellets; 3: mult VI 1 min / VI 4 min VT 1.33 min; 4: mult VI 2 min /VI 2 min VR 9). They found that the manipulations in the first three conditions, which manipulated motivational variables, all affected bout initiation rate but did not change bout length. Decreasing reinforcement rate decreased bout initiations, as did delivering non-contingent pellets. Increasing the reinforcer magnitude increased the bout initiation rate. Adding a tandem requirement to the VI 2 min, a schedule definition manipulation, increased the length of the bouts without affecting bout initiation rates. These data seem to verify that variables can differentially affect the components of response rate, with "motivational" variables affecting initiations, and schedule requirements affecting bout length.

Shull and Grimes (2003) systematically replicated the findings from Shull et al (2001). They changed the operant response to a lever press, to see if the structure of distribution of key poking would generalize to other operant behaviors. An exponential function was fit like that described in relation to the Shull et al (2001) paper, but a double-exponential fit was also used to determine a within bout response rate (see equation 2).

$$r(t) = (1-p)\, e^{-wt} + pe^{-bt} \qquad (2)$$

r(t) = proportion of IRTs > t (equivalent to t in equation 1)

p = proportion of responses that are bout initiations

(1-p) = proportion of responses that are within bout responses

w = the rate of within bout responding (responses/second)

b = bout initiation rate (responses/second)

Analysis of lever pressing yielded similar results as to those found in nose poking for bout initiation rate, and bout length and total response rate in relation to motivational and schedule definition manipulations. Additionally, within-bout response rate was found to be fairly insensitive to manipulations; only two of four rats showed increased response rates with an addition of a VR requirement to a VI schedule. The lever press as an operant rather than the previously used key poking resulted in a more curved log survivor plot. This is due to more overlap of the distribution of IRTs for within bout responding and pausing, perhaps due to the topography or "effortfulness" of lever pressing versus key poking.

Shull, Gaynor and Grimes (2002) used the log survivor plot analysis to evaluate stimulus control, and resistance to change in both a multiple VI 1 min VI 4 min and a multiple VI 4 min VI 4 min + VT 1.33 min schedule of food reinforcement for key poking. Forty 50s components alternated each session. After behavior became stable for several sessions (varying from 12-43 sessions), a session was run in which the first 10 cycles proceeded according to the established schedule, but the last 30 cycles were run under extinction. Previous research had indicated that total response rates decline more

slowly when a discriminative stimulus associated with a higher reinforcement rate is presented than when a stimulus associated with a lower reinforcement rate. This finding was replicated, and further broken down into components using log survivor plots (using $y = a \exp^{bt}$) and the cutoff IRT method. A log relative resistance index evaluated the extent to which the rich schedule was more resistant to extinction than the lean schedule (see equation 3).

$$\textbf{Log } (R_x/R_0) - \textbf{Log } (L_x/L_0) \qquad \textbf{(3)}$$

R_x = response rate during the rich reinforcement signal during EXT

R_0 = response rate during the rich reinforcement signal during baseline

Lx = response rate during the lean reinforcement signal during EXT

L_0 = response rate the lean reinforcement signal during baseline

The difference between the log ratios indicates the difference between the resistances for the two discriminative stimuli. Bout initiation rates decreased more slowly in the presence of the rich-reinforcement signal. Bout length was not affected by the discriminative stimuli or by extinction.

Summary

This literature review has built a case for examining motor deficits in rats differentially exposed to methylmercury and selenium. Schedule-controlled operant behavior has been shown to be quite sensitive to effects of methylmercury effects previously. A percentile IRT schedule reinforces high rates of behavior, relative to an animal's own ability. It is hoped that this arrangement improves upon previous research

using a DRH schedule, where animals obtained different reinforcement rates with different response rates. Behavior under the percentile schedule, a new arrangement, must be characterized independent of toxicant and dietary manipulations. After a molecular characterization of behavior under the schedule is obtained, the schedule can be evaluated as a tool for toxicological experimentation.

To examine interactions between methylmercury and selenium, it is important to have a level of methylmercury which will certainly result in behavioral deficits (5 ppm), and a level that produces less robust behavioral effects, and sometimes only in the most sensitive animals (0.5 ppm). These levels allow for subtle changes that may deviate when a diet rich or deficient in selenium is provided. The most interesting group comparisons should between diets in the lower methylmercury groups.

If selenium is in fact protective against motor deficits with methylmercury exposure, it is possible that the low selenium group will show detriments, while the high selenium group shows fewer, more delayed, or no motor deficits.

References

Armus, H. L. M., Denise C. (1990). Response effort and interresponse time: Effect of additional, response-produced stimulus feedback. *Bulletin of the Psychonomic Society, 28*(2), 133-134.

Axtell, C. D., Cox, C., Myers, G. J., Davidson, P. W., Choi, A. L., Cernichiari, E., et al. (2000). Association between methylmercury exposure from fish consumption and child development at five and a half years of age in the Seychelles Child Development Study: an evaluation of nonlinear relationships. *Environmental Research, 84*(2), 71-80.

Bornhausen, M., Musch, H. R., & Greim, H. (1980). Operant behavior performance changes in rats after prenatal methylmercury exposure. *Toxicology and Applied Pharmacology, 56*, 305-310.

Branch, M. N. (1991). Behavioral pharmacology. In F. v. Haaren (Ed)., *Techniques in the Behavioral and Neural Sciences: Experimental Analysis of Behavior. Part 2* (pp. 21-78). Amsterdam: Elsevier.

Burbacher, T. M., Rodier, P. M., & Weiss, B. (1990). Methylmercury developmental neurotoxicity: A comparison of effects in humans and animals. *Neurotoxicology and Teratology, 12*, 191-202.

Burbacher, T. M., Sackett, G. P., & Mottet, N. K. (1990). Methylmercury effects on the social behavior of Macaca fascicularis infants. *Neurotoxicology & Teratology, 12*(1), 65-71.

Castoldi, A., Coccini, T., Ceccatelli, S., & Manzo, L. (2001). Neurotoxicity and molecular effects of methylmercury. *Brain Research Bulletin, 55*(2), 197-203.

Castoldi, A., Coccini, T., & Manzo, L. (2003). Neurotoxic and molecular effects of methylmercury in humans. *Reviews on Environmental Health, 18*(1), 19-31.

Catania, A. C., & Reynolds, G. S. (1968). A quantitative analysis of the responding maintained by interval schedules of reinforcement. *Journal of the Experimental Analysis of Behavior, 11*, 327-383.

Coscina, D. V., Yehuda, S., Dixon, L. M., Kish, S. J., & Leprohon-Greenwood, C. E. (1986). Learning is improved by a soybean oil diet in rats. *Life Sciences, 38*(19), 1789-1794.

Critchfield, T. S., & Lattal, K. A. (1993). Acquisition of a spatially defined operant with delayed reinforcement. *Journal of the Experimental Analysis of Behavior, 59*, 373-387.

Crump, K. S., Kjellstrom, T., Shipp, A. M., Silvers, A., & Stewart, A. (1998). Influence of prenatal mercury exposure upon scholastic and psychological test performance: benchmark analysis of a New Zealand cohort. *Risk Analysis, 18*(6), 701-713.

Davidson, P. W., Kost, J., Myers, G. J., Cox, C., Clarkson, T. W., & Shamlaye, C. F. (2001). Methylmercury and neurodevelopment: reanalysis of the Seychelles Child Development Study outcomes at 66 months of age. *JAMA, 285*(10), 1291-1293.

Davidson, P. W., Myers, G. J., Cox, C., Axtell, C., Shamlaye, C., Sloane-Reeves, J., et al. (1998). Effects of prenatal and postnatal methylmercury exposure from fish consumption on neurodevelopment: outcomes at 66 months of age in the Seychelles Child Development Study [see comments]. *JAMA, 280*(8), 701-707.

Dews, P. B., & Wenger, G. R. (1977). Rate dependency and the Behavioral Effects of Amphetamine. In T. Thompson & P. B. Dews (Eds)., *Advances in Behavioral Pharmacology* (Vol. 1, pp. 167-228). New York: Academic Press.

Di Lollo, V. E., W. D; Notterman, J. M. (1965). Response force as a function of amount of reinforcement. *Journal of Experimental Psychology: Animal Behavior Processes, 70*(1), 27-31.

Elsner, J. (1991). Tactile-kinesthetic system of rats as an animal model for minimal brain dysfunction. *Archives of Toxicology, 65*(6), 465-473.

Elsner, J., Suter, D., & Alder, S. (1990). Microanalysis of ultrasound vocalizations of young rats: assessment of the behavioral teratogenicity of methylmercury. *Neurotoxicology & Teratology, 12*(1), 7-14.

Elsner, J., Suter, K. E., Ulbrich, B., & Schreiner, G. (1986). Testing strategies in behavioral teratology: IV. Review and general conclusions. *Neurobehavioral Toxicology & Teratology, 8*(5), 585-590.

EPA. (1997). *Mercury Report To Congress: Health Effects of Mercury and Mercury Compounds* (No. EPA-425/R-97-007). Washington, DC: United States Environmental Protection Agency.

Evans, H. L., Garman, R. H., & Laties, V. G. (1982). Neurotoxicity of Methylmercury in the Pigeon. *NeuroToxicology, 3*, 21-36.

Evenden, J. (1999). The pharmacology of impulsive behavior in rats V: the effects of drugs on responding under a discrimination task using unreliable visual stimuli. *Psychopharmacology, 143*, 111-122.

Findley, J. D., & Brady, J. V. (1965). Facilitation of large ratio performance by use of conditioned reinforcement. *Journal of the Experimental Analysis of Behavior, 8*, 125-129.

Fowler, S. C., & Notterman, J. M. (1974). Some effects upon response force and response rate of variations in amount of sucrose reinforcement. *Journal of General Psychology, 90*(1), 25-38.

Fowler, S. C., Skjoldager, P. D., Liao, R. M., Chase, J. M., & Johnson, J. S. (1991). Distinguishing between haloperidol's and decamethonium's disruptive effects on operant behavior in rats: use of measurements that complement response rate. *J Exp Anal Behav, 56*, 239-260.

Galbicka, G., & Platt, J. R. (1984). Interresponse-time punishment: A basis of shock-maintained behavior. *JEAB, 41*, 291-308.

Galbicka, G., & Platt, J. R. (1986). Parametric manipulation of interresponse-time contingency independent of reinforcement rate. *Journal of Experimental Psychology: Animal Behavior Processes, 12*(4), 371-380.

Ganther, H., Goudie, C., Sunde, M., Kopecky, M., & Wagner, P. (1972). Selenium: relation to decreased toxicity of methylmercury added to diets containing tuna. . *Science, 175*(26), 1122-1124.

Gilbert, S. G., Rice, D. C., & Burbacher, T. M. (1996). Fixed interval/fixed ratio performance in adult monkeys exposed in utero to methylmercury. *Neurotoxicology & Teratology, 18*(5), 539-546.

Gunderson, V. M., Grant, K. S., Burbacher, T. M., Fagan, J. F. d., & Mottet, N. K. (1986). The effect of low-level prenatal methylmercury exposure on visual recognition memory in infant crab-eating macaques. *Child Development, 57*(4), 1076-1083.

Gunderson, V. M., Grant-Webster, K. S., Burbacher, T. M., & Mottet, N. K. (1988). Visual recognition memory deficits in methylmercury-exposed Macaca fascicularis infants. *Neurotoxicology & Teratology, 10*(4), 373-379.

Hake, D. F., & Azrin, N. H. (1969). (1969). A response-spacing effect: An absence of responding during response-feedback stimuli. *Journal of the Experimental Analysis of Behavior, 12*, 17-25.

Harada, M. (1995). Minamata disease: methylmercury poisoning in Japan caused by environmental pollution. *Critical Reviews in Toxicology, 25*(1), 1-24.

Herrnstein, R. J. (1970). On the law of effect. *JEAB, 13*, 243-266.

Imura, N., & Naganuma, A. (1991). Possible mechanisms of detoxifying effect of selenium on the toxicity of mercury compounds. In T. Suzuki, N. Imura & T. Clarkson (Eds.), *Advances in mercury toxicity* (pp. 490). New York: Plenum Press.

Iwata, H., Okamoto, H., & Ohsawa, Y. (1973). Effect of selenium on methylmercury poisoning. *Research Communications in Chemical Pathology & Pharmacology, 5*(3), 673-680.

Julien, R. M. (2001). *A Primer of Drug Action* (9th ed).. New York: Worth Publishers.

Kandel, E. R., Schwartz, J. H., & Jessell, T. M. (1991). *Principles of Neural Science*. Norwalk, CT: Appleton and Lange.

Kandel, E. R., Schwartz, J. H., & Jessell, T. M. (1995). *Essentials of Neural Science and Behavior*. Stamford, CT: Appleton and Lange.

Komsta-Szumska, E., Reuhl, K., & Miller, D. (1983). The effect of methylmercury on the distribution and excretion of selenium by the guinea pig. *Archives of Toxicology, 54*(4), 303-310.

Kuch, D. O. P., John R. (1976). Reinforcement rate and interresponse time differentiation. *Journal of the Experimental Analysis of Behavior, 26*(3), 471-486.

Kulig, B. M., & Jaspers, R. M. A. (1999). Assessment techniques for detecting neurobehavioral toxicity. In R. J. M. Niesink, R. M. A. Jaspers, L. M. W. Kornet, J. M. van Ree & H. A. Tilson (Eds)., *Introduction to Neurobehavioral Toxicology* (pp. 70-113). Boca Raton, Florida: CRC Press.

Laties, V. G. (1978). How operant conditioning can contribute to behavioral toxicology. *Environmental Health Perspectives, 26*, 29-35.

Lattal, A. (1991). Scheduling positive reinforcers. In *Techniques in the Behavioral and Neural Sciences: Experimental Analysis of Behavior.* (pp. 87-134). Amsterdam: Elsevier.

Lattal, K. A. (1991). Scheduling positive reinforcers. In I. H. E. L. Iversen, Kennon A. (Ed). (1991). (Ed)., *Experimental analysis of behavior, Parts 1 & 2. Techniques in the behavioral and neural sciences* (Vol. 6, pp. 87-134). Amsterdam: Elsevier.

Machado, A. (1989). Operant conditioning of behavioral variability using a percentile reinforcement schedule. *Journal of the Experimental Analysis of Behavior, 52*, 155-166.

Marr, J. (1979). Second-order schedules and the generation of unitary response sequences. In M. D. Zeiler & P. Harzem (Eds)., *Reinforcement and the organization of behaviour*. Chichester, New York: Wiley.

Marr, M. J. (1970). Effects of chlorpromazine in the pigeon under a second-order schedule of food presentation. *Journal of the Experimental Analysis of Behavior, 13*, 291-299.

Michael, J. (1993). Establishing operations. *Behavior Analyst, 16*(2), 191-206.

Myers, G. J., Davidson, P. W., Cox, C., Shamlaye, C., Cernichiari, E., & Clarkson, T. W. (2000). Twenty-seven years studying the human neurotoxicity of methylmercury exposure. *Environmental Research, 83*(3), 275-285.

National Research Council. (1992). *Environmental neurotoxicology*. Washington, D. C.: National Academy Press.

National Research Council. (2000). *Toxicological Effects of Methylmercury*. Washington, D.C.: National Academy Press.

Newland, M. C. (1997). Quantifying the molecular structure of behavior: separate effects of caffeine, cocaine, and adenosine agonists on interresponse times and lever-press durations. *Behavioural Pharmacology, 8*, 1-16.

Newland, M. C., & Marr, M. J. (1985). The effects of chlorpromazine and imipramine on rate and stimulus control of matching to sample. *J Exp Anal Behav, 44*, 49-68.

Newland, M. C., & Paletz, E. M. (2000). Animal studies of methylmercury and PCBs: what do they tell us about expected effects in humans? *Neurotoxicology, 21*(6), 1003-1027.

Newland, M. C., & Rasmussen, E. B. (2000). Aging unmasks adverse effects of gestational exposure to methylmercury in rats. *Neurotoxicology and Teratology, 22*, 819-828.

Newland, M. C., Yezhou, S., Logdberg, B., & Berlin, M. (1994). Prolonged behavioral effects of in utero exposure to lead or methyl mercury: reduced sensitivity to changes in reinforcement contingencies during behavioral transitions and in steady state. *Toxicology & Applied Pharmacology, 126*(1), 6-15.

Pinkston, J. W., & Branch, M. N. (2004). Repeated post- or presession cocaine administration: Roles of dose and fixed-ratio schedule. *Journal of Experimental Analysis of Behavior, 81*, 169-188.

Prohaska, J., & Ganther, H. (1977). Interactions between selenium and methylmercury in rat brain. *Chemico-Biological Interactions, 16*(2), 155-167.

Rasmussen, E., & Newland, M. (1999). Acquisition of a multiple DRH Extinction schedule of reinforcement in rats exposed during gestation to methylmercury. *Society of Toxicology Abstracts*.

Rasmussen, E. B., & Newland, M. C. (2001). Developmental exposure to methylmercury alters behavioral sensitivity to *d* amphetamine and pentobarbital in adult rats. *Neurotoxicology and Teratology, 23*, 45-55.

Rice, D. C. (1989). Delayed neurotoxicity in monkeys exposed developmentally to methylmercury. *Neurotoxicology, 10*(4), 645-650.

Rice, D. C. (1992). Effects of pre- plus postnatal exposure to methylmercury in the monkey on fixed interval and discrimination reversal performance. *Neurotoxicology*, *13*(2), 443-452.

Rice, D. C. (1996). Evidence for delayed neurotoxicity produced by methylmercury. *Neurotoxicology*, *17*(3-4), 583-596.

Rice, D. C. (1996). Sensory and cognitive effects of developmental methylmercury exposure in monkeys, and a comparison to effects in rodents. *Neurotoxicology, 17*(1), 139-154.

Rice, D. C. (1998). Lack of effect of methylmercury exposure from birth to adulthood on information processing speed in the monkey. *Neurotoxicology & Teratology, 20*(3), 275-283.

Rice, D. C., & Gilbert, S. G. (1982). Early chronic low-level methylmercury poisoning in monkeys impairs spatial vision. *Science*, *216*(4547), 759-761.

Rice, D. C., & Gilbert, S. G. (1990). Effects of developmental exposure to methyl mercury on spatial and temporal visual function in monkeys. *Toxicology & Applied Pharmacology, 102*(1), 151-163.

Rice, D. C., & Gilbert, S. G. (1992). Exposure to methyl mercury from birth to adulthood impairs high-frequency hearing in monkeys. *Toxicology & Applied Pharmacology, 115*(1), 6-10.

Rice, D. C., & Gilbert, S. G. (1995). Effects of developmental methylmercury exposure or lifetime lead exposure on vibration sensitivity function in monkeys. *Toxicology & Applied Pharmacology, 134*(1), 161-169.

Rice, D. C., & Hayward, S. (1999). Comparison of visual function at adulthood and during aging in monkeys exposed to lead or methylmercury. *NeuroToxicology, 20*, 767-784.

Ross, L., & Schaal, D. W. (2002). Time of supplemental feeding alters the effects of cocaine on lever pressing of rats. *Journal of the Experimental Analysis of Behavior, 77*, 199-208.

Shull, R. L., & Grimes, J. A. (2003). Bouts of responding from variable-interval reinforcement of lever pressing by rats. *Journal of the Experimental Analysis of Behavior, 80*, 159-171.

Shull, R. L. G., Scott T; Grimes, Julie A. (2001). Response rate viewed as engagement bouts: Effects of relative reinforcement and schedule type. *Journal of the Experimental Analysis of Behavior, 75*(3), 247-274.

Shull, R. L. G., Scott T; Grimes, Julie A. (2002). Response rate viewed as engagement bouts: Resistance to extinction. *Journal of the Experimental Analysis of Behavior, 77*(3), 211-231.

Slifkin, A. B., & Brener, J. (1998). Control of operant response force. *Journal of Experimental Psychology: Animal Behavior Processes, 24*(4), 431-438.

Snodgrass, S. H., Hardin, J. L., & McMillan, D. E. (1997). Behavior of rats under fixed consecutive number schedules: Effects of drugs of abuse. *Journal of the Experimental Analysis of Behavior, 68*, 117-132.

Staddon, J. E. R. (1969). The effect of informative feedback on temporal tracking in the pigeon. *Journal of the Experimental Analysis of Behavior, 12*, 27-38.

Steuerwald, U., Weihe, P., Jorgensen, P. J., Bjerve, K., Brock, J., Heinzow, B., et al. (2000). Maternal seafood diet, methylmercury exposure, and neonatal neurologic function. [see comments]. *Journal of Pediatrics, 136*(5), 599-605.

Stubbs, D. A. (1971). Second-order schedules and the problem of conditioned reinforcement. *Journal of the Experimental Analysis of Behavior, 16*, 289-313.

