Influences of Drug and Toxicant Exposure on the Microstructure of Responding

by

Joshua E. Johnson

A dissertation submitted to the Graduate Faculty of
Auburn University
in partial fulfillment of the
requirements for the Degree of
Doctor of Philosophy

Auburn, Alabama
August 6, 2011

Approved by

M. Christopher Newland, Chair, Alumni Professor of Psychology
Christopher Correia, Alumni Associate Professor of Psychology
Martha Escobar, Associate Professor of Psychology
Alejandro Lazarte, Assistant Professor of Psychology

Abstract

A recent quantitative model has been used to examine the microstructure of behavior, and has described a bout of responding by three separate measures: within-bout response rate, bout initiation rate, and bout length. These measures have been shown to be affected by different types of variables. The studies that follow use this model as a tool to examine the microstructure of behavior. The first study examined the microstructure of behavior after haloperidol administration aiming to differentiate the anhedonic and motor effects of haloperidol. Both wheel running and nose-poking behavior was examined for BALB/c and C57BL/6 mice. Haloperidol decreased nose poking in a dose-dependent fashion with BALB/c mice being more sensitive to the rate depressing effects. The bout parameter most affected by haloperidol administration was bout initiation rate, which mirrored the decrease of overall nose poking. However, the same strain difference did not exist for wheel running. Wheel running was relatively unaffected by haloperidol administration for both strains. The statistical technique was also used to examine the microstructure of behavior after methylmercury (MeHg) exposure. Male BALB/c mice were exposured to 0 or 15ppm of Hg as methylmercuric chloride dissolved in drinking water. Nose-poking was maintained under a multiple schedule arrangement using two approaches to establishing high-rate responding. MeHg lowered within-bout response rate after about three months of exposure in both schedules. Effects on bout initiation rate were less

consistent and appeared to have interacted with time of day and schedule type. The partitioning technique is a promising way of separating motor and motivational influences.

Table of Contents

List of Tables

List of Figures

Literature Review

Behavior is often examined as a rate measure. Response rate is derived by dividing the total number of responses emitted over a period of time by that period of time. This method can be misleading as the rate measure derived may not produce an accurate temporal description of what occurs on a momentary basis during an experimental session. Responding, contrary to what is assumed by a response rate measure, does not occur at a constant rate across an experimental session, but rather occurs in bouts. Bouts of responding, or periods of engagement, are followed by periods of disengagement creating a two-state conceptualization of behavior (Gilbert, 1958). The two-state conceptualization of behavior has existed for over fifty years, but it was not until recently a model was developed to help understand the dynamics of a bout of responding (Shull et al, 2001).

Log Survivor Analysis

Responses are separated by inter response times (IRTs). The time between responses is inversely related to response rate. Short IRTs are associated with high response rates or frequent responding, while long IRTs are associated with low response rates or infrequent responding. A quantitative model was developed to use the information provided by IRTs to separate a response from a bout of responding into two separate measures (Shull et al., 2001). The goal was to examine the distribution of IRTs and quantitatively decide, based on the IRT that preceded a response, what the type of response was. To oversimplify, responses that were preceded by a short IRT were categorized as one type of response while responses that were preceded by a long IRT were categorized as another type of response.

More specifically, Shull, Gaynor, and Grimes (2001) described a method for describing a bout of responding using a "log survivor analysis." According to the model, two independent composite measures of response rate can be derived by fitting a two-mode exponential equation to the distribution of IRTs. The two composites of response rate are within-bout response rate and bout initiation rate. A log survivor analysis provides a good template for highlighting the two-mode component of responding. Equation 1 is used to model the data:

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

The log survivor analysis begins with the cumulative proportion of responses plotted as a function of the IRT duration. In other words, the log survivor plot shows the proportion of IRTs that are longer than some duration. For the example log survivor plot (Figure 1), the y-axis represents the cumulative proportion of IRTs, 1.0 represents 100% of the IRTs, 0.8 represents 80% and so on. The x-axis is the length of the IRT in seconds. The open circles in the example represent the different IRT lengths and are plotted according to shortest (highest response rate) to longest (lowest response rate) length in a survival function. The decay function shows that a small percentage of the IRTs were longer than 1s. For example, fewer than approximately 5% of IRTs were greater than 10s, and 85% were shorter than 1 second. The event record chart that is inset into Figure 1 depicts four examples of response bouts. Each line represents an individual response, and the lines are separated by varying lengths of inter-bout intervals.

Figure 1 also features a line that is derived from the sum of two negative exponential functions in equation 1, where $Y(t)$ equals the proportion of IRTs that are greater than some specified duration (t). The first term ($(1-p)\, e^{-wt}$) represents the within-bout responses component

of the plot, while the second term (pe^{-bt}) represents the bout-initiation responses component of the plot. For each of the terms *e* is the natural log base, and *t* is the unit of time specified by the x-axis. The parameter *w* is the within-bout response rate, and *b* is the bout initiation rate. The equation provided for Figure 1 describes the within-bout response rate as 3.5 responses/s, and a bout initiation rate of .11/s, or roughly one bout per 9 s. The proportion of all IRTs that are within-bout responses is 91% leaving 9% of the responses as bout initiations. The bout length can be estimated using one divided by *p* (1/.09) or roughly 11 responses per bout in the example.

Shull et al. (2001) used a log survivor plot to classify responses as either bout initiations or within-bout responses. This proved to be an excellent method for classifying responses as the y-axis clearly represented within-bout responses and the slope represented different bout initiation rates. The important aspect of these survivor plots was the appearance of a "broken stick" figure. The broken stick appearance represented the two state conceptualization of behavior, and appeared as two relatively straight lines that intersected at an angle less than 180°. The initial steep limb of the survivor plots represented the short IRTs or bouts of operant behavior, while the second limb represented longer IRTs or the time between bouts of responding. In contrast, if the IRT distribution produced a straight line this method of analysis would fail to support a two state conceptualization of responding.

While the estimates will never provide a perfect description of responding, Shull provided support for this method of analysis using several lines of evidence (Shull et al., 2001). First, Shull used computer simulations where responses could be coded as bout initiations or within-bout responses. As such, bout initiation rates and within-bout response rates could be calculated directly. These direct calculations compared favorably to the estimates provided by the model supporting the overall validity of the model. Second, Shull compared actual rat

behavior to the simulated behavior. He found reasonable agreement as rat behavior produced a similar broken stick or dual slope appearance. Additional support can be derived from experiments showing that bout-initiation rate and within-bout response rates were influenced by different variables (Shull et al., 2001; Shull et al., 2004). Specifically, the bout initiation term has been affected by deprivation level (Shull, 2004), rate of reinforcement, amount of reinforcement, and by altering the percentage of reinforcers that were contingent on key poking (Shull et al., 2001) while these same variables exerted little to no effect on within-bout response rate. Alternatively, the addition of a response requirement to the end of a variable interval (VI) schedule has affected within-bout response rate while leaving bout initiation rate unaffected (Shull et al., 2004; Shull et al., 2001).

Bout Initiation Rate

Shull et al. (2001) contended that operant behavior can be viewed as periods of engagement in response bouts separated by periods of disengagement. Thus, the traditional definition of response rate actually blurs two distinct states. One measure is bout initiation rate, which is a measure of how frequently an animal initiates a bout of responding, or how often an animal changes from a period of disengagement to a period of responding. The idea that periods of disengagement alternate with periods of responding on the reinforced activity is a common way of distinguishing the reinforced responding that is being measured directly from other, unmeasured, operants that might be occurring (Herrnstein, 1970; Mechner, 1992; Shull, 1991). Several different motivational or incentive-based variables can affect the bout initiation rate variable (Shull et al., 2001). Specifically, Shull orchestrated these motivational variable changes by changing the schedule of reinforcement from a VI 4m to a VI 1m (increasing rate of reinforcement), increasing the number of pellets delivered from 1 to 4 (increasing the amount of

the reinforcer). In addition, work in our laboratory has indicated that transitioning from food restriction to free feeding reduces bout initiation rate, while reversing the photoperiod from light to dark and administration of low to moderate doses of pentobarbital increases bout initiation rate (Johnson et al., 2010; Johnson et al., 2009). The idea that was suggested by Shull and colleagues (2001) was that while these are different variables, they all have a similar effect on behavior. That is, they are motivational variables that affect the propensity to engage in an activity by altering the relative reinforcement of the activity (Herrnstein, 1970).

Within-Bout Responding

As proposed by Shull, the other composite variable of response rate is within-bout responding. Within-bout responding is controlled by motor variables, reinforcement contingencies that produce high response rates, and reflect the physics of the response device itself (Shull et al., 2001). A simple way that may affect within-bout response rate that has been suggested is to make the operant response more difficult (e.g. increasing the amount of force required to depress a lever) or change the response device (Shull et al., 2001). By making the response more difficult, one would expect a decrease in within-bout responding. If within-bout responding truly taps motor influences of behavior, another variable that could affect within-bout responding is the administration of pharmacologically active compounds that affect motor behavior.

Shull and colleagues (2001) were able to manipulate within-bout responding and bout initiation rate variables separately of one another. Thus, while altering an independent variable thought to affect motivation (e.g. increasing rate of reinforcement), bout initiation rate increased while within-bout responding remained constant. Similarly, within bout response rate was increased when a tandem schedule was changed from a tandem VI VT to a tandem VI VR

schedule of reinforcement. This change is clearly different than altering a motivational variable. The change does not affect an animal's likelihood to initiate a response, rather it alters what the animal learns to do to obtain a reinforcer. By adding the VR to the end of the tandem schedule the animal learns to respond in a burst to obtain a reinforcer (Shull et al., 2001). This suggests that there are two different measures within our traditional measure of response rate that are controlled by two separate types of variables.

Challenges to the Log Survivor Analysis Method

Despite the initial success of the model, this method for partitioning response rate has experienced varying degrees of success across different laboratories and species. With rats, this method of analysis has been successful in partitioning bouts of responding into composite measures (Shull et al., 2001; Shull, 2004; Shull et al., 2004). Conversely, this log survival method for partitioning response bouts has produced difficulties when applied to pigeons' keypecking (Bennett et al., 2007; Bowers et al., 2008; Davison, 2004; Podlesnik et al., 2006). These studies measured pigeon key-pecking on single and concurrent schedules of reinforcement, and have failed to reliably produce survivor plots with a clear 'broken stick' appearance. More specifically, these survivor plots do not indicate two separate composite measures of a response bout, but rather a single term, indicative of an uninterrupted stream of responses. There have been several hypotheses as to why the log survival analyses have failed for pigeons. One of the more notable being that response rates are so high in pigeon's key pecking that they may be insensitive to changes in reinforcement rate that are commonly used (Bennett et al., 2007; Bowers et al., 2004; Davidson, 2004; Podlesnik et al., 2006; Shull, 2005). Similarly, Shull (2005) proposed that access to alternate behavior may play a role in the discrepancies between pigeon and rat responding. Drawing from Herrnstein (1970), responding

is always a choice between engaging in the experimenter's response and doing something else, and which occurs is influenced by the programmed reinforcement rate and the rate (normalized) by which other behavior is reinforced. If a pigeon's response rate is extremely high, or in this case higher than that of a rat, then theoretically the pigeon would have less time to engage in other behaviors because it is spending more time on the experimenter's programmed response. All theories provide a reasonable explanation for the disparate results of partitioning rat versus pigeon responding. Clearly, species differences and response type need to be carefully considered, and extending the model to another species could provide a further test of the model's validity.

Percentile Schedule

Through a single, multiple or concurrent schedule arrangement, the majority of studies using rats or pigeons used variable or ratio interval schedules of reinforcement (Bennett et al., 2007; Bowers et al., 2004; Davidson, 2004; Podlesnik et al., 2006; Shull et al., 2004; Shull et al., 2001). In the following studies high-rate behavior was examined in part to use such responding to identify motor deficits associated with drug or toxicant exposure, but also because it presented a further test of the log-survivor model. The main high-rate schedule of reinforcement that was used in the present studies was a percentile schedule of reinforcement.

The percentile schedule can be viewed as an automated approach to shaping that offers a way to keep reinforcement rate constant while some targeted dimension of behavior varies (Galbicka, Kautz, & Jagers, 1993). We have targeted high rate behavior by reinforcing short IRTs to provide an operant measure of motor behavior. Reinforced IRTs are based on the specific animals' previous distribution of IRTs. An IRT is eligible for reinforcement if it is shorter than some pre-determined number of the previous IRTs. There are two components of

this version of the percentile schedule. The first is the look-back window, which specifies how many previous IRTs are considered when determining whether the current one qualifies for reinforcement. The second component is the percentile criterion, which sets a value for which the current IRT must be shorter than a specific percentage of the previous IRTs. For example, on a percentile 20: 0.75 schedule of reinforcement, responses are reinforced if the current IRT is shorter than 75% of the previous 20 IRTs (this nomenclature was developed in the Newland lab to facilitate discussing these schedules).

