The Temporal Patterning of Spontaneous High-Rate Behavior: An Evaluation of Bout Partitioning Methods for Behavior Under Neurotoxicant Challenge

by

Daniel James Hoffman

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
May 09, 2015

Keywords: bout analysis, change-point, methylmercury, nimodipine, spontaneous running

Approved by

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

Abstract

Across species, much free-operant behavior exhibits a temporal pattern characterized by bouts of activity and periods of disengagement. Analysis of interevent time distributions has been widely used to characterize bout structure. As an alternative to such methods, we propose a novel bout analysis technique that uses change-point detection to partition a cumulative record into activity epochs directly. The utility of the change-point bout analysis was examined in two studies. The first study directly compared the change-point analysis to the two most widely used bout analysis methods, log-survivor analysis with a biexponential model and log-interval analysis with a Gaussian mixture model. All three methods provided similar descriptions of bout structure for data simulated to follow the standard theoretical model, but the log-interval analysis produced less accurate parameter estimates for several bout structures. When applied to spontaneous wheel running data, the change-point analysis proved as good as or better than the conventional methods. The log-survivor consistently overestimated response rate and the log-interval analysis produced an implausible description of the bout structure. All three methods detected a significant methylmercury-related decrease in within-bout rate, but the change-point analysis also detected a significant reduction in bout length. In the second study, the change-point analysis was used to characterize changes in the bout structure of spontaneous wheel running in BALB/c mice chronically exposed to 0 or 15 ppm methylmercury. Study two also examined the potential role of calcium in methylmercury neurotoxicity using co-treatment with the calcium channel blocker nimodipine. Methylmercury exposure increased mortality rates and

substantially decreased total running. The change-point analysis revealed that the decreases in global running were the result of declines in within-bout running rate and the length of response bouts. Nimodipine dose-dependently protected against those effects.

Acknowledgements

I want to thank the members of my committee, Drs. Chris Newland, Martha Escobar, Alejandro Lazarte, and Christopher Correia, for their formative feedback on the work presented in this dissertation, as well as their professional guidance during my time in the Auburn Psychology Department. Special thanks are due to Chris Newland, my committee chair and major advisor, for his mentorship and for knowing both when to be patient and when to prod—this manuscript would not have been completed otherwise. I also want to express appreciation to Dr. Blake Hutsell for being an eager sounding board for some of the ideas contained in this dissertation, and on behavioral science in general. Lastly, I want to thank the various, many graduate and undergraduate researchers with whom I've worked in the behavioral toxicology lab for their day to day efforts, big and small, that too often go unremarked.

Table of Contents

List of Tables

List of Figures

Chapter 1: Overview of Bout Analysis Methodology and the Effects of Methylmercury Exposure on Behavior

Response strength has been an important theoretical construct in the scientific study of behavior for over fifty years. Skinner (1938) emphasized visual analysis of cumulative records and advocated absolute response rate of a target behavior as a measure of response strength. In contrast to Skinner, Herrnstein (1961) characterized behavior as choice because all behavior occurs in the context of competing alternatively available behaviors. Accordingly, Herrnstein emphasized the relative response rate—the rate of a target behavior relative to the rate of some other measured behavior—as the index of response strength. Both approaches have been enormously successful and influential in the maturation of a rigorous behavioral literature.

Average response rate is commonly calculated as the total number of responses divided by available time, but average response rate is a function of the interevent times (IETs) that separate consecutive responses and so response rate can also be calculated as the reciprocal of the average IET. High rate behavior produces short IETs on average and low-rate behavior produces long IETs on average. Calculating response rate as the total number of responses per available time implicitly assumes that all interevent times occur as a result of a single generating process and therefore can belong to a single probability distribution (Sibly, Nott, & Fletcher, 1990).

A single-process account is inconsistent with Herrnstein's conception of behavior as choice. IETs that span two alternative behavior are qualitatively different from IETs that separate responses within a bout of a single behavior. Slater (1974) showed that these qualitatively different IETs differ quantitatively as well and suggested that the microstructure of responding may be an important variable in its own right. As discussed below, Shull and colleagues (2001;

2002) have demonstrated that the two classes of IETs are functionally different, with each representing different behavioral processes. Figure 1 shows a frequency histogram of hypothetical data conforming to a two-process model; the overlying curve plots the expected height based on a single exponential process. The single-process model underestimates the proportion of long IETs (which is greater than expected because of the contributions of the unaccounted for second distribution). The result is an average IET that underestimates the absolute rate of responding during response bouts while also providing a poor estimate of the relative rate of responding when compared to concurrently available behavioral alternatives. Thus, average response rate is a gross measure of behavior that may obscure important differences in the temporal structure of responding across a behavioral record.

Ethologists have long recognized that the single-process account does not provide an adequate account of behavior in natural environments (Slater, 1972; Machlis, 1977). Across species, much behavior occurs as bursts of responding (bouts of behavior) separated by periods of disengagement during which the organism may engage in alternative behavior. As a result, many have treated the bout as the behavioral unit of interest (Machlis, 1977; Slater, 1974). Bouts are defined across time by the intervals that punctuate bursts of responding for a given behavior, as opposed to the intervals that separate individual responses within bouts. Despite the seemingly simple definition of a bout, identifying response bouts in behavioral records has proven to be a challenging endeavor in practice.

A number of sophisticated visual and quantitative methods of partitioning behavior into bouts have been examined, but no method has yet distinguished itself as clearly superior to the alternatives (Sibly et al., 1990; Yeates et al., 2001). Empirically based guidelines for determining which method to apply in the case of particular behaviors or schedules of reinforcement have yet

to be established. The choice of quantitative method is complicated further by the fact that the models are often applied in different types of preparations, for different types of behaviors, and over different time scales. Although some studies (e.g., Sibly, et al., 1990; Yeates, et al., 2001) have directly compared multiple approaches on a common data set, systematic comparisons of bout analysis methods for high-rate behavior are lacking in the literature. Furthermore, no published study has yet directly compared the models' sensitivity to drug or toxicant effects on the temporal patterning of behavior.

The experiments described in this dissertation were designed to address these weaknesses in the literature by comparing three methods for partitioning behavior using both simulated data and spontaneous wheel-running in adult mice. Spontaneous wheel-running occurs at high rates in rodents (Sherwin, 1998), and is sensitive to motor effects of heavy metal neurotoxicants (Heath et al., 2010), as well as several classes of drugs (Johnson, Bailey, & Newland, 2009). Then, after initial comparisons, a suitable model will be applied to characterize the effects of chronic exposure to methylmercury on bout structure.

Bout Analysis Methods

Within a bout of behavior, responses tend to be separated by interevent times shorter than the overall mean IET and with lower variability than the overall IET distribution (Machlis, 1977). These within-bout responses are often viewed as a motor component of behavior (Shull, Gaynor, & Grimes, 2001) and are differentially affected by drugs with known motor effects (Johnson, Pesek, & Newland, 2009). The bouts are separated by inter-bout intervals (IBIs) comprising relatively longer and more variable IETs.

Early forms of bout analysis were based on visual inspection of a histogram of the IET frequency distribution (see Figure 1). The researcher identified the bout-criterion interval—the

IET duration above which IETs are unlikely to be part of a bout—based on a large drop in relative frequency. The bout-criterion interval (BCI) was then used to divide the IETs into two separate populations for subsequent analysis (Machlis, 1977). Visual analysis of IET histograms is subjective and prone to misclassification of IETs. More sophisticated visual methods and quantitative models for partitioning behavior into bouts have since been developed (Sibly et al., 1990; Shull et al., 2001; Tolkamp et al., 1998). The quantitative methods apply curve-fitting algorithms to estimate the best-fitting parameters of an underlying theoretical distribution, allowing for less biased estimation of the bout properties. The remainder of this section will discuss different approaches to bout analysis and describe manipulations known to differentially influence bout parameters.

Log-survivor analysis

Log-survivor analysis is both a visual technique for estimating the bout-criterion interval (Slater, 1974) and the basis for a common quantitative approach to estimating parameters of the separate IET distributions (Shull et al., 2001). For a log-survivor analysis, interevent times from throughout the measurement period are accumulated and the resulting complementary cumulative distribution function (hereafter referred to as the survivor function) is plotted against time in semi-log space; see Figure 2 (described below) for an example. In the most common quantitative method, the survivor function is modeled as a mixture of two negative exponential distributions, each of which corresponds to a separate response-generating process. One process generates the short interevent times within a bout and the other generates the long interevent times between bouts. Each generating function is considered a Poisson process, giving rise to a mixture of two exponential distributions that produce the characteristic "broken-stick" pattern of the log-survivor distribution (Kessel & Lucke, 2008). The details of the log-survivor method are

summarized in Table 1 below.

The ideal, broken-stick pattern of the log-survivor function is illustrated in Figure 2. The distribution is composed of two distinct limbs, each of which corresponds to IETs from a different generating process. The steep initial portion of the plot represents short IETs that belong to the within-bout distribution; the long tail of the function represents the longer IETs that belong to the bout-initiation distribution (Slater, 1974). The point where the two exponential functions meet corresponds to the approximate bout-criterion interval (Machlis, 1977). The slopes of the two limbs correspond to the rate parameters of the two exponential functions (and thus to the average IET for that subpopulation), and the Y-intercept of the tail corresponds to the proportion of total IETs that are between-bout IETs. The function shown in Figure 2 was generated using a biexponential model with a within-bout response rate equal to 3.5 responses per second, with a bout-initiation rate of 0.2 bouts per second (meaning an average of 10 seconds separating bouts), and a Y-intercept at 0.10 (meaning that 10% of all IETs are between-bout IETs). It is important to note that these interpretations of the visual elements are predicated upon the biexponential model. If the IETs are not well described by an exponential mixture model, then these interpretations will be invalid and should be modified to conform to the appropriate probability distribution.

Visual analysis of log-survivor plots was the predominant method of bout analysis until recently (Sibly et al., 1990). The advent and wide availability of personal computers and, especially, advances in nonlinear curve fitting algorithms has advanced the log-survivor approach by facilitating the estimation of the bout parameters numerically, rather than subjectively or via tedious curve-stripping algorithms. The log-survivor function can be modeled quantitatively as a sum of negative exponentials, hereafter referred to simply as the biexponential

distribution:

$$P(y \geq t) = p_w e^{-\lambda_w t} + p_b e^{-\lambda_b t} \quad (1)$$

where $P(y \geq t)$ is the proportion of IETs greater than time t, λ_w is the within-bout response rate, λ_b is the between-bout response rate, p_w is the proportion of IETs that occur within bouts, and p_b is the proportion of IETs that occur between bouts. The average bout length is estimated by $1+1/p_b$ (1 is added to the reciprocal because every IET must consist of two responses). The bout-criterion interval that minimizes misclassification of IETs can be calculated by solving equation 1 for the condition where the first term equals the second term (Slater & Lester, 1982), resulting in

$$t_c = \frac{1}{\lambda_w - \lambda_b} ln \frac{p_w}{p_b} \quad (2)$$

where t_c is the criterion interval, λ_w and λ_b are as in equation 1, and p_w and p_b are the number of within and between-bout IETs, respectively.