Su, M., Wakabayashi, K., Kakita, A., Ikuta, F., & Takahashi, H. (1998). Selective involvement of large motor neurons in the spinal cord of rats treated with methylmercury. *Journal of the Neurological Sciences, 156*(1), 12-17.

Uauy-Dagach, R., Valenzuela, A. (1996). Marine oils: the health benefits of n-3 fatty acids. [*Nutrition Reviews, 54*(11 PT 2), S102-108.

Wakabayashi, K., Kakita, A., Sakamoto, M., Su, M., Iwanaga, K., & Ikuta, F. (1995). Variability of brain lesions in rats administered methylmercury at various postnatal development phases. *Brain Research, 705*(1-2), 267-272.

Watanabe, C., Yin, K., Kasanuma, Y., & Satoh, H. (1999). In utero exposure to methylmercury and Se deficiency converge on the neurobehavioral outcome in mice. *Neurotoxicology & Teratology, 21*(1), 83-88.

Whanger, P. (1992). Selenium in the treatment of heavy metal poisoning and chemical carcinogenesis. [Review]. *Journal of Trace Elements & Electrolytes in Health & Disease, 6*(4), 209-221.

Williams, B. A., & Dunn, R. (1991). Preference for conditioned reinforcement. *JEAB, 55*, 37-46.

Yasutake, A., Nakano, A., Miyamoto, K., & Eto, K. (1997). Chronic effects of methylmercury in Zenick, H. (1974). Behavioral and biochemical consequences in methylmercury chloride toxicity. *Pharmacology, Biochemistry & Behavior, 2*(6), 709-713.

CHAPTER 2: USING THE LOG-SURVIVOR PLOT ANALYSIS TO EXAMINE THE PERCENTILE IRT 10:0.5 SCHEDULE OF REINFORCEMENT

Abstract

The percentile IRT 10:0.5 schedule reinforces an IRT if it is shorter than the median of the previous ten. The schedule produces high response rates, and minimizes direct effects of reinforcement rate as observed, for example, in a DRH schedule. The resulting response pattern of bouts separated by periods of disengagement was characterized using a log survivor plot analysis (Shull and Grimes, 2003) to provide independent measures of within-bout response rate, bout initiation rate and bout length. For 8 rats, overall response rates ranged from under 50 to over 100 responses/min. These overall rate differences were most closely correlated with within-bout response rates and boutlengths, with a smaller contribution from bout-initiation rates. Intercorrelations among these measures were low, indicating that they contribute independently to overall response rates.

Introduction

The production of high response rates can be achieved by manipulating response contingencies using several types of schedule arrangements. Ratio schedules can produce steady, high rates by delivering a reinforcer after a specific number of responses. These particular schedules do not *require* that response rates are high, but they do reinforce higher rates of behavior, making the high rate an indirect schedule effect (Zeiler, 1977).

For example, under a variable ratio 30 (VR 30), an average of 30 responses must be emitted to receive reinforcement, and the faster that those responses occur, the higher the reinforcement rate. Moreover, responses are likely to be reinforced during a string of consecutive responses, or a response bout, so that short inter-response times are also reinforced. For interval schedules, the link between response bouts and reinforcer delivery is much weaker. Interval schedules set up and hold a reinforcer until a response occurs, so there may be a significant pause just prior to the response that produces the reinforcer. This contributes to a negatively accelerating relationship between response and reinforcer rate under interval schedules, which can be characterized by a hyperbolic function (Catania and Reynolds, 1968; Herrnstein, 1970). Response rate increases result in reinforcer rate increases until an "asymptote" of reinforcement is reached, where no further increase in reinforcer rate occurs. Longer interresponse times (IRTS) may be differentially reinforced in interval schedules, since the longer the subject waits to respond, the more likely a reinforcer will be available (Catania and Reynolds, 1968.) However, the distribution of IRTs associated with either ratio or interval schedules have been altered by deprivation (McDowell and Dallery, 1999), and direct schedule arrangements (Galbicka and Platt, 1986.)

Schedules such as differential reinforcement of high rates of behavior (DRH), as well as IRT < t schedules, set the criteria for reinforcement specifically on high rates of responding. As often employed, a DRH schedule designates a number of responses that must occur in a set amount of time. Newland and Rasmussen (2000) introduced a terminology specifying that a DRH 9:4 s means that 9 responses must occur in four

seconds. IRT < t schedules place contingencies specifically on interresponse times. An IRT < 1 s schedule will deliver reinforcement if a response occurs after an interresponse time of less than 1 second. Both arrangements tend to generate high rates of responding (Lattal, 1991.) Like ratio schedules, reinforcement rates are higher when response rates are higher.

Percentile Schedules

Percentile schedules maintain specific response rates by targeting a specific class of interresponse times, and they do so while holding overall reinforcement rates constant. Therefore, they can break the relationship between response rate and reinforcement rate. In order to minimize the effect of reinforcement rate on behavior in schedules that generate high-rates of behavior, a percentile IRT schedule can be use

Kuch and Platt (1976) developed a schedule arrangement that holds reinforcement rate constant, but differentially reinforces particular IRT classes. Pigeons received reinforcement for emitting either long IRTs or short IRTs, relative to their own baseline IRT distribution. Reinforcement rate remained constant throughout the sessions. Behavior tracked the contingency in place, with either IRTs getting longer or shorter. Therefore, IRTs were differentially reinforced without the effect of reinforcement rate altering responding. When long IRTs were followed by food, the mean IRT rate reached asymptotic levels of about 10 seconds. Response rates increased with increasing reinforcement rate. When short IRTs were followed by food, reinforced IRTs averaged between 0.1 and 0.2 seconds. These IRT values greatly differed from baseline IRT values, which clustered around 1.0 seconds.

Galbicka and Platt (1986) manipulated the degree to which reinforcement depended on IRT duration. Pigeons received food reinforcers for key pecking. A phi coefficient calculation was modified to alter the relationship between reinforcement and IRT duration. Increasing reinforcement rate generally increased response rate when IRT contingencies were weak. By increasing the contingency between reinforcement and long interresponse times, lower response rates and longer IRT durations were produced. With weaker IRT contingencies, where reinforcement was independent of IRT length, response rates tended to increase with increasing reinforcement rates. It was concluded that IRT contingencies account for a majority of the control of responding in VI schedules, with minimal effects of reinforcement rates.

For the present study, contingencies were arranged to hold reinforcement rate constant, at about 2 reinforcers per minute while reinforcing short IRTs. A median interresponse time was calculated for every successive 10 IRTs, and this formed the basis of the reinforcement contingency; only IRTs shorter than the median were eligible for reinforcement. This schedule was intended to create high rates of behavior while breaking the molar relationship between response rate and reinforcer rate. We term this a Percentile IRT (10:0.5) schedule.

Because a specific rate of behavior was not targeted, a range of different response rates (1/IRTs) were obtained but they fell into distinct patterns that could be described as periods of disengagement and periods of engagement, or bouts. Log survivor plots were used to "partition" response rate into components that describe this pattern of behavior: bout-initiation rates, within-bout response rates, and bout lengths (Shull and Grimes, 2003.) Shull, Gaynor and Grimes (2001) proposed that independent variables may be

grouped functionally according to their effects on these components of responding. They hypothesized that motivation or incentive variables affect bout initiation rates, while more molecular schedule type variables would influence bout lengths and within bout responding.

Feedback

When particular dimensions of behavior such as force, rate, IRT length, or duration, are targeted by a reinforcement schedule, acquisition and maintenance can be altered by the presence of feedback stimuli. An animal receives proprioceptive sensory feedback when a certain behavior is performed and reinforcement follows. This feedback is essential to shaping and maintaining behavior. External feedback stimuli can also supplement the sensory feedback that the animal receives. These stimuli, which are presented contingent upon behavior, indicate the "effectiveness" of that response.

In most experimental preparations, feedback can be a tone or a light contingent on a response that meets a contingency. (A primary reinforcer delivery also acts as feedback for correct behavior as well). This feedback has been found to accelerate acquisition of behavior during shaping procedures, and to increase response strength of steady state operant behavior (eg. Armus and Mikesell, 1990; Williams and Dunn, 1991;) Feedback has been shown to affect the dimensions of force (Slifkin and Brener, 1998; DiLollo, Ensminger and Notterman, 1965; Fowler and Notterman, 1974), IRT distribution (Hake and Azrin, 1969, Staddon, 1969), topography (Critchfield and Lattal, 1993) and variability (Machado, 1989). The presence of contingent, informative feedback in these studies has resulted in steady state behavior being acquired more quickly, less behavioral variability at steady state, and less perturbation by various interventions. In other words,

informative feedback facilitates “quicker” learning and “stronger” behavior. The feedback is thought to serve the functions of both discriminative stimuli and conditioned reinforcers.

Summary

The present experiment was designed to engender high rates of behavior while breaking the molar relationship between response rate and reinforcement rate. A schedule that differentially reinforces relatively “short” IRTs without altering reinforcement rate affords the isolation of schedule effects. The study employed a multiple schedule, to examine the effect of external feedback that signaled criterion or “correct” responses in one component and no feedback in the other component. The molecular structure of the behavior emitted in both components was described using log survivor plots, which allows a precise description of the relationships between the components of response rate to overall response rate.

Method

Subjects

Subjects were 7 Long-Evans rats obtained at six weeks of age from Harlan and used as "controls" for an experiment examining the neurotoxicity of methylmercury. Rats were exposed to a 12-hour light-dark cycle, with lights on at 0600 h. Experimental sessions were conducted during the light period. The colony room was environmentally controlled with temperature maintained at 24° C. Four animals maintained on a coconut oil diet, and 3 animals maintained on a fish oil diet began testing at approximately 40 weeks of age. Rats were housed in 22 x 20 x 45cm plexiglass shoebox cages with wire

tops, with free access to water. Aspen chip bedding was provided and changed weekly. Two rats were housed in each cage, separated by a plexiglass partition.

These rats were mated between 17 and 25 weeks of age. Rats were subject to motor test batteries at 7, 11, 14, and 18 months of age, as well as one 12 hour overnight period of access to a running wheel at 12 and 30 months of age (Day, Reed, & Newland, in press.) Throughout the experiment, weights were maintained at 260 g (±10 g.)

Diet

Upon arrival at the laboratory, the 12 week old rats were allowed free access to tap water and rodent chow diet. One week after arriving, the rats were divided into 2 diet groups, equalizing the groups according weight distributions. Four rats began receiving a customized, AIN-93 semi-purified diet containing no docosahexaenoic acid (DHA) (coconut oil diet), while the four rats were fed a semi-purified diet, which was rich in DHA (fish oil diet.) Diets were purchased from either Dyets, Inc (Bethlehem, PA) or Research Diets, Inc (New Brunswick, NJ.) Both mixtures contained 42.8% palm oil, 9.2% safflower oil, and 15.0% soybean oil. The remainder of the diet consisted of either 33% coconut oil or 33% fish oil (which contained a blend of fish, primarily menhaden oil.) The n-3 fatty acids contained in the fish oil diet consisted of 1.6% α-linolenic acid, 5.4% eicosapenaenoic acid (EPA), and 11.2% docosahexaenoic acid (DHA). The coconut oil diet contained 1% α-linolenic acid, with no EPA or DHA. The fish oil diet contained 23.2% of n-6 PUFAs, resulting in an n-6 to n-3 fatty acid ratio of 2.1. The coconut oil diet consisted of 18.1% n-6 PUFAs, with a ratio of 16.5 to n-3 fatty acids.

Dams were exposed to this diet, with consumption monitored, for 4 weeks until breeding started. Weights were maintained at 270 ± 5 g. Upon breeding, dams were

exposed to a coconut or fish oil pregnancy growth diet, which they remained on until weaning of pups. At that time, they were again placed on either the maintenance Fish oil or Coconut oil diet.

Apparatus

A tall modular operant test chamber (Med ENV 007) was used for sessions. Dimensions of the chamber measure 12"L x 9½"W x 11½"H. Aluminum walls were located in the front and back of the chamber, while clear polycarbonate was used for the side and door of the chamber. The typical grid flooring in the chamber was covered from the front of the chamber to within an inch of the back of the chamber with a piece of secured plastic. (This ensured that rats with motor disorders, also being run in the chambers during this time period, would not slip off of the bars.) Two levers were located on either side of the front of the chamber, with a height of 7 cm from the floor. A feeder was located between the levers. Signal lights were located over each lever, at 13.5 cm from the floor. A house light was located above the sipper tube at 13.5 cm from the floor. The operant chamber was enclosed in a sound-attenuating dark chamber.

Procedure

Lever-pressing was established using an autoshaping protocol when the rats were eight months old. Once one-hundred responses occurred under an FR 1 schedule, a second order multiple RI 30" (Percentile 10:0.5 – S^d) RI 30" (percentile-IRT 10:0.5) schedule of reinforcement for lever pressing was implemented. Throughout the session, every IRT was recorded with centisecond resolution. In the first session of the percentile schedule, the first 10 responses in each component were reinforced under an RI 5" schedule. After the 10^{th} response in and for every subsequent response, a criterion IRT was calculated

determining the median of the previous 10 IRTs, and requiring that a response be emitted with an IRT of less than the median in order to be eligible for reinforcement. For example, if the last 10 IRTs were 10", 9", 9", 6", 5" 5", 4", 3", 2", 1", and 1", the criterion for reinforcement for the next response would be a response with an IRT < 5 seconds. With the next response, a new distribution of IRTs is obtained, and a new criterion calculated.

A "feedback" and "no-feedback" component were presented according to a multiple schedule. A feedback component was presented during the first two minutes of the session. The house light was on, and criterion responses were signaled by a 4500 Hz (high) feedback tone was presented for 0.05 seconds. After this two-minute component, a 20 second blackout occurred; all lights were extinguished and lever pressing produced no tones or reinforcement deliveries. A two-minute no-feedback component began after the 20-second blackout elapsed. During this condition, the houselight was off, and the light over the left lever was illuminated but, apart from the absence of a feedback tone, all other contingencies were the same as in the feedback component.

In both conditions, a sucrose pellet reinforcer was delivered after a criterion response under a random interval schedule. Initially the schedule delivered reinforcers an average of every 5 seconds, and this increased up to a terminal value of 30 seconds over the course of eleven sessions. Each of the following RI values was used for one session, and then increased to the higher value for the following session: 5", 6", 7.2", 8.6", 10.4", 12.4", 14.9", 17.9", 21.5", 25.8", and 30". The RI 30" schedule remained in place for the

remainder of the experiment. The feedback and no-feedback components, separated by 20" blackouts, alternated six times in a 28-minute session. Sessions were run Monday through Friday.

Log Survivor Plots

Figure 1 shows an example of a distribution of IRTs from a single session, arranged sequentially from the shortest to the longest. Elapsed time (t) in seconds is graphed along the X-axis, which represents the IRT duration. The proportion of responses with IRTs greater than *t* is graphed on the Y-axis. Note the "broken-stick" appearance; the initial decline in the slope is very steep, and a second portion declines more slowly. This phenomenon reflects two different types of responding. The first, steep portion represents within-bout responding characterized by IRTs less than about 2" in this example. The second shallower portion represents pausing in responding. A two-exponential curve was fit to the distribution of IRTs using a non-linear least squares calculation. From this equation bout-initiation rate, within-bout response rate, and bout length can be calculated (see equation 1).

$$\mathbf{r(t) = (1\text{-}p)\, e^{-wt} + pe^{-bt}} \qquad \mathbf{(1)}$$

r(t) = proportion of IRTs > t (equivalent to t in equation 1)

p = proportion of responses that are bout initiations (.18 in figure 1)

(1-p) = proportion of responses that are within a bout (.82 in figure 1)

w = the rate of within bout responding (responses/second) (.24 r/s in figure 1)

b = bout initiation rate (responses/second) (.01 r/s in figure 1)

1/p = bout length (responses) (5.6 r in figure 1)

Data Analysis

Overall response rate, reinforcer rate, bout-initiation rate, within-bout response rate, bout-length, and median post-reinforcement pause length were analyzed both independently via time-series graphs, and interdependently, using correlations, x-y plots and a linear regression to examine the relationships between the variables. Steady state was only assessed for overall response rates.

Results

Feedback

The presentation of a feedback stimulus contingent on the emission of criterion responses did not change behavior. Figure 2 shows plots of feedback versus no-feedback conditions. For the key measures of response rate, within-bout response rate, bout initiation rate and bout length, behavior during the feedback portion of the multiple schedule looked no different than behavior that occurred during the no-feedback portion. Correlations are specified in the lower right hand corner of each graph. The line in each of these plots indicates the where data would lie if there were a perfect correlation between behavior under the feedback and no-feedback conditions. There is variation of behavior, as indicated by the distribution of data points, but there is no systematic difference in the relationship between the behaviors under each component. Since there are no differences between the conditions, only the feedback component data will be presented.

Overall Response Rate

Average response rates for the feedback component are presented by sessions for each rat in figure 3. Points indicate the average response rate for a particular session.

The line is a lowess curve, which locally weights data points to create a smoothed line (Cleveland, 1981). The top four graphs are rats that received a high-DHA diet, and the bottom four graphs are rats which received a low-DHA diet. Response rates increase to maximum, steady-state rates over the first 20 to 60 sessions. The response rates of all animals except rat 3 stabilized at an asymptotic rate between 40 and 80 responses/min. Rat 3 quickly acquired an asymptotic rate of nearly 150 responses/min, which eventually decreased to a rate which is within the range of other animals. The low-DHA diet was associated a higher response rate (65.3±25.52 r/m) than the high dha diet (47.1±18.6 r/m), even when excluding rat 3 (55.4±17.6 r/m) ($p<.01$). This wide range of rates allows for an examination of how the components of response rates, such as post-reinforcement pause, within-bout response rate, bout initiation rate, and bout length contribute to overall response rate.

Reinforcement Rate

The percentile schedule was designed to minimize the dynamic relationship between reinforcement rate and overall responding, while maintaining high local response rates. Figure 4 shows reinforcement rate as a function of response rate. There is a direct relationship between the two variables at response rates below 30 responses/min (Pearson's $r = .673$), with any faster response rate not resulting in faster reinforcement rates. Therefore, the schedule succeeds in minimizing the effects of reinforcer rate on response rate (and vice-versa) when response rates greater than 30 responses per minute are produced (Pearson's $r = .09$). The average response rates of all animals are well above this level, so animals are receiving ~ 2 reinforcer/min across the experiment.

Post Reinforcement Pauses

Reinforcers were not always collected immediately upon delivery. Because of the high-rate behavior engendered by the schedule, the rat often responded several times after the reinforcer was delivered before consuming the pellet. Therefore, the definition of the post reinforcement pause, as being initiated by the delivery of a reinforcer, could not be used. After inspection of data records, it was decided that the first IRT that lasted longer than 3 seconds after the delivery of a reinforcer was coded to be coded as a post-reinforcement pause.

The boxplot in figure 5 shows variation within rats and between rats of PRP lengths. The error bars represent 95% confidence intervals. The box includes the 25^{th} to 75^{th} percentiles, with the line within the box indicating the median PRP duration. There is some variation in length for each rat, but there are very few outliers, indicating that PRP is fairly stable both within and across rats throughout acquisition and steady state responding.

Log Survivor Plots

Not all interresponse time distributions were amenable to a log survivor plot analysis. The analysis relies on a two-component distribution on IRTs, and if there is only one component, or more than two components, the log survivor plots are typically not good fits. Also, if response rates were very low, and therefore there were few IRTs, the analysis did not work well. Overall, 572 out of 687 (83%) of the log survivor plots represented good "fits" of the two-component conception of responding. All of the rats, except R40, had over 80% of their distributions the log survivor plots. R40 had only 58% distributions fit.

Figure 6 shows an example of a poor-fitting log survivor curve. Because of the insufficient number of responses, there is not a broken-stick appearance similar to figure 1. There is not evidence of engagement versus disengagement in responding for this example. Notice that the bout lengths derived from this IRT distribution would be nonsensical. The inverse of -3.91, or -.26, should represent the length of bursts, but obviously does not represent a real possibility. Also note that the within-burst response rate (1.24 r/s) and bout initiation rate (1.03 r/s) are not very different, which would also indicate that there are not different components of responding.

Log survivor functions which produce data similar to this example is dropped from analysis. The computer automatically excludes this data based on the following rules:

- Either intercept (p or (p-1)) <0 or >1
- A slope of the fast component (w) <0 or >15
- A slope of the slow component (b) <0 or > 3
- The bout length (1/p) <0 or > 100

Within-Bout Response Rates

The acquisition of within-bout response rates for each rat is presented in figure 7. Variation of this measure occurs both within and between rats. The lowest within-bout response rates, between .8 and 2 responses/second are evident in R39. This rat also has some of the lowest overall response rates (figure 3.) The highest within-bout response rates reach over 5 responses/sec in R6. Most within-bout response rates occur between 2.5 and 4 responses/second.