Most of the studies that have attempted the log survival analysis of response bouts have investigated random or variable interval responding in both pigeon and rat behavior (Bennett et al., 2007; Bowers et al., 2004; Davidson, 2004; Podlesnik et al., 2006; Shull et al., 2004; Shull et al., 2001). In recent studies in our laboratory, the model has been extended to include a new species (mouse) and a different type of reinforcement schedule (high-rate percentile). The log survival method was successful in partitioning bouts of mouse nose-poking into two composite measures (Johnson et al., 2009). In this study, several behavioral challenges were introduced over the course of the study including a photoperiod reversal, the addition of a running wheel during experimental sessions, and altering food deprivation. Reversing the photoperiod did not affect overall nose-poke rate, but bout initiation rate was higher in the dark period. Free-feeding animals reduced nose-poking by reducing bout initiation rate, while adding a wheel decreased total nose-poking and bout length but increased initiation rate. The log survival method proved useful in assessing mouse-strain differences in responding as BALB/c mice produced more bouts of nose-poking that were longer, more frequent, and contained a higher within-bout response rate than C57BL/6 mice. Not only was the log survival model valid for characterizing mouse

behavior, it provided support for extension as this model could be useful in describing the effects of drugs and toxicants on behavior.

The aim of the following studies is to use the model to answer questions about the effects of different drugs and toxicants on the structure of a high-rate response bout. A recent study (Johnson et al, 2010) sought to further test the independence of the model's parameters by examining the effects of pentobarbital on the structure of a response bout. Pentobarbital was chosen because of its bi-phasic effects produced under certain schedule arrangements (Dews, 1955; Herrnstein & Morse, 1957). At low to moderate doses, nose-poking increased due to increases in bout initiation rate and bout length, while at the same doses, within-bout response rate was unaffected. Within-bout response rates were not affected until the highest dose, where they were reduced up to 50%. The quantitative model was successful in separating the motivational and motor effects of pentobarbital and therefore could be useful in examining other drugs.

The aforementioned study (Johnson et al., 2010) produced an additional interesting result when examining the effects of pentobarbital on two primary response alternatives: wheel running and nose-poking. While low to moderate doses of pentobarbital increased nose-poking for both strains, wheel running was relatively unaffected for both strains. One hypothesis for this discrepant effect of pentobarbital across response alternatives was the influence of the type of reinforcement (extrinsic or intrinsic) provided by each response. Intrinsic reinforcers have a natural relation to the response (e.g. wheel running), while extrinsic reinforcers have an arbitrary relation to the response (e.g. sucrose pellets for nose-poking) (Catania, 2007). Sherwin (1998) discussed in a review article a novel interpretation for wheel running. In short, studies that examined motivation provide support for the notion that wheel running is self reinforcing, or

provides intrinsic reinforcement. This is an important distinction when compared to an arbitrary behavior that is associated with sucrose reinforcement as the environmental contingencies that maintain each of these two behaviors may be completely different. Examining the effect of a different drug may provide further insight on this drug effect of reinforcement type.

Haloperidol

Haloperidol offers some interesting possibilities because it has long been established that animals working for positive reinforcement show decrements in operant performance after pretreatment with haloperidol (Dews & Morse, 1961; Wise, 2004; Salamone et al., 1999; Salamone et al., 2002). Further, two, sometimes competing, possible causes for decreased operant behavior have been proposed; anhedonic or motoric effects of haloperidol. The general idea of the anhedonia hypothesis is that neuroleptics (like haloperidol) block the dopamine motivational system and one consequence of this can be rate reductions. The anhedonia hypothesis was proposed (Wise, 1982) and subsequently defended (Wise, 2008) after it met considerable criticism (Aparicio, 2007; Freed & Zec, 1982; Gramling et al., 1984). As originally offered, it blended subjective effects of drugs and their reinforcing efficacy, but this has been shown to be overly simple (Salamone et al., 2009. It has also been proposed that, at low doses, dopamine antagonists do not necessarily reduce motivation, but rather reduce the allocation of behavior to reinforcing events (Salamone & Correa, 2002).

In addition to the disruption of reinforcement processes caused by haloperidol, motor disruptions have been observed following haloperidol administration, especially high doses (Fowler et al., 2001; MeKerchar et al., 2005). These semi-competing effects of haloperidol offer an interesting opportunity for the quantitative model that purports to generate bout parameters that are differentially affected by motor and motivational variables (Shull et al., 2001).

Specifically, because operant rate decreases are expected at all doses, it may be difficult to disentangle the motor from the motivational effects of the drug. The primary goal of the present study, then, is to identify different dose-related changes in motivational and motor endpoints.

Strain differences between BALB/c and C57BL/6 mice on operant tasks are well documented (Fowler et al., 2001; Heyser et al., 1997; Johnson et al., 2009; McKerchar et al., 2005; Wang & Fowler, 1999) as are other behavioral measures (Crabbe, 1986; Crawley et al., 1997; Owen et al., 1997; Roullet & Lassalle, 1995), but differences in sensitivity to motor and motivational influences of drugs, especially haloperidol, has garnered less attention. Several reports have examined the 50% effective dose, or the dose that produces half of the maximal effect (ED50) on several response types (Fowler et al., 2001; Kanes et al., 1993; Wang & Fowler, 1999; Wenger, 1979). A study that assessed the (ED50) values of haloperidol-induced catalepsy reveled that C57BL/6 mice had an ED50 of 3.8 mg/kg while BALB/c mice were among the most sensitive strains with an ED50 of 0.3 mg/kg (Kanes et al., 1993). While catalepsy is not of particular interest to the present study, it is a motor impairment that should be reflected by changes in the within-bout response rate. As a result, one would expect a decrease in overall responding when an effective (catalepsy-inducing) dose of haloperidol is administered. However, both C57BL/6 and BALB/c mice have been shown to be relatively resistant to the rate suppressing effects of haloperidol (Fowler et al., 2001) suggesting the 10-fold difference in ED50 of catalepsy to haloperidol may not translate to different operant tasks. A study that examined operant lick suppression in C57BL/6 and BALB/c mice showed that BALB/c mice were five times more sensitive than C57BL/6 mice (Wang & Fowler, 1999). Said another way, the difference in sensitivity between the two strains was half of that found by Kanes and colleagues (1993). Another conflicting report examined nose-poking in C57BL/6 mice and found

an ED50 dose of 0.3 mg/kg of nose poke suppression (Wenger, 1979), a dose more than 10 times lower than the ED50 dose for inducing catalepsy (Kanes et al., 1993).

In addition to the research on the ED50 of operant rate suppression/catalepsy, several studies have examined the difference in strain sensitivity to the rate decreasing effects of haloperidol. Previously cited studies found BALB/c mice more sensitive to the rate suppressing effects of haloperidol on licking behavior (Fowler et al., 2001; Wang & Fowler, 1999), but another study (McKerchar & Fowler, 2005) that measured disk-pressing in these two strains found BALB/c mice to be more resistant to rate suppressing effects of haloperidol than C57BL/6 mice. This is a reminder that the operant task in question can play an important role in assessing drug effects.

The following study should answer several questions concerning haloperidol's effect on behavior. First, it will provide a further test of Shull's quantitative model and its ability to separate motivational from motor effects of a drug. It should also provide more information concerning the role of the nature of reinforcement (intrinsic or extrinsic) in determining a drugs effect on behavior.

Methylmercury (MeHg)

While this log survival model has been shown to be useful in describing the effect of a drug (pentobarbital) on the structure of response bouts, rarely has the method been used to characterize the effects of an environmental toxicant. Given that this model has been sensitive in detecting drug effects, it is potentially useful in characterizing subtle effects of environmental neurotoxicants. Methylmercury (MeHg) provides an opportunity to further test the model. As a neurotoxicant that many humans are exposed to, further information can be acquired as to how exposure to MeHg affects behavior, and in detecting the earliest signs of exposure.

Mercury (Hg) is a ubiquitous environmental toxicant that exists in both natural and anthropogenic sources in the environment. Volcanoes and other natural sources release some elemental mercury into the environment, but the majority is released by coal-burning power generating facilities and other industrial sources. Particular concern has been expressed in the United States concerning the emission of Hg from coal burning power plants (EPA, 1998). Once Hg enters water sources it is converted by aquatic biota to an organic form known as methylmercury (MeHg). MeHg bioaccumulates in marine food chains such that large, long-lived predatory fish contain the highest levels of MeHg. Humans that consume these fish, then, are exposed to MeHg. Thus, there are three main ways that a human can be exposed to Hg: disasters, occupational exposure, and the consumption of contaminated fish. The main route of human exposure is through consumption of contaminated fish and shellfish.

MeHg is a known neurotoxicant that produces detrimental health effects whose severity depends on the dose. In humans, fatalities and severe neurological impairment have been observed in high-exposure poisoning episodes in Minamata, Japan (Harada, 1995) and Iraq (Marsh et al., 1987). For lower environmentally relevant doses, the developing organism has been sensitive. Populations that primarily consume fish or marine mammals have shown cognitive deficits at age 7 (Grandjean et al., 1997), and delayed brainstem auditory evoked potential latencies at age 14 (Murata et al., 2004). The importance of this has been addressed in a recent paper that examined the economic ramifications due to a loss of IQ points have been suggested as a potential consequence of low-dose MeHg exposure (Trasande et al., 2005). The idea of the study was that the loss of IQ affects an individual's productivity across their lifespan, and that loss of productivity was estimated at $8.7 billion annually. The neurotoxicity of MeHg, however, may be more complex than originally thought as a cohort from the Seychelles has

failed to show developmental outcomes to low-dose MeHg exposure (Davidson et al., 2006; Davidson et al., 1998; Myers et al., 2009).

While MeHg exposure during development has garnered a fair amount of attention (Grandjean et al., 1997; Murata et al., 2004), adult-onset exposure has not been examined as frequently. Individuals exposed to the poisoning episode in Minimata, Japan have provided an opportunity to assess the effects of low to high MeHg exposure across the lifespan. In the Minimata population, many of the effects of MeHg exposure persist throughout aging (Ninomiya et al., 2005; Takaoka et al., 2004), and normal age-related deficits in daily living activities appear sooner than in unexposed individuals in neighboring villages (Kinjo et al., 1993).

Fortunately, disaster-type exposure to MeHg is rare. More commonly, humans are exposed to MeHg through fish consumption, which provides a low chronic exposure to MeHg. Therefore, chronic low-dose MeHg exposure is more environmentally relevant and provides an opportunity to assess the potential threat to human health. Chronic low-dose exposures may not result in the overt signs of MeHg toxicity that a disaster-level exposure may cause, but careful neuropsychological testing has revealed more subtle effects including subclinical sensory deficits, and impaired manual dexterity (Beuter et al., 1999; Dolbec et al., 2000; Lebel et al., 1998).

These subtle effects can be detected in an animal model using sensitive operant tasks. In particular, operant schedules of reinforcement that target high rate behavior are of interest if they can be used as a tool to detect the motor deficits associated with MeHg exposure, which has been argued (Newland, 1995, 1997, 2010). The present study aims to juxtapose, under a multiple schedule arrangement, two such reinforcement schedules, that may be differentially sensitive to MeHg exposure. The two schedules that were selected are both second-order schedules designed

to generate high rates of responding. In one, the unit schedule is a differential reinforcement of high rates (DRH 9:4) schedule that requires an animal to respond nine times in four seconds to qualify for reinforcement. Sucrose pellets will be delivered under the FR 5 schedule to limit the number of reinforcers that an animal could earn during a session. This arrangement is denoted a FR5 (DRH 9:4) to show that the DRH unit is reinforced under a FR 5 schedule. Under certain conditions, BALB/c mice can have a fairly abrupt threshold of sucrose consumption. Response rates begin to decline during a 30 minute session after approximately 50 reinforcers are earned.

The other second-order schedule is the RI 60s (Percentile 10:0.5) schedule of reinforcement. The percentile schedule has two components; a look-back window, and a percentile criterion. When a nose poke occurs, the IRT that it terminates is compared to the previous 10 IRTs (look-back window). If the current IRT is shorter than 50% (percentile criterion) of the previous 10 IRTs then it qualifies for reinforcement (criterion response). A 0.05s tone is produced by each criterion response Sucrose reinforcement is then provided under a random interval (RI) 60s schedule of reinforcement. A criterion response that occurred after the termination of the RI 60s resulted in the delivery of a sucrose pellet, and this occurred roughly once every 60s.

The percentile and the DRH schedules are similar in that they are second-order schedules that produce high rates of responding, but they differ in crucial aspects. The DRH schedule is based on a rigid time-based response requirement that has been shown to be sensitive to MeHg exposure (Newland & Rassmussen, 2000; Bornhausen, 1980). Further, reinforcement is delivered under a response-based (FR) schedule of reinforcement. This arrangement should be sensitive to a downward spiral that has been described elsewhere (Johnson et al., 2009; Newland & Rasmussen, 2000). In short, the idea is that after an exposed animal begins to show motor

deficits a corresponding deficit in responding will occur. If the animal is responding at lower rates, it will come in contact with fewer reinforcers. If the animal is contacting fewer reinforces it will respond less thus creating the downward spiral. In contrast, the percentile schedule of reinforcement is based on an individual's recent performance and reinforcement is delivered under a time-based schedule. Because the response criterion is adjusted dynamically, the percentile schedule prevents the aforementioned downward spiral by keeping reinforcement rate relatively constant over a broad range of response rates. However, since it is a sort of shaping schedule, it could promote adjustment to impairment and therefore be less sensitive to MeHg exposure. As such, it is likely that deficits would emerge in responding under the DRH schedule than the percentile schedule.