Figure 3 shows the effects of the various parameters on the appearance of the log-survivor function. The solid black line serves as a reference for the other three functions, each of which shows the result of changing a single parameter while keeping others consistent with the reference line. The black reference line shows a case where the within-bout rate is 3 responses per second, the bout-initiation rate is 0.5 bouts per second (*i.e.*, an average of 2 seconds separates bouts), and 85% of IETs (1.0 – 0.15) occur within bouts. The blue line shows the effect of increasing the within-bout rate from 3.0 to 6.0 responses per second while keeping other parameters constant; note the steeper slope of the survivor function's initial limb relative to the reference line. The red line shows the effect of decreasing the bout-initiation rate from 0.5 to 0.15 (*i.e.*, increasing the average inter-bout interval from 2 seconds to 6.7 seconds); note the shallower slope of the survivor function's tail relative to the reference line. The green line shows

the effect of increasing percentage of IETs that occur within bouts from 85% to 95% (*i.e.,* increasing the average bout length from 6.7 to 20 responses); note the greater length of the initial limb relative to the reference line, and the decrease in Y-intercept of the tail.

Machlis (1977) provided an early application of the log-survivor approach described by Slater (1974) in a seminal paper on the temporal patterning of pecking by chicks. In Machlis' study, food-deprived Ross chicks pecked at colored pins on the floors of their home cages. Machlis estimated the bout parameters from the log-survivor curve of inter-peck intervals using a curve-peeling technique in which each negative exponential function was fit to the log-survivor curve iteratively. On the first iteration, a fit was performed for all time intervals greater than some value *X*, where *X* = a very short IET. The Kolmogorov-Smirnov one-sample t-test was used to assess goodness of fit by the exponential. If the model resulted in a poor fit, a larger value of *X* was chosen and the fit and evaluation performed again. This process continued until a good fit was achieved and all intervals to the right of the *X* value were then trimmed from the initial log-survivor curve. The separate components were analyzed as separate negative exponential distributions. Machlis used this iterative algorithm to estimate bout parameters for two models of the temporal patterning of chicks' pecking.

According to Machlis's model I, a two-state model, the organism exists in one of two states at any given time: a pecking state or a not-pecking state. IETs during the pecking state are within-bout IETs, and very long IETs between-bout intervals (BBI) during which the chick is in a non-pecking state. Additionally, the model assumes that state transitions occur as a low-rate Poisson process in which bouts are initiated randomly and the transition from a bout to disengagement is also random. The two processes' IET distributions overlap and sum to produce the survivor function. The degree of overlap is especially important—the larger the overlap, the

greater the number of intervals that would be misclassified by the bout-criterion (Machlis; Slater & Lester, 1982).

According to Machlis's model II, a three-state model, the chick cycles among a pecking state, a between-bout state, and a third not-pecking state of alternative behavior, such as resting. Thus, with a three-state model bouts of bouts may occur. As with model I, model II assumed that switching between the pecking state and the between-bout state occurs as a Poisson process and that within-bout IETs are generated by a higher-rate Poisson process than between-bout IETs. The additional not-pecking state in model II captures alternative behavior, and can be considered a "meta-bout" state wherein bouts cluster together, similar to the clustering of single responses within a bout. Switching between the between-bout state and the not-pecking state also occurs as a Poisson process. Figure 4 illustrates the difference in appearance between two-state and three-state log-survivor plots. Machlis's results generally supported the three-state, but the two-state model has been more commonly used—perhaps because of difficulties in estimating the third component reliably. However, other factors (discussed below in detail) that influence the microstructure of behavior may cause a two-state process to appear to have three states on a log-survivor plot.

Shull, Gaynor, and Grimes (2001) used the log-survivor method to support a theoretical model of operant behavior. According to Shull's two-state Markov model, an organism alternates between two behavioral states: a disengagement state and an engagement, or visit, state. A response during the visit state continues the visit with probability $1 - p$, which is assumed to be constant for all within-visit responses. A response during the disengagement state transitions to the visit state with probability p, which is assumed to be constant for all responses during the disengagement state. As with earlier two-state log-survivor models, Shull et al.'s (2001) model

assumes that the response-generating processes are Poisson and, consequently, that the resulting interevent time distribution is biexponential. In addition to the IET distribution, the distribution of bout lengths also follows a negative exponential distribution. Thus, Shull et al.'s model is similar to Machlis' (1977) two-state model, with the principal difference being Machlis' inclusion of a dead-time constraint. The dead-time constraint accounts for the minimum IET possible, which is a function of the physiological limitations of the animal and the mechanical limitations of the recording device. On a log-survivor plot, dead-time appears as a flat section (slope = 0) of the initial limb spanning from the origin to the minimum IET. To avoid complications when fitting an exponential model to an IET distribution, the distribution is commonly shifted to the left by subtracting the minimum obtained IET from each IET; thus, technically behavior is partitioned into bouts using a shifted biexponential distribution.

Perhaps the most important contribution of Shull and colleagues to the bout analysis literature has been their demonstration across several studies that the bout parameters are independent and sensitive to different experimental manipulations. Manipulations that affect establishing operations (Michael, 1982) affect the bout-initiation term, but not the within-bout term. Deprivation level and changes in probability of reinforcement, two manipulations that should influence motivation rather than motor processes, selectively influence bout-initiation rate (Shull et al., 2004). The within-bout component is sensitive to changes in the response requirement (Shull et al.), as would be expected if the visit state is a functional response unit. These findings support the interpretation of the visit as a response unit whose within-bout IET distribution is related to motor processes and whose interbout interval distribution is related to motivation.

Slater (1974) identified log-frequency analysis as a closely related alternative to log-

survivor analysis. Similar to the log-survivor method, log-frequency analysis begins by accumulating the interevent times from throughout the measurement period into a single IET distribution. Rather than the survivor distribution, the log-transformed frequency distribution of IETs is plotted against IET duration in semi-log space. The resulting distribution is then modeled with the probability density function of the biexponential distribution:

$$P(y = t) = p_w \lambda_w e^{-\lambda_w t} + p_b \lambda_b e^{-\lambda_b t} \quad (3)$$

where all parameters are as in equation 1. The critical difference is that log-frequency analysis uses the probability density function, whereas log-survivor analysis uses the survivor distribution. Table 1 presents side-by-side summaries of the two methods for easy comparison. Figure 5 shows a comparison of a log-survivor plot (top panel) and a log-frequency plot (middle panel) for the same interevent time data.

Although their underlying mathematical model is the same, log-frequency method may make departures from exponentiality more visually apparent (Sibley et al., 1990). The log-survivor visual analysis, by virtue of using a cumulative function, creates interdependency among data points and always results in the appearance of a monotone decreasing function, making departures from exponentiality difficult to detect visually in some cases. In contrast, log-frequency plots preserve the independence of individual IETs and therefore can show increases and decreases in the frequency distribution when applicable. However, log-survivor plotting remains the standard at least in part because of the inherent subjectivity of identifying distribution forms visually. As can be seen in Figure 5, the log-survivor plot also has the advantage of making separate populations of exponentially distributed IETs clearly visible whereas detecting separate populations of IETs can be difficult using log-frequency plots (see the middle panel of Figure 5 for an example).

As Kessel and Lucke (2008) pointed out, Shull's two-state model of operant behavior neither requires nor implies a log-survivor approach. Shull's model states that the IETs are distributed according to a negative biexponential distribution, but the model can be applied to either the complementary cumulative distribution function (as in log-survivor analysis), or the probability distribution function (as in log-frequency analysis). Brackney et al. (2011) have recently applied Shull's theoretical model to a log-frequency analysis of IET distributions. Brackney et al. made use of both methods by providing log-survivor plots for visual analysis while modeling the data using log-frequency analysis to obtain maximum likelihood estimates of parameter values. Thus, these two biexponential methods can be used in conjunction, but at the cost of consistency between visual and quantitative analysis.

Biexponential Mixture Model Assumptions. Traditional bout analysis models (e.g., Machlis, 1977 and Shull et al., 2001) assume that the response-generating processes are Poisson and, therefore, that the resulting component IET distributions are exponential. The assumed Poisson counting processes are stationary, meaning that the bout parameters do not vary systematically as a function of time. In other words, the rate of within-bout and between-bout responses are stable throughout the measurement period. The stationarity assumption is a necessary assumption to justify aggregating IETs from the beginning and end of the measurement period into a single distribution.

The distribution of bout lengths is also assumed to be exponential. The exponential distribution is the only continuous distribution with the memoryless property. A distribution is memoryless if and only if $P(X > t + \Delta t \mid X > t) = P(X > \Delta t)$, for all non-negative values of t and Δt. For an IET distribution, the conditional probability that the current IET (X) will be greater than 15 seconds ($t + \Delta t$), given that more than 10 seconds (t) have already elapsed since the

previous response, must be equal to the probability of observing an IET longer than 5 seconds (Δt). To qualify as memoryless—and thus be exponential—the same must be true for all values of t and Δt greater than or equal to zero. For example, the probability that a bout terminates on a 3 second IET should be equal to the probability that a bout terminates on a 5 second IET given that 2 seconds have already elapsed since the previous IET. Because the same process governs transitions into and out of response bouts, the same property must hold for transitions from interbout intervals to response intervals. To the extent that the behavior under investigation does not adhere to the properties of the underlying theoretical distributions, exponential mixture models may be inappropriate.

Log-Interval Analysis

A number of common behaviors do not appear to have constant bout initiation and termination rates and thus may be ill-described by the traditional biexponential model (Tolkamp et al., 1998). For example, feeding patterns display reliable temporal patterning that suggests that suggests satiety influences both the initiation and duration of feeding bouts. Satiety models suggest that the likelihood of ending a meal does depend on the current elapsed time in the meal state and that the likelihood of initiating a meal increases as the time elapsed since the previous meal increases. If satiety changes the rate of initiating a bout of eating, then the behavior does not result from a Poisson process and there is no theoretical basis for treating the IET distribution as exponential. Mixture models based on distributions other than the exponential distribution (and thus without the constant bout initiation assumption) may provide better descriptions behavior influenced by satiety, as well as other behavioral and physiological processes such as habituation and motor fatigue (Yeates et al., 2001). Although other mixture models can be applied to log-survivor and log-frequency transformed interevent times, alternatives to

biexponential models have relied instead on log-transforming the interevent times themselves resulting in the class of non-exponential models being collectively referred to as log-interval analysis (Yearsley, Tolkamp, & Illius, 2001).

The log-interval approach to bout analysis differs from the conventional approach in several ways. Rather than log-transforming Y (either the survivor distribution or the frequency distribution), the X variable (raw interevent time) is log-transformed. Analyzing the resulting probability density function preserves independence of data points and allows parameter estimation using modern expectation-maximization algorithms (Redner & Walker, 1984). The probability density function is then commonly modeled as a mixture of Gaussians (see bottom panel of figure 5; refer to table 1 for a summary and side-by-side comparison of all three partitioning methods). The resulting probability density function is described as a mixture of two Gaussian distributions:

$$P(y = t) = p_w \left(\frac{1}{\sigma_w\sqrt{2\pi}}\right) e^{-((t)-\mu_w)^2/2{\sigma_w}^2} + p_b \left(\frac{1}{\sigma_b\sqrt{2\pi}}\right) e^{-((t)-\mu_b)^2/2{\sigma_b}^2} \qquad (4)$$

where p_w and p_b represent the proportions of IETs that belong to the within-bout and between-bout states respectively, μ_w and μ_b are the respective means of the log-transformed IETs, and σ_w^2 and σ_w^2 the corresponding variances. The two-state log-interval method can be readily extended to model processes with three or more states. (Yeates, Tolkamp, Allcroft, & Kyriazakis, 2001). Log-interval analysis as a class benefits from sophisticated and flexible quantitative methods.