Figure 8 shows the direct relationship between within-bout response rate and overall response rate. The correlation between the two measures is r = 0.59, accounting for 36%

of the variability. As within bout response rates increase from 0.5 to 6 responses/sec, overall response rates increase from ~20 responses/min to 150 responses/min.

Bout Initiation Rate

The acquisition of bout initiation rates are presented for each rat in figure 9. This measure, which represents the rate at which new bouts of responding are initiated, seems to be the component of response rate that is least stable for each rat. The highest initiation rates reach about 1 initiation every 2 to 2.5 seconds (0.5 to 0.4 initiations/s). Note that the anomalous shape of the time-series graph for overall response rates in R03 (figure 3) is replicated in this figure. This more than likely indicates that the increasing-then-decreasing overall response rates in R03 were primarily due to bout-initiation rates. Shape-similarities between overall response rates and initiation rates also exist for R39 and R10.

Figure 10 presents total response rate as a function of bout initiation rate for all rats. There is a positive relationship between these two variables, with a moderate correlation between the two (Pearson's r = .387). Most initiation rates fall between .1 and .3 r/s (x-axis), which are associated with overall response rates varying from 30 to 120 response/min (y-axis).

Bout Length

Bout lengths vary greatly among rats and sometimes within a single rat over time (see figure 11.) Bout lengths tend to average less than 10 responses, but can reach lengths of 40 to 50 at various times. Again, the curve of R3 differs from the curves of other animals, which appears to be inversely related to both overall response rate and bout initiation rate. As rates increase in R3, bout lengths decrease. The relationship between

bout rate and overall response rate is curvilinear, with increasing bout lengths having less impact on overall response rates. In figure 12, the log (base 10) of bout length has been plotted against response rates. A doubling of bout length results in an average increase in overall response rate of ~10 r/m. The line ($y = -1.42 + 56.8 * \log_{10}(x)$) represents the regression of the logged bout length against overall response rate. Overall, there is a positive relationship ($r = 0.654$) between logged bout lengths and overall response rate.

Relationships among all variables

The primary variables being examined: overall response rate, reinforcer rate, within bout response rate, initiation rate, bout length, and post reinforcement pause length, may have various levels of interrelatedness or independence to each of the other variables. Table 1 shows the Pearson's r correlations among all variables. Overall response rate is highly correlated to all components of response rates derived from the partition analysis, and a low correlation with reinforcer rate, as described in previous sections. Reinforcement rate is weakly correlated with all measures, confirming its independence from response rate. Large changes in response rates or in any of its components do not produce comparable changes in reinforcer rates. Within bout response rate, initiation rate, bout length and post-reinforcement pause are all weakly correlated with one another, indicating that each makes a separate contribution to response rate. The highest correlation amongst these measures is between bout length and initiation rate, at -.306. This means that as bout lengths get longer, initiation rates get slower. This could be because when engaging longer bouts, there is necessarily less opportunity to initiate a response, or it could be due to longer pauses after longer bouts.

Regression

To determine the importance of the contributions of within-bout response rate, bout initiation rate, bout length and post reinforcement pause to overall response rate, a linear regression was performed. Linear regression uses data to predict a single value, in this case, overall response rate. An equation is produced can be used to predict the values of one dependent variable (response rate) from known values of one or more independent variables (the components of response rate.)

The log conversion of bout length was used because of the curvilinear relationship between bout length and response rate. All measures were converted to units of responses and responses/second. Two equations are derived from the linear regression. Equation 2 shows the regression line that best fits the available data. Actual values of contributing variables are used to predict overall response rate. Equation 3, the "standardized" equation, indicates the relative importance of the variables contributing to the dependant variable. Values are converted to z-scores for each measure. The two equations follow:

Regression equation

Overall Response Rate (r/s) = -.86 + [-0.03*PRP(s)] + [0.10*Within Bout Rate (r/s)] + [2.48*initiation rate (r/s)] + [1.10* Log_{10} (Bout length)(r)] **(2)**

Standardized Regression

Overall Response Rate (r/s) = [0.28*Within Bout Response Rate (r/s)] + [0.55*Bout Initiation Rate (r/s)] + [0.76*Log_{10} (Bout Length)(r)] – [0.10*PRP (s)] **(3)**

Equations 2 accounts for 89.4% of the variability of response rate, showing that these components account for most of the variability found both between and within a subject. Most individual R^2 values range from .83 to .90 (accounting for 83 – 90 % of the variability), except for rat 42 (R2 = .79). The regression equation fit individual subjects, as well as data across subjects. Figure 13 shows the values predicted by the regression equation (x-axis) plotted against the actual response rates. The line indicates where data points would be located with a perfect correlation. Data falls very close to this line, with most of the variability occurring at high rates of behavior.

Discussion

The percentile IRT schedule was designed so as to minimize the dynamic interaction between response rate and reinforcement rate. The correlation between these two measures was quite low, and was virtually zero at response rates greater than 30 r/min, indicating that the relationship between response rate and reinforcer rate were independent of one another. At the lowest response rates (less than 30 r/min), there was a positive correlation between the two measures. The low response rates were only exhibited during the acquisition phase of the experiment, and not during steady state responding. Reinforcement rate, therefore, contributed to acquisition of behavior under the schedule, but not to the maintenance of steady state behavior.

The addition of feedback contingent on the emission of criterion responses had no effect on behavior. This could be due to a carryover effect from the feedback to the no-feedback portion of the multiple schedule. Because both were presented in the same session, the feedback that signaled relatively high rates of responding during one component may have also acted as a discriminative stimulus in general for the production

of short IRTs. It is also possible that the stimulus was totally neutral, and behavior would have looked the same if no stimulus had been presented at all.

The conceptualization of response rate as a measure consisting of several components was helpful in explaining the structural differences between response rates that can not otherwise be explained. Responding was broken into measures representing periods of engagement, pauses to consume reinforcers, and pauses to engage in "other" behaviors. The analysis of structural differences in response rates revealed orderly relationships that were obscured in the molar rate measure. For all animals, responding occurred in bouts of behavior separated by periods of pausing.

Scatterplots and correlations, suggest that bout length and within bout response rate are the strongest determinants of response rate. Post-reinforcement pauses do not vary greatly between or within subjects, and were not highly correlated with overall rate. Initiation rates are moderately correlated with response rates.

These analyses confirm that analysis of bouts using log survivor plots can be useful for explaining the relationships between molecular components of behavior. Shull, Gaynor and Grimes (2001), postulated that 'schedule-type variables' would primarily influence within-bout response rate, and bout-lengths. The percentile IRT 10:0.5 schedule arrangement appeared to primarily target within-bout response rates, which resulted in a relatively small amount of variability as indicated by the standardized regression equation. A small change in within bout response rate, equal to ±0.28*standard error, results in a change of ± 1 standard error of overall response rates. Other measures, such as bout length, require a much larger change in value (±0.76*standard error) to result in the same change in overall response rate. Bout

initiation rates and the bout lengths contributed the most to overall response rates, as indicated by the regression equation. Post reinforcement pause rates were stable both within and between subjects, and therefore did not contribute much to overall response rate differences. Finally, although highly correlated with overall response rate, the contribution of within-bout response rates to the variability in total response rate was only moderate, most likely due to the small amount of variability both within and between animals for this measure.

The animals from this experiment served as control animals in a chronic methylmercury exposure experiment. Animals exposed to methylmercury typically have lower response rates than control animals, and analysis of the components of response rate may be able to elucidate the nature of the different structures of behavior emitted by these different groups of animals. The feedback/no-feedback manipulation was used because of the implication that feedback could act as a "behavioral prosthesis." With feedback, there may less discrepancy between the behavior of a normal animal and an affected animal. Perhaps a difference between the two components will be evidenced with disabled animals.

References

Armus, H. L., & Mikesell, D. C. (1990). Response effort and interresponse time: Effect of additional, response-produced stimulus feedback. *Bulletin of the Psychonomic Society, 28*(2), 133-134.

Catania, A. C., & Reynolds, G. S. (1968). A quantitative analysis of the responding maintained by interval schedules of reinforcement. *Journal of the Experimental Analysis of Behavior, 11, 327*-383.

Cleveland, W. S. (1981). LOWESS: A program for smoothing scatterplots by robust locally weighted regression. *The American Statistician, 35, 54, 35*, 54.

Critchfield, T. S., & Lattal, K. A. (1993). Acquisition of a spatially defined operant with delayed reinforcement. *Journal of the Experimental Analysis of Behavior, 59*, 373-387.

Day, J., Reed, M., & Newland, M. C. (In review). Neuromotor Deficits in Aging Rats Exposed to Methylmercury and n-3 Fatty Acids. *Neurotoxicology and Teratology.*

Di Lollo, V. E., W. D; Notterman, J. M. (1965). Response force as a function of amount of reinforcement. *Journal of Experimental Psychology: Animal Behavior Processes, 70*(1), 27-31.

Fowler, S. C., & Notterman, J. M. (1974). Some effects upon response force and response rate of variations in amount of sucrose reinforcement. *Journal of General Psychology, 90*(1), 25-38.

Galbicka, G., & Platt, J. R. (1986). Parametric manipulation of interresponse-time contingency independent of reinforcement rate. *Journal of Experimental Psychology: Animal Behavior Processes, 12*(4), 371-380.

Hake, D. F., & Azrin, N. H. (1969). (1969). A response-spacing effect: An absence of responding during response-feedback stimuli. *Journal of the Experimental Analysis of Behavior, 12*, 17-25.

Herrnstein, R. J. (1970). On the law of effect. *Journal of the Experimental Analysis of Behavior, 13*, 243-266.

Kuch, D. O., & Platt, J. R. (1976). Reinforcement rate and interresponse time differentiation. *Journal of the Experimental Analysis of Behavior, 26*(3), 471-486.

Lattal, A. (1991). Scheduling positive reinforcers. In *Techniques in the Behavioral and Neural Sciences: Experimental Analysis of Behavior.* (pp. 87-134). Amsterdam: Elsevier.

Machado, A. (1989). Operant conditioning of behavioral variability using a percentile reinforcement schedule. *Journal of the Experimental Analysis of Behavior, 52*, 155-166.

Shull, R. L. G., Scott T; Grimes, Julie A. (2001). Response rate viewed as engagement bouts: Effects of relative reinforcement and schedule type. *Journal of the Experimental Analysis of Behavior, 75*(3), 247-274.

Shull, R. L., & Grimes, J. A. (2003). Bouts of responding from variable-interval reinforcement of lever pressing by rats. *Journal of the Experimental Analysis of Behavior, 80*, 159-171.

Slifkin, A. B., & Brener, J. (1998). Control of operant response force. *Journal of Experimental Psychology: Animal Behavior Processes, 24*(4), 431-438.

Staddon, J. E. R. (1969). The effect of informative feedback on temporal tracking in the pigeon. *Journal of the Experimental Analysis of Behavior, 12*, 27-38.

Williams, B. A., & Dunn, R. (1991). Substitutability between conditioned and primary reinforcers in discrimination acquisition. *Journal of the Experimental Analysis of Behavior, 55, 21-35., 55*, 21-35.

Figure and Table Captions

Figure 1: Example of a log-survivor plot. Time (t) (in seconds) is expressed on the x-axis, and the proportion of IRTs greater than t is represented on the logged y-axis. Points indicate individual IRT lengths. The line is a double-exponential fit of the IRT distribution. Note the two-component shape of the function. The slope of the first portion represents the within-bout response rate, while the slope of the second portion represents bout-initiation rates. The inverse of the Y-intercept of the second slope represents the bout-length.

Figure 2: Total response rate, within bout initiation rate, bout initiation rate, and bout length plotted for the feedback component (x-axis) versus the non-feedback component (y-axis). Pearson's correlations indicated in lower right hand corner of each graph. Line indicates a slope of 1, or where points would lie if Pearson's r=1.0.

Figure 3: Overall response rates for 100 session. The top four graphs are animals exposed to a high DHA diet, and the lower four graphs are animals exposed to the low DHA diet. Points represent response rate averages for each session. The line is a lowess smoother to make the acquisition of responding easier to detect.

Figure 4: Reinforcement rate as a function of response rate. Each point represents data, with a smoothed lowess line to show the trend in the relationship between the two variables. The correlation between the variables is .189 (Pearson's r value.)

Figure 5: Boxplot of post reinforcement pause lengths in seconds for each rat. The error bars represent 95% confidence intervals. The box includes the 25^{th} to 75^{th} percentiles, with the line within the box indicating the median PRP duration.

Figure 6: Example of a poor-fitting log survivor. For this IRT distribution, there is no evidence of two-component responding (there is no bout-then-pause pattern.)

Figure 7: Within-bout response rate (r/sec) for each rat over sessions. Points represent the slope (w) of the "fast" portion of the log survivor plot, with a lowess smoothed line. Some sessions have no points because a good fit could not be obtained for the log-survivor analysis.

Figure 8: Overall response rate as a function of within-bout response rate. There is a strong correlation between these two measures (Pearson's r = .594).

Figure 9: Bout initiation rates (r/sec) for each rat across sessions. Points represent the slope (b) of the "slow" portion of the log survivor plot, with a lowess smoothed line. Some sessions have no points because a good fit could not be obtained for the log-survivor analysis.

Figure 10: Overall response rate as a function of bout-initiation rate. There is a moderate correlation between these two measures (Pearson's r = .387).

Figure 11: Bout lengths for each rat across sessions. Points represent individual bout lengths, with a lowess smoothed line. Some sessions have no points because a good fit could not be obtained for the log-survivor analysis.

Figure 12: Overall response rates as a function of bout length. The bout lengths have been plotted on a log axis. The correlation between the logged bout length and response rates is higher than all other measures (r = .654)

Figure 13: Overall response rate values as predicted by the regression equation are plotted against actual overall response rates. The correlation between the two measures is r = 0.95.

Table 1: Correlations amongst overall response rate, reinforcer rate, within-bout response rate, bout-initiation rate, bout length, and post-reinforcement pause length.

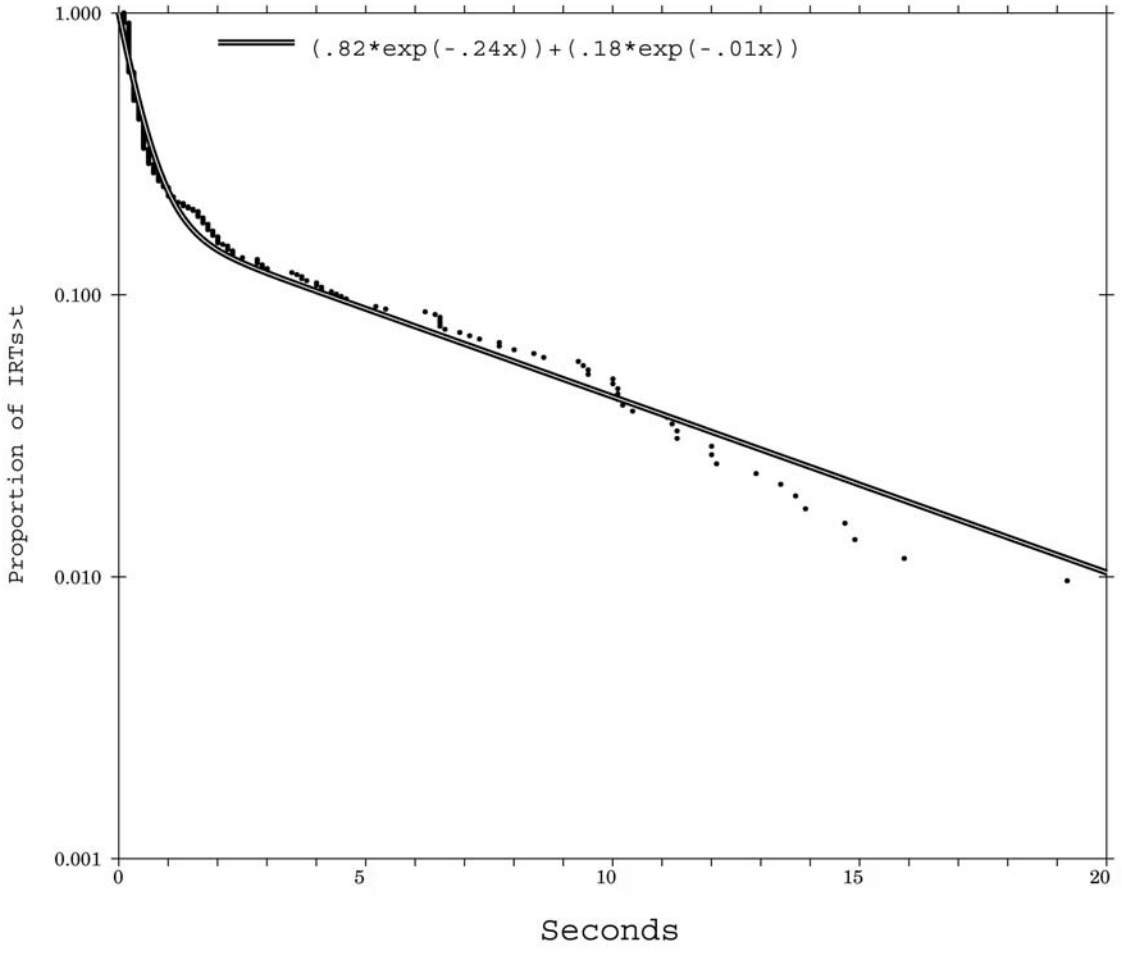
(.82*exp(-.24x))+(.18*exp(-.01x))
1.000
0.100
0.010
0.001
Proportion of IRTs>t
0
5
10
15
20
Seconds

Figure 1

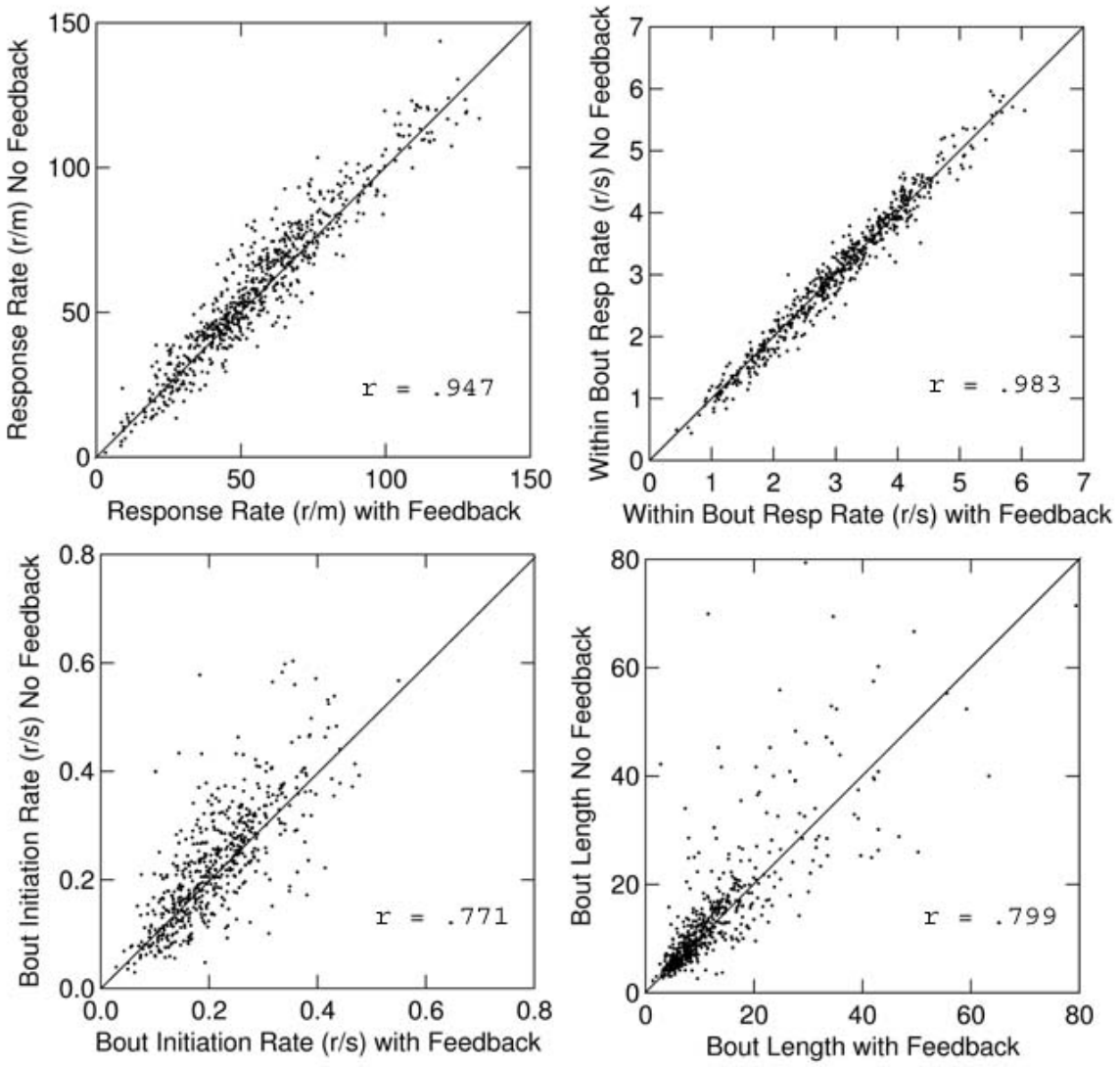
Response Rate (r/m) No Feedback
Response Rate (r/m) with Feedback
r = .947
Within Bout Resp Rate (r/s) No Feedback
Within Bout Resp Rate (r/s) with Feedback
r = .983
Bout Initiation Rate (r/s) No Feedback
Bout Initiation Rate (r/s) with Feedback
r = .771
Bout Length No Feedback
Bout Length with Feedback
r = .799