An analysis of response bouts on the DRH schedule (Newland & Rassmussen, 2000) showed that MeHg exposure did not cause a disruption in the structure of the nine-response bursts. Instead rather the time between these response bursts increased after gestational exposure to MeHg. With a response-based reinforcement schedule it is more likely that a decrease in nine-response bursts (due to increased inter-burst interval) would be reflected in a decrease of reinforcer deliveries, initiating the downward spiral. An analysis of response bouts on the percentile schedule could be equally interesting. As previously stated, it is hypothesized that deficits in overall response rate may appear later for the percentile schedule than those observed for the DRH schedule.

However, changes in the partitioned measures of response rate (bout initiation rate, within-bout response rate) may occur in the absence of changes in the global measure. This scenario is of particular interest since we are interested in characterizing the earliest signs of exposure. Within-bout response rate should be the most sensitive parameter to MeHg exposure if

it is influenced by motor variables as sensory and motor deficits are some of the hallmark consequences of MeHg exposure (Day et al., 2005; Heath et al., 2010).

Summary

Two specific studies follow. One uses acute doses of haloperidol to provoke motor deficits, while the other uses chronic MeHg exposure. Both studies were designed to examine the microstructure of responding through the use of a quantitative model based on the frequency of IRTs. Generally speaking, both studies seek to extend this quantitative model to describing the effects of a drug and a toxicant.

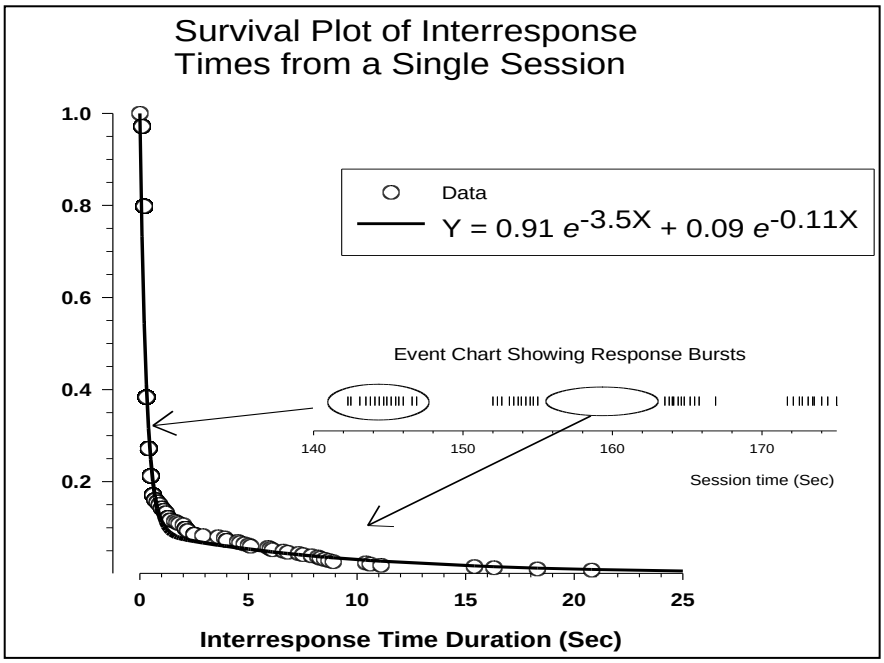

Figure 1. Example of a partition analysis that breaks responding into periods of bouts separated by inter-bout intervals. An event record (inset) shows four individual response bouts, and one stray response following the third bout. Each vertical line shows a lever-press. Open circles show the survival plot of interresponse times and the line shows a fit of Equation 1. Approximately 85% (330 of 386) interresponse times are less than one sec in this example. To generate the survival function, all interresponse times are sorted from shortest (representing high response rates) to longest and plotted as a shown as a survival function (open circles). The equation describes responding as bouts of 3.5 responses/sec and a bout initiation rate of 0.11/sec, or one bout every 9 seconds. The average bout length is 1/0.09 or about 11 responses in this example.

Differential Effects of Haloperidol on Operant versus Locomotor Tasks in BALB/c and C57BL/6 Mice

Joshua E. Johnson, Jordan M. Bailey, M. Christopher Newland

Auburn University

Introduction

Measures of behavior based on overall response rate do not capture the microstructure of operant behavior or wheel-running very effectively because this behavior often occurs in bouts that are characterized by runs of high-rate responding and separated by within-bout intervals. The bout structure can be estimated from a log-survival plot that is based on interresponse times (IRTs) (Shull et al., 2001). This method of partitioning response bouts has been successful in identifying environmental contingencies that affect specific bout parameters including increasing reinforcer rate, increasing reinforcer amount, requiring additional responses at the end of a variable interval schedule, food deprivation and the availability of alternate reinforcement (Johnson et al., 2009; Shull et al., 2001, 2004).

Pentobarbital's effect on mouse operant behavior also showed that the parameters are independent indicators of responding (Johnson et al., 2011). It further showed that nose-poking and wheel-running are affected differently. Several hypotheses were offered for this discrepant effect of pentobarbital across response alternatives. One of these hypotheses was that whether the response was intrinsically reinforcing (i.e., the reinforcing consequence was embedded in the response itself) or extrinsically reinforcing was potentially important. Salamone and colleagues

(2009), in examining the anhedonic effects of haloperidol, have posited a distinction that may be analogous. Salamone posited that in choice scenarios animals with DA depletions reallocate food-maintained responding to less effortful responding. This is potentially important considering the present study can be viewed as a choice scenario where an animal can choose to nose-poke or run in an activity wheel. Further, one of the bout parameters, bout initiation rate, can be viewed as a measure of switching between nose-poking and doing something else.

Haloperidol offers some interesting possibilities because it has long been established that animals working for positive reinforcement show decrements in operant performance after pretreatment with haloperidol (Dews & Morse, 1961; Wise, 2004; Salamone et al., 1999; Salamone et al., 2002). Further, two, sometimes competing, possible causes for decreased operant behavior have been proposed: anhedonic or motoric effects of haloperidol. The general idea of the anhedonia hypothesis is that neuroleptics (like haloperidol) block the dopamine motivational system and one consequence of this can be rate reductions. The anhedonia hypothesis was proposed (Wise, 1982) and subsequently defended (Wise, 2008) after it met considerable criticism (Aparicio, 2007; Freed & Zec, 1982; Gramling et al., 1984). As originally offered, it blended subjective effects of drugs and their reinforcing efficacy, but this has been shown to be overly simple (Salamone et al., 2009). It has also been proposed that, at low doses, dopamine antagonists do not necessarily reduce motivation, but rather reduce the allocation of behavior to reinforcing events (Salamone & Correa, 2002).

In addition to the disruption of reinforcement processes caused by haloperidol, motor disruptions have been observed following haloperidol administration, especially high doses (Fowler et al., 2001; MeKerchar et al., 2005). These semi-competing effects of haloperidol offer an interesting opportunity for the quantitative model that purports to generate bout parameters

that are differentially affected by motor and motivational variables (Shull et al., 2001). Specifically, because operant rate decreases are expected at all doses, it may be difficult to disentangle the motor from the motivational effects of the drug. The primary goal of the present study, then, is to identify different dose-related changes in motivational and motor endpoints.

In addition, two different strains were selected in order to determine the generality of motor v. motivational effects of haloperidol. Strain differences between BALB/c and C57BL/6 mice on operant tasks are well documented (Fowler et al., 2001; Heyser et al., 1997; Johnson et al., 2009; McKerchar et al., 2005; Wang & Fowler, 1999) as are other behavioral measures (Crabbe, 1986; Crawley et al., 1997; Owen et al., 1997; Roullet & Lassalle, 1995). Further, differences in sensitivity to motor and motivational influences of drugs, especially haloperidol, have garnered some attention. BALB/c mice were 10 times more sensitive than C57BL/6 mice to the cataleptic effects of haloperidol (Kanes et al., 1993), 5 times more sensitive to the effects on operant licking (Wang & Fowler, 1999), and more resistant to the effects on operant disk pressing (Fowler et al., 2001). Overall, the operant tasks identified effects at much lower doses than those that produce catalepsy. Considering the disparate effects on different operant tasks, this is a reminder that the behavioral task in question can play an important role in assessing drug effects.

By partitioning response rates into bouts, the present study may be able to identify separate effects on motor and motivational endpoints. In addition, it will provide a further test of Shull's quantitative model and its ability to separate motivational from motor effects of a drug. It should also provide more information concerning the role of the nature of reinforcement (intrinsic or extrinsic) in determining a drugs effect on behavior. Finally, it should provide

additional information concerning the strain differences in sensitivity to the effects of haloperidol on different response devices.

Method

Subjects

Male BALB/c (*n*=6) and C57BL/6 (*n*=8) mice obtained from Harlan Laboratories (Indianapolis) were individually housed and allowed free access to water under a 12 hr light-dark cycle in an AAALAC accredited facility. The animals were kept at 24-27 grams, approximately 85% of their expected free feeding weight, based on growth curves, for the duration of the experiment. All sessions occurred during the day (8am-12pm) during the light portion of a standard light-dark cycle. The use of animals and all experimental procedures were approved by the Auburn University Institutional Animal Care and Use Committee.

Apparatus

MedPC (St. Albans, VT) rat operant chambers fitted to accommodate mice were used in the present study. Each chamber was surrounded by a sound attenuating ventilated shell. Each chamber contained a photo-beam-based nose poke device (model no. ENV-313M), a lever which was not used in the present study, and a 7-inch diameter running wheel. The nose-poke device was located to the left of the food tray. The running wheel was located in the back of the operant chamber. Signal lights were located above both the nose poke device and the lever. A houselight was located near the top of the chamber directly above the food tray. A pellet dispenser delivered 20 mg sucrose pellets into a food tray. MEDPC was used to program the experiments and collect data with 0.01s resolution.

Procedure

Experimental sessions occurred five days a week (Monday-Friday) and were 30 minutes in duration. Animals had previously been trained to nose poke for sucrose under a second order Random Interval (RI) 60s (Percentile 10:0.5) schedule of reinforcement (see Johnson et al., 2009). This schedule reinforces short IRTs (high-response rates) as follows: to qualify for reinforcement the animal's current IRT had to be faster than 50% of the previous 10 IRTs (see Johnson et al., 2009). The percentile schedule is defined by two parameters; the percentile criterion (0.5) and the look back window (10 IRTs). Reinforcement was available on the average of 1 per minute (RI 60s). A running wheel was freely available in the rear of the chamber during all experimental sessions.

Haloperidol (Sigma-Aldrich Co., St Louis, Missouri, USA) was dissolved in a dilute citric acid solution, mixed with physiological saline and buffered to a pH of approximately 5.5) Drug doses were administered in an ascending fashion, *i.p.*, at the following doses: 0.06, 0.1, 0.3 and 0.6 mg/kg. All doses were administered 45 minutes prior to the start of an experimental session. Haloperidol was only administered once per week on Fridays.

Log-survivor analysis

A log-survivor analysis was applied to the IRTs from a single session in order to differentiate between bout initiations and within-bout responses (Shull et al., 2001, Shull et al., 2004). IRTs following reinforcer delivery (i.e. post-reinforcer pause times) were removed from the IRT distribution of nose-pokes before analysis as follows. Sometimes a mouse produced a few IRTs in the 0.25 to 0.50 s range after the sucrose pellet dispenser was activated (and coded as such in the session record). These IRTs resembled the high within-bout response rate. To locate the post-reinforcer pause, the five IRTs following the activation of the sucrose pellet dispenser were reviewed and the pause time was considered to be either any value greater than 2

s or the longest of those five IRTs. This process was arrived at after inspection of many sequences of IRTs that followed activation of the pellet dispenser. All the IRTs (exclusive of post reinforcer pauses) from a session were recorded in deciseconds, collated and sorted in ascending fashion, from shortest to longest. The longest 0.5% and 1% of the IRTs were removed for wheel running and nose poking respectively because preliminary analyses indicated that they exerted excessive influence over the parameter estimates and resulted in visually poorer fits. A two-exponential function (Equation 1) was fitted to this survival function of IRTs

$$Y(t) = (1-p)^{e-wt} + p^{e-bt} \qquad \text{(Equation 1)}$$

using nonlinear least squares regression. Y(t) represents the proportion of IRTs> t seconds; p is the proportion of responses that initiate a bout, and (1-p) is the proportion of responses that are within a bout; b represents the bout-initiation rate in bouts/s ; w represents the within-bout response rate in responses/s. Bout length is 1/p. Both sides of the equation were logged (base 10) prior to performing the fit. RS/1 software (Brooks Automation, Chelmsford, MA) was used for data management and to perform the non-linear regressions required to estimate the bout parameters automatically each day.

This technique was also used to determine the microstructure of wheel-running. Each quarter-wheel revolution generated a pulse that was treated as an individual response, and the sum of all ¼ wheel revolution for an individual session was converted to a distance. Inter-pulse intervals were sorted and subjected to a log-survival analysis as described for nose poke IRTs.

Inferential statistics

All error bars represent standard error of the mean, and all cases are included in each analysis unless specified otherwise. The dependent measures analyzed here included: nose poke

rate, bout-initiation rate, within bout response rate, bout length and distance run (as previously described). If zero nose pokes were emitted during a session, zeroes were recorded for bout parameter values. Statistical significance was assessed at an alpha level of .05. Figures are presented as percent of control responding due to strain differences in baseline responding and in order to facilitate comparisons of relative drug effects on different bout parameters.