However, the theoretical model of behavior is much less developed than the model associated with the log-survivor class of bout analysis (Shull, 2001; Yeates, et al., 2001). The lack of established theoretical framework is especially evident in the choice of distribution family. No *a priori* basis for choosing the probability distribution family—or the number of those component distributions—exists. Although the two-state Gaussian mixture is common,

three-state Gaussian and two- and three-state Weibull mixtures, as well as Gaussian-Weibull mixtures are also frequently applied to the same data, and when adequate statistical fits are not achieved other statistical distributions are also tested (Yeates et al., 2001). Thus, although the log-interval approach to bout analysis represents an important quantitative advance, the approach as currently used in the literature remains closer to pure curve-fitting rather than a theoretical model of behavior.

The top panel of Figure 6 shows a log-interval plot of data simulated to meet a log-normal mixture model. The two IET distributions are evident as separate peaks whose location corresponds to the average IET of that distribution. The relative height of the peaks is a function of the proportion of IETs belonging to each distribution (and thus of the bout length). The bottom panel of Figure 6 plots the same data using the more familiar log-survivor plot. On the log-survivor plot, the log-normal data still appear to follow a broken-stick pattern with the important exception of a change in slope early in the within-bout limb. The early slope change is common in published log-survivor plots of behavioral data (Shull & Grimes, 2003; Davison, 2004; Podlesnik, Jimenez-Gomez, Ward, & Shahan, 2006). The initial portion is often described as a plateau caused by a non-zero minimum IET that results from mechanical limitations of the response operandum and the physical capabilities of the behaving organism (Brackney, Cheung, Neisewander, & Sanabria, 2011). However, the minimum IET is only a factor if the slope of the segment equals 0 and performing a linear shift by subtracting the minimum IET from all IETs would eliminate the appearance of the plateau. Although such linear shifts are commonly employed (e.g., Johnson, Bailey, & Newland, 2011, the apparent plateau remains evident in many cases. Such a plateau is inconsistent with exponentially distributed IETs, but is consistent with IETs that follow a log-normal distribution. For log-normally distributed within-bout IETs,

the length of the plateau and the location of its end are controlled by the mean and variance of the within-bout IETs. Low within-bout variability would produce a segment of low slope (a nearly horizontal segment) with an abrupt change, whereas high variability would result in the appearance of a rolling curve with slowly accelerating slope.

Change-point Bout Analysis

The log-survivor and log-interval approaches to partitioning behavior discard information about the temporal sequencing of events by aggregating IETs. As a result, both bout analysis methods assume stationary bout parameters over time and will therefore systematically misestimate bout parameters when behavior is in transition. For example, if motor fatigue causes a decrease in the within-bout rate over the course of a session then both the log-survivor and log-interval methods will produce a parameter estimate that underestimates the initial within-bout rate at the beginning of the session and overestimates the within-bout rate at the end of the session. The greater the motor fatigue then the greater the within-session change and the greater the misestimation; in such cases, the problem becomes more pronounced as session length increases.

Systematic changes in bout-initiation rate over time can give a two-state process the false appearance of containing three or more components (Slater & Lester, 1982). Direct observation of subject's behavior and visual inspection of event records may help distinguish a true three-state process from an nonstationary two-state process (Machlis, 1977), but these methods are inherently subjective, as well as impractical for large data sets. One alternative approach is to segment the measurement period into equal duration periods and then partition IETs within each segment separately, comparing across segments to determine temporal stability. The segment width is a critical issue, however, and its optimal value may differ for different behaviors and

different reinforcement schedules—and perhaps across individuals measured under the same conditions. A quantitative method sensitive to individual differences, but easily summarized at the group level, for evaluating temporal stability of bout characteristics is therefore highly desirable.

Gallistel, Mark, King, and Latham (2001) described the application of a change-point detection analysis to individual learning curves. The change-point analysis is uses an iterative algorithm to detect changes in a cumulative record's gradient. The change-points are then evaluated using a user-specified decision criterion to determine whether the change in slope is significant. The algorithm carries no assumptions about the process or processes that generate responding, nor about the shape of the interevent time distribution. The change-point algorithm (described in more detail below) does not aggregate interevent times and therefore preserves information about the temporal sequence of events, making a change-point approach to bout analysis a viable potential alternative to traditional methods based on IET distribution analysis. Studies have used the change-point algorithm to evaluate individual and group learning curves for a number of behaviors across species (Gallistel et al., 2001; Gallistel, Fairhurst, & Balsam, 2004), but thus far it has not been explicitly applied to bout analysis. Figure 7 shows the change-point algorithm applied to a sample cumulative record. The average within-bout rate is calculated from the slope of the bout segments. The average bout length is calculated from the number of responses contained in the bout segments. The interbout interval is calculated as the time between the termination of a bout segment and the beginning of the subsequent bout segment.

Each change-point identified by the algorithm corresponds to a transition between states. The section of the cumulative record between any two consecutive change-points is either a response bout or an interbout pause. The bout parameters can be estimated by analyzing the

appropriate segments. The change-point bout analysis should provide bout estimates comparable to those from the log-survivor and log-interval analyses when their respective assumptions are met. However, the change-point analysis may also provide acceptable bout parameter estimates when the constant termination rate assumption is invalid. By preserving the temporal sequence of IETs, the change-point analysis may also be applied when the bout structure is not stationary. The change-point bout analysis is flexible but its utility has not previously been tested against more common methods of partitioning behavior into response bouts. To address that gap in the literature, the experiments presented in this dissertation compare the three bout analysis methods using common datasets.

Methylmercury

Mercury is a heavy metal that is found in both inorganic and organic forms in the environment (EPA, 1998). Inorganic mercury in the lower atmosphere is regularly released via rainwater into bodies of water, where benthic organisms can then methylate it to produce methylmercury, an organic compound. (National Research Council, 2000); inorganic mercury from coal plants and other industrial applications also contributes to environmental levels of mercury. Methylmercury is a known developmental neurotoxicant that produces an array of impairments, including sensorimotor and cognitive deficits (Takeuchi et al., 1962). Methylmercury readily crosses the blood-brain and placental barriers. Therefore the developing organism—especially the fetus—is particularly sensitive to methylmercury toxicity.

Exposure in adulthood can also produce in neurotoxic effects, with the severity depending on exposure level and duration. In the 1950s, industrial contamination of Minamata Bay in Minamata, Japan led to an epidemic of "Minamata Disease," which was eventually linked to high-level methylmercury exposure (Harada, 1995). Minamata residents presented with any of

a host of symptoms, including sensorimotor deficits, impaired vision or blindness, loss of motor control, and cognitive decline consistent with old age—while in middle-age (Kinjo & Nakano, 1991). Nearly two-thirds of Minamata victims reported partial or complete loss of sensation in the lower portions of their arms and legs. Muscle weakness, uncontrollable tremor, and gait disturbances were also common (Harada, 1995).

Methylmercury bioaccumulates in the food chain. Humans become exposed to methylmercury by eating long-lived predator species from high trophic levels, such as shark and king mackerel. Humans are also at risk of exposure from eating methlymercury-contaminated grain products, most notably rice (Barrett, 2010). Consequently, animal models based on the acute, high-level exposure of Minamata, and similar tragedies, are poor analogues for studying common methylmercury toxicity. Chronic low-level exposure produces subtle effects that are more difficult to detect. Effects of adult-onset exposure also do not occur immediately, but instead after a latent period (Weiss et al., 2002). Detecting the effects of low-level exposure is therefore challenging; this is especially true of effects during the early stage of impairment. However, operant reinforcement schedules that generate high rates of behavior have provided a number of preparations sensitive to these subtle effects (Newland, 1995). Heath et al. (2010) found that chronic, low-level exposure resulted in a decrease of spontaneous running in rats. Unfortunately, mechanical limitations of the running wheels precluded a bout analysis of wheel running. Log-survivor analysis has been effective at isolating motor effects of methylmercury toxicity in other operant behavior tasks, however.

Calcium and Methylmercury Toxicity

Methylmercury toxicity is associated with neuronal damage in several areas of the central nervous system. MeHg toxicity damages neurons in the primary motor cortex, which can cause

sensorimotor impairment of the distal extremities (Naito, 2004). Methylmercury causes neural degeneration in the cerebellum, as well as damage to dorsal root ganglia (Sakamoto, Ikegami, & Nakano, 1996). Mercury adversely impacts the cerebellum in multiple ways. Methylmercury impairs the development of granule cells, leading to a reduction in the granule cell layer of the cerebellum (Sager, Aschner, & Rodier, 1984). Methylmercury toxicity adversely impacts Purkinje cells, resulting in diminished dendritic branching (Choi et al., 1981)—but at higher exposure levels than are required for granule cell neurotoxicity (Edwards, Marty, and Atchison, 2005). The mechanism of methylmercury's neurotoxicity remains unclear, but calcium dysregulation may be a contributing factor.

Disruptions in calcium homeostasis have been implicated in cell damage in the periphery (Beaton et al., 2002) and the central nervous system (Limke, Bearsnhj, & Atchison, 2004; Piacentini et al., 2008). Calcium channel blockers have proven effective at attenuating disruptions of calcium homeostasis and mitigating cell damage and neuronal loss in vitro. Prolonged or intense physical activity results in skeletal muscle tissue damage. This damage presents with a disruption in calcium homeostasis, and calcium channel blockers have demonstrated effectiveness at mitigating the disruption in homeostasis. Beaton et al. (2002) showed that pretreatment with the calcium channel blocker amlodipine was effective at attenuating and delaying the onset of muscle damage.

The role of calcium homeostasis in methylmercury toxicity is of particular interest in the present study. Sakamoto, Ikegami, and Nakano(1996) demonstrated protection by calcium channel blockers against methylmercury toxicity that was evidenced by an increased survival rate and improved health outcomes in exposed rats treated with the calcium channel blockers flunarizine, nifedipine, and verapamil.

Verapamil, nimodipine, and other L-type calcium channel blockers prevent the influx of calcium into the cell. L-type calcium channels are found in the cardiovascular system and the dorsal root ganglia (Bowles et al., 1997; Dobremez et al., 2005), as well as in high concentrations in the cerebellum. Given the protective effects of calcium channel blockers (Beaton et al., 2002; Sakamoto et al., 1996) and the distribution of L-type calcium channels, the L-type CCB nimodipine can be expected to delay—or possibly prevent—the onset of methylmercury induced motor impairment. MeHg-exposed animals treated with nimodipine should show delayed onset of decrements in within-bout response rate. Thus experiment two will examine the calcium homeostasis theory of methylmercury neurotoxicity by evaluating the extent to which compounds that enhance calcium homeostasis in the neuron also protect against MeHg's neurotoxicity in a behavioral preparation sensitive to MeHg effects.