Figure 2

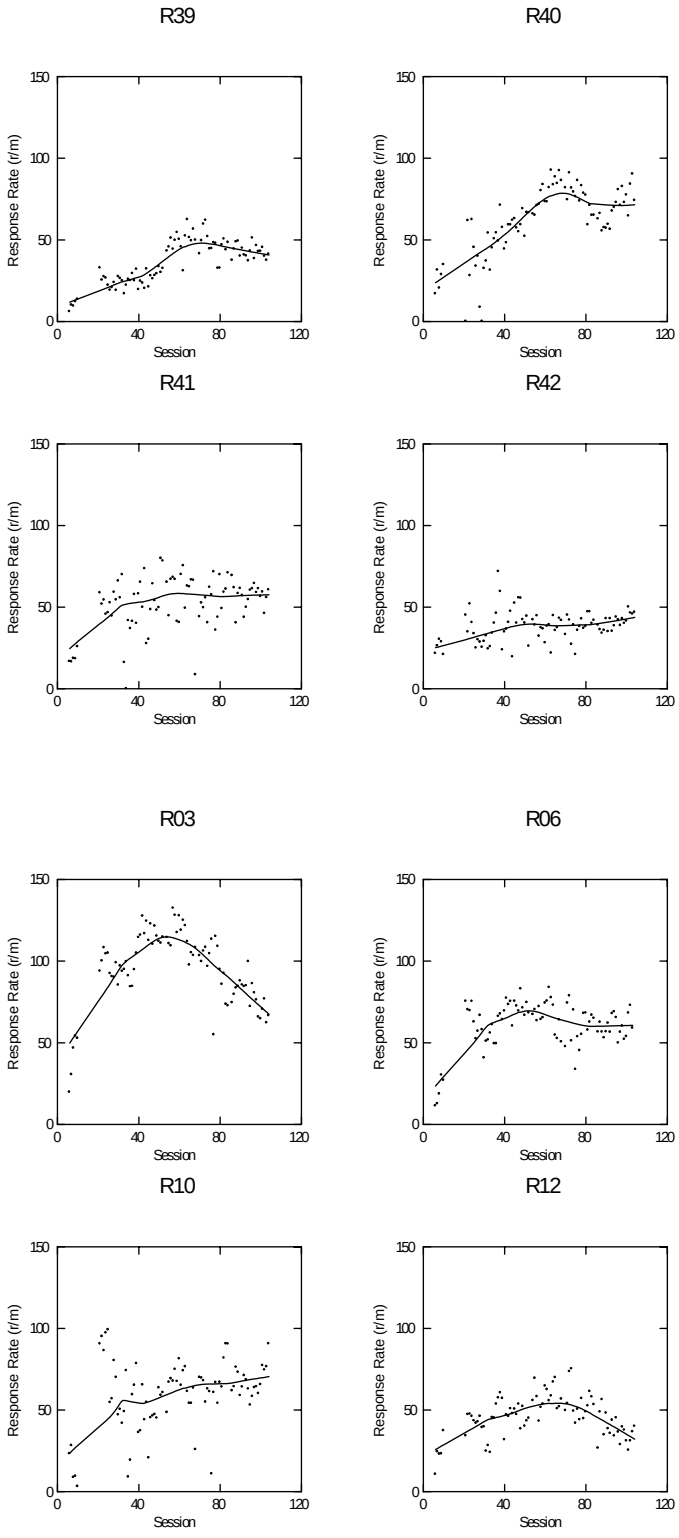
R39
R40
R41
R42
R03
R06
R10
R12
Response Rate (r/m)
Session
0
40
80
120
50
100
150

Figure 3

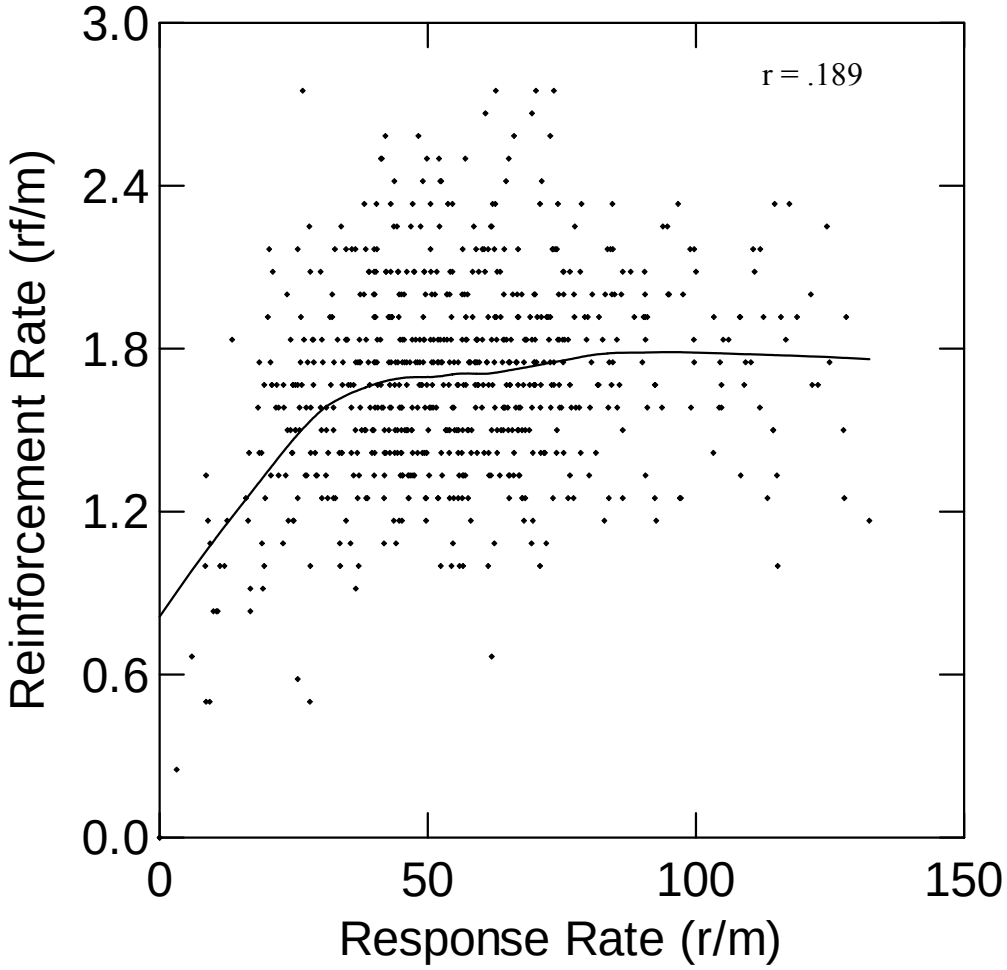
r = .189
Reinforcement Rate (rf/m)
Response Rate (r/m)
3.0
2.4
1.8
1.2
0.6
0.0
0
50
100
150

Figure 4

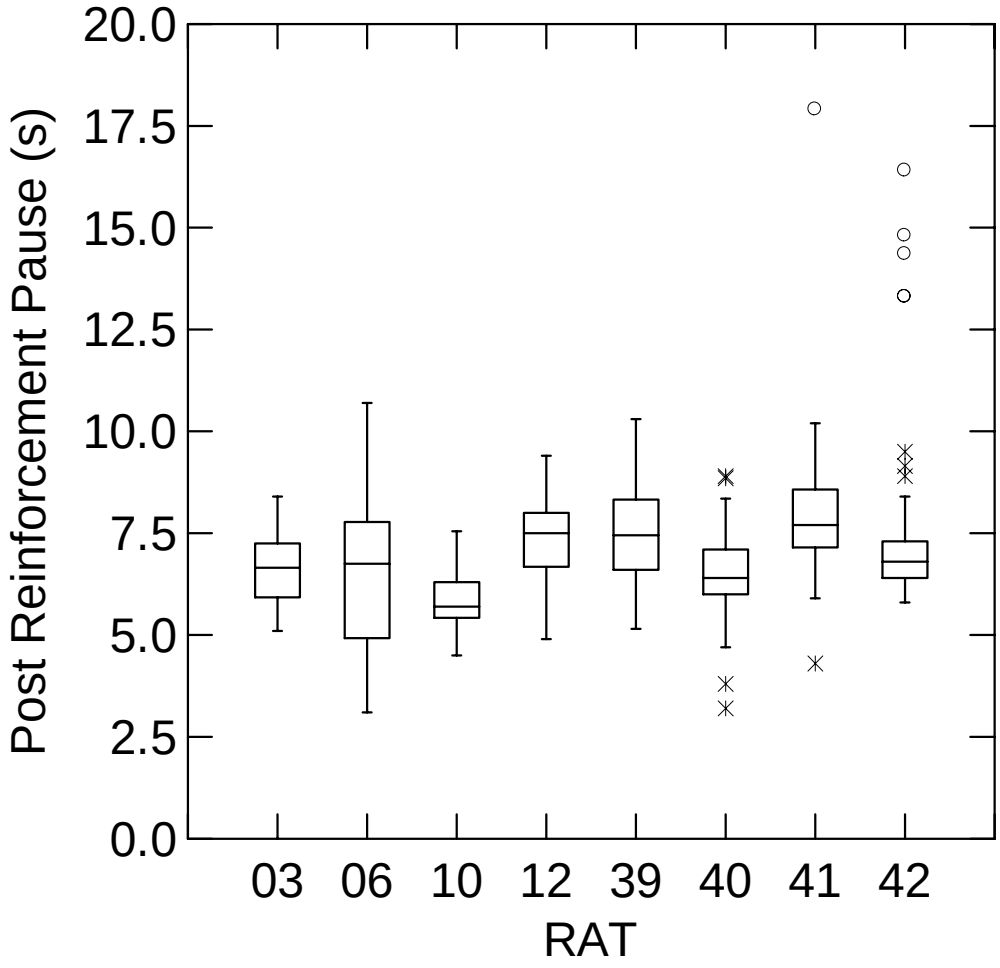
Post Reinforcement Pause (s)
20.0
17.5
15.0
12.5
10.0
7.5
5.0
2.5
0.0
03
06
10
12
39
40
41
42
RAT

Figure 5

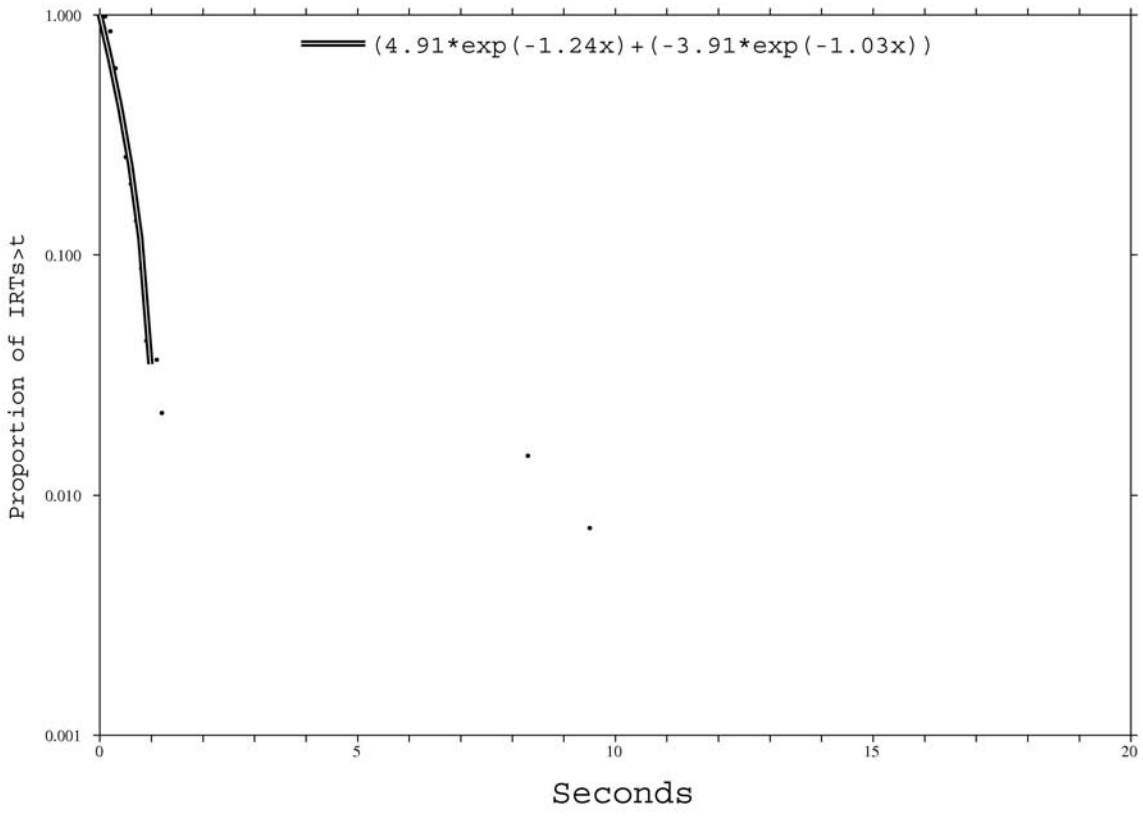
(4.91*exp(-1.24x)+(-3.91*exp(-1.03x))
Proportion of IRTs>t
1.000
0.100
0.010
0.001
0
5
10
15
20
Seconds

Figure 6

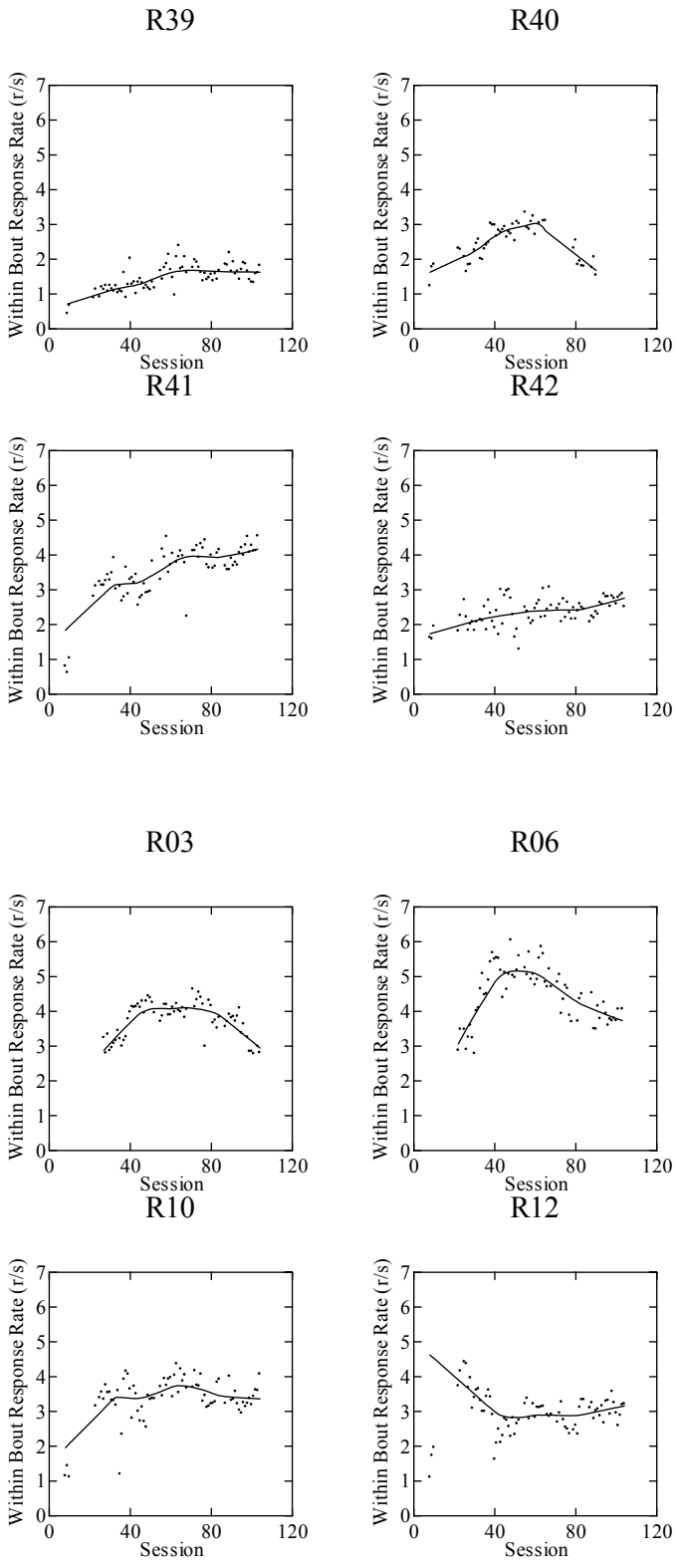
R39
R40
R41
R42
R03
R06
R10
R12
Within Bout Response Rate (r/s)
Session
0
40
80
120
1
2
3
4
5
6
7

Figure 7

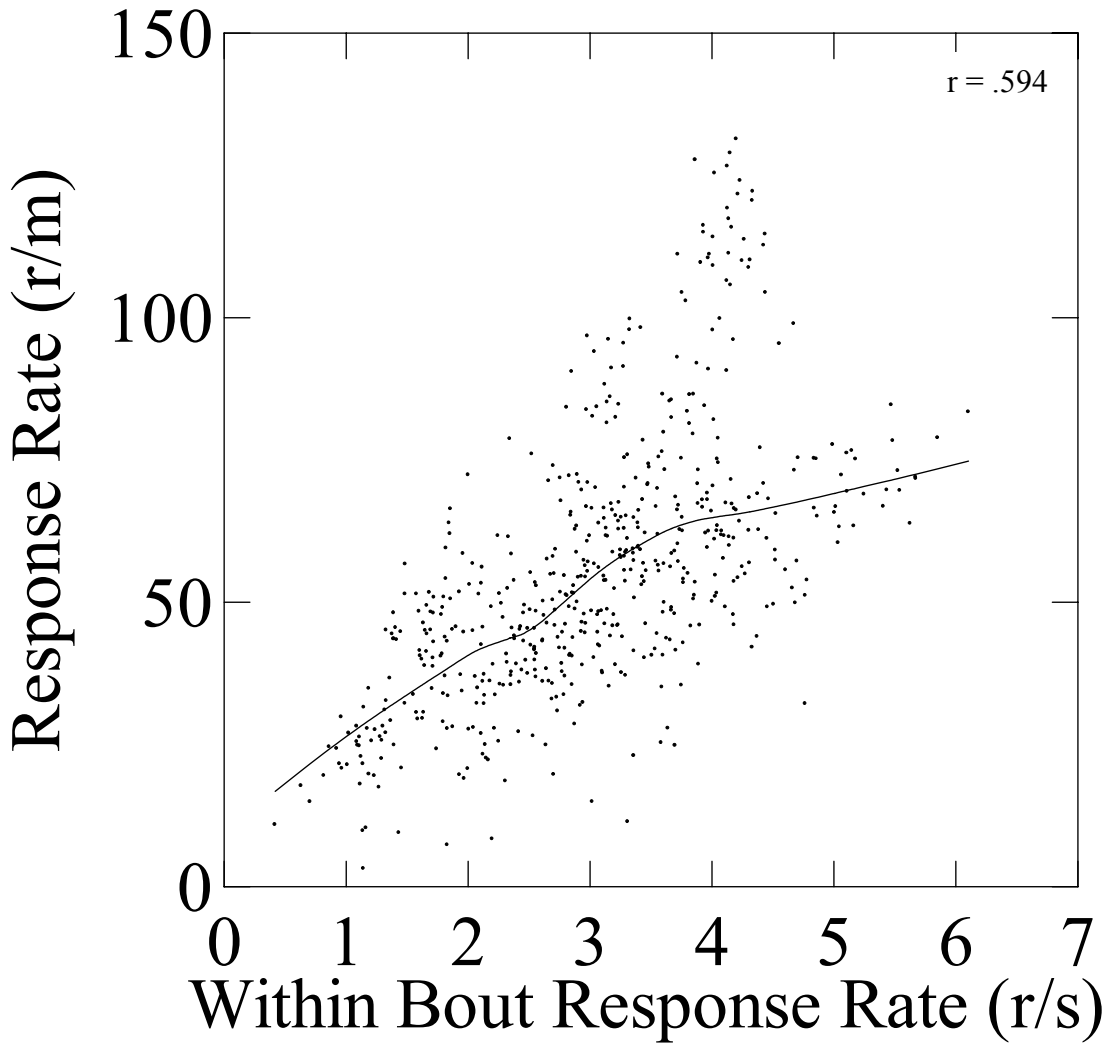
150
100
50
0
r = .594
Response Rate (r/m)
0
1
2
3
4
5
6
7
Within Bout Response Rate (r/s)