To evaluate strain differences on baseline performance, independent samples *t*-tests were conducted on response rates for total nose pokes and wheel running. Separate two-way repeated measures ANOVAs were used to analyze the effects of haloperidol on nose poke rate, distance run, and the bout parameters, with strain as the between subjects variable and the dose as the within subjects variable. Post hoc testing was applied to determine which doses were significantly different from vehicle sessions.

Results

Baseline

The left panels of Figure 2 show that, during baseline, BALB/c mice had higher nose-poke rates ($t(14) = 4.49$, $p<.001$), higher bout initiation rates ($t(14) = 2.75$, $p= .016$), and longer bouts ($t(14) = 5.49$, $p< .001$) than C57BL/6 mice but similar within-bout rates. The right panel of Figure 2 shows overall wheel-running and the bout parameters associated with it. There were no significant differences between BALB/c and C57BL/6 mice in the baseline rates of wheel running or any of the bout parameters, although C57BL/6 mice showed more variability on all measures.

Haloperidol effects

As seen in the left panel of Figure 3, haloperidol decreased total nose-poking for both strains in a dose-dependent fashion ($F(4,56) = 67.88$, $p<.001$). BALB/c mice were more

sensitive to the rate-decreasing effects of the drug ($F(1, 14)= 21.90$, $p<.001$). Further, there was a greater decline in total nose poking for BALB/c mice ($F(4, 56) = 20.31$, $p<.001$, dose X strain interaction).

Both strains showed a dose-related decline in bout-initiation rate ($F(4,56)= 42.85$, $p<.001$), with an interaction indicating BALB/c mice initiated bouts more slowly than C57BL/6 mice as the dose increased ($F(4,56)= 4.89$, p=.008). Similarly, within bout response rate decreased in a dose-related manner for both strains ($F(4,56) = 23.41$, $p<.001$) with BALB/c mice displaying a sharper decline in within-bout response rates ($F(4,56) = 5.25$, $p=.001$). The shape of the dose-response curves was different for within-bout response rate and bout initiation rate. Both total nose-pokes and bout initiation rate featured monotonically decreasing dose-response curves, while all doses of haloperidol produced a roughly similar decrease in within-bout response rate. This was tested using multiple regression analyses. After controlling for strain, the slope of the dose-response curve for bout initiation rate ($\beta=-.65$, $p<.001$) was steeper than for within bout response rate ($\beta=-.43$, $p<.001$). Bout length decreased as a function of dose for both strains ($F(4,56) = 25.66$, $p<.001$) and response bouts were significantly longer for C57BL/6 mice ($F(1,14) = 17.65$, $p=.001$). Finally postreinforcer pause (PRP) increased as a function of dose ($F(4,52) = 34.34$, $p<.001$) and a interaction revealed that this increase was larger for BALB/c mice ($F(4,52) = 4.14$, $p= 0.019$). The baseline PRP average was 1.01s for BALB/c mice and 2.59s for C57BL/6 mice, these averages increased to 3.28s and 3.19s for BALB/c and C57BL/6 mice respectively at the highest haloperidol dose.

The right panel of Figure 3 shows the effect of haloperidol administration on wheel running and its bout parameters. Haloperidol administration decreased wheel running for the C57BL/6 mice ($F(4,56) = 3.21$, $p=.019$). Bout initiation rate was also significantly reduced by

haloperidol administration ($F(4,56) = 5.38$, $p=.001$). Within-bout response rate was increased by haloperidol administration ($F(4,56) = 24.03$, $p<.001$), while bout length was unaffected.

Discussion

The log-survival analysis partitioned response rate into three components, within-bout rate, bout-initiation rate, and the length of response bouts. These three separate measures were differentially affected by haloperidol administration, and the nature of the effect depended on the response type studied. For nose-poking, the haloperidol dose-effect curves for total responses most closely resembled that of bout initiation rate suggesting that motivational influences likely played a major role in the reductions in total response rate. However, reductions in response rate observed at the lowest two doses of haloperidol (especially for BALB/c mice) can be attributed to reductions in bout length. There was a fairly large decline in bout length after administration of the lowest dose of haloperidol that persisted and was fairly unchanged across the other doses, and a similar pattern was observed with within-bout response rate. What was striking was how similar the dose-effect curves looked for total responding and bout initiation rate. They were very similar suggesting that bout initiation rate had a large influence over the global measure of total responding.

Haloperidol appeared to shift response allocation from nose-poking to wheel running. A similar interpretation has been discussed in previous research (Johnson et al., 2009). The idea is that a choice was arranged between the explicitly reinforced activity (nose-poking) and the non-explicitly reinforced activity (wheel running). Thus, one could view this arrangement as a concurrent schedule where behavior is allocated such that time devoted to one alternative is related to its relative value (Baum & Rachlin, 1969; Herrnstein, 1970). Switching in a concurrent schedule arrangement is at its highest when the two alternatives are equally valued (Myerson &

Hale, 1988; Newland et al., 2004) and decreases as preference for one alternative develops. Because bout initiation rate can be viewed as switching from nose-poking (Shull et al., 2001), it is possible that haloperidol administration shifted the preference to wheel running. This idea is supported by the reduction in bout initiation rate and bout length of nose poking observed after administration of haloperidol, and the corresponding tendency for bout length and bout initiation rate to increase for wheel running.

These findings are similar to a recent review that examined DA depletions and effort (Salamone, 2009). The idea was that, as previously discussed, DA depletions or antagonism did not alter an animal's motivation for food (Koob et al., 1978; Salamone et al., 1993). Rather, when given a choice situation behavior would migrate to the less effortful response, or the response that was not maintained by scheduled food presentation (Salamone, 2009). Here, the response maintained by scheduled food delivery (nose-poking) saw decreases after administration of several doses of haloperidol, while wheel-running showed no effect until the highest dose where motor behavior was likely compromised.

Wheel running may not be a more effortful response than nose-poking, but a common theme is developing: behavior is shifting away from a response that was separate from the reinforcer and toward the condition where the response and the consequence were inseparable. It may be possible that the effect seen here is related to the idea that dopamine antagonists, at low doses, reduce the allocation of behavior to reinforcing events (Salamone & Correa, 2002). Further, based on the current and previous findings (Johnson et al., 2011), it is possible that behavior maintained by intrinsic reinforcement is more difficult to perturb than behavior maintained by extrinsic reinforcement. This may be a distinction that builds on Salamone and colleagues' (2009) analysis on food consumption v. instrumental activities. Wheel running may

be like consuming food in his analysis since both activities can be considered intrinsically reinforcing. Behavioral task has been a variable to consider when evaluating drug effects (Crabbe, 2002), but how reinforcement is derived from the behavioral task (intrinsically, extrinsically) may also be an important variable to consider.

A recent paper has used a quantitative model to assess the effects of DA antagonists and suggests a greater role for motor disturbances in operant behavior reduction for typical antipsychotics like haloperidol (Zhang et al., 2005). In this study, haloperidol increased the minimum response time, which is interpreted to be a "motor debilitating effect". However, the animals were not in a cataleptic state as responding persisted even at the highest dose examined; they simply have increased response time. Is this due to a motor debilitating effect, or has behavior moved from a food-reinforced operant to something else? Examining the results of Zhang et al. (2005) in light of the review on DA depletions and effort (Salamone et al., 2009) it is possible that the increased response time is a function of behavior shifting towards a less effortful response not maintained by food reinforcement. In the present study it may have been easy to make a similar claim based on the bout parameter thought to be affected by motor variables (within-bout response rate) being reduced by haloperidol administration. However, in the present study where two competing measured responses were examined (nose-poking, wheel running) it appears that haloperidol may have shifted behavior toward wheel running as it was not affected by haloperidol until the highest dose where motor behavior was clearly affected. Had there been a similar less effortful second behavior being recorded in the Zhang et al. (2005) paper, it is possible a similar phenomenon may have occurred.

Similar to previous research, there were strain differences in the baseline rates of nose-poking, but not wheel running (Johnson et al., 2010; Johnson et al., 2009). BALB/c mice nose-

poked more, initiated more bouts of nose-poking, and had longer bouts of nose-poking. Previous reports also indicated BALB/c mice produce higher rates of nose-poking (Heyser, 1997) and higher numbers of licks, lick peak force, and lick rhythm than C57BL/6 mice (Wang & Fowler, 1999). Further, the strain differences observed in both disk-pressing and nose-poking occurred during true baselines void of pharmacologic or motivational influences, suggesting a different genetically-based behavioral trait. These results indicate subtle behavioral and motor differences between BALB/c and C57BL/6 mice that should be taken into account when selecting a mouse model.

In summary, the quantitative model was successful in partitioning response rate into three different measures that were differentially affected by haloperidol. Also, the role of type of reinforcement (intrinsic, extrinsic) was again determined to be an important determinant of a drug's effect, underscoring not only the importance of considering the behavioral task in question when evaluating drug effects, but also considering the how reinforcement is derived by the behavioral task. Haloperidol caused both motivational and motor effects on nose-poking, while the effects on wheel running were less pronounced. Finally, further support was given to the idea that differences in operant behavior between BALB/c and C57BL/6 mice are due to differences in genetically-based behavioral traits between the two inbred strains of mice.

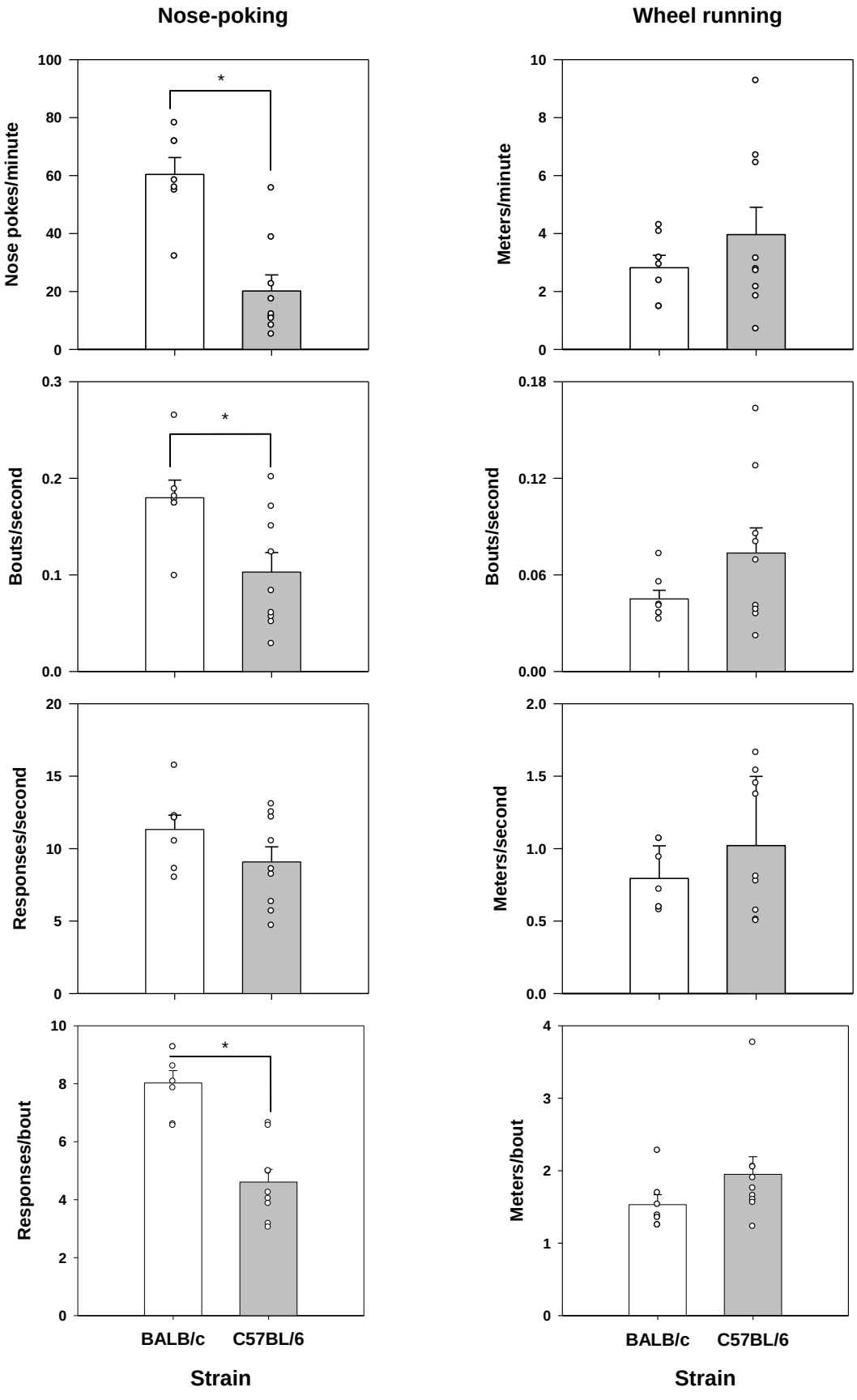
Nose-poking
Wheel running
Nose pokes/minute
Meters/minute
Bouts/second
Bouts/second
Responses/second
Meters/second
Responses/bout
Meters/bout
*
BALB/c
C57BL/6
Strain

Figure 2. Baseline measures of nose-poking (left panels) and wheel running (right panels) for both strains. Total responding and individual bout parameters are in separate panels. Session duration is 30 min. Asterisks (*) denote significant main effects of strain.