Purpose

Spontaneous wheel running is an excellent preparation for directly comparing the various bout-analysis methods, as well as for evaluating their respective sensitivities to toxicant effects. A number of studies have found that rats and mice run at high rates for extended periods of time even when doing so results in adverse health consequences or is otherwise maladaptive (for an in-depth review, see Sherwin, 1998). In contrast, Aoyama and McSweeney (2001) have found within-session decreases in running rates using short (30-min) sessions. Running rate can be restored to early session levels by introducing a dishabituating stimulus (*e.g.*, briefly removing an animal from and then returning it to the chamber)—findings not consistent with decreased running due to muscle fatigue. Fatigue and habituation may separately or jointly contribute to spontaneous running, resulting in a violation of the constant initiation rate assumption or a non-stationary bout structure.

Eikelboom and Mills (1988) examined the bout structure of rodent wheel-running in a 12-hr overnight session using visual analysis of log-survivor plots. The log-survivor plots for approximately half of all sessions appeared to show three IET distributions instead of the typical two. Unfortunately, Eikelboom and Mills elected to trim the longest IETs from the data to fit a 2-state biexponential model rather than pursue an in-depth analysis. It is thus unclear whether those cases were actually the result of a three-state process, or whether the shape of the log-survivor function was due to changes in the bout-initiation rate that occurred within a session (e.g., as a result of habituation, muscle fatigue, or even periods of sleep), or some other cause.

This dissertation contains two studies that advance the bout analysis literature. Study one was designed to compare the bout analysis methods on common datasets in a series of three experiments. In the first experiment, bout analyses were conducted on data simulated to meet the assumptions of the biexponential model. In the second experiment, the three bout analyses were compared using spontaneous wheel running data where the validity of the biexponential assumptions was not assured. In the third experiment, the three bout analyses were compared using behavior perturbed by chronic exposure to methylmercury to examine their sensitivity to motoric effects. Study two used the results of study one to select a bout analysis method to analyze the effects of chronic methylmercury exposure on the bout structure of spontaneous running over the course of exposure. An additional aim of study two was to examine the ability of the calcium channel blocker nimodipine to mitigate methylmercury's effects on bout structure in accordance with the current theory that methylmercury neurotoxicity is mediated by disruptions in calcium homeostasis.

Tables

Table 1
Comparison of Bout Partitioning Methods

	Method			
	Log-survivor	Log-frequency	Log-interval	Change-point
Y(t) =	$p_w e^{-\lambda_w t} + p_b e^{-\lambda_b t} + \cdots$	$p_w \lambda_w e^{-\lambda_w t} + p_b \lambda_b e^{-\lambda_b t} + \cdots$	$p_w \left(\frac{1}{\sigma_w \sqrt{2\pi}}\right) e^{-(\log(t)-\mu_w)^2/2} + p_b \left(\frac{1}{\sigma_b \sqrt{2\pi}}\right) e^{-(\log(t)-\mu_b)^2/2\sigma} + \cdots$	N/A
Constraints	$1-(p_w+p_b+\cdots)=0,$ $\{p_w, p_b, \ldots\} > 0,$ $\lambda_w > \lambda_b$	$1-(p_w+p_b+\cdots)=0,$ $\{p_w, p_b, \ldots\} > 0,$ $\lambda_w > \lambda_b$	$1-(p_w+p_b+\cdots)=0,$ $\{p_w, p_b, \ldots\} > 0,$ $\mu_w < \mu_b$	N/A
Theoretical distribution	Exponential Mixture	Exponential Mixture	Log-normal mixture	N/A
Form	Survivor (1 – CDF)	PDF	PDF	Cumulative record, step function
Shape	Monotone Decreasing, Broken-stick	Bi-/multi-modal, skewed right	Bi-/multi-modal	Slope ≥ 0 for all $x \geq 0$
Transformation	Log (Y)	Log (Y)	Log (X)	N/A
Free parameters	$2k-1$	$2k-1$	$3k-1$	N/A
Assumptions	Stationary Memoryless	Stationary Memoryless	Stationary	N/A

Note: For the log-interval method, all μ and σ are for the log-transformed IRTs.

Figures

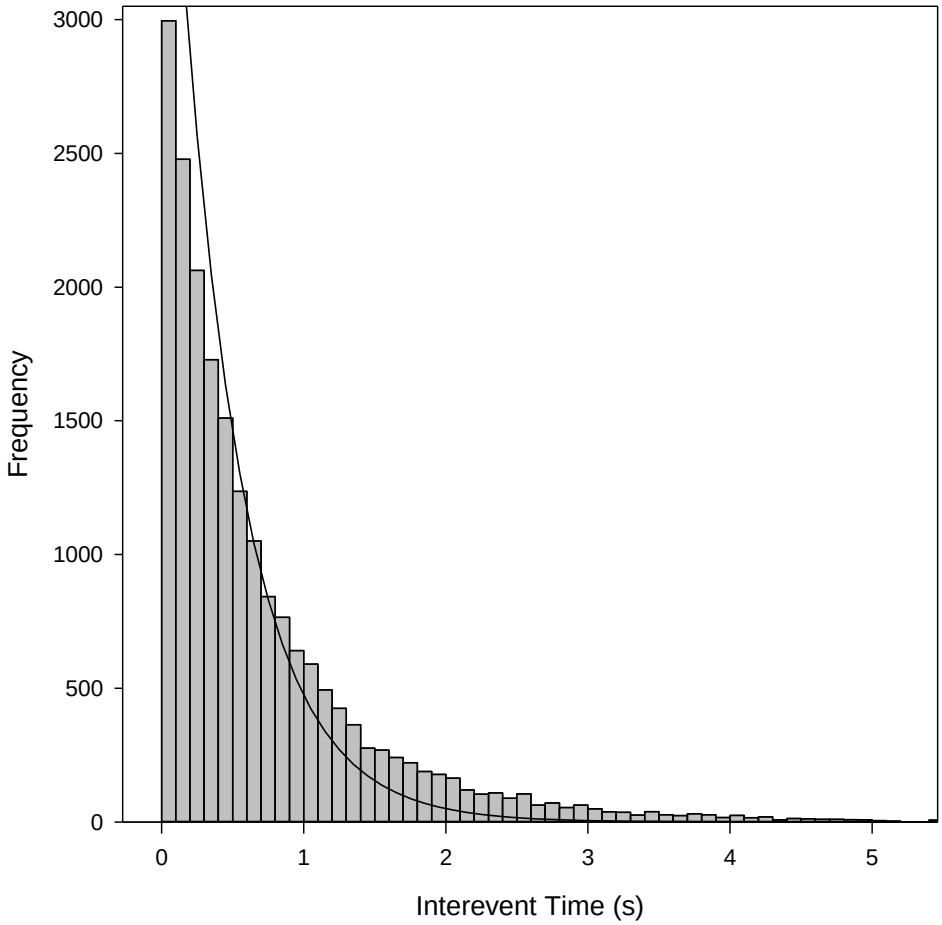

Figure 1. A histogram of simulated inter-responses times from a biexponential distribution. Beginning around 1-sec, the tail of the distribution contains more IRTs than predicted from a single exponential distribution. The difficulty in determining precisely where the two distributions intersect illustrates the weakness of the visual histogram analysis.

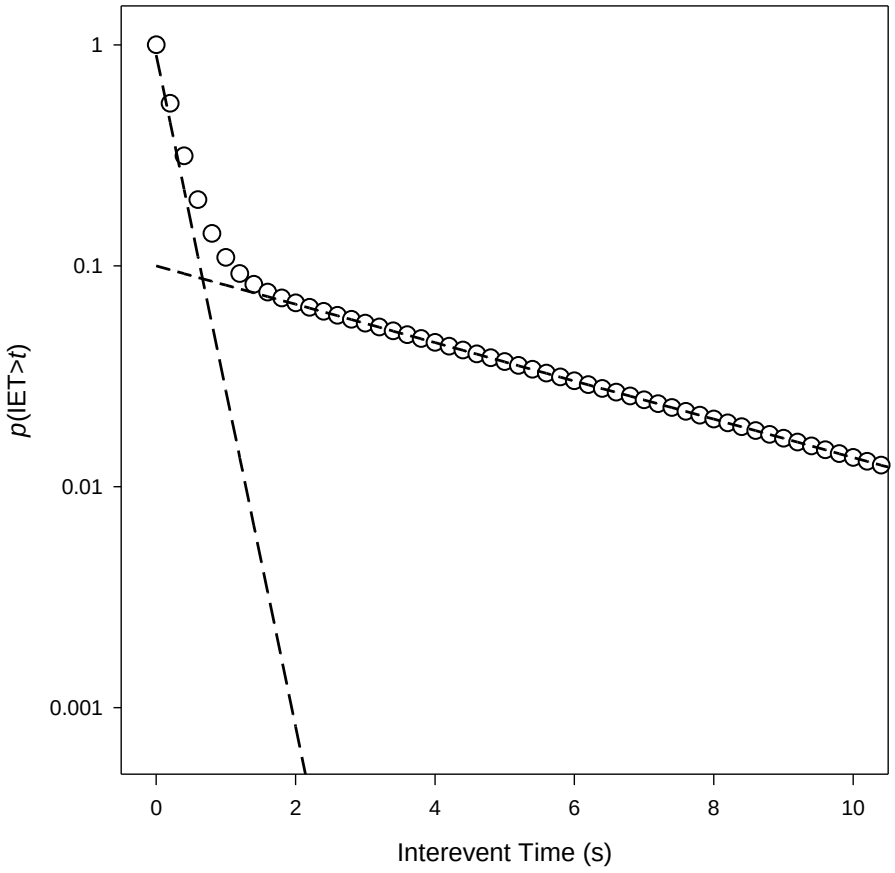

Figure 2. A log-survivor plot of a two-state process. The biexponential survivor distribution is a mixture of two negative exponentials, one related to a high-rate Poisson process and the other to a low-rate Poisson process. The short, within-bout IETs contribute the steep initial limb of the function, and the long, between-bout IETs contribute the tail.

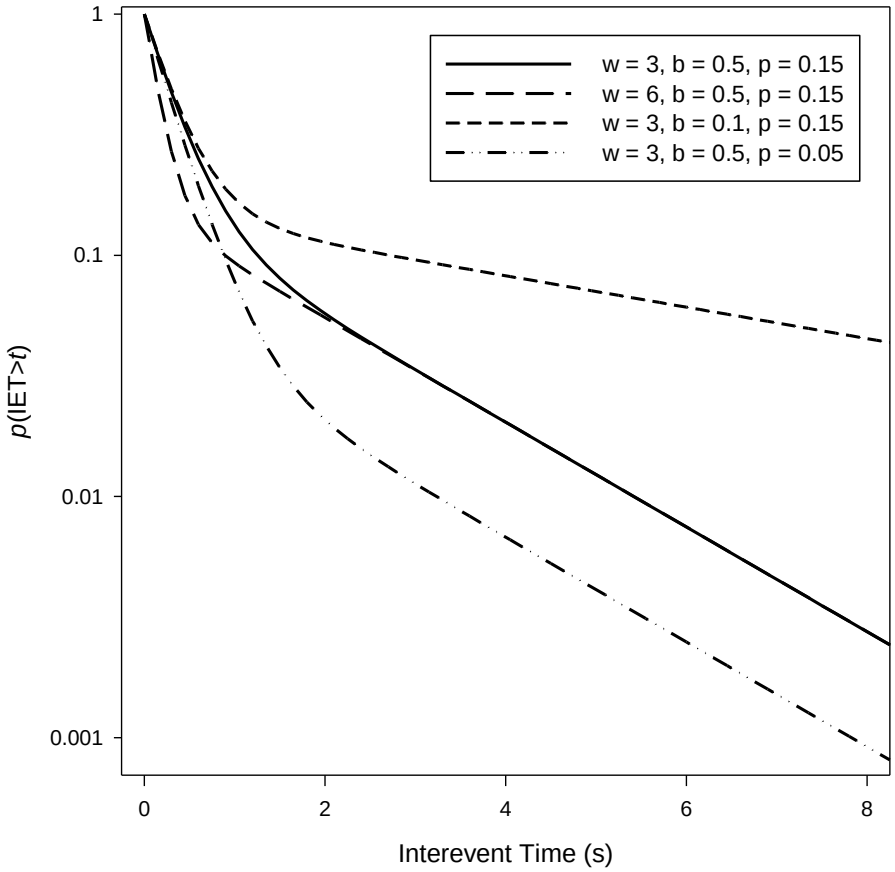

Figure 3. A comparison of two-state log-survivor functions with different parameter values (listed in the legend for ease of comparison).