Figure 8

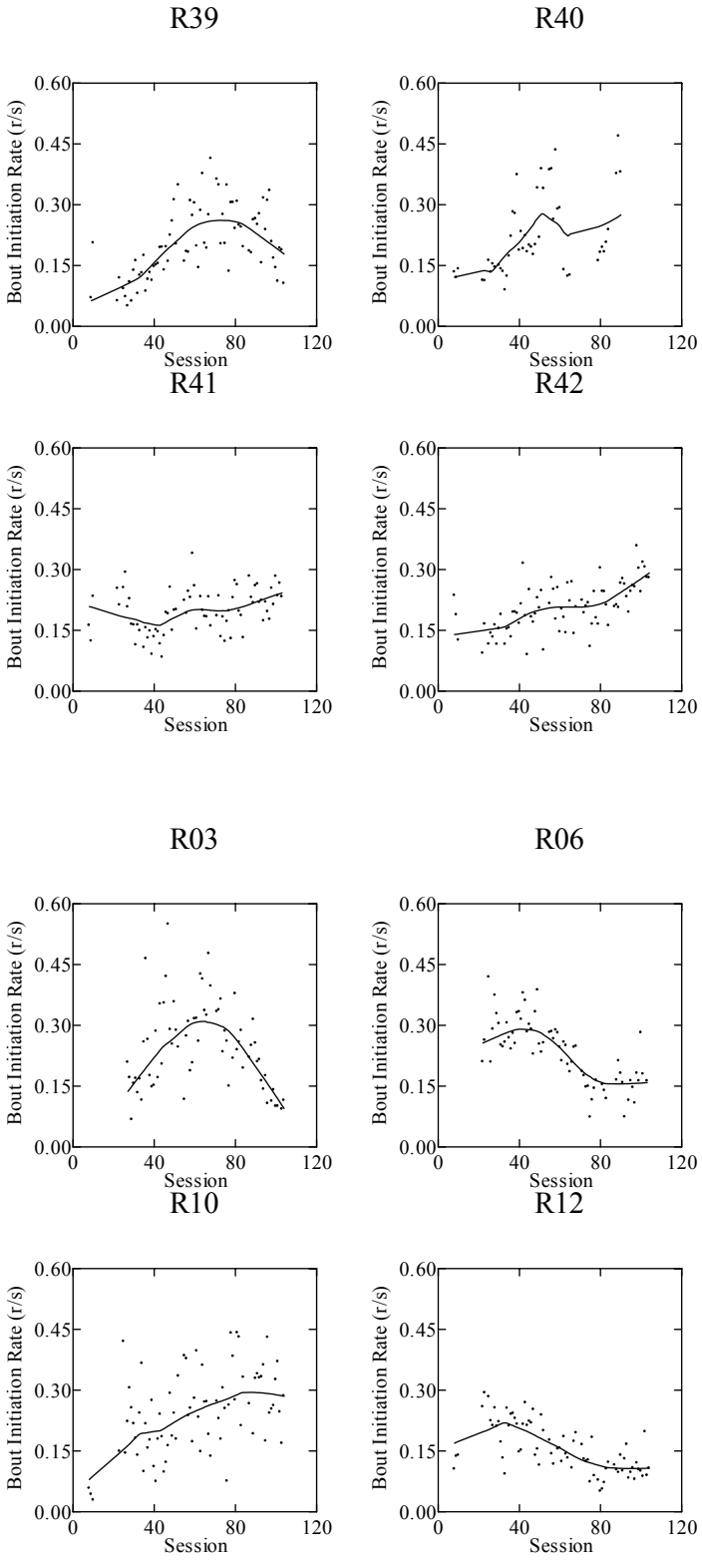
R39
R40
R41
R42
R03
R06
R10
R12
Bout Initiation Rate (r/s)
0.00
0.15
0.30
0.45
0.60
0
40
80
120
Session

Figure 9

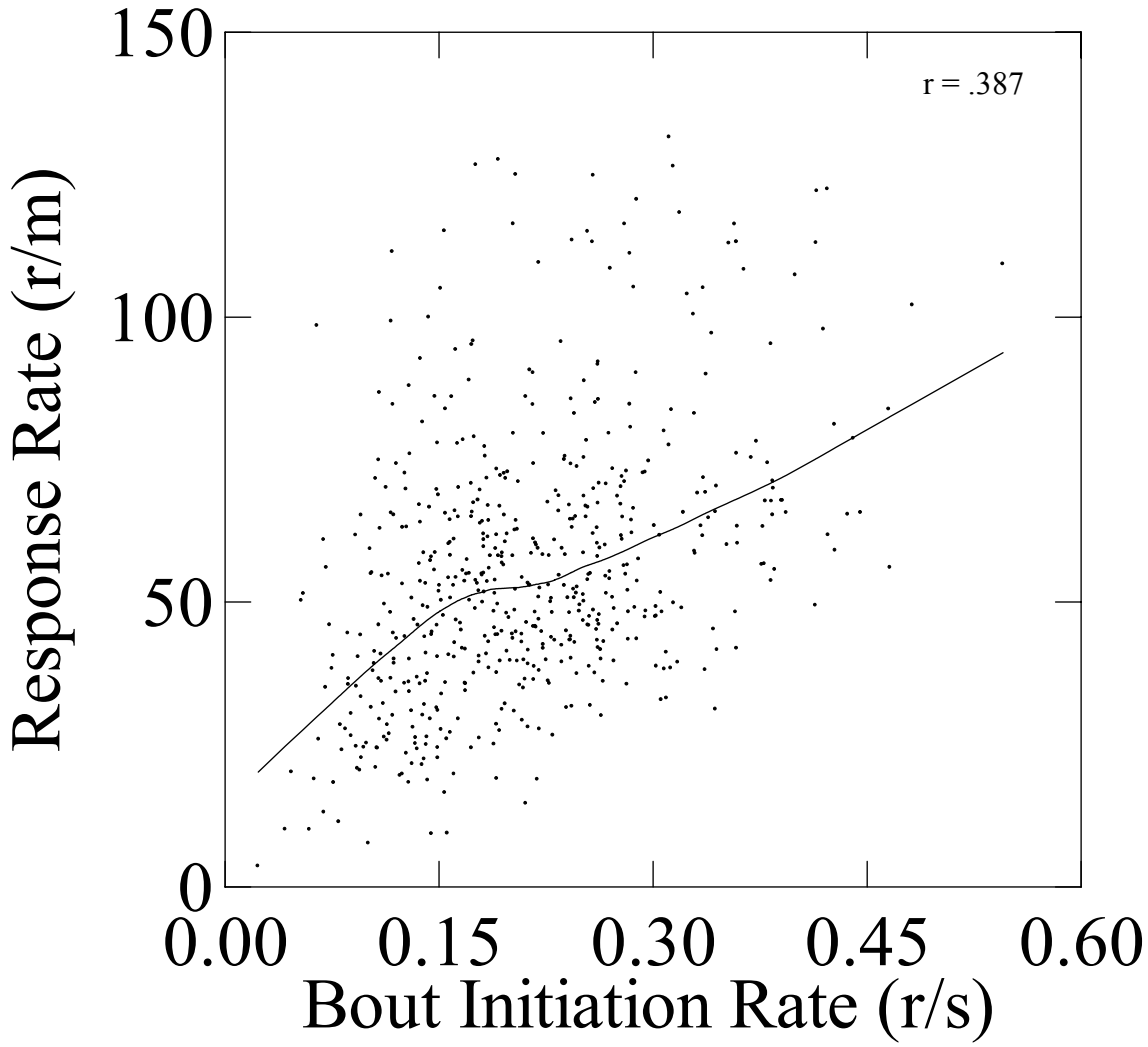
150
100
50
0
r = .387
Response Rate (r/m)
0.00
0.15
0.30
0.45
0.60
Bout Initiation Rate (r/s)

Figure 10

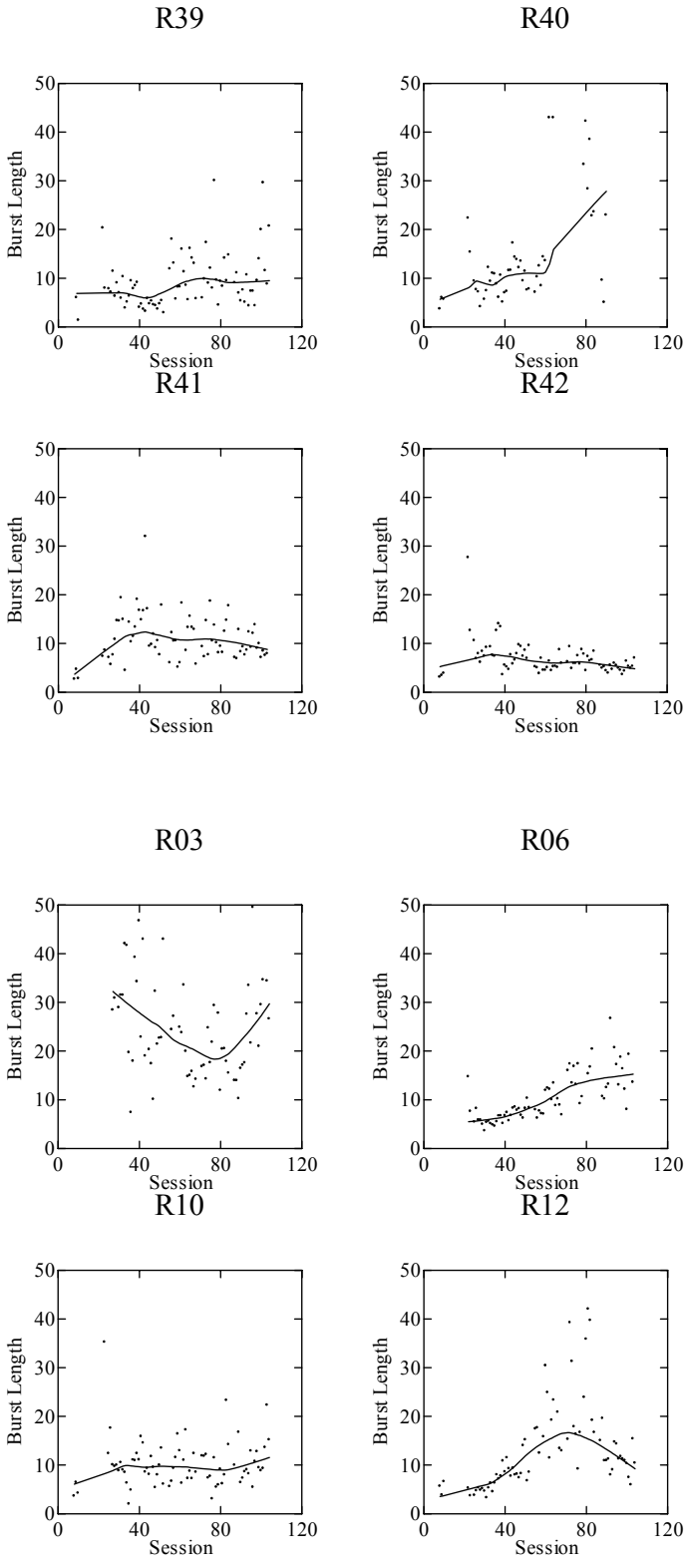
R39
R40
R41
R42
R03
R06
R10
R12
Burst Length
Session
0
10
20
30
40
50
0
40
80
120

Figure 11

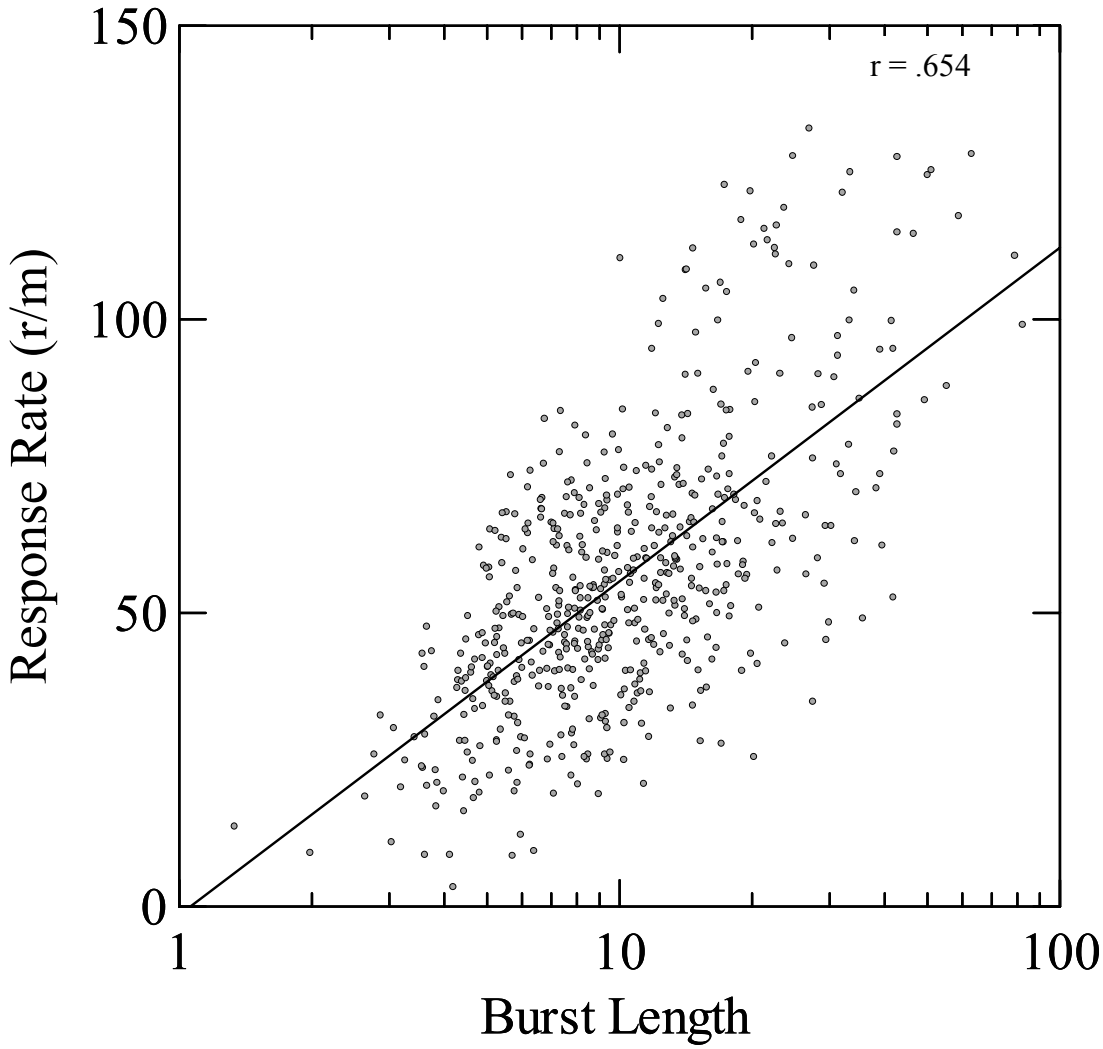

Figure 12

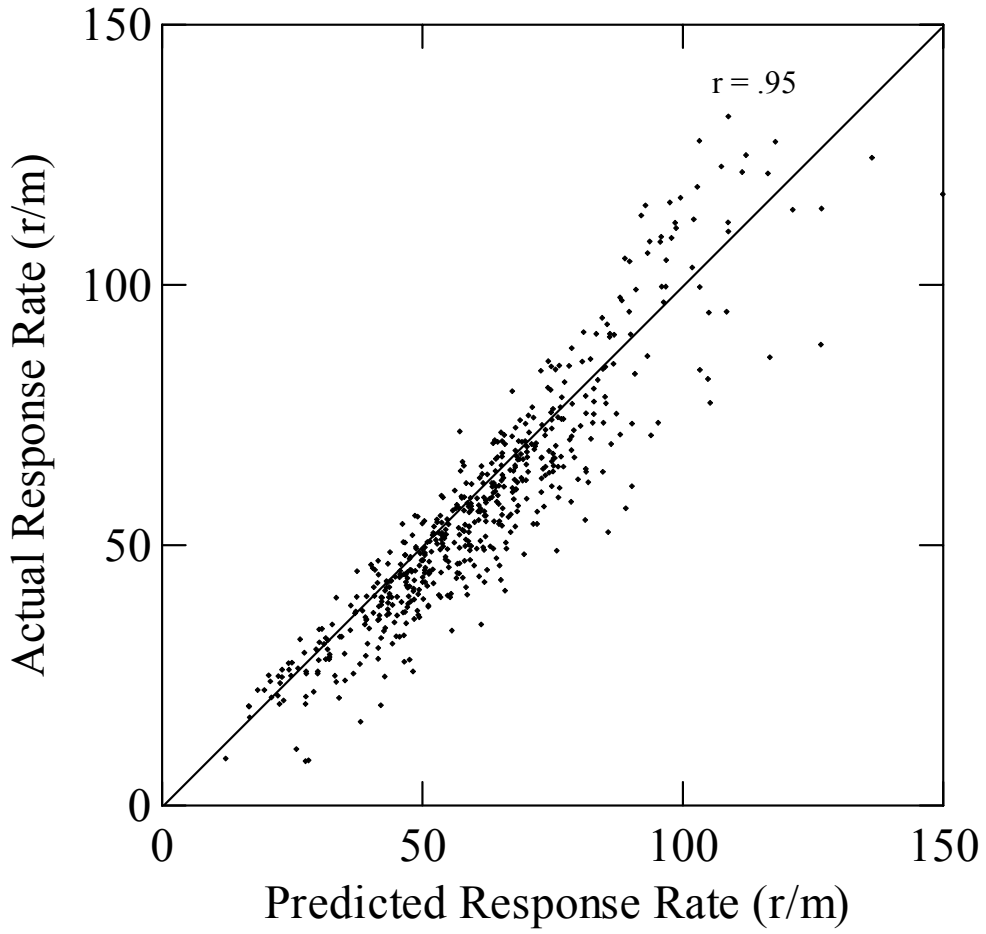
150
100
50
0
0
50
100
150
r = .95
Actual Response Rate (r/m)
Predicted Response Rate (r/m)

Figure 13

Table 1

	Overall Response Rate	Reinforcer Rate	Within Bout Rate	Initiation Rate	Bout Length	L_{10}(Bout Length)	PRP
Overall Response Rate	1						
Reinforcer Rate	0.189	1					
Within Bout Rate	0.594	0.119	1				
Initiation Rate	0.387	0.174	0.245	1			
Bout Length	0.598	0.144	0.147	-0.306	1		
L_{10}(Bout Length)	0.654	0.174	0.270	-0.273	0.904	1	
PRP	-0.240	-0.036	-0.169	-0.222	-0.120	0.048	1

CHAPTER 3: THE EFFECTS OF METHYLMERCURY AND SELENIUM ON HIGH RATE BEHAVIOR IN THE RAT

Abstract

Chronic exposure to methylmercury has been associated with motor dysfunction and selenium has been shown to counteract some effects of methylmercury exposure. The current experiment uses a percentile inter-response time (IRT) 10:5 schedule to generate high-rate operant behavior while holding overall rate of reinforcement constant. An IRT is defined as the length of time between two lever presses. An IRT is reinforced if it is shorter than the median of the previous ten IRTs (for each individual animal.) Rats were chronically exposed to 0, 0.5 or 5 ppm of methylmercury through drinking water daily. Half of each group of rats was exposed to a diet that contained low levels of selenium (0.04 PPM), and the other half received diets that contained high levels of selenium (0.4 PPM). Responding was characterized as bouts of engagement separated by pauses. Methylmercury exposure resulted in slower behavioral acquisition, lower steady state response rates, and longer post-reinforcement pause durations. Selenium deficiency resulted in slower behavioral acquisition and lower steady state response rates. High selenium levels were associated with decreasing bout initiation rates over time. There were no statistically significant interactions between methylmercury and selenium.