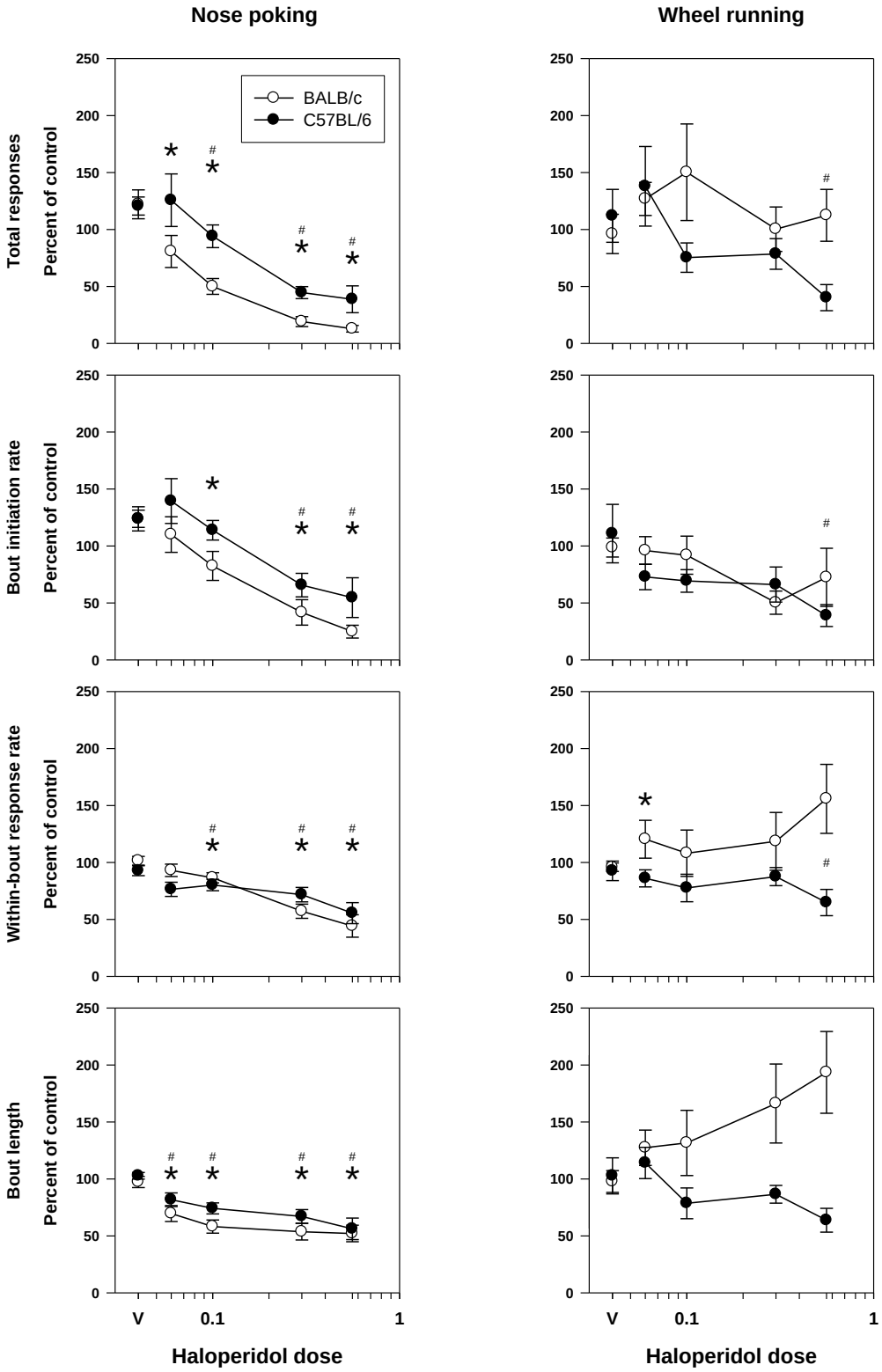

Figure 3. Haloperidol's effect on nose-poking rates and bout parameters (left) and wheel running and bout parameters (right) is displayed. The data are expressed as a proportion of control to

highlight strain differences in Haloperidol's effects independent of control rate differences. The letter V represents experimental sessions where the vehicle (saline) was administered prior to the start of the session. Individual doses that significantly differ from vehicle for BALB/c mice are denoted by the symbol (*). Individual doses that significantly differ from vehicle for C57BL/6 mice are denoted by the symbol (#).

The Microstructure of Behavior Reveals Motor Deficits Associated with Adult-Onset Exposure to Methylmercury

Joshua E. Johnson, M. Christopher Newland

Auburn University

Introduction

MeHg is a well-known developmental neurotoxicant (Newland, 2008; Newland, 2006; Rice, 1996), but long-term chronic low-dose exposure has garnered less attention both in epidemiological studies and in experimental models, which often use high-dose acute exposures. Chronic low-dose exposures may not result in the overt signs of MeHg toxicity that a disaster-level exposure may cause, but careful neuropsychological testing has revealed subclinical sensory deficits, and impaired manual dexterity (Beuter et al., 1999; Dolbec et al., 2000; Lebel et al., 1998).

These subtle effects might be detected in an animal model using sensitive operant tasks. Operant schedules of reinforcement that target high rate behavior are of interest because they can be used as a tool to detect sensory-motor deficits such as those associated with chronic MeHg exposure (Newland, 1995, 1997, 2010). With such preparations, motor and motivational influences over behavior are sometimes conflated. The present study aims to juxtapose, under a multiple schedule arrangement, two reinforcement schedules that may be differentially sensitive to MeHg exposure. The two schedules, a percentile and a Differential Reinforcement of High Rate (DRH) schedule, were selected because they generate high rates of responding but differ in how reinforcement rate is linked to motor competence.

Operant behavior under certain schedules of reinforcement occurs in bouts that can be described by three separate measures: within-bout response rate, bout initiation rate, and bout length (Johnson et al., 2009; Shull et al., 2001; Shull, 2004; Shull et al., 2004). By analyzing the distribution of interresponse times (IRTs), these previous studies have shown that the rate at which bouts are initiated is influenced by motivational variables while the high-rate within-bout responding reflect motor influences. Recently, this quantitative model has been useful in describing changes in behavior brought on by administration of behaviorally active drugs (Johnson et al., 2010). Subtle changes in behavior could be observed in within-bout response rate while bout initiation rate remained relatively stable. The success of distinguishing motivational from motor influences over operant behavior after behavioral and pharmacological interventions suggests that it might have utility in detecting subtle effects of environmental neurotoxicants.

The percentile and the DRH schedules are similar in that they produce high rates of responding, but they differ in crucial aspects. The DRH schedule is based on a rigid time-based response requirement. As implemented here, the animal must respond 9 times within 4 seconds for reinforcement and if it failed to meet that criterion then no reinforcer was delivered. (Newland & Rassmussen, 2000; Bornhausen, 1980). This arrangement could produce a downward spiral in which an exposed animal experiencing motor deficits has difficulty meeting the response criterion (Johnson et al., 2009; Newland & Rasmussen, 2000). If, as a result, overall reinforcement rate decreases then this could further decrease responding, and a downward spiral ensues until responding ceases. In contrast, the percentile schedule of reinforcement is based on an individual's recent performance and reinforcement is delivered under a time-based schedule. A response is reinforced if the current interresponse time (IRT) is shorter than half of the previous 10 IRTs. Because the response criterion is adjusted dynamically, the percentile schedule

may prevent the aforementioned downward spiral by constantly adjusting the reinforcement criterion. Moreover, criterion responses were reinforced randomly about once every minute, so overall reinforcement rate was held relatively constant over a broad range of response rates. Since it is a sort of shaping schedule, however, it could promote adjustment to impairment and might be less sensitive to MeHg exposure. As such, deficits could emerge sooner in responding under the DRH schedule than the percentile schedule. .

The primary objective of the present study was to examine the microstructure of responding under these two reinforcement schedules after chronic MeHg exposure using the log-survival technique developed by Shull and colleagues (2001). The primary hypothesis is that MeHg-related deficits will emerge on a bout parameter (within-bout response rate) before the global measure (response rate) providing an early sign of exposure. In addition to the primary hypothesis, it is hypothesized that deficits in overall response rate will emerge sooner on the DRH schedule of reinforcement than the percentile schedule of reinforcement due to the therapeutic nature of the latter. A secondary objective was to examine the possibility that an L-type calcium channel blocker might prevent MeHg's neurotoxicity. Several such drugs were shown to prevent severe neurological signs associated with MeHg when the drugs were administered orally (Sakamoto et al., 1996). Here, we delivered the drug via a sustained-release pellet implanted subcutaneously.

Method

Subjects

Male BALB/c mice (*n*=96), approximately 7-8 weeks in age obtained from Harlan Laboratories (Indianapolis, IN) were individually housed in a room that featured a 12 hr light-dark cycle (lights on at 6am). Mice were maintained at 25-27 g, approximately 85% of free-

feeding weight throughout the duration of the study. All mice were implanted subcutaneously with a 90-day time-release pellet that contained either nimodipine in one of three doses (2.5, 10, 20mg/kg/day) or a non-drug vehicle obtained from Innovative Research of America (Sarasota, FL) after animals reached a weight range of 25-27g.

Nimodipine exposure

Fourteen days after arriving in the colony, experimental subjects were randomly assigned to one of four possible nimodipine groups. One group was implanted with a placebo pellet and the other three groups were implanted with a 90-day release cellulose pellet containing nimodipine such that the nominal rate of delivery was 0.5, 2.0, or 20 mg/kg/day. All pellets were obtained from Innovative Research of America (Sarasota, FL). The pellets were implanted subcutaneously on the lateral dorsal side of the animal under isoflurane anesthesia. Initial behavioral testing commenced 30 days after pellet implantation so plasma could reach the desired level and animals could be shaped to nose poke (Fanelli et al., 1993).

MeHg exposure

Exposure to 0 or 15 ppm of Hg, as methylmercuric chloride, dissolved in drinking water began 30 days after implantation of the time-release pellets, after autoshaping of the operant response was completed. Subjects from each of the time-release pellet groups were randomly assigned to one of the MeHg groups. The 15 ppm group consumed about 1200 mg/kg/day of mercury based on daily water consumption.

Operant chamber

Sixteen MedPC (St. Albans, VT) rat chambers fitted to accommodate mice were situated inside sound-attenuating ventilated shells. Each chamber contained two nose poke devices (model no. ENV-313M) located in a recessed space on the front wall to the left and right of the

food tray. LED signal lights were located above both nose poke devices, and a houselight was located near the top of the chamber directly above the food tray. A pellet dispenser delivered 20mg pellets into the food tray. MedPC was used to program the experiment and collect data with 0.01s resolution.

Behavioral testing

Eight experimental sessions were conducted per day, six days per week (Monday-Saturday), and sessions lasted a total of 30 minutes. Unexposed mice were run in the first four sessions. Animals were trained to nose poke through an autoshaping procedure similar to Johnson et al. (2009) and under a multiple schedule with two components. The first, denoted as FR5 (DRH 9:4), was a second-order differential reinforcement of high rates (DRH) schedule of reinforcement. The other component, denoted as RI 60" (Percentile 10:0.5), was a second order percentile schedule of reinforcement. The schedule of reinforcement alternated in five minute intervals between the DRH schedule and the percentile schedule. The DRH and Percentile components were signaled by illumination of both the houselight and a LED located directly above the right nose poke device (DRH) and the absence of the houselight and the illumination of a LED located directly above the left nose poke device (Percentile), respectively. The 9:4 DRH schedule of reinforcement was arranged to count a criterion response if nine responses occurred in four seconds. Each criterion nine-response burst resulted in a conditioned reinforcer: a 0.5s tone that was also paired with primary reinforcement when delivered. The nine-response bursts, which constituted a response unit, were reinforced under a FR 5 schedule of reinforcement.

The percentile schedule also engenders high-rate responding, but differs from the DRH in that the criterion is constantly adjusting to an individual animal's recent performance. The

percentile schedule features two important elements: a look-back window and a percentile criterion. The current study used a look-back window of 10 and a criterion of 0.5. Functionally, an animal's current IRT needed to be shorter (faster) than 50% of the previous 10 IRTs (Johnson et al., 2009).

A "low-rate challenge" was introduced during exposure week 11: for three weeks the percentile schedule was inverted so that an IRT was eligible for reinforcement if it was longer than half of the previous 10 IRTs. Otherwise, the percentile parameters remained the same. The DRH component was unchanged during this period.

Log-survivor analysis

A log survivor analysis was applied to the IRTs from a single session in order to differentiate between bout initiations and within-bout responses (Shull et al., 2001, Shull et al., 2004). IRTs immediately following reinforcer delivery (post-reinforcer pause times) were removed from the IRT distribution of nose-pokes before analysis. Sometimes a mouse produced a few short IRTs after the sucrose pellet dispenser was activated (and coded as such in the session record). These IRTs were on the order of 60 to about 500 ms and, in this, resembled the high within-bout response rate rather than the longer post-reinforcer pause, which was at least 2sin length and usually much longer. To ensure that these very short IRTs were not counted as a post-reinforcer pause, the IRTs following reinforcer delivery were screened so that a reasonable candidate for a post-reinforcer pause could be identified. Specifically, an algorithm was written to review the five IRTs following the activation of the sucrose pellet dispenser. A pause time was considered to be either any value greater than 2 s or the longest of those five IRTs. This process was arrived at after manual inspection of many sequences of IRTs that followed activation of the pellet dispenser. All the IRTs (exclusive of post reinforcer pauses) from a session were recorded

in deciseconds, collated and sorted in ascending fashion, from shortest to longest. The longest 1% of the IRTs were trimmed because preliminary analyses indicated that they exerted excessive influence over the parameter estimates and resulted in visually poorer fits. A two-exponential function (Equation 1) was fitted to this survival function of IRTs using nonlinear least squares regression. The Y(t) term represents the proportion of

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

IRTs> t seconds; p is the proportion of responses that initiate a bout, and (1-p) is the proportion of responses that are within a bout; b represents the bout-initiation rate in bouts/s ; w represents the within-bout response rate in responses/s. Bout length is 1/p. Both sides of the equation were logged (base 10) prior to performing the fit. RS/1 software (Brooks Automation, Chelmsford, MA) was used for data management and to perform the non-linear regressions required to estimate the bout parameters automatically each day when the data was transferred to RS/1.