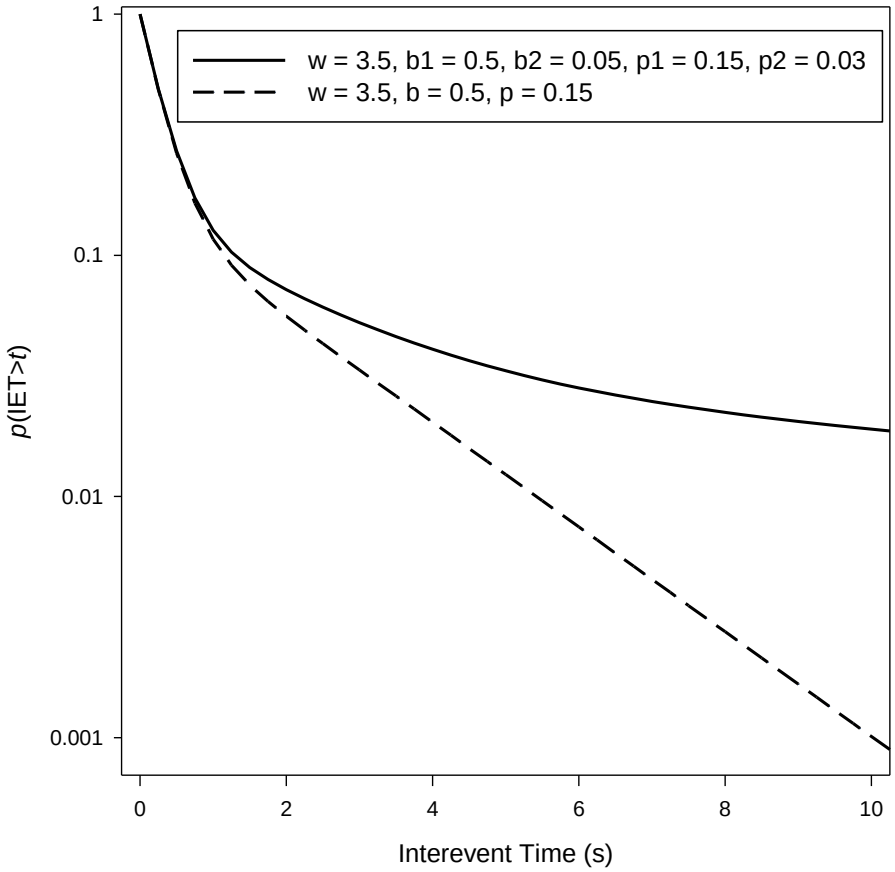

Figure 4. A comparison of log-survivor functions for two-state and three-state processes. The within-bout rate and bout initiation rate are equal for the two functions. Note the concavity of the triexponential distribution's tail, and the absence of a clear break between distributions.

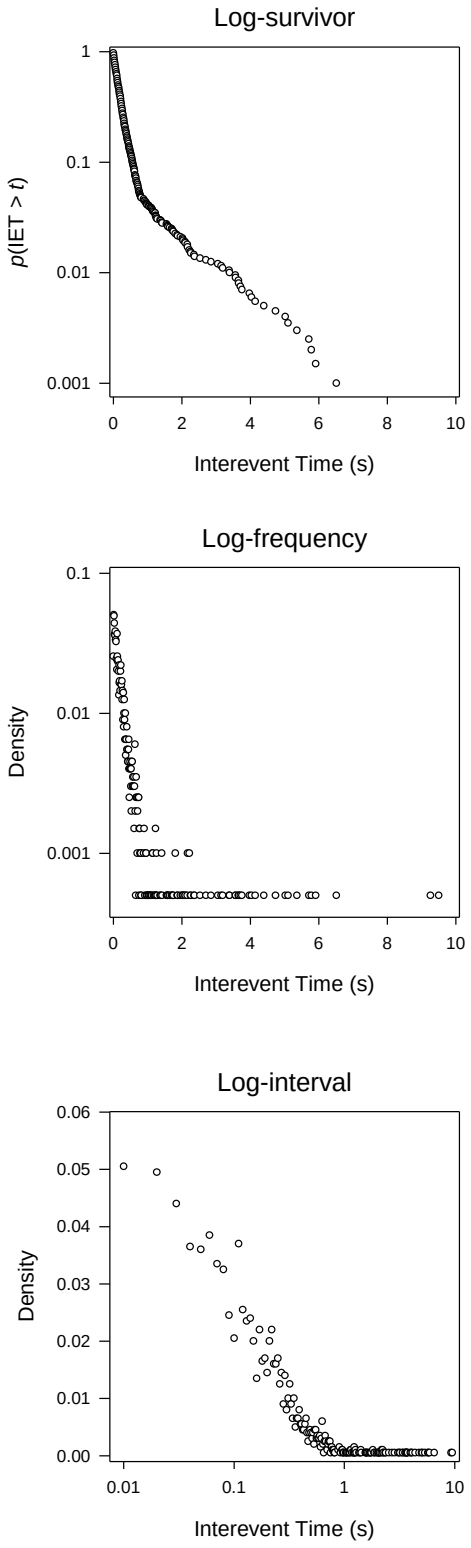

Figure 5. A comparison of log-survivor (top), log-frequency (middle), and log-interval (bottom) plots of simulated biexponential IETs. Although clear in the log-survivor plot, the second distribution of IETs is difficult to discern in the log-frequency and log-interval plots.

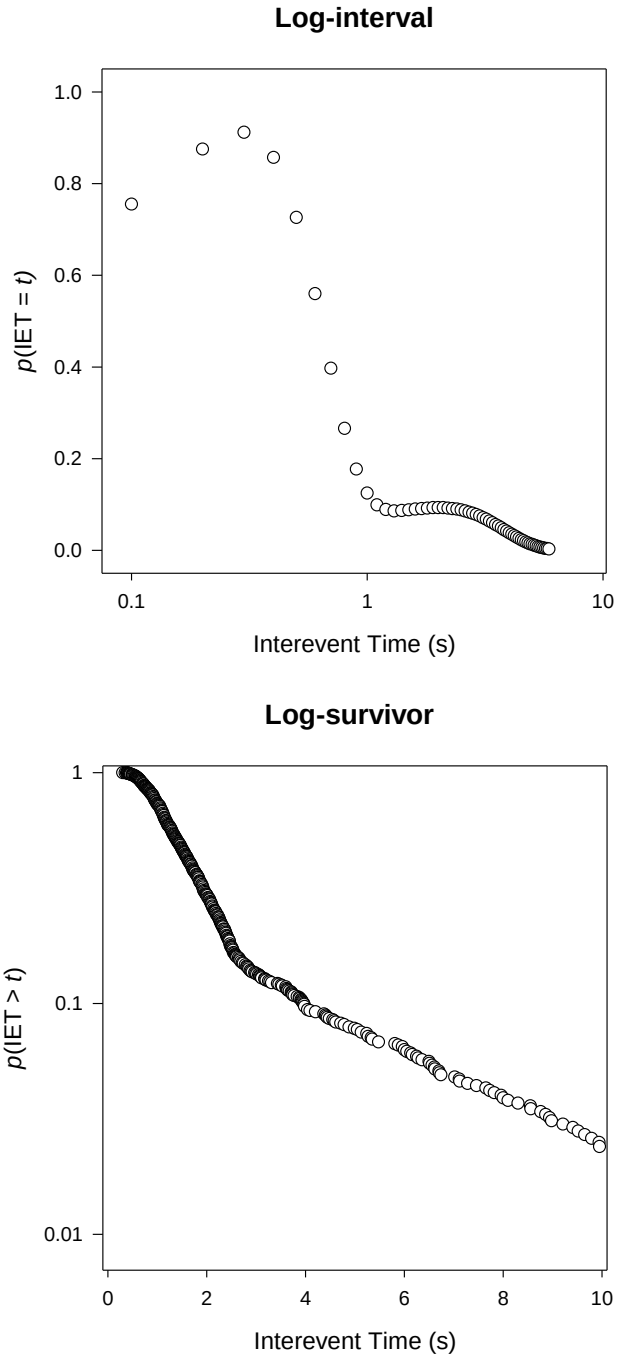

Figure 6. Data simulated according to a 2-state log-normal mixture model plotted as a log-interval plot (top) and as a log-survivor plot (bottom). In the log-interval plot, the interbout intervals are apparent as a second peak between 1 and 10 seconds. In the log-survivor plot, the function approximates a broken-stick pattern, but with a plateau at the beginning of the within-bout component.

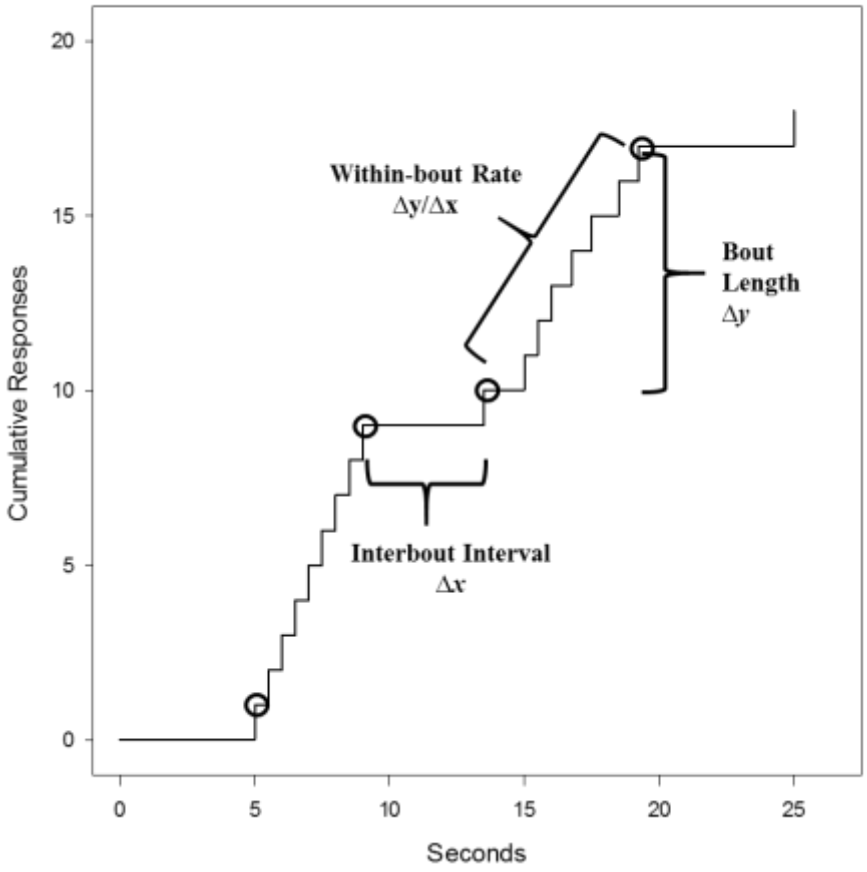

Figure 7. A simulated cumulative record showing four change-points. The change-points partition the record into successive segments that alternate between bouts and interbout intervals. For any given bout segment, the within-bout rate (speed of responding) is calculated as the slope of that segment and the bout length as the number of responses. The interbout interval is calculated as the time elapsed between the end of one bout and the beginning of the next.