1. Introduction

Chronic exposure to methylmercury, a potent neurotoxicant and teratogen, has been associated with various problems with motor function (Evans, Garman, & Laties, 1982; Rice, 1989; Wakabayashi et al., 1995). Newland and Paletz (2000) reviewed the endpoints most sensitive to prenatal methylmercury exposure, and found that operant, high-rate behavior was disrupted at relatively low levels of developmental exposure. High rate behavior was obtained using a differential reinforcement of high rates (DRH) 9:4 schedule of food reinforcement, a schedule that requires motor endurance. This schedule is sensitive to prenatal methylmercury exposure, presumably (at least partially) because of motor deficits (Newland & Rasmussen, 2000). Behavioral differences between the control group and methylmercury exposed groups could also be due to an interaction with the rate of reinforcement associated with the schedule; faster responding produces higher reinforcement rates. Isolating motor effects from reinforcement effects requires that differences between individual animal's reinforcement rates under the schedule to be minimized as much as possible.

Selenium, a natural element, confers protection against methylmercury exposure. Consumption of mercury via tuna containing selenium is less toxic than other instances of methylmercury poisoning (Ganther, Goudie, Sunde, Kopecky, & Wagner, 1972). This protection may be due to selenium binding to mercury (Imura & Naganuma, 1991), or through its antioxidant properties(Park, Lim, Chung, & Kim, 1996). Research indicates that there is a chemical reaction between selenium and methylmercury, which leads to a BMS complex. This complex results in different distributions of both elements throughout the body, which ultimately results in effects on brain, cellular, renal and

cardiovascular functioning. Selenium supplementation ameliorates the prenatal effects of methylmercury on early motor function in rodents. However, interactions have not been examined for chronic low level exposure to methylmercury and selenium. Binding results in varying distributions of both selenium and mercury concentrations throughout the body, as compared to controls. Selenium concentrations are lower in most organs, except the liver, where there are elevated concentrations relative to conditions in which mercury exposure did not occur (Komsta-Szumska, Reuhl, & Miller, 1983). Mercury concentrations in brain (Prohaska & Ganther, 1977), blood, liver and testes (Whanger, 1992) are elevated when selenium and methylmercury are co-administered relative to mercury-only administration.

Selenium also seems to ameliorate neurobehavioral effects of methylmercury. It reduces weight loss that is a function of age and methylmercury exposure, as well as delaying the onset of signs of neurotoxicity (Iwata, Okamoto, & Ohsawa, 1973). Watanabe, Yin, Kasanuma and Satoh (1999) examined the behavior of mice exposed prenatally to 0, 5 or two doses of 3 mg/kg Hg and a diet either sufficient (0.4 µg Se/kg) or deficient (<0.02 µg Se/kg) in selenium. The rats exposed to high doses of methylmercury showed dose-dependant deficiencies in the righting reflex, walking ability, and open-field locomotion, as well as increased thermal preference what were amplified by selenium deficiencies. These differences disappeared as the animal approached weaning.

High rate behavior as the major behavioral endpoint was used in the present study to examine the interactions of chronic methylmercury exposure and dietary selenium intake because of its sensitivity to methylmercury exposure. In a previous report, a DRH 9:4 schedule of operant reinforcement was used to produce high rates of lever pressing in rats

exposed prenatally to methylmercury (Newland & Rasmussen, 2000). Under this arrangement, there was multiple control of behavior by the rate contingency and the reinforcement rate. The deficits in behavior exhibited by animals in the methylmercury exposure group could have been a result of either motor deficits or because of differential reinforcement rates. To isolate these influences, short IRTs were be reinforced according to the animals own performance criterion, and reinforcement rate can be held constant both across an experiment for a single animal and across subjects. This type of schedule is referred to as a "percentile" schedule. Removal of the indirect effects of reinforcement rates on responding allowed the direct effects of methylmercury exposure to become more visible. This focused on the motor ability of the rat to emit lever presses at a high rate and its sensitivity to the consequences of behavior. The structure of the resulting behavior, characterized by periods of responding and periods of non-responding, was examined using a log survival plot analysis. This analysis produces measures of behavior which correspond "engaged" lever-pressing behavior. The number of times that the animal successively responds is called "bout length", and the rate of those specific responses it termed "within-bout response rates." After disengaging in lever pressing, the animal eventually re-engages in the lever-pressing behavior. The rate at which this re-engagement occurs is called "initiation rate" (Shull, 2003).

Motor execution and feedback is disrupted by methylmercury exposure, although the underlying mechanism is not known. One possibility is through cell loss in the granular layer of the cerebellum, and a loss of large motor neurons in the spinal cord (Castoldi, Coccini, & Manzo, 2003). This could result in a loss of proprioception, and that loss of feedback during any motor task could result in less refined movements, as well as a

possible loss in the emission of the behavior. Providing external feedback to indicate successful emission of a behavior could act as a "behavioral prosthesis" that could lessen the effects of methylmercury exposure. Accordingly, two alternating components, one containing feedback for correct emissions of behavior, and one with no feedback, were used to examine this possibility.

2. Method

2.1. Subjects

Forty eight female Long-Evans rats were used in the current experiment. They were chosen from a pool of 114 rats obtained at 115 days of age from Harlan based on successful breeding. Rats were exposed to a 12-hour light-dark cycle in an environmentally controlled colony room. These subjects were divided into six experimental groups, with two diet variations and 3 methylmercury variations (a 2x3 design of diet by methylmercury), as described in following sections. Females, ranging from 24 to 43 weeks of age, were mated with unexposed males. Rats were housed individually in Plexiglas home cages until pups were weaned, at which time they shared one cage with another rat, divided by a Plexiglas partition. These subjects remained on the diet and mercury levels described, throughout the course of the entire operant experiment.

2.2. Exposure

2.2.1. Diet

Upon arrival to the laboratory, rats were allowed free access to tap water and a standard rodent chow diet. Eleven days later (at age 125 days) rats were placed on one of two selenium diets. The "low selenium" group had less than 0.05 ppm selenium total in

their diet. The “high selenium” group had supplemental selenium, in the form of sodium selenite, added to both growth and maintenance diets, resulting in 0.5 ppm selenium. During mating and pregnancy, animals were maintained on an AIN 93 growth diet containing 7% fat. Otherwise, the animals received a maintenance diet of an AIN 93 diet with 5.9% fat, for mature rodents. Both diets were obtained from Research Diets Inc (New Brunswick, NJ.)

2.2.2. Methylmercury

At 145 days of age, after 20 days on the special selenium diets, dams were further divided into three groups, creating 6 experimental groups. One third of the dams were given water with no methylmercury (0 Hg), 1/3 were exposed to water containing 0.5 ppm methylmercury, yielding approximately 40 μg/kg/day Hg (0.5 Hg), and the final 1/3 of the dams were given water containing 5.0 ppm methylmercury, yielding approximately 400 μg/kg/day Hg (5.0 Hg). Rats were not exposed to methylmercury through water from day 16 to 21 after giving birth, in order to prevent offspring from experiencing methylmercury exposure from the water bottle. After weaning, methylmercury exposure via drinking water resumed.

2.3. Behavioral Procedures

2.3.1 Apparatus

An extra tall modular operant test chamber (Med ENV 007) was used for sessions. Dimensions of the chamber measure 12"L x 9½"W x 11½"H. Aluminum walls are located in the front and back of the chamber, while clear polycarbonate is used for the side and door of the chamber. The typical grid flooring in the chamber was covered from the front of the chamber to within an inch of the back of the chamber with a piece of

secured plastic. This insured that rats with motor disorders would not slip through the bars. Two levers were located on either side of the front of the chamber, with a feeder in the middle of the levers. Signal lights were located over each lever. A sipper device was located in the center of the back of the chamber, with a house light above it. White noise was generated from a speaker 7.2 cm above the feeder. The operant chamber was enclosed in a sound-attenuating dark chamber. Sessions were controlled and data were collected on a computer in an adjacent room, using Med-PC software.

2.3.2. Training

Lever-pressing was established using an autoshaping protocol at fifty weeks of age. They were placed in the experimental chamber for approximately 12 hours overnight. Water was provided during these training sessions. Rats were placed in the chambers with the plexiglass doors shut and the chamber doors open. House lights were disconnected from the chamber, and illumination was supplied from the room light in the adjacent room. A fan ran continuously in each chamber, providing both fresh air and background noise similar to white noise.

During the first session, rats were delivered 12 sucrose pellets paired with a 0.5 second, 4500 Hz tone, according to a fixed time 60-second schedule (one reinforcer delivered every 60 seconds regardless of behavior). Following the delivery of the twelfth non-contingent reinforcer, a 0.5 second 2900Hz tone sounded and the left lever extended and the light over that lever was lit. A continuous reinforcement schedule for lever pressing began. After 30 seconds the lever was retracted, the stimulus light was turned off, and a 0.5 second 4500 Hz tone signaled the delivery of a sucrose pellet. After a 5-minute inter-trial interval, the lever was again extended for 30 seconds. This pattern

repeated until the lever was pressed 10 times, at which time the "maintenance" component began.

The left lever was extended and out throughout the maintenance component. Sucrose pellets were delivered on a continuous reinforcement schedule, as described above, except that non-contingent pellets were no longer delivered. Sessions ended once 100 lever presses accumulated in a session, or 12 hours elapsed. If the rat did not lever press the requisite 100 times in 1 session, the training was repeated, and responses were cumulated across sessions. Training continued until 10 autoshaped responses and 100 maintenance responses were emitted.

2.3.3. Percentile IRT 10:0.5 schedule of reinforcement

At 56 weeks of age, rats were placed under a multiple feedback/no-feedback percentile-IRT schedule of reinforcement for lever pressing. Every response was recorded, and the interresponse time (IRT) duration between each response was calculated with 0.1" resolution. The first 10 responses were reinforced under the random interval session in place, because no criterion had been set yet. After the 10^{th} response in the session, a criterion IRT was calculated for each rat with every response. The previous 10 IRTs were sorted, and the median of those IRTs was designated as the criterion for reinforcement. For example, if the last 10 IRTs were 10", 9", 9", 6", 5" 5", 4", 3", 2", 1", and 1", the criterion for reinforcement for the next response would be a response with an IRT < 5 seconds. With the next response, a new distribution of IRTs is obtained, and a new criterion determined.

For the first two minutes of the session, the feedback component was presented. The house light was on, and criterion responses were signaled by a 4500 Hz (high) tone was

presented for 0.05 seconds. This tone is used as feedback and a conditioned reinforcer for responses that meet criterion. After this two-minute component, a 20 second blackout occurs. All lights are turned out and lever pressing produces no tones or reinforcement deliveries. A two-minute no-feedback component begins after the 20-second blackout elapses. During this condition, the houselight is off, and the light over the left lever is illuminated. The reinforcement schedule works the same was as in the feedback component except that no feedback tone is sounded for criterion responses.

In each condition, a sucrose pellet reinforcer is delivered for criterion responses under a random interval schedule. For six sessions, an RI 0.5" was in effect, then two sessions of RI 15" were implemented. Finally, an RI 30" schedule was implemented for the remainder of the experiment. The feedback and no-feedback components, with separating 20" blackouts, alternate six times, for a 28-minute session. Sessions were run in the morning Monday through Friday.

2.2.4. Data Collection

Each reinforcer delivery, response, and the corresponding interresponse time (IRT) were recorded throughout the entire session. Average reinforcement and response rates were calculated for both the feedback and no-feedback condition. Percentile IRT distributions were calculated for each condition as well.

2.2.4.1 Partitioning response rate

Figure 1 shows an example of a distribution of IRTs from a single session, arranged sequentially from the shortest to the longest. Elapsed time (t) in seconds is graphed along the X-axis, which represents the IRT duration. The proportion of responses with IRTs greater than *t* is graphed on the Y-axis. Note the "broken-stick" appearance; the initial

decline in the slope is very steep, and a second portion declines more slowly. This phenomenon reflects two different types of responding. The first, steep portion represents within-bout responding characterized by IRTs less than about 2" in this example. The second shallower portion represents pausing in responding. A two-exponential curve was fit to the distribution of IRTs using a non-linear least squares calculation. From this equation bout-initiation rate, within-bout response rate, and bout length can be calculated (see equation 1).

$$\mathbf{r(t) = (1\text{-}p)\, e^{-wt} + pe^{-bt}} \qquad \mathbf{(1)}$$

In the example, the exponential function yields the following values:

$$\mathbf{r(t) = 0.82{*}e^{(-0.24x)} + 0.18{*}e^{(-01x)}} \qquad \mathbf{(2)}$$

The following shows the definition of the variables, and values derived from the log survivor plot:

r(t) = proportion of IRTs > t

p = proportion of responses that are bout initiations (.18 in equation 2)

(1-p) = proportion of responses that are within a bout (.82 in equation 2)

w = the rate of within bout responding (r/s) (.24 r/s in equation 2)

b = bout initiation rate (r/s) (.01 r/s in equation 2)

1/p = bout length (responses) (5.6 r in equation 2)

2.2.4.2. Statistical Analysis

RMANOVAs were performed separately for acquisition and steady state performance for each endpoint. Weekly averages for each rat were calculated for each dependant variable. Week 0 included an average for the sessions prior to the RI 30s schedule being implemented. Week 1 included an average for the following 5 sessions, and so on for 18 weeks. When variability was related to the magnitude of the mean, the data were log transformed to stabilize variance.

Post reinforcement pause durations were calculated beginning in week 3. Therefore, acquisition was weeks 3-10 for PRP. The log survivor plot analysis was used beginning in week 4, when a pattern of response bouts appeared reliably. Thus, initiation rates, within bout response rates, and bout lengths are presented for weeks 4-17.

A few rats died during the experiment, and others performed at such low rates that log-survivor plots could not be generated to fit their data. These animals had to be omitted from RMANOVAs if they had insufficient data. See the table 1 for death and omission records.

3. Results

3.1. Autoshaping

All rats acquired lever pressing through autoshaping. There were no differences between exposure groups on the acquisition of lever-pressing.

3.2 Feedback

No differences were found between the feedback and no-feedback component. Correlations between the two components ranged from r = .92 to r = .99. Only the feedback condition data are presented for the remainder of the results section.

3.3 Overall Responding

The acquisition of overall response rates was sensitive to both selenium and mercury exposure during the first ten weeks of the experiment. Figure two shows aggregated average response rates for each exposure group by week. The top graph shows the rates for the low selenium group, and the bottom the high selenium groups. All high selenium animals had similar patterns of acquisition and maintenance patterns of behavior, regardless of methylmercury exposure. Average response rates of all high selenium animals reached 35 responses/min within 5 weeks of experience on the percentile schedule. Low selenium controls also reached steady state responding over 35 responses/minute within 5 weeks. The pattern of acquisition for the low-selenium, mercury-exposed groups differed. These groups required more than ten weeks to reach stable response rates of approximately 30 responses/minute.

Separate RMANOVAs were used to examine the effects of selenium and mercury on acquisition (weeks 0-10) and steady state responding (weeks 14-17). In acquisition, there was a main effect of week ($F_{10,410} = 52$, $p=0.000$), and an interaction between methylmercury and week ($F_{20, 410} = 1.66$, $p=0.036$). Log-transforming the average response rates also revealed a main effect of selenium during this period ($F_{1,41} = 4.815$, $p=0.034$). During steady-state there were neither significant effects of selenium, mercury, week nor any interactions.

3.3 Reinforcement Rate

Figure 3 illustrates a direct relationship between response rate and reinforcement rate when rates were lower than 15 responses/min (Pearson's r = 0.813) but no correlation when rates exceeded 30 responses/min (Pearson's r = 0.079.)

Figure 4 shows reinforcement rates for low (top) and high (bottom) selenium groups. Reinforcement rates were highest during week 0, because programmed reinforcer availability was 1 reinforcer/min (see description of the percentile schedule.) RMANOVA revealed a main effect of selenium during acquisition ($F_{1,41}$= 5.001, p=0.031), but not for steady state ($F_{1,39}$ = 1.877, p=0.179) Reinforcement rates were lower in low selenium groups (1.43 ±0.66 rf/min) than in high selenium groups (1.77±0.68 rf/min) during acquisition.

3.4 Within-bout response rate

Figure 5 shows within bout response rates over weeks for low (top) and high (bottom) selenium groups. Within bout response rates could not be calculated reliably until week 4 because the nonlinear least-squares analysis of the log survivor plots provided poor fits when behavior does not exhibit a pattern of engagement bouts and pauses. Therefore, weeks 4-10 were included in the analysis of acquisition, and 14-17 were analyzed as steady state responding. During weeks 4-10, there was a main effect of selenium ($F_{1,39}$ = 4.875, p=0.033), and methylmercury ($F_{2,39}$ = 6.024, p=0.005), but no interaction ($F_{2,39}$ = .574, p=0.568). During this period, selenium deficiency and methylmercury were associated with lower within-bout response rates. For weeks 14-17, the effects of mercury, selenium and week were no longer significant.

3.5 Bout initiation rate

Bout initiation rates are presented in figure 6, with low selenium groups in the top panel, and high selenium in the bottom panel. There were no main effects of selenium or mercury either during acquisition or steady-state. There was a selenium by week interaction during acquisition after the data were log-transformed ($F_{6,234}$ = 3.328,

p=0.004.) With increasing weeks during acquisition, high selenium initiation rates declined below the average initiation rate associated with low selenium exposure. During weeks 14-17, their initiation rates were stable. When initiation rate is analyzed as a function of week over the course of the entire experiment, there was a Se x week interaction ($F_{13,468}$ = 1.985, p=0.02.)

3.6 Bout Length

Bout length, presented in figure 7, was unrelated to selenium or mercury exposure, and did not change as a function of time (p>0.1).

3.7 Post-reinforcement pause

Values for post-reinforcement pause length were calculated for weeks 3 through 17. During acquisition, there was a main effect of selenium ($F_{1,40}$ = .047, p=0.047). No main effect of mercury was detected during this period, but there was a mercury by week interaction ($F_{14,280}$ = 1.770, p=0.043). For steady state, there was a marginal main effect of methylmercury (F2,39 = 3.069, p=0.058), as well as an effect of week ($F_{3,117}$ = 3.218, p=0.025). During acquisition, low selenium PRPs (9.7±3.2 sec) were longer than high selenium PRPs (8.11±1.9 sec). Over the course of the entire experiment, methylmercury exposure is associated with increasing post reinforcement pause durations ($F_{28,532}$ = 1.738, p=0.012.)

4. Discussion

The experiment was designed to examine whether or not selenium confers protection against the behavioral toxicity of methylmercury on operant high rate behavior. There were no detected interactions between selenium and mercury, although each was associated with different patterns of behavior. Selenium deficiency was linked to longer

acquisition periods of responding, lower overall response rates as a function of week, lower reinforcement rates, lower within-bout response rates during acquisition, higher initiation rates as a function of week, and longer post-reinforcement pause durations during acquisition.

Methylmercury exposure decreased overall response rate during acquisition, but in the latter part of the experiment, mercury exposure did not affect steady-state response rates. Lower within bout response rates occurred over the course of the entire experiment in animals exposed to methylmercury, although, as evidenced by figure 5, this relationship was not dose-dependant. Finally, longer post-reinforcement pauses were associated with methylmercury exposure as a function of week both during acquisition and throughout the entire experiment.

Selenium deficiency and methylmercury exposure affected behavior similarly. Selenium deficiency resulted in slower acquisition of behavior, and even lower steady state rates, but these relationships did not intensify over time. Strangely, initiation rates seem to decline as a function of week in only high selenium animals. Mercury, was associated with slower acquisition of behavior, lower steady state levels, as well as detriments that appear or intensify as a function of exposure to methylmercury. Post reinforcement pause duration increased as a function of the duration of methylmercury exposure. It also appears that initiation rates were on a declining trend which was nearing significance in high selenium, high mercury animals. Previous studies have reported motor deficits such as hindlimb crossing and gait abnormalities with long term exposure to methylmercury (Day, Reed, & Newland, In press; Rice, 1996). By the time that operant testing began for the rats in the current study, three low-selenium, high

mercury animals were already exhibiting evidence of these motor dysfunctions. By The end of the experiment, most of the high mercury rats were exhibiting motor dysfunction. This dysfunction may mediate some of the detriments found in operant dependant variables.