Data analysis

Some MeHg-exposed mice did not complete the study. Since a repeated measures ANOVA would remove those animals from all analyses, a hierarchical regression model was used for data analysis, with exposure week entered as a random-effects variable. The weeks where the percentile schedule was inverted (weeks 11-13) were removed from the hierarchical regression model. Dependent measures were total nose-pokes, within-bout response rate, bout initiation rate, bout length, and post-reinforcer pause under each the two schedules (DRH and Percentile) separately. Fixed main effects were MeHg exposure week, time of experimental session, nimodipine dose, and MeHg-week^2 (since some relationships to exposure duration were

nonlinear). The interactions examined were MeHg * Week, MeHg * Exposure-Week^2 and MeHg * Nimodipine* Week. Other interactions were uninformative and did not significantly improve the model's fit, as determined by a decrease in the log likelihood ratio. All terms were fixed with the exception of MeHg week and, when it provided a significantly lower log likelihood, the intercept. Nimodipine was removed from the model for percentile and DRH nose-poke rate and DRH reinforcers after it was determined that there were neither main effects nor interactions involving nimodipine. It is not clear how best to conduct post hoc analyses on hierarchical analyses. We chose to do simple *t* tests comparing the two mercury groups at each week. The *'s show the results of these tests after applying a conservative Bonferonni correction, formed by dividing the family-wise error rate of .05 by the number of comparisons (12). Because this is a very conservative approach, we added another symbol to show comparisons that are between .004 and .05, a value that was chosen because it captured differences between exposure groups that visually appeared reasonable. Table 1 provides a summary of the mixed regression analyses performed on the dependent measures generated by the multiple DRH percentile schedule of reinforcement.

A repeated measures ANOVA was used to identify significant interactions between responding on a percentile schedule that reinforced high-rate responding and a percentile schedule that reinforced low-rate responding. A 2 (MeHg group) X 4 (week) model was used. Nimodipine group was initially included in the model but was dropped after no significant effects were found. The week prior to the start of the low rate percentile challenge was used to compare against the three weeks of the low rate percentile challenge. For the low-rate challenge the Bonferonni correction was formed by dividing 0.05 by 4.

Results

Figure 4 shows the effect of MeHg exposure group and exposure duration on the measures derived from the two schedules. After behavior stabilized under the multiple schedule animals were responding 30-40 and 70-130 times per minute under the percentile component and DRH component respectively. Within-bout response rates were roughly 12 responses/s under both DRH and percentile schedules. Bouts were about 5-6 responses in length for the percentile schedule and 30 responses for the DRH schedule. Bouts of responding were initiated more frequently during the percentile component (1.5 every 10s in percentile versus 1 every 10s in DRH). There was no effect of nimodipine and no interactions of nimodipine with any variable so graphs in Figure 4 were collapsed across all nimodipine groups, and only effects and interactions involving MeHg will be described.

Percentile responding

Unless stated otherwise, all *p* values described in this narrative are for a two-way interaction. There was a significant interaction between MeHg exposure and $HgWeek^2$ (quadratic term) ($p<.01$) under the percentile schedule. Percentile responding (Figure 4, left panel) was higher for the animals exposed to 15ppm in the early weeks of exposure. Bout initiation rate was slightly higher for exposed animals during some weeks of MeHg exposure ($p<.01$). Within-bout response rate increased to an asymptote of about 12 responses/s and remained at that rate for unexposed mice but decreased for exposed mice beginning week 8 of MeHg exposure ($p<.01$ for the interaction between within-bout rate and $HgWeek^2$). For percentile schedule responding, there were no effects of MeHg exposure on bout length ($p=.17$). Post reinforcer pause (not shown) increased for 15ppm exposed animals at week 13 ($p<.01$) from roughly 0.12s to 0.15s while 0ppm animals remained relatively constant at 1.2s.

DRH responding

The effect of MeHg exposure on the DRH schedule reinforcement can be seen in the right columns of Figure 4 and Table 1 respectively. Again, unless stated otherwise, all *p* values described in this narrative are for a two-way interaction. For controls, DRH response rate rose to an asymptote of about 100 responses/min and remained fairly constant for the remainder of the study. For exposed animals, this measure showed an inverted-U shaped curve, with high rates during weeks 4-8 that declined as the study progressed ($p<.01$ for interaction between DRH total responses and both HgWeek and $HgWeek^2$). For total response rate, there was a significant effect of session time because animals in the first experimental sessions emitted fewer DRH responses than animals in subsequent sessions ($p<.01$, data not shown). The coefficient was positive indicating that as session time-of-day increased so did overall response rate, suggesting that response rate was faster for animals that experimental were later in the day. There was also a main effect of session time such that animals in the first experimental group had shorter bout lengths than those in later experimental sessions ($p<.01$). Animals exposed to 15ppm initiated more bouts under the DRH schedule in the early weeks of MeHg exposure ($p<.01$). Animals in the 15ppm exposure group experienced declines in within-bout response rate beginning as early as week 9 of exposure ($p<.01$). The effect of MeHg exposure on DRH bout length was similar to the effect observed on total DRH responses. A sharp drop in bout length was observed for the 15ppm animals beginning at week 13 ($p<.01$).

Low-rate challenge

For the low-rate challenge response rate during the percentile component was faster for exposed animals ($F(1,73) = 6.78$ $p=.011$) and declined during the weeks where long IRTs were reinforced ($F(3, 219)= 25.99$ $p<.01$). Additionally, bout initiation rate ($F(1,70)$ 14.76 $p<.01$) was faster for exposed animals while within-bout response rate ($F(1,70)$ 15.13 $p<.01$) was faster for

unexposed animals. Bout initiation rate also decreased during the weeks where long IRTs were reinforced (F(3,210) 5.99 p<.01). The length of bouts was shorter for unexposed mice when long IRTs were reinforced (F(3, 210)= 2.9, p=.036).

Reinforcers earned

Figure 5 shows the reinforcers earned for the 0 and 15ppm exposure groups for both percentile and DRH responding. The left panel, showing reinforcers earned under the percentile schedule, remained remarkably consistent until the final weeks and even then the decrease was fairly minor. In contrast, DRH reinforcers earned peaked during exposure week 7 at around 42 reinforcers/session and subsequently began to steadily decline for exposed animals to well under 50% of asymptotic levels (p<.01).

Discussion

Two approaches to generating high-rate operant behavior were used to examine chronic, adult-onset MeHg exposure. Under the DRH schedule, response and reinforcer rates were closely linked but in the percentile schedule these two important variables could be disassociated from each other. MeHg exposure decreased within-bout response rate under both schedules beginning after about 9 weeks of exposure. Insofar as the within-bout response rate reflects the ability to produce high response rates, this result is consistent with other studies showing that motor function is impaired by chronic MeHg exposure (Day et al., 2005; Heath et al., 2010; Newland & Rasmussen, 2000**)**. Thus, by examining the microstructure of behavior using the model developed by Shull and colleagues (2001), a measure was developed that was sensitive to the subtle motor effects of MeHg exposure.

The pattern of effects seen here differs from that observed with lever-pressing under a DRH 9:4 schedule after developmental MeHg exposure (Newland & Rasmussen, 2000). In the

present study, with chronic adult-onset exposure to MeHg, the short IRTs that make up within-bout responding were lengthened by MeHg exposure. In contrast, with developmental exposure within-bout response rate remained intact even as overall responding declined substantially. The lower response rates in that study were due largely to longer between-bout IRTs, or increased pausing, between bouts. If the principle holds that short and longer IRTs reflect motor and motivational variables, respectively, then that would suggest more of a motivational influence over the response rate changes after developmental exposure. Since increased pausing also occurs with more effortful response classes, such as large fixed ratios (Perone & Courtney, 1992), the result from Newland and Rasmussen (2000) could reflect greater perceived effort to produce the high-rate nine-response sequence, even as the integrity of the sequence remained intact

One of the secondary hypotheses was that responding under the DRH schedule would deteriorate sooner than responding on the percentile schedule. This hypothesis was supported as the expected differentiation occurred and is displayed in Figure 5. The decline became clearly evident during the last 4 weeks of MeHg exposure before the end of the study. This result provides support for the "downward spiral" notion. . Animals experienced an initial decline in responding which, in turn, provided fewer reinforcers. Earning fewer reinforcers likely exacerbated the initial decline in responding causing a further decline in the number of reinforcers earned causing the downward spiral of behavior. This decline was observed much earlier in DRH responding as shown in Figure 5. Percentile responding, however, did not experience the same decline observed with DRH responding. Under the percentile schedule used here, the criterion for reinforcement adjusts according to an individual animal's recent performance, and reinforcers are delivered under a random interval schedule of reinforcement

that holds overall reinforcement rates constant. This dynamically changing criterion maintained responding even as the ability to respond at a high rate changed. Apparently there was some adjustment to impairment in the form of increased bout lengths and modest changes in bout initiation rate.

The low-rate challenge produced some interesting findings concerning responding during the percentile component. While overall response rate declined for both exposure groups, the determinants of this effect were different. The 0ppm exposed animal's behavior conformed to the changing demands of the percentile schedule as the length of bouts was significantly reduced producing a lower overall response rate. At the same time, the within-bout response rate was unaffected suggesting that reinforcing longer IRTs did not affect motor behavior. In contrast, within-bout response rate began to decline for 15ppm exposed animals during the low rate challenge and was significantly lower than that of the 0ppm exposed animals. While the 15ppm exposed animals appeared to have adjusted to the new demands of the inverted percentile schedule, there were also clear motor disturbances that likely played a role in the decline of overall response rate.

The higher rates observed for DRH nose-poking and DRH bout initiation for the exposed animals may be at odds with the delayed neurotoxicity of chronic MeHg exposure frequently seen. Animals exposed to 15ppm of MeHg both completed more nose-pokes and initiated more bouts of nose-poking in the beginning of the experiment. However, this result may be due to an interaction with time of day effects. As revealed in the mixed regression analysis, the time of day a session occurred was a significant factor for DRH responding. Specifically, animals in the first experimental session of the day (all in the 0ppm exposure group) completed fewer total DRH responses than animals in subsequent sessions. The positive coefficient for time-of-day effects

indicates that response rate increased through the day. Examination of the residuals (not reported) revealed that the increase in rates through the day approximated the difference between the exposed and unexposed animals, and the unexposed animals' sessions were conducted earlier than those of the exposed animals. The higher rates associated with session time were only seen in DRH rate. Session time was not a significant factor for any measure under the percentile schedule.

Within-bout response rate proved to be a sensitive indicator of early and subtle motor deficits associated with MeHg exposure. The quantitative model shows promise for not only differentiating the motor from the motivational influences of a neurotoxicant, but also for providing a sensitive measure in detecting the motor deficits associated with MeHg and other neurotoxicants. A percentile schedule that holds reinforcement rates constant even as the ability to respond at high rates deteriorates was both therapeutic, in that overall responding continued, and sensitive to neurotoxic effects, since decreases in the high response rates seen within bouts could still be detected.