References

Aoyama, K., & McSweeney, F. K. (2001). Habituation contributes to within-session changes in free wheel running. *Journal of the Experimental Analysis of Behavior*, *76*(3), 289-302.

Barrett, J. R. (2010). Rice is a significant source of methylmercury: research in china assesses exposures. *Environmental Health Perspectives*, *118*(9), 1183-1188.

Beaton, L. J., Tarnopolsky, M. A., & Phillips, S. M. (2002). Contraction-induced muscle damage in humans following calcium channel blocker administration. *The Journal of Physiology*, *544*(3), 849-859.

Bennett, J. A., Hughes, C. E., & Pitts, R. C. (2007). Effects of methamphetamine on response rate: A microstructural analysis. *Behavioural Processes*, *75*(2), 199-205.

Bowles, D. K., Hu, Q., Laughlin, M. H., & Sturek, M. (1997). Heterogeneity of L-type calcium current density in coronary smooth muscle. *The American Journal of Physiology*, *273*(2), 2083-2089.

Brackney, R. J., Cheung, T. H. C., Neisewander, J. L., & Sanabria, F. (2011). The isolation of motivational, motoric, and schedule effects on operant performance: a modeling approach. *Journal of the Experimental Analysis of Behavior*, *96*(1), 17-38.

Choi, B. H., Kudo, M., & Lapham, L. W. (1981). A Golgi and electron-microscopic study of cerebellum in methylmercury-poisoned neonatal mice. *Acta Neuropathologica*, *54*(3), 233-237.

Dobremez, E., Bouali-Benazzouz, R., Fossat, P., Monteils, L., Dulluc, J., Nagy, F., & Landry, M. (2005). Distribution and regulation of L-type calcium channels in deep dorsal horn neurons after sciatic nerve injury in rats. *The European Journal of Neuroscience*, *21*(12), 3321-3333.

Dolbec, J., Mergler, D., Sousa Passos, C.-J., Sousa de Morais, S., & Lebel, J. (2000). Methylmercury exposure affects motor performance of a riverine population of the Tapajos river, Brazilian Amazon. *International Archives of Occupational and Environmental Health*, *73*(3), 195-203.

Edwards, J. R., Marty, M. S., & Atchison, W. D. (2005). Comparative sensitivity of rat cerebellar neurons to dysregulation of divalent cation homeostasis and cytotoxicity caused by methylmercury. *Toxicology and Applied Pharmacology*, *208*(3), 222-232.

Eikelboom, R., & Mills, R. (1988). A microanalysis of wheel running in male and female rats. *Physiology & Behavior*, *43*(5), 625-630.

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.

Eto, K., Tokunaga, H., Nagashima, K., & Takeuchi, T. (2002). An Autopsy Case of Minamata Disease (Methylmercury Poisoning)—Pathological Viewpoints of Peripheral Nerves. *Toxicologic Pathology*, *30*(6), 714 -722.

Gallistel, C. R., Mark, T. A., King, A. P., & Latham, P. E. (2001). The rat approximates an ideal detector of changes in rates of reward: implications for the law of effect. *Journal of Experimental Psychology. Animal Behavior Processes*, *27*(4), 354-372.

Gallistel, C. R., Fairhurst, S., & Balsam, P. (2004). The learning curve: Implications of a quantitative analysis. *Proceedings of the National Academy of Sciences of the United States of America*, *101*(36), 13124-13131.

Harada, M. (1995). Minamata disease: methylmercury poisoning in Japan caused by

environmental pollution. *Critical Reviews in Toxicology*, *25*(1), 1-24.

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*(2), 169-179.

Herrnstein, R. J. (1961). Relative and absolute strength of response as a function of frequency of reinforcement. *Journal of the Experimental Analysis of Behavior*, *4*, 267-272.

Howie, J. A., Tolkamp, B. J., Avendaño, S., & Kyriazakis, I. (2009). A novel flexible method to split feeding behaviour into bouts. *Applied Animal Behaviour Science*, *116*(2-4), 101-109.

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 strains. *Pharmacology, Biochemistry and Behavior*, *97*(3), 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*(2), 309-315.

Kessel, R., & Lucke, R. L. (2008). An analytic form for the interresponse time analysis of Shull, Gaynor, and Grimes with applications and extensions. *Journal of the Experimental Analysis of Behavior*, *90*(3), 363-386.

Kinjo, Y., Higashi, H., Nakano, A., Sakamoto, M., & Sakai, R. (1993). Profile of Subjective Complaints and Activities of Daily Living among Current Patients with Minamata Disease after 3 Decades. *Environmental Research*, *63*(2), 241-251.

Langton, S. D., Collett, D., & Sibly, R. M. (1995). Splitting Behaviour into Bouts; A Maximum Likelihood Approach. *Behaviour*, *132*(9/10), 781-799.

Limke, T. L., Bearss, J. J., & Atchison, W. D. (2004). Acute exposure to methylmercury causes Ca2+ dysregulation and neuronal death in rat cerebellar granule cells through an M3

muscarinic receptor-linked pathway. *Toxicological Sciences: An Official Journal of the Society of Toxicology*, *80*(1), 60-68.

Michael, J. (1982). Distinguishing between discriminative and motivational functions of stimuli. *Journal of the Experimental Analysis of Behavior*, *37*(1), 149-155.

Machlis, L. (1977). An analysis of the temporal patterning of pecking in chicks. *Behaviour*, *63*(1), 1-70.

National Research Council. (2000). *Toxicological effects of methylmercury*. Washington D.C.: National Acadamy press.

Naito, E. (2004). Sensing Limb Movements in The Motor Cortex: How Humans Sense Limb Movement. *The Neuroscientist*, *10*(1), 73 -82.

Newland, M.C. (1995) Motor function and the physical properties of the operant: screening and advanced applications. In Chang, L.W., and Slikker, W. (Eds) Neurotoxicology: Approaches and Methods. San Diego, Academic Press. pp 265-300.

Piacentini, R., Gangitano, C., Ceccariglia, S., Del Fà, A., Azzena, G. B., Michetti, F., & Grassi, C. (2008). Dysregulation of intracellular calcium homeostasis is responsible for neuronal death in an experimental model of selective hippocampal degeneration induced by trimethyltin. *Journal of Neurochemistry*, *105*(6), 2109-2121.

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*(3), 329-347.

Sager, P. R., Aschner, M., & Rodier, P. M. (1984). Persistent, differential alterations in developing cerebellar cortex of male and female mice after methylmercury exposure. *Developmental Brain Research, 12*(1), 1-11.

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

Sherwin, C. M. (1998). Voluntary wheel running: a review and novel interpretation. *Animal Behaviour*, *56*(1), 11-27.

Shull, R. L. (2004). Bouts of Responding on Variable-Interval Schedules: Effects of Deprivation Level. *Journal of the Experimental Analysis of Behavior*, *81*(2), 155-167.

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

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

Shull, R. L., & Grimes, J. A. (2003). Bouts of Responding from Variable-Interval Reinforcement of Lever Pressing by Rats. *Journal of the Experimental Analysis of Behavior*, *80*(2), 159-171.

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*(1), 65-83.

Skinner, B. F. (1938). *The behavior of organisms: an experimental analysis*. Oxford, England: Appleton-Century.

Sibly, R. M., Nott, H. M., & Fletcher, D. J. (1990). Splitting behaviour into bouts. *Animal Behaviour*, *39*(1), 63-69.

Sibly, R. M., Nott, H. M., & Fletcher, D. J. (1994). "Splitting behaviour into bouts": Erratum.

Animal Behaviour, *48*(2).

Slater, P. J. (1974). The temporal pattern of feeding in the zebra finch. *Animal Behaviour*, *22*(2), 506-515.

Slater, P. J., & Lester, N. P. (1982). Minimising errors in splitting behaviour into bouts. *Behaviour*, *79*(2-4), 153-161.

Takeuchi, N., Morikawa, N., Matsumoto, H., & Shiraishi, Y. (1962). A pathalogical study of Minamata disease in Japan. *Acta Neuropathologica, 2*, 40-57.

Tolkamp, B. (1999). To split behaviour into bouts, log-transform the intervals. *Animal Behaviour*, *57*(4), 807-817.

Tolkamp, B. J., Allcroft, D. J., Austin, E. J., Nielsen, B. L., & Kyriazakis, I. (1998). Satiety Splits Feeding Behaviour into Bouts. *Journal of Theoretical Biology*, *194*(2), 235-250.

Weiss, B., Clarkson, T. W., & Simon, W. (2002). Silent latency periods in methylmercury poisoning and in neurodegenerative disease. *Environmental Health Perspectives*, *110 Suppl 5*, 851-854.

Yeates, M. P., Tolkamp, B. J., Allcroft, D. J., & Kyriazakis, I. (2001). The use of Mixed Distribution Models to Determine Bout Criteria for Analysis of Animal Behaviour. *Journal of Theoretical Biology*, *213*(3), 413-425.

Chapter 2: Partitioning behavior into activity bouts: A comparison of log-survivor analysis, log-interval analysis, and change-point analysis.

Abstract

The present study evaluated 3 bout analysis methods, the log-survivor with biexponential model, the log-interval with Gaussian mixture model, and a novel method based on change-point analysis. In experiment 1, the three analyses were applied to data simulated to meet the assumptions of the most common theoretical model, the two-state exponential model. Ten samples of 1000 events were simulated for each of eight different bout structures. As expected, the log-survivor analysis estimated the original bout parameters most accurately. The change-point analysis provided adequate bout estimates but tended to overestimate the overall rate by 5-10%. The log-interval analysis tended to systematically overestimate bout initiation rate and the probability of ending a bout for microstructures characterized by long average bout lengths. In experiment 2, the three analyses were applied to spontaneous wheel running data from a sample of adult BALB/c mice. One group of mice was chronically exposed to methylmercury (MeHg). The change-point bout parameters best recaptured the overall response rate and the log-survivor bout parameters consistently overestimated overall rate by more than 40%. The log-interval analysis mischaracterized the actual bout structure as consisting of extremely short bouts with extremely short interbout pausing—a characterization that disagreed with both other bout analyses. All three methods were sensitive to MeHg's sensorimotor effects, as evidenced by significantly slower within-bout rates for exposed animals. Additionally, the change-point analysis—but not the others—also detected a significant MeHg-related decrease on bout length. These findings support the utility of the change-point approach to bout analysis and support the general utility of bout analysis techniques for behavioral toxicology.