There are a few possibilities why no interactions between mercury and selenium were found. The mercury levels were chosen based upon previous evidence that the low dose would produce effects as a function of aging, and that the high dose would produce fairly early detriments in motor skills without profoundly affecting the overall health of the rat (Newland & Rasmussen, 2000). The moles of methylmercury consumed per rat per day are ~0, 0.2, and 2.0 μmoles/kg. Selenium levels were chosen based on previous research that showed interaction effects with prenatal methylmercury exposure (Watanabe et al., 1999). These levels, however, were not extreme; they represented small deviations from recommended selenium intake levels (0.32 μmoles/kg) (National Research Council, 1995). The moles of selenium consumed per day are either 0.04 μmoles/kg or 0.4 μmoles/kg. If all possible binding occurs between methylmercury and selenium, there are varying degrees of selenium and methylmercury availability. See the appendix for possible bioavailability of each of these elements if 100% binding occurs. According to this table, all low selenium, and the high selenium high mercury group has virtually no selenium available. This total selenium depletion allows for "leftover" mercury, which is then available to exert toxic effects only in these groups. It is possible that there is virtually no mercury "free" in the high selenium, low methylmercury group. This complex interaction between selenium and methylmercury may not be sufficiently detectable using statistics designed for a 2x3 factorial design.

Another possibility is that with aging, the interaction of selenium and methylmercury may become evident. Previously, behavioral deficits have worsened as a function of age, and since the animals were only 19 months old at the conclusion of this experiment, there may not have been sufficient time to detect any subtle interactions as a function of aging.

In summary, both selenium deficiency and methylmercury exposure were associated with detriments in the acquisition of high rate responding under the percentile schedule. No interaction between methylmercury and selenium was detected No endpoint was affected by the presentation or omission of feedback stimuli for criterion responses. Selenium deficiency primarily affected the acquisition of response rates, and lower steady state within-bout response rates. Mercury affected acquisition rates of overall responding, and was only associated with lower steady state response rates in the low selenium group (although no interaction was detected.) Both selenium deficiency and methylmercury exposure resulted in longer post reinforcement pauses. This detriment increased as a function of week for methylmercury.

The particular schedule of reinforcement chosen is a very “forgiving” schedule, in that fairly consistent reinforcement rates are maintained despite fluctuations in response rates. This schedule was chosen to try to isolate motor performance from reinforcement sensitivity. Animals in this experiment are currently aging and participating in a schedule arrangement which includes both a percentile schedule like presented in this paper, as well as a component which requires 9 responses in 4 seconds in order for a reinforcer to be delivered. Perhaps behavioral will be more sensitive to exposure with aging, and under different schedule requirements.

References

Castoldi, A., Coccini, T., & Manzo, L. (2003). Neurotoxic and molecular effects of methylmercury in humans. *Reviews on Environmental Health, 18*(1), 19-31.

Council, National Research (1995). *Nutrient Requirements of Laboratory Animals*. Washington, D.C.: National Academy Press.

Day, J., Reed, M., & Newland, M. C. (In press). Neuromotor Deficits in Aging Rats Exposed to Methylmercury and n-3 Fatty Acids. *Neurotoxicology and Teratology*.

Evans, H. L., Garman, R. H., & Laties, V. G. (1982). Neurotoxicity of Methylmercury in the Pigeon. *NeuroToxicology, 3*, 21-36.

Ganther, H., Goudie, C., Sunde, M., Kopecky, M., & Wagner, P. (1972). Selenium: relation to decreased toxicity of methylmercury added to diets containing tuna. . *Science, 175*(26), 1122-1124.

Imura, N., & Naganuma, A. (1991). Possible mechanisms of detoxifying effect of selenium on the toxicity of mercury compounds. In T. Suzuki, N. Imura & T. Clarkson (Eds.), *Advances in mercury toxicity* (pp. 490). New York: Plenum Press.

Iwata, H., Okamoto, H., & Ohsawa, Y. (1973). Effect of selenium on methylmercury poisoning. *Research Communications in Chemical Pathology & Pharmacology, 5*(3), 673-680.

Komsta-Szumska, E., Reuhl, K., & Miller, D. (1983). The effect of methylmercury on the distribution and excretion of selenium by the guinea pig. *Archives of Toxicology, 54*(4), 303-310.

Newland, M. C., & Paletz, E. M. (2000). Animal studies of methylmercury and PCBs: what do they tell us about expected effects in humans? *Neurotoxicology, 21*(6), 1003-1027.

Newland, M. C., & Rasmussen, E. B. (2000). Aging unmasks adverse effects of gestational exposure to methylmercury in rats. *Neurotoxicology & Teratology, 22*(6), 819-828.

Park, S. T., Lim, K. T., Chung, Y. T., & Kim, S. U. (1996). Methylmercury-induced neurotoxicity in cerebral neuron culture is blocked by antioxidants and NMDA receptor antagonists. *Neurotoxicology, 17*(1), 37-45.

Prohaska, J., & Ganther, H. (1977). Interactions between selenium and methylmercury in rat brain. *Chemico-Biological Interactions, 16*(2), 155-167.

Rice, D. C. (1989). Delayed neurotoxicity in monkeys exposed developmentally to methylmercury. *Neurotoxicology, 10*(4), 645-650.

Rice, D. C. (1996). Evidence for delayed neurotoxicity produced by methylmercury. *Neurotoxicology, 17*(3-4), 583-596.

Shull, R. L., & Grimes, J. A. (2003). Bouts of responding from variable-interval reinforcement of lever pressing by rats. *Journal of the Experimental Analysis of Behavior, 80,* 159-171.

Wakabayashi, K., Kakita, A., Sakamoto, M., Su, M., Iwanaga, K., & Ikuta, F. (1995). Variability of brain lesions in rats administered methylmercury at various postnatal development phases. *Brain Research, 705*(1-2), 267-272.

Watanabe, C., Yin, K., Kasanuma, Y., & Satoh, H. (1999). In utero exposure to methylmercury and Se deficiency converge on the neurobehavioral outcome in mice. *Neurotoxicology & Teratology, 21*(1), 83-88.

Whanger, P. (1992). Selenium in the treatment of heavy metal poisoning and chemical carcinogenesis. [Review]. *Journal of Trace Elements & Electrolytes in Health & Disease, 6*(4), 209-221.

Figure and Table Captions

Figure 1: Example of a log-survivor plot. Time (t) (in seconds) is expressed on the x-axis, and the proportion of IRTs greater than t is represented on the logged y-axis. Points indicate individual IRT lengths. The line is a double-exponential fit of the IRT distribution. Note the two-component shape of the function. The slope of the first portion represents the within-bout response rate, while the slope of the second portion represents bout-initiation rates. The inverse of the Y-intercept of the second slope represents the bout-length.

Figure 2: Overall response rates for each group over 18 weeks. Weeks are graphed along the x-axis, and response rates (r/min) are graphed along the y-axis. Lines represent the mean and standard error for each week for each group. Low-selenium exposure groups are presented in the top graph, and high-selenium exposure is presented in the bottom graph. Groups that received no methylmercury are indicated by a filled circle, the 0.5 PPM methylmercury groups are indicated by a filled square, and the 5.0 PPM methylmercury groups are indicated by a filled triangle.

Figure 3: Reinforcement rate as a function of response rate. Response rates (r/min) are represented on the x-axis and corresponding reinforcer rates (rf/min) on the y-axis. Each point represents one rat for one day.

Figure 4: Reinforcement rates for each group over 18 weeks. Weeks are graphed along the x-axis, and reinforcer rates (rf/min) are graphed along the y-axis. Lines represent the mean and standard error for each week for each group. Low-selenium exposure groups are presented in the top graph, and high-selenium exposure is presented in the bottom graph. Groups that received no methylmercury are indicated by a filled circle, the 0.5 PPM methylmercury groups are indicated by a filled square, and the 5.0 PPM methylmercury groups are indicated by a filled triangle.

Figure 5: Within bout response rates for each group from week 3 to 17. Weeks are graphed along the x-axis, and within bout response rates (r/sec) are graphed along the y-axis. Lines represent the mean and standard error for each week for each group. Low-selenium exposure groups are presented in the top graph, and high-selenium exposure is presented in the bottom graph. Groups that received no methylmercury are indicated by a filled circle, the 0.5 PPM methylmercury groups are indicated by a filled square, and the 5.0 PPM methylmercury groups are indicated by a filled triangle.

Figure 6: Initiation rates for each group from week 3 to 17. Weeks are graphed along the x-axis, and initiation response rates (r/sec) are graphed along the y-axis. Lines represent the mean and standard error for each week for each group. Low-selenium exposure groups are presented in the top graph, and high-selenium exposure is presented in the bottom graph. Groups that received no methylmercury are indicated by a filled circle, the 0.5 PPM methylmercury groups are indicated by a filled square, and the 5.0 PPM methylmercury groups are indicated by a filled triangle.

Figure 7: Bout Lengths for each group from week 3 to 17. Weeks are graphed along the x-axis, and bout lengths (r) are graphed along the y-axis. Lines represent the mean and standard error for each week for each group. Low-selenium exposure groups are presented in the top graph, and high-selenium exposure is presented in the bottom graph. Groups that received no methylmercury are indicated by a filled circle, the 0.5 PPM methylmercury groups are indicated by a filled square, and the 5.0 PPM methylmercury groups are indicated by a filled triangle.

Figure 8: Post reinforcement pause durations for each group from week 3 to 17. Weeks are graphed along the x-axis, and the durations of the post reinforcement pause (sec) are graphed along the y-axis. Lines represent the mean and standard error for each week for each group. Low-selenium exposure groups are presented in the top graph, and high-selenium exposure is presented in the bottom graph. Groups that received no methylmercury are indicated by a filled circle, the 0.5 PPM methylmercury groups are indicated by a filled square, and the 5.0 PPM methylmercury groups are indicated by a filled triangle.

Table 1: The mercury and selenium dosing matrix is located in the top grid. Rats excluded analyses are presented in the bottom three grids.

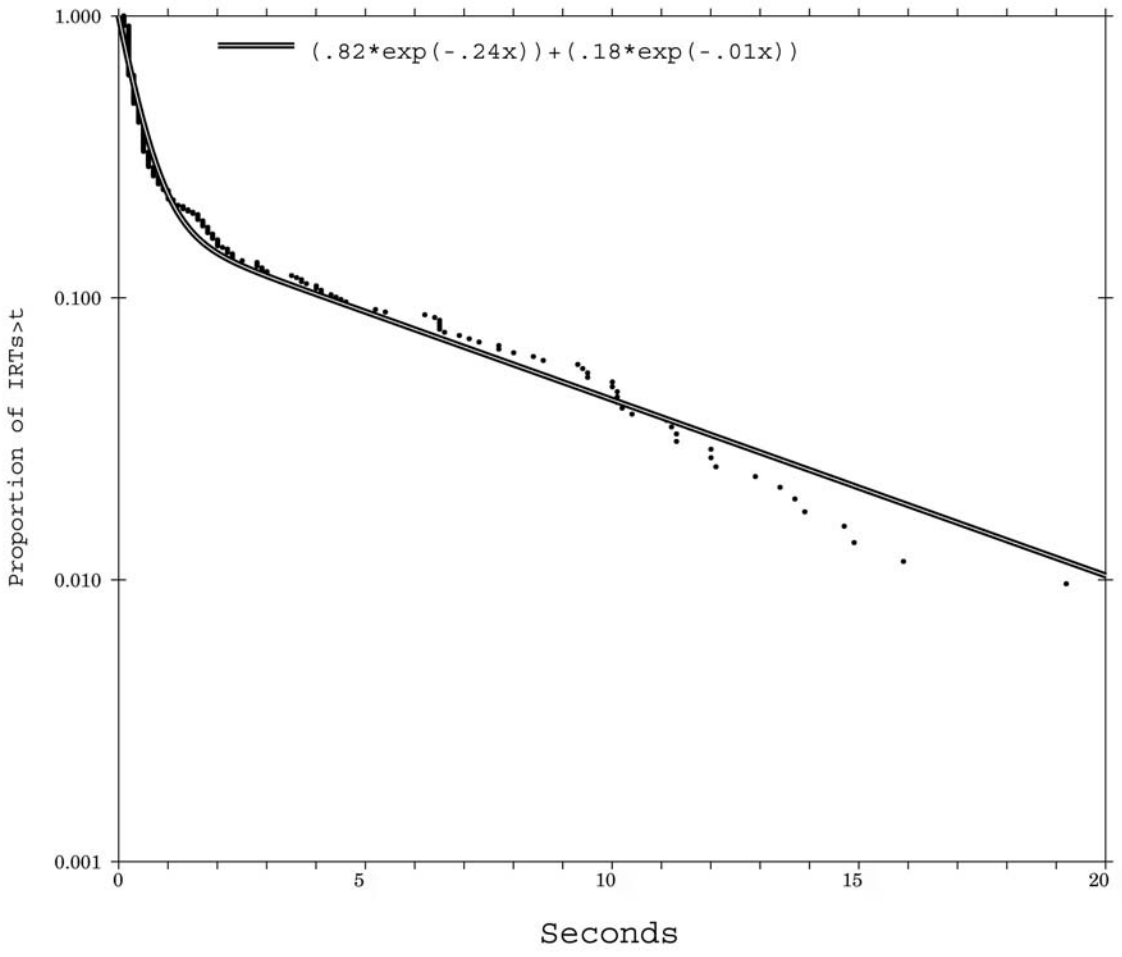
(.82*exp(-.24x))+(.18*exp(-.01x))
1.000
0.100
0.010
0.001
Proportion of IRTs>t
0
5
10
15
20
Seconds

Figure 1

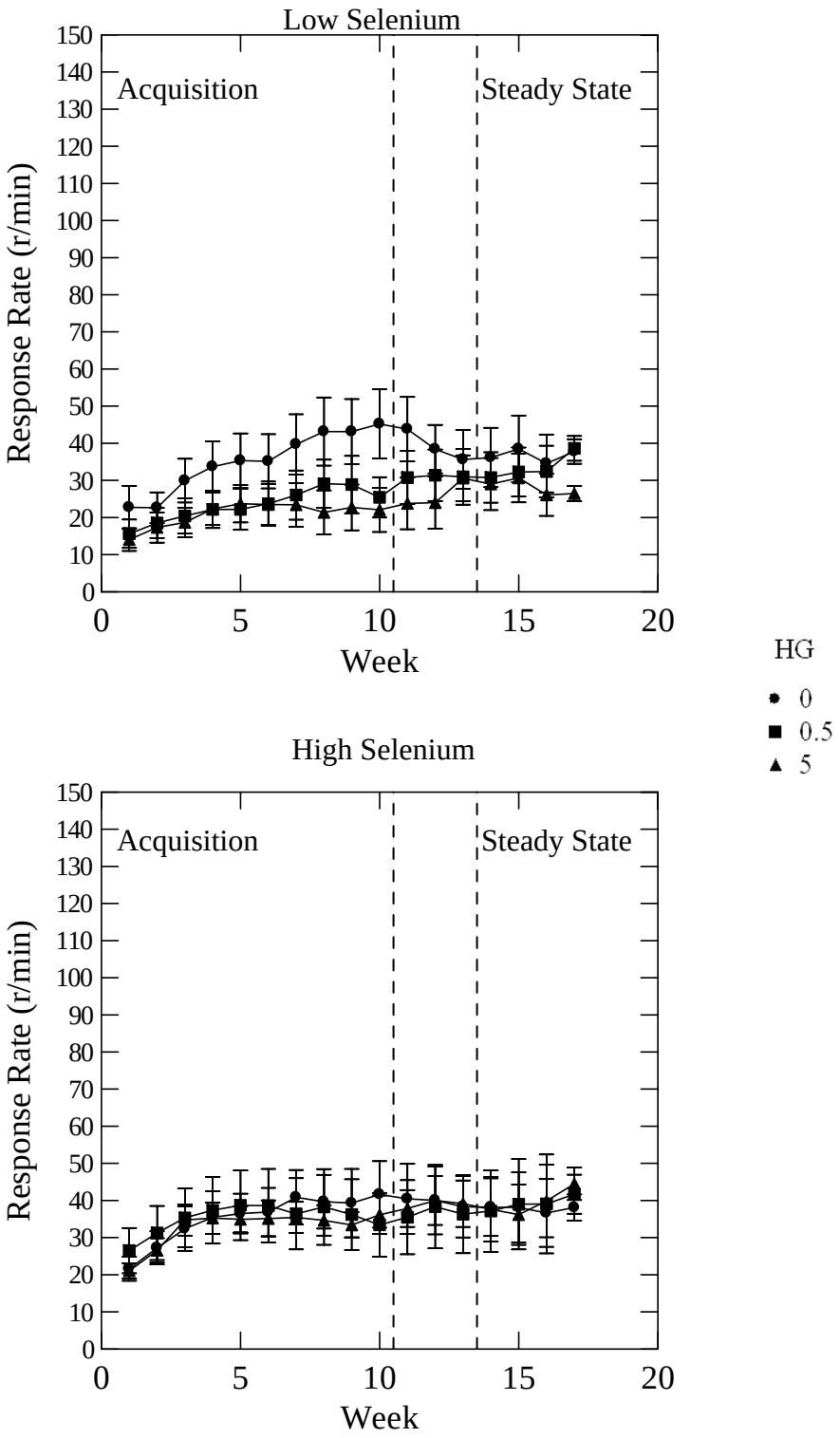
Low Selenium
Acquisition
Steady State
Response Rate (r/min)
Week
HG
0
0.5
5
High Selenium
Acquisition
Steady State
Response Rate (r/min)
Week

Figure 2

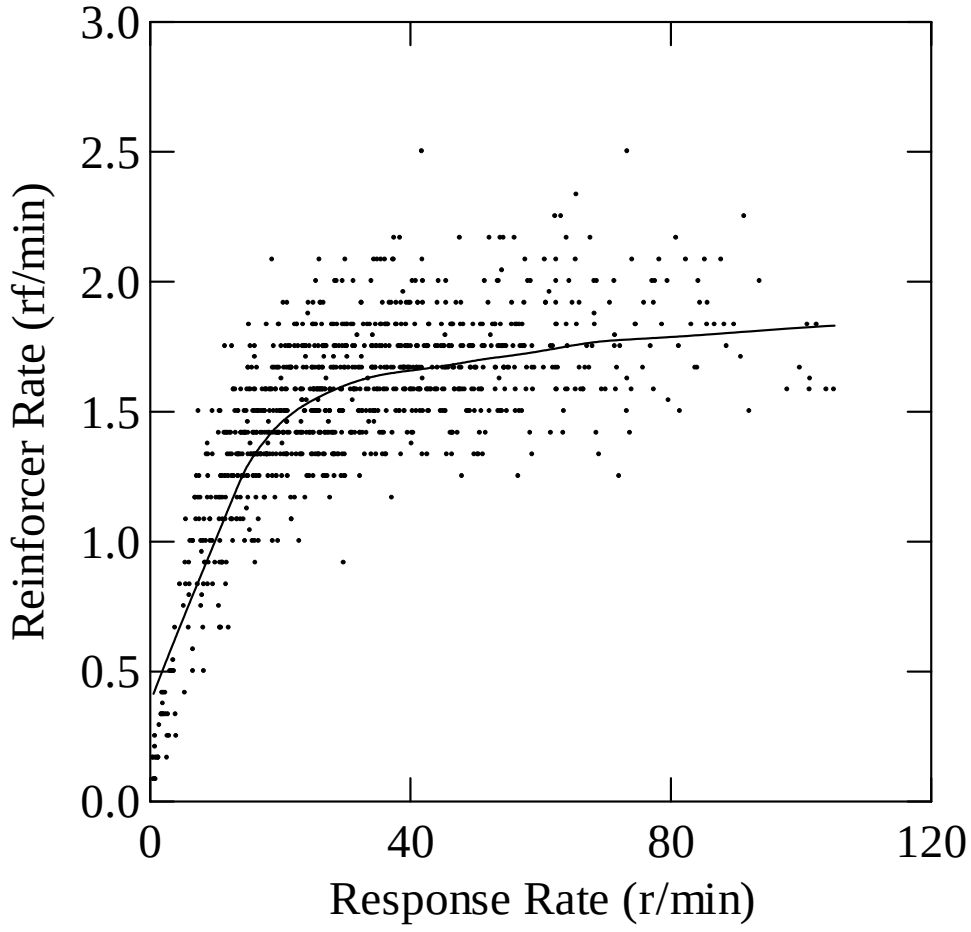
3.0
2.5
2.0
1.5
1.0
0.5
0.0
Reinforcer Rate (rf/min)
0
40
80
120
Response Rate (r/min)