Table 1

Effect of MeHg on bout parameters

Variable	Effect	Coefficient	SE	*p*	Variable	Effect	Coefficient	SE	*p*
Percentile Response Rate					DRH Response Rate				
	Intercept	2.98	.24	< .001		Intercept	425.48	234.93	.07
	Hg Week	.09	.02	< .001		Hg Week	386.77	23.57	< .001
	Hg WeekSQ	-.006	.001	< .001		Hg WeekSQ	-20.51	1.76	< .001
	Session Time	.012	.023	.594		Session Time	-59.87	22.59	0.008
	Nimodipine	---	---	---		Nimodipine	---	---	---
	Hg*Hg Week	.005	.001	< .001		Hg*Hg Week	14.61	2.18	< .001
	Hg*Hg WeekSQ	$7.0*10^{-4}$	$1.3*10^{-4}$	< .001		Hg*Hg WeekSQ	-1.4	.17	< .001
	Hg*Hg Week*Nimodipine	$2.3*10^{-5}$	$2.1*10^{-5}$	.27		Hg*Hg Week*Nimodipine	.01	.02	.6
Percentile Bout Initiation Rate					DRH Bout Initiation Rate				
	Intercept	.159	.037	< .001		Intercept	.067	.033	.043
	Hg Week	-.003	.002	.11		Hg Week	.005	.002	.035
	Hg WeekSQ	$1.0*10^{-4}$	$1.3*10^{-4}$	.452		Hg WeekSQ	$3.0*10^{-4}$	$1.3*10^{-4}$	.024
	Session Time	$3.2*10^{-4}$	.004	.931		Session Time	$4.6*10^{-4}$	.003	.889
	Nimodipine	$4.6*10^{-6}$	$1.7*10^{-4}$	.786		Nimodipine	$9.5*10^{-5}$	$1.5*10^{-4}$	.536
	Hg*Hg Week	$5.3*10^{-4}$	$2.0*10^{-4}$	.009		Hg*Hg Week	$6.1*10^{-4}$	$2.0*10^{-4}$	.002
	Hg*Hg WeekSQ	$5.1*10^{-5}$	$1.2*10^{-5}$	< .001		Hg*Hg WeekSQ	$5.0*10^{-5}$	$1.2*10^{-5}$	< .001
	Hg*Hg Week*Nimodipine	$3.1*10^{-6}$	$1.6*10^{-6}$	.057		Hg*Hg Week*Nimodipine	$2.1*10^{-7}$	$1.5*10^{-6}$	.891
Percentile Within-Bout Response Rate					DRH Within-Bout Response Rate				
	Intercept	5.27	2	.008		Intercept	4.72	1.75	.007
	Hg Week	1.16	.127	< .001		Hg Week	1.04	.133	< .001
	Hg WeekSQ	-.049	.008	< .001		Hg WeekSQ	-.049	.008	< .001
	Session Time	-.099	.199	.619		Session Time	-.094	.175	.587
	Nimodipine	.008	.01	.416		Nimodipine	.009	.008	.235
	Hg*Hg Week	.027	.012	.02		Hg*Hg Week	.009	.012	.446
	Hg*Hg WeekSQ	-.004	$8.0*10^{-4}$	< .001		Hg*Hg WeekSQ	.002	$7.6*10^{-4}$	.004
	Hg*Hg Week*Nimodipine	$8.0*10^{-5}$	$1.1*10^{-4}$	.498		Hg*Hg Week*Nimodipine	$1.3*10^{-4}$	$1.1*10^{-4}$	.242
Percentile Bout Length					DRH Bout Length				
	Intercept	2.68	1.07	.012		Intercept	.722	4.52	.873
	Hg Week	.268	.074	< .001		Hg Week	6.11	.412	< .001
	Hg WeekSQ	-.019	.004	< .001		Hg WeekSQ	-.294	.032	< .001
	Session Time	.162	.107	.13		Session Time	.046	.453	.918
	Nimodipine	$-4.7*10^{-5}$	.006	.939		Nimodipine	.021	.025	.409
	Hg*Hg Week	.006	.007	.329		Hg*Hg Week	.129	.039	.001
	Hg*Hg WeekSQ	$4.5*10^{-4}$	$4.6*10^{-4}$	.324		Hg*Hg WeekSQ	-.015	.003	< .001
	Hg*Hg Week*Nimodipine	$4.9*10^{-5}$	$6.4*10^{-5}$	.439		Hg*Hg Week*Nimodipine	$2.3*10^{-4}$	$4.3*10^{-4}$	.592
PRP					DRH Reinforcers				
	Intercept	137.36	23.72	< .001		Intercept	15.65	6.31	.013
	Hg Week	-4.73	1.36	< .001		Hg Week	4.93	.59	< .001
	Hg WeekSQ	.24	.11	.026		Hg WeekSQ	-.26	.04	< .001
	Session Time	-1.02	2.37	.665		Session Time	-5.12	.6	.397
	Nimodipine	.04	.12	.775		Nimodipine	---	---	---
	Hg*Hg Week	-.46	.13	< .001		Hg*Hg Week	.25	.05	< .001
	Hg*Hg WeekSQ	.06	.01	< .001		Hg*Hg WeekSQ	-.02	.003	< .001
	Hg*Hg Week*Nimodipine	$1.8*10^{-2}$	.002	.279		Hg*Hg Week*Nimodipine	$3.1*10^{-3}$	$5.4*10^{-3}$	.558

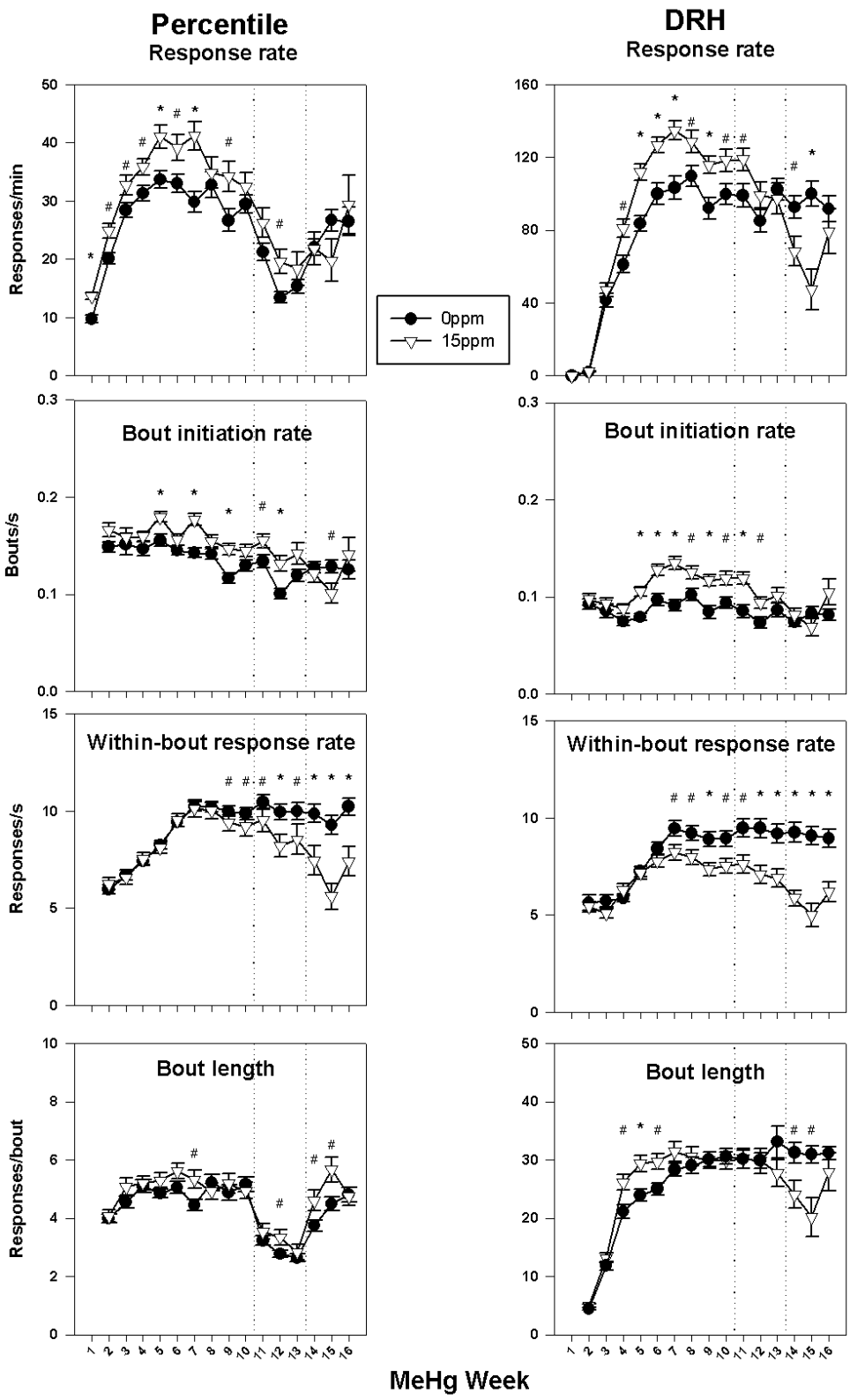

Figure 4. MeHg induced changes over time in nose poke rate and the bout parameters associated with nose-poke rate under percentile (left) and DRH schedules of reinforcement. Animals exposed to 0ppm of MeHg are denoted by a filled circle, and animals exposed to 15ppm of

MeHg are denoted by an upside down open triangle. Significant differences between exposure groups during a given week are highlighted by the symbol (*) when *p* values are <.0045, and the symbol (#) when *p* values are >.0045 and <.05.

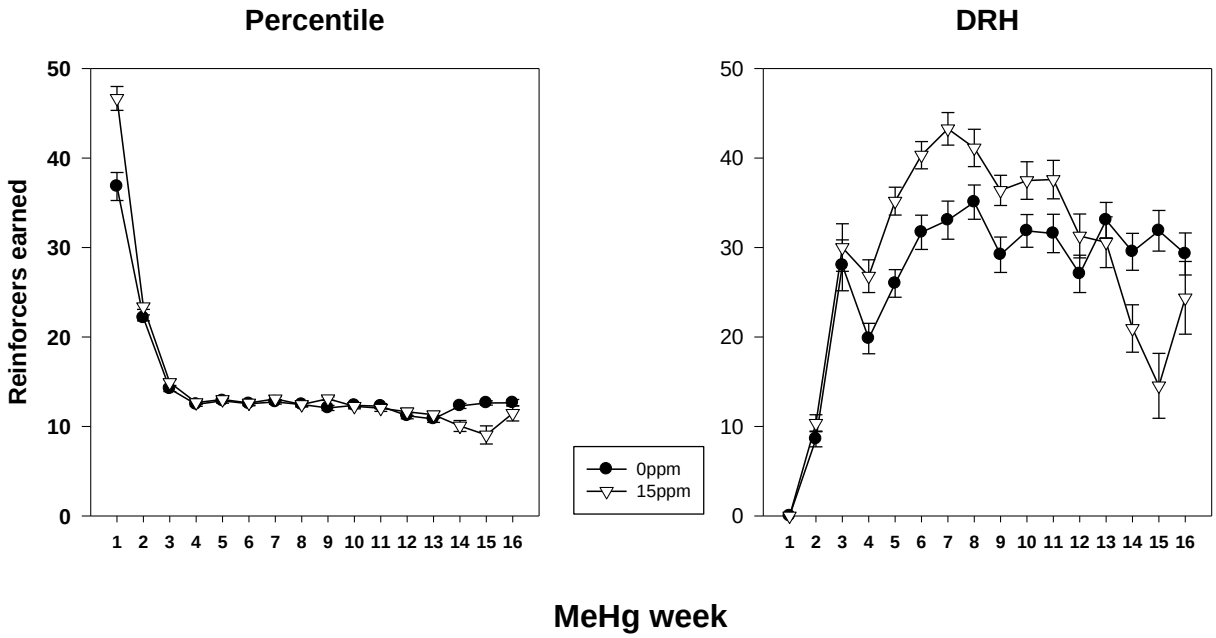

Figure 5. Reinforcers earned during an experimental session are contrasted for responding under a percentile schedule of reinforcement and a DRH schedule of reinforcement over time. Filled circles represent animals exposed to 0ppm MeHg and open upside down triangles represent animals exposed to 15ppm MeHg.

References

Aparicio, C. F. (2007). Haloperidol, dynamics of choice, and the parameters of the matching law. *Behavioural Processes, 75*, 206-212.

Baum, W. M., & Rachlin, H. C. (1969). Choice as time allocation. *Journal of the Experimental Analysis of Behavior, 12*, 861-874.

Bennett, J. A., Hughes, C. E., & Pitts, R. C. (2007). Effects of methamphetamine of response rate: A microstructural analysis. *Behavioural Processes, 75*, 199-205.

Beuter, A., de Geoffroy, A., Edwards, R. (1999). Analysis of rapid alternating movements in Cree subjects exposed to methylmercury and in subjects with neurological deficits. *Environmental Research, 80*, 64-79.

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.

Bowers, M. T., Hill, J., & Palya, W. L. (2008). Interresponse time structures in variable-ratio and variable-interval schedules. *Journal of the Experimental Analysis of Behavior, 90*, 345-362.

Crabbe, J. C. (1986). Genetic differences in locomotor activation in mice. *Pharmacology Biochemistry & Behavior, 25*, 289-292.

Crawley, J. L., Belknap, J. K., Collins, A., Crabbe, J. C., Frankel, W., Henderson, N., Hitzemann, R. J., Maxson, S. C., Miner, L. L., Silva, A. J., Wehner, J. M., Wynshaw-Boris, A., Paylor, R. (1997). Behavioral phenotypes of inbred mouse strains: implications and recommendations for molecular studies. *Psychopharmacology, 132*, 107-124.

Davidson, P. W., Myers, G. J., Cox, C., Axtell, C. D., Shamlaye, C. F., Sloane-Reeves, J.,

Cernichiari, E., Needham, L., Choi, A., Wang, Y., Berlin, M., & Clarkson, T. W. (1998). Effects of prenatal and postnatal methylmercury exposure from fish consumption at 66 months of age: The Seychelles Child Development Study. *Journal of the American Medical Association, 280*, 701–707.

Davidson, P. W., Myers, G. J., Cox, C., Wilding, G. E., Shamlaye, C.F., Huang, L., Cernichiari, E., Sloane-Reeves, J., Palumbo, D., & Clarkson, T. W. (2006). Methylmercury and development: longitudinal analysis of the Seychelles child development cohort. *Neurotoxicology and Teratology,* 28, 529–535.

Davison, M. (2004). Interresponse times and the structure of choice. *Behavioural Processes, 66,* 173-187.

Day, J. J., Reed, M. N., & Newland, M. C. (2005). Neuromotor deficits and mercury concentrations in rats exposed to methyl mercury and fish oil. *Neurotoxicology and Teratology, 27,* 629-641.