Introduction

Across species, much of voluntary behavior is organized into bouts of activity separated by periods of disengagement during which an organism engages in alternative behaviors (Machlis, 1977; Shull & Grimes, 2003). Response rate can be viewed as a weighted average of the rate of within-bout behavior and the frequency of bout initiations, with the weight of the two components determined by the proportion of events that occur within bouts (Shull, Gaynor, & Grimes, 2001). Within activity bouts, inter-event times (IETs) tend to be relatively short with relatively low variability (Shull, Gaynor, & Grimes, 2001). In contrast, the inter-bout intervals (IBIs) tend to be relatively long with greater variability (Machlis, 1977). The within-bout response rate and bout-initiation rate are differentially sensitive to biologically relevant variables. The bout-initiation rate is primarily affected by motivational variables, including rate of reinforcement (Shull, Gaynor, & Grimes, 2001; Shull & Grimes, 2003), availability of alternative reinforcement (Johnson, Pesek, & Newland, 2009), and level of deprivation of the primary reinforcer (Shull, 2004). The within-bout rate is affected by motoric variables, such as response effort (Brackney, Cheung, & Sanabria, 2011) and motoric effects of drugs (Johnson, Bailey, & Newland, 2011). Analysis of the bout structure, or microstructure, of behavior is often more revealing than analysis of response rate alone and, as such, high-fidelity methods for partitioning behavior into bouts are desirable.

Two classes of quantitative methods for partitioning behavior into activity bouts, one based on the survivor distribution and the other on the probability density function of inter-event times, are common in the literature (Tolkamp & Kyriazakis, 1999). Log-survivor analysis has been the most widely used in the operant literature (Shull, 2011). For a log-survivor bout analysis, the log-transformed survivor distribution (1 – cumulative distribution function) of IETs

is analyzed using a mixture model. When plotted in semi-log space, the log-survivor distribution of IETs often follows a "broken stick" pattern (Shull & Grimes, 2003) with two visually distinct limbs corresponding to the two types of IETs. The biexponential mixture model has been so commonly used in conjunction with log-survivor analysis that the log-survivor method and the biexponential model have become almost synonymous in the literature (Kessel & Lucke, 2008). The second common class of bout analysis fits a mixture model to the probability distribution function rather than the survivor distribution, usually after first log-transforming the IETs (Tolkamp, 1999). Variants of the biexponential mixture model have been used with log-interval analysis (Sibly, Nott, & Fletcher, 1990; Brackney, Cheung, & Sanabria, 2011), but Gaussian and Gaussian-Weibull mixtures are more common (Yeates, Tolkamp, Allcroft, & Kyriazakis, 2001).

The purpose of the present study was to directly compare bout analysis methods to determine which provides the best description of high-rate behavior. This study consists of two experiments in which the most common bout analysis methods, the log-survivor analysis with the biexponential model and the log-interval analysis with the Gaussian mixture model, were compared to each other and a third, novel bout analysis method based on a change-point detection algorithm. In experiment one, the three methods were examined using data simulated to meet the assumptions of a two-state biexponential mixture. In experiment two, the three methods were examined using spontaneous wheel running data collected from two groups of adult mice measured at two time points (pre- and post-exposure). To examine the sensitivity and specificity of bout parameters to experimental manipulation, one of the two groups was chronically exposed to the neurotoxicant methylmercury, which produces sensorimotor impairment that affects wheel running (Heath, et al., 2010).

Experiment 1

A critical (and typically untested) assumption of the biexponential model is that the conditional probability of initiating a bout is constant throughout the interbout interval; similarly, the conditional probability of terminating a bout is constant throughout the bout. However, the constant initiation rate assumption is untenable for behavior affected by physiological or behavioral processes such as satiation (Tolkamp, et al., 1998), habituation (Aoyama & McSweeney, 2001), or motor fatigue (Eikelboom & Mills, 1988). For such behavior, alternative mixture models with less restrictive assumptions may be better suited (Tolkamp et al., 1998). A number of alternative models have been evaluated, but Gaussian mixture models have been the most widely used (Yeates, et al., 2001; Davison, 2004).

Regardless of the mixture model used, both log-survivor analysis and log-interval bout analysis methods are similar in that they rely on a cumulated IET distribution to quantify the microstructure of responding. Accordingly, both assume a stationary bout structure to justify combining IETs from throughout an entire measurement period. Such an assumption is inappropriate for behavior in transition. Whenever one or more bout properties change systematically over time, methods based on aggregate IET distributions may produce biased estimates of bout parameters. A recent biexponential variant, the dynamic bi-exponential refractory model (Brackney et al., 2011; Cheung, Neisewander, & Sanabria, 2012) addresses the stationarity concern by incorporating additional exponential decay parameters for each of the first-order bout parameters. The dynamic model allows each bout parameter to decay at separate rates. Thus, the model allows for any combination of parameters to change over time, but only for monotonic changes. No published studies have applied a similar modification to the Gaussian mixture models, though the same re-parameterization is possible.

Not all behavior is well described by the common bout analysis methods. In particular,

high-rate operant behavior often appears not to conform to the familiar broken-stick pattern (Podlesnik, Jimenez-Gomez, Ward, & Shahan, 2006), suggesting in those cases that behavior is not well described by the biexponential mixture model. On concurrent reinforcement schedules, log-survivor analysis suggests that high-rate behavior may consist of three or more IET components (Davison, 2004). Although mixture models can accommodate more than two distributions, theoretical interpretation of the parameters is problematic. A third component may be accounted for as a measure of bout cycling (producing cycles of activity bouts, paralleling the arrangement of within-bout IETs and interbout intervals), but meaningful interpretation of further components cannot be so easily integrated into existing bout theory. These theoretically intractable components would be more commonly discussed in the literature if they were not frequently omitted from analysis by systematically trimming some predetermined proportion (e.g., 1%) of the longest IETs (Shull, 2004; Johnson, Pesek, & Newland, 2009) or by removing a growing proportion of the longest IETs until a suitable fit is achieved (Eikelboom & Mills, 1988).

In response to practical and theoretical issues with common bout analysis methods, we propose a novel method for partitioning behavior directly into activity bouts using sequential IETs rather than aggregate IET distributions. This novel approach is based on the detection and analysis of local changes in response rates identifiable from individual cumulative records (Ferster & Skinner, 1957). Unlike aggregate IET methods, the nonparametric change-point partitioning method preserves the temporal sequence of events and therefore requires no stationarity assumption. By partitioning behavior directly, the change-point analysis creates a new dependent measure—the activity epoch—for subsequent analysis. Each epoch corresponds to either an engagement period or a disengagement period, consistent with the traditional 2-state

model of activity bouts. The change-point analysis is explained in more detail in the Methods section below.

Although the validity of the 2-state biexponential model's assumptions is questionable in some free-operant contexts, the model has proven enormously useful in quantifying behavior and produces bout parameters that are differentially sensitive to behavioral manipulations (Shull, 2011). Thus, the biexponential model is a worthwhile reference analysis for judging the utility of alternative bout analysis techniques. In fact, the log-survivor analysis with 2-state biexponential model has been the standard against which new bout analysis methods are judged (Sibly, Nott, & Fletcher, 1990; Yeates, et al., 2001). Because of the uncertainty of the model's assumptions for behavioral data, experiment one compared the methods using data simulated to conform to the model's assumptions. Shull, Gaynor, and Grimes (2001) used a similar approach, simulating data to conform to certain reinforcement schedule-specific behavior patterns, to demonstrate the utility of the log-survivor biexponential analysis on schedule-controlled operant behavior. In the present paper, we take the alternative approach of simulating data according to a random biexponential generating process without regard to the schedule of reinforcement to provide a more general solution. Given that the bi-exponential model's assumptions were satisfied by design, the log-normal model and change-point partitioning method are not expected to outperform the bi-exponential. Rather, experiment one serves to establish a context for evaluating the differences seen when applying the about analysis methods to actual behavioral data in experiment two.

Method

Data Simulations

Inter-event times (IETs) were simulated according to the conventional bi-exponential

mixture model (equation 1 below) with the mixing parameter modeled by a binomial distribution with p equal to the probability of disengaging from a bout, p. Eight distinct response microstructures were simulated using two values for each bout parameter: fast or slow within-bout responding, long or short bouts, with frequent or infrequent bout initiations. The true values used in the simulations for each of the eight bout topographies are presented in Table 2. The average overall response rate ranged from 1 response per second to 5 responses per second. For each microstructure, 10 random samples (each generated using a different seed number) of 1000 IETs were generated. All data simulations and subsequent bout analyses were performed in R (R Core Development Team, 2013).

Data Analysis

Log-survivor Analysis. For each simulated sample, IETs were cumulated to form a survivor distribution. The individual log-survivor distributions were modeled with a bi-exponential function:

$$P(y \geq t) = p_w e^{-\lambda_w t} + p_b e^{-\lambda_b t} \quad (1)$$

where $P(y \geq t)$ is the proportion of IETs greater than time t, λ_w is the within-bout rate, λ_b is the bout initiation rate, p_w is the proportion of IETs that occurs within bouts, and p_b is the proportion of IETs that occur between bouts; p_w and p_b were constrained to sum to 1.0.

Log-interval analysis. For each simulated sample, IETs were cumulated into a frequency distribution and log (x) transformed. Bout parameters were then estimated by fitting a Gaussian mixture model to the log-transformed IETs:

$$P(y = t) = p_w \left(\frac{1}{\sigma_w\sqrt{2\pi}}\right) e^{-(t-\mu_w)^2/2\sigma_w{}^2} + p_b \left(\frac{1}{\sigma_b\sqrt{2\pi}}\right) e^{-(t-\mu_b)^2/2\sigma_b{}^2} \quad (2)$$

where p_w and p_b represent the proportions of IETs that belong to the within-bout and between-bout states respectively, μ_w and μ_b are the respective means of the log-transformed IETs, and $\sigma_w{}^2$

and σ_w^2 the corresponding variances; p_w and p_b were constrained to sum to 1.0. The mixing parameters p_w and p_b directly correspond to the log-survivor mixing parameters, however μ_w and μ_b (after being transformed back to linear space) correspond to the reciprocals of λ_w and λ_b; for all fits, both variance parameters were allowed to freely vary. Fits were performed in R (R Core Development Team, 2013) using the package mixtools (Bengalia, Chauveau, Hunter, & Young, 2009).

Change-point Partitioning Method. Details of the change-point bout analysis are illustrated in Figure 1. For each simulated sample, cumulative records were constructed by summing the successive IETs to create a time track and plotting the running total of events against the time track. Each cumulative record was parsed into epochs using a custom change-point detection algorithm written in R and based on a change-point detection algorithm previously applied to both response acquisition in Pavlovian preparations and free-operant behavior (Gallistel, Mark, King, & Latham, 2001; Gallistel, Fairhurst, & Balsam, 2004). The algorithm scans through an individual cumulative record looking for statistically significant changes in the record's gradient. Each pass of the algorithm begins at the origin (time = 0 and cumulative responses = 0) and the evaluation window grows by 1 with each successive event until a significant change-point is identified. At each increment (i.e., new response), the algorithm evaluates whether the obtained number of events in the target segment deviates significantly from the number that would be expected assuming a constant event rate within that segment. To do this, the algorithm calculates the logit (strictly speaking, the algorithm uses the pseudo-logit; see Gallistel et al., 2001 for a discussion) against a significant change and compares that value to a decision-criterion chosen *a priori*. After identifying a significant change-point, the algorithm re-anchors the record at the change-point which is then treated as the

origin for the algorithm's next pass through the record. Each epoch parsed by the algorithm is either a period of activity (a bout) or a period of disengagement (an interbout interval). The partitioned epochs can therefore be analyzed directly to examine bout structure. This approach requires no *a priori* assumptions about either the processes generating behavior or governing transitions between states.