Figure 3

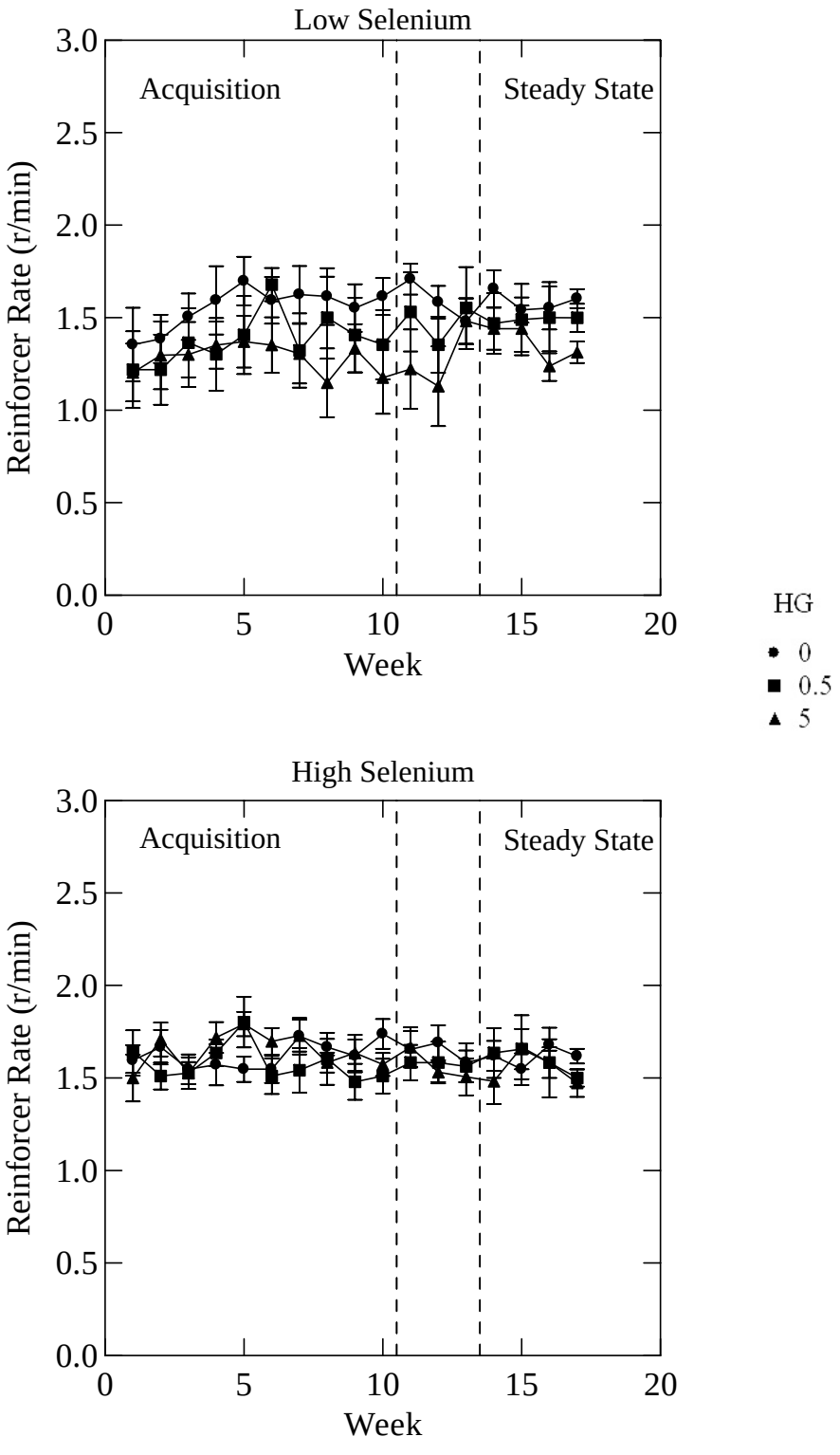
Low Selenium
Acquisition
Steady State
Reinforcer Rate (r/min)
Week
HG
0
0.5
5
High Selenium
Acquisition
Steady State
Reinforcer Rate (r/min)
Week

Figure 4

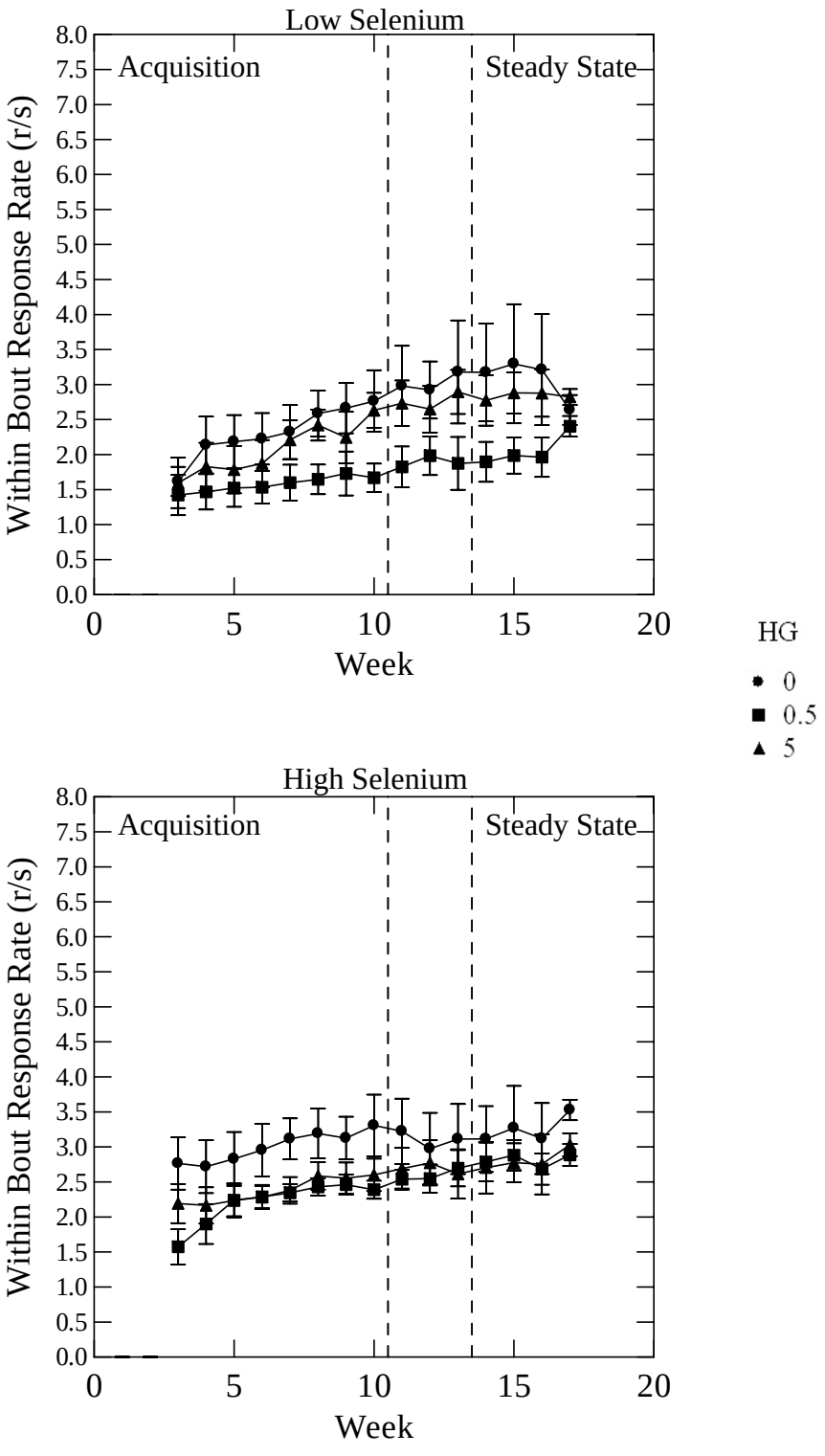
Low Selenium
Acquisition
Steady State
Within Bout Response Rate (r/s)
Week
High Selenium
Acquisition
Steady State
Within Bout Response Rate (r/s)
Week
HG
0
0.5
5

Figure 5

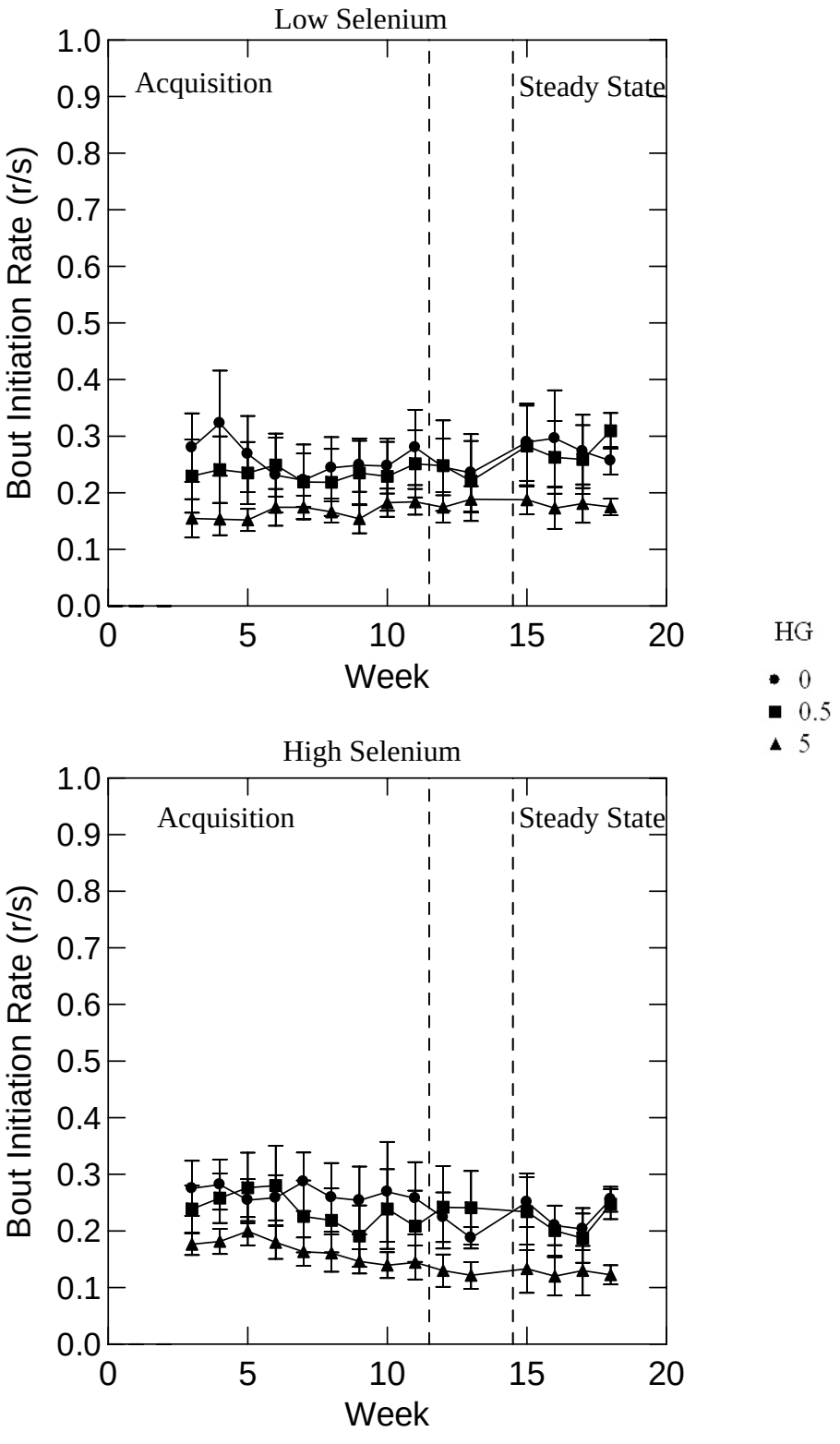
Low Selenium
Acquisition
Steady State
Bout Initiation Rate (r/s)
Week
HG
0
0.5
5
High Selenium
Acquisition
Steady State
Bout Initiation Rate (r/s)
Week

Figure 6

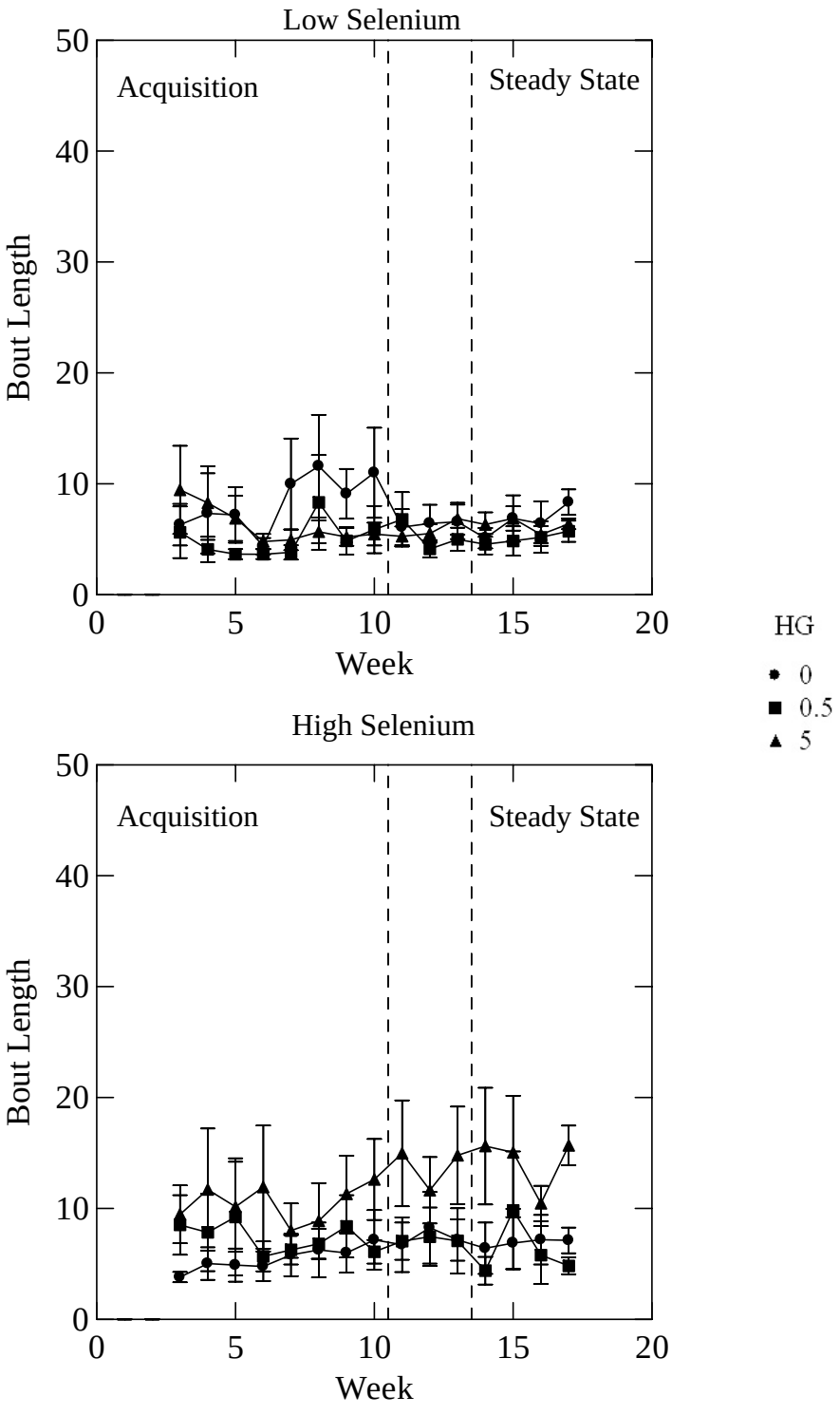
Low Selenium
Acquisition
Steady State
Bout Length
Week
High Selenium
Acquisition
Steady State
Bout Length
Week
HG
0
0.5
5

Figure 7

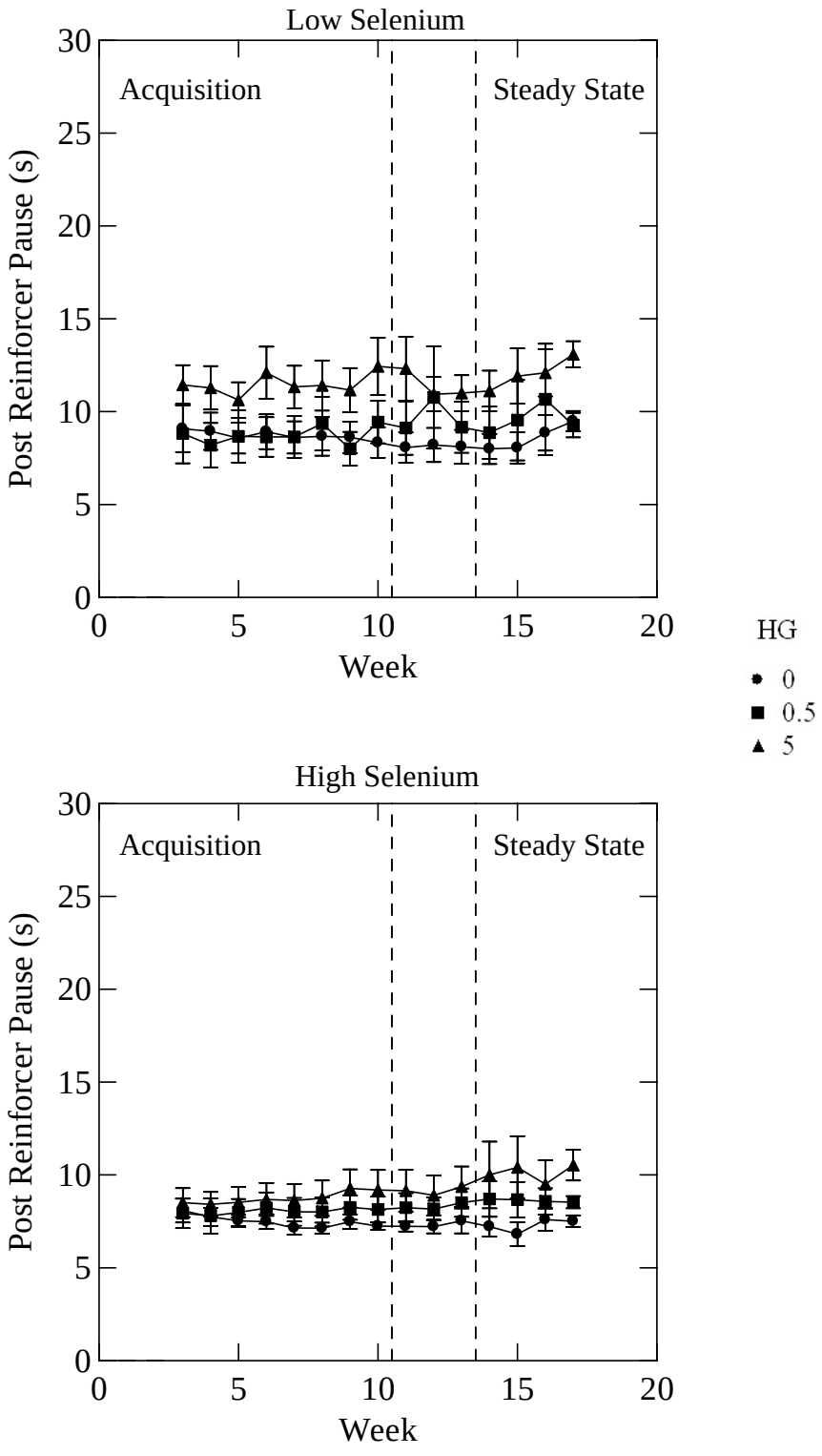
Low Selenium
Acquisition
Steady State
Post Reinforcer Pause (s)
Week
0
5
10
15
20
25
30
HG
0
0.5
5
High Selenium
Acquisition
Steady State
Post Reinforcer Pause (s)
Week

Figure 8

Table 1

Mercury and Selenium Levels

Selenium Group	Hg Group	Selenium µmoles/kg/day	Hg µmoles/kg/day	Ratio moles Hg:Se	Available Selenium µmoles/kg/day	Available Hg µmoles/kg/day
LOW	Zero	0.04	0	0	0.04	0
LOW	Low	0.04	0.2	5	0	0.16
LOW	High	0.04	2	50	0	1.96
HIGH	Zero	0.4	0	0	0.4	0
HIGH	Low	0.4	0.2	0.5	0.2	0
HIGH	High	0.4	2	5	0	1.6

Excluded Rats

Acquisition

Selenium	Mercury	Response Rate	Reinforcer Rate	Within Bout Rate	Initation Rate	Bout Length	PRP
Low	0			1	1	1	
	0.5			1	1	1	
	5			1	1	1	
High	0						
	0.5						
	5						

Steady State

Selenium	Mercury	Response Rate	Reinforcer Rate	Within Bout Rate	Initation Rate	Bout Length	PRP
Low	0			1	1	1	
	0.5			1	1	1	
	5	2	2	2	2	2	2
High	0						
	0.5	1	1	1	1	1	1
	5	2	2	2	2	2	2

2	poor log survivor plot fits
4	died