Dews, P. B. (1955). Studies on behavior. I. Differential sensitivity to pentobarbital of pecking performance in pigeons depending on the schedule of reward. *Journal of Pharmacology and ExperimentalTherapeutics, 113,* 391-401.

Dews, P. B., & Morse, W. H. (1961). Behavioral pharmacology. *Annual Review of Pharmacology, 1,* 145-174.

Dolbec, J., Mergier, D., Sousa Passos, C. J., Sousa de Morias, S., & Lebel, J. (2000). Methylmercury exposure affects motor performance of a riverine population of the Tapajos river, Brazilian Amazon. *International Archives of Occupational Environmental Health, 73,* 195-203.

Donlin, W. D. (2005). The percentile IRT schedule: High- rate behavior as a tool for examining

the motor effects of methylmercury exposure, in Psychology, Auburn University: Auburn.

EPA (U.S. Environmental Protection Agency). (1998). Study of hazardous air pollutant emissions from electrical steam generating units. Final report to congress. EPA-453/R-98-004a,-b. U.S. Environmental Protection Agency, Office of Air Quality Planning and Standards and Office of Research and Development.

Fisher, C., Schonberger, F., Mugk, W., Heuck, K., & Eichelbaum, M. (1993). Simultaneous Assessment of the Intravenous and Oral Disposition of the Enantiomers of Racemic Nimodipine by Chiral Stationary-Phase High-Performance Liquid chromatography and Gas Chromatography/Mass Spectroscopy Combined with a Stable isotope technique. *Journal of Pharmaceutical Science, 82* (3) 244 - 250.

Fowler, S. C., Zarcone, T. J., & Vorontsova, E. (2001). Haloperidol-induced microcatalepsy differs in CD-1, BALB/c, and C57BL/6 mice. *Experimental and Clinical Psychopharmacology, 9* (3), 277-284.

Freed, W. J., & Zec, R. F. (1982). Criteria for ruling out sedation as an interpretation of neuroleptic effects. *Behavioral and Brain Sciences, 5*, 57-59.

Galbicka, G., Kautz, M. A., & Jagers, T. (1993) Response acquisition under targeted percentile schedules: A continuing quandary for molar models of operant behavior. *Journal of the Experimental Analysis of Behavior, 60*(1), 171-184.

Gilbert, T. F. (1958). Fundamental dimensional properties of the operant. *Psychological Review, 5*, 529-530.

Gramling, S. E., Fowler, S. C., & Collins, K. R. (1984). Some effects of pimozide on

nondeprived rats licking sucrose solutions in an anhedonia paradigm. *Pharmacology Biochemistry and Behavior, 21*, 617-624.

Grandjean, P., Weihe, P., White, R. F., Debes, F., Araki, S., Yokoyama, K., Murata, K., Sorensen, N., Dahl, R., & Jorgensen, P.J. (1997). Cognitive deficit in 7-year-old children with prenatal exposure to methylmercury. *Neurotoxicology and Teratology, 19*(6), 417–428.

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

Heath, J. C., Banna, K. M., Reed, M. N., Pesek, E. F., Cole, N., Li, J., & Newland, M. C. (2010). Dietary selenium protects against selected signs of aging and methylmercury exposure. *Neurotoxicology, 31*, 169-179.

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

Herrnstein, R. J., & Morse, W. H. (1957). Effects of pentobarbital on intermittently reinforced behavior. *Science, 125*, 929-31.

Heyser, C. J., McDonald, J. S., Beauchamp, V., Koob, G. F., & Gold, L. H. (1997). The effects of cocaine on operant responding for food in several strains of mice. *Psychopharmacology, 132*, 202-208.

Hinson, J., Lambert, D. C. & Levere, T. E. (1996). Recovery of function after brain damage: Differential effects of blocking calcium uptake during the recovery of a learned behavior and the performance of the recovered behavior following neocortical brain injury. *Brain Research Bulletin, 39*, 311-316.

Johnson, J. E., Bailey, J. M., & Newland, M. C. (2011). Using pentobarbital to assess the

sensitivity and independence of response-bout parameters in two mouse stains. *Pharmacology Biochemistry and Behavior, 97,* 470-478.

Johnson J. E., Pesek E. F., & Newland M. C. (2009). High rate operant behavior in two mouse strains: A response-bout analysis. *Behavioural Processes, 81*, 309-15.

Kanes, S. J., Hitzemann, B. A., & Hitzemann, R. J. (1993). On the relationship between D2 receptor density and neuroleptic-induced cataplexy among eight inbred strains of mice. *Journal of Pharmacology and Experimental Therapeutics, 267*, 538-547.

Kinjo, Y., Higashi, H., Nakano, A, Sakamoto, M, & Sakai, R. (1993). Profile of subjective complaints and activities of daily living amount current patients with Minamata disease after 3 decades. *Environmental Research, 63,* 241-251.

Kitahana, K., & Valatx, J. L. (1979). Strain differences in amphetamine sensitivity in mice. *Psychopharmacology, 66*, 291-295.

Krol, G. J., Noe, A. J., & Yeh, S. C. (1984). Gas and Liquid Chromatographic Analyses of Nimodipine Calcium Antagonist in Blood and Cerebrospinal Fluid. *Journal of Chromatography, 305* 105 -118.

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

Lebel, J., Mergler, D., Branches, F., Lucotte, M., Amorim, M., & Larribe, F. (1998). Neurotoxic effects of low-level methylmercury contamination in the Amazonian Basin. *Environmental Research, 79,* 20-32.

Marsh, D. O., Clarkson, T. W., Cox, C., Myers, G. J., Amin-Zaki, L., & Al-Tikriti, S. (1987). Fetal methylmercury poisoning: Relationship between concentration in single strands of maternal hair and child effects. *Archives of Neurology, 44,* 1017-1022.

McKerchar T. L, & Fowler S. C. (2005). Dissimilar effects of subchronicclozapine and haloperidol on operant lever pressing in C57BL/6J, BALB/cJ and LP/J mice. *Behavioural Pharmacology,16*, 585-9.

Mechner, F. (1992). *The revealed operant: A way to study the characteristics of individual occurrences of operant responses.* Cambridge, Ma: Cambridge Center for Behavioral Studies.

Moser, V. C. (1990). Approaches for assessing the validity of a functional observational battery. *Neurotoxicology and Teratology, 12*, 483-488.

Myers, G.J ., Thurston, S. W., Pearson, A. T., Davidson, P.W ., Cox, C., Shamlaye, C. F., Cernichiari, E., & Clarkson, T. W. (2009). Postnatal Exposure to Methyl Mercury from Fish Consumption: a Review and New Data from the Seychelles Child Development Study. *Neurotoxicology, 30*, 338-349.

Myerson, J., & Hale, S. (1988). Choice in transition: A comparison of melioration and the kinetic model. *Journal of the Experimental Analysis of Behavior, 49***,** 291-302.

Newland, M. C. (1995). Motor function and the physical properties of the operant: applications to screening and advanced techniques. In L. W. Chang, & W. Slikker (Eds.), *Neurotoxicology: Approaches and methods* (pp. 265-299). San Diego: Academic Press.

Newland, M. C. (1997). Neural, behavioral, and measurement considerations in the detection of motor impairment. In K. R. Reuhl, & H. E. Lowndes (Eds.), *Nervous system and behavioral toxicology* (Vol. 11, pp. 247-269). NewYork: Pergamon/Elsevier.

Newland, M. C. (2010). Neural, Behavioral, and Measurement Considerations in the Detection of Motor Impairment. In M. Philbert (Ed.), *Comprehensive toxicology: Nervous system and behavioral toxicology* (2 ed., Vol. 13, pp. 289-315). New York: Academic Press.

Newland, M. C., Donlin, W. D., Paletz, E. M., & Banna, K. M. (2006). Developmental behavioral toxicity of methylmercury. In Levin, E. D., & Buccafusco, J. J. (Eds.), *Animals models of cognitive impairment* (pp. 101-146). Boca Raton, FL: CRC Press.

Newland, M. C., Paletz, E. M., & Reed, M. N. (2008). Methylmercury and nutrition: adult effects of fetal exposure in experimental models. *Neurotoxicology, 29*, 783-801.

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., & Reile, P. A. (1999). Blood and brain mercury levels after gestational exposure. *Toxicological Sciences, 50*, 106-116.

Newland, M. C., Reile, P. A., & Langston, J. L. (2004). Gestational exposure to methylmercury retards choice in transition in aging rats. *Neurotoxicology and Teratology, 26,* 179-194.

Ninomiya, T., Imamura, K., Kuwahata, M., Kindaichi, M., Susa, M., & Ekino, S. (2005). Reappraisal of somatosensory disorders in methylmercury poisoning. *Neurotoxicology and Teratology, 27*, 643-653.

Owen, E. H., Logue, S. F., Rasmussen, D. L., & Wehner, J. M. (1997). Assessment of learning by the morris water task and fear conditioning in inbred mouse strains and F_1 hybrids: Implications of genetic background for single gene mutations and quantitative trait loci analyses. *Neuroscience, 80*, 1087-1099.

Perone, M., & Courtney, K. (1992). Fixed-ratio pausing: Joint effects of past reinforcer magnitude and stimuli correlated with upcoming magnitude. *Journal of the Experimental Analysis of Behavior, 57*(1), 33-46.

Podlesnik, C. A., Jimenez-Gomez, C., Ward, R. D., & Shahan, T. A. (2006). Resistance to change of responding maintained by unsignaled delays to reinforcement: A

response bout analysis. *Journal of the Experimental Analysis of Behavior, 85,* 329-347.

Rice, D. C. (1996). Sensory and cognitive effects of developmental methylmercury exposure in monkey, and a comparison to effects in rodents. *Neurotoxicology, 17,* 139-54.

Roullet, P., & Lassalle, J. M. (1995). Radial maze learning using exclusively distant visual cues reveals learners and nonlearners among inbred mouse strains. *Physiology and Behavior, 58,* 1189-1195.

Sakamoto, M., Ikegami, N., & Nakano, A. (1996). Protective effects of Ca2+ channel blockers against methyl mercury toxicity. *Pharmacology & Toxicology, 78*(3), 193-199.

Salamone, J. D., Aberman, J. E., Sokolowski, J. D., & Cousins, M. S. (1999). Nucleus accumbens dopamine and rate of responding: neurochemical and behavioral studies. *Psychobiology, 27,* 236–247.

Salamone, J. D., & Correa, M. (2002) Motivational views of reinforcement: implications for understanding the behavioral functions of nucleus accumbens dopamine. *Behavioral Brain Research, 137,* 3-25.

Sherwin, C. M. (1998). Voluntary wheel running: a review and novel interpretation. *Animal Behaviour, 56,* 11-27.

Shull, R. L. (1991). Mathematical description of operant behavior: An introduction. In I. H. Iversen, & K.A. Lattel (Eds.), *Techniques in the behavioral and neural sciences: Experimental analysis of behavior* (Vol. 6, part 2, pp. 243-282). Amsterdam: Elsevier.

Shull, R. L. (2004). Bouts of responding on variable-interval schedules: Effects of deprivation level. *Journal of the Experimental Analysis of Behavior, 84,* 99-110.

Shull, R. L. (2005). The sensitivity of response rate to rate of variable –interval reinforcement for

pigeons and rats: A review. *Journal of the Experimental Analysis of Behavior, 84*, 99-109.

Shull, R. L., Gaynor, S. T., & Grimes, J. A. (2001). Response rates viewed as engagement bouts: Effects of relative reinforcement and schedule type. *Journal of the Experimental Analysis of Behavior, 75*, 247-274.

Shull, R. L., Grimes, J. A., & Bennett, J. A. (2004). Bouts of responding: The relation between bout rate and the rate of variable-interval reinforcement. *Journal of the Experimental Analysis of Behavior, 81*, 65-83.

Takaoka, S., Fujino, T., Sekikawa, T., & Miyaoka, T. (2004). Psychophysical sensory examination in individuals with a history of methylmercury exposure. *Environmental Research, 95*, 126-132.

Tilson, H. A. (1993).Neurobehavioral methods used in neurotoxicological research. *Toxicology Letters, 68*, 231-240.

Trasande, L., Landrigan, P. J., & Schechter, C. (2005). Public health and economic consequences of methyl mercury toxicity to the developing brain. *Environmental Health Perspectives, 113*, 590-596.

Wang, G., & Fowler, S. C. (1999). Effects of haloperidol and clozapine on tongue dynamics during licking in CD-1, BALB/c and C57BL/6 mice. *Psychopharmacology, 147*: 38–45.

Wenger, G. R. (1979). Effects of clozapine, chlorpromazine and haloperidol on schedule-controlled behavior. *Pharmacology Biochemistry & Behavior, 11*, 661-667.

Wise, R. A. (1982) Neuroleptics and operant behavior: the anhedonia hypothesis. *Behavioral Brain Sciences, 5*, 39-87.

Wise, R. A. (2004). Dopamine, learning and motivation. *Nature Reviews Neuroscience, 5*, 483–

494.

Wise, R. A. (2008). Dopamine and Reward: The Anhedonia Hypothesis 30 years on. *Neurotoxicity Research, 14,* 169-183.

Zhang, Z., Rickard, J. F., Asgari, K., Body, S., Bradshaw, C. M., & Szabadi, E. (2005). Quantitative analysis of the effects of some “atypical” and “conventional” antipsychotics on progressive ratio schedule performance. *Psychopharmacology, 179,* 489-497.