The choice of decision-criterion, however, is important and its selection mirrors considerations of the effect on alpha level and Type I and Type II error in null hypothesis significance testing. A decision-criterion that is too liberal or too conservative will mischaracterize the response microstructure. Gallistel and colleagues (2004) have suggested that a decision-criterion of 3 (corresponding to an alpha level of 0.001) should be considered extremely conservative, while a criterion of 1.0 (corresponding to an alpha of 0.10) should be considered liberal for low-rate behavior. A criterion that is too liberal will result in a high frequency of false positive transitions out of bouts and, as a result, would underestimate the bout length and overestimate both the within-bout rate and the bout initiation rate. A criterion that is too conservative will have the opposite effect, overestimating the bout length while underestimating the within-bout bout rate. Because change-point algorithms have not previously been applied to bout analysis problems, no standard decision-criterion has been established. For the present experiment, cumulative records were parsed using a decision-criterion of 1.5, which corresponds approximately to an alpha level of 0.05.

Model Comparisons. The bout parameters produced by all three partitioning methods have the same theoretical interpretation but are estimated differently. Unlike the log-survivor and log-interval analysis, the change-point bout analysis does not estimate bout properties by fitting a mixture model to the interevent time distribution. As such, standard approaches to model

comparisons (e.g., AIC) could not be used to compare parameters in the present study. However, because the true parameter values were known in all cases, the partitioning methods were directly compared by the degree to which bout parameters recaptured the original parameter value. For each simulated record, a separate recovery ratio for every bout parameter as the ratio of the estimated parameter value to the true parameter for each bout analysis method. A recovery ratio of 1.0 indicates a perfect estimate of the true value. Ratios above and below 1.0 correspond to over-estimation and under-estimation, respectively. For example, a recovery ratio of 1.25 would indicate that the true value was overestimated by 25%. The recovery ratios produced by the three bout analysis methods were then compared directly using a 3 (bout analysis method) by 8 (microstructure) factorial ANOVA.

Results

Representative plots for each of the eight simulated microstructures are shown in Figure 2. The top row of Figure 2 shows log-survivor plots divided into microstructures with fast within-bout rates in the left panel and slow within-bout rates in the right panel. The broken-stick pattern is clearly evident for each of the 8 simulated bout structures, with the defining visual features of the function controlled by the bout parameters. The slope of the initial limb is steeper in the left panel than the right panel, corresponding to the faster within-bout rates. Within each panel, the intersection of the two limbs point is higher for high values of p than low values of p; the higher values of p correspond to shorter average bout lengths. The slope of the second limb corresponds to the bout initiation rate, with shallower functions indicating longer average pauses between bouts.

The second row of Figure 2 shows log-interval plots of the same simulated records used in the first row, again separated by fast (left) and slow (right) within-bout rates. The difference in

within-bout rates is apparent in the shorter peaks in the right panel. Unlike the log-survivor plots, differences in bout initiation rate and bout length are difficult to discern. However, that poor discriminability is not surprising because the log-survivor method has long been recognized as having better visual economy for bout analysis (Slater, 1974).

The third row of Figure 2 shows cumulative records of the same simulated records used in the first and second rows. Cumulative records end at different time points for each microstructure because the simulations had the same sample sizes but different average overall event rates (consistent with the different bout topographies). All the records show a steady event rate as indicated by the relatively linear trajectory from the origin to each record's termination. While not evident in the figure, it can be noted that a systematic change in the response rate over time would appear as a bowing of the cumulative record relative. For example, a concave function would occur if the rate were higher than the overall average near the beginning of the record and decreased over time to be consistently lower than the overall average near the end of the record.

For each fit, parameter estimates were combined to predict the overall rate for the target record and a recovery ratio was calculated as the ratio of predicted rate to obtained rate (number of simulated responses divided by total simulated time). Figure 3 shows the recovery ratios for the bout partitioning methods as a function of microstructure for overall rate (top panel), within-bout rate (second panel), bout initiation rate (third panel) and the probability of terminating a bout (bottom panel). The recovery ratios for each measure were analyzed separately using a 3x8 (method by microstructure) factorial ANOVA.

For the overall rate recovery ratio (Figure 3, top left panel), there was a significant main effect of partitioning method, $F(2, 216) = 55.71$, $p < 0.001$, and a significant main effect of

microstructure, $F(7, 216) = 4.28$, $p < 0.001$, but no significant method by microstructure interaction, $F(14, 216) = 1.57$, $p = 0.09$. Post hoc testing revealed that the change-point method produced significantly higher estimates of overall rate than either the log-survivor method ($p < 0.01$) or the log-interval method ($p < 0.01$); the log-interval and log-survivor methods did not differ significantly ($p = 0.21$). Both the log-survivor and log-interval methods produced parameter estimates that systematically underestimated overall rate by approximately 3% to 10% across microstructures. The change-point method predicted comparable overall rates for select microstructures, but overestimated event rate by 3% to 15% in the majority of cases, including all microstructures with slow within-bout rates.

For within-bout rate (Figure 3, top right panel), there were significant main effects of method ($F(2, 216) = 13.47$, $p < 0.001$) and microstructure ($F(7, 216) = 23.29$, $p < 0.001$), as well as a significant method by microstructure interaction ($F(14, 216) = 11.61$, $p < 0.001$). Post hoc testing revealed differences among the methods on six of the eight microstructures. None of the three methods differed significantly from each other on the fast-long-infrequent or fast-long-frequent microstructures (all p's > 0.10). The change-point method estimated significantly higher within-bout rates than the log-survivor method for four microstructures: fast-short-infrequent ($p = 0.02$), fast-short-frequent ($p = 0.01$), slow-long-frequent ($p < 0.001$), and slow-short-frequent ($p < 0.001$). The change-point method also estimated significantly higher within-bout rates than the log-interval method for three microstructures (fast-short-infrequent, fast-short-frequent, and slow-short-frequent, all p's < 0.001) and significantly lower estimates for slow-long-infrequent, $p < 0.001$). The log-interval method estimated significantly higher within-bout rates than the log-survivor for two microstructures (slow-long-frequent and slow-long-infrequent, both p's < 0.001) and a significantly lower estimate for one microstructure (fast-short-infrequent, $p = 0.04$). Across

microstructures, the log-survivor method tended to produce estimates of within-bout rate that were slightly lower than the true within-bout rate by approximately 2% – 4%. In cases where the change-point method differed from the log-survivor, the change-point method overestimated the within-bout rate by 2% – 10%. In cases where the log-interval method differed from the log-survivor method, the log-interval overestimated the within-bout rate by approximately 4% – 10% for microstructures with slow within-bout rates and underestimated it by approximately 8% for microstructures with fast within-bout rates.

For bout initiation rate (Figure 3, bottom left panel) there were significant main effects of method ($F(2, 216) = 25.82$, $p < 0.001$) and microstructure ($F(7, 216) = 4.47$, $p < 0.001$), as well as a significant method by microstructure interaction ($F(14, 216) = 5.33$, $p < 0.001$). Post hoc testing revealed differences among the methods on seven of the eight microstructures. None of the three methods differed significantly from each other on the slow-short-infrequent microstructures (p's > 0.10). The change-point method estimated significantly higher bout initiation rates than the log-survivor method for two microstructures: fast-short-frequent ($p = 0.008$) and slow-short-frequent ($p = 0.02$). The change-point method also estimated significantly higher bout initiation rates than the log-interval method for two microstructures (fast-short-frequent, $p = 0.03$, and slow-short-frequent, $p = 0.01$) and significantly lower bout initiation rates for four microstructures (fast-long-infrequent, $p = 0.02$, fast-long-frequent, $p < 0.001$, fast-short-infrequent, $p = 0.02$, and slow-long-frequent, $p = 0.04$). The log-interval method estimated significantly higher bout initiation rates than the log-survivor for four microstructures (fast-long-frequent, $p < 0.001$, fast-long-infrequent, $p = 0.02$, slow-long-frequent, $p < 0.001$, and slow-long-infrequent, $p = 0.006$). Across microstructures, the log-survivor method tended to produce estimates of bout initiation rate that were approximately equal to the true bout initiation rate,

with the exception of the slow-long-frequent microstructure, which was underestimated by nearly 20%. In the two cases where the change-point's estimated bout initiation rate differed from the log-survivor's estimated bout initiation rate, the change-point method overestimated the bout initiation rate by 2% – 15%. In cases where the log-interval method differed from the log-survivor method, the log-interval overestimated the bout initiation rate by approximately 25% – 40%.

For the probability of terminating a bout (Figure 3, bottom right panel), there were significant main effects of method ($F(2, 216) = 71.48$, $p < 0.001$) and microstructure ($F(7, 216) = 13.15$, $p < 0.001$), as well as a significant method by microstructure interaction ($F(14, 216) = 16.02$, $p < 0.001$). Post hoc testing revealed differences among the methods on six of the eight microstructures. None of the three methods differed significantly from each other on either the fast-short-frequent or the slow-short-frequent microstructures (all p's > 0.10). The change-point method estimated significantly lower values of p (corresponding to significantly longer bout lengths) than the log-survivor method for one microstructure: fast-short-infrequent ($p < 0.001$). The change-point method also estimated significantly lower values of p (corresponding to significantly longer bout lengths) than the log-interval method for five microstructures: (fast-long-infrequent, $p = 0.006$, fast-long-frequent, $p < 0.001$, slow-long-infrequent, $p < 0.001$, slow-long-frequent, $p < 0.001$, and slow-short-infrequent, $p = 0.02$. The log-interval method estimated significantly higher values of p (corresponding to significantly shorter bout lengths) than the log-survivor method for fast-long-infrequent, $p = 0.04$, fast-long-frequent, $p < 0.001$, slow-long-infrequent, $p < 0.001$, and slow-long-frequent, $p < 0.001$. For microstructures with slow within-bout rates, the log-survivor method tended to produce estimates of p that were approximately equal to the true value—with the exception of the slow-long-frequent microstructure. However,

the log-survivor method systematically overestimated the probability of terminating a bout (corresponding to underestimating the average bout length) by 10% – 20% for microstructures with fast within-bout rates. The change-point method estimated values of p that were comparable to or more accurate than those from the log-survivor method. The log-interval approach differed significantly from other methods on the four microstructures with relatively long bouts (low true probability of bout termination). In those cases, the log-interval estimates of p ranged from 40% to 100% higher than the true values, suggesting a tendency to substantially underestimate the average bout length for microstructures characterized by long bouts of activity.

Discussion

For data simulated to meet the assumptions of the 2-state biexponential bout model, the log-survivor method produced generally good estimates of all three bout parameters across the range of microstructures. The log-survivor method tended to underestimate within-bout rate by a small amount (less than 5%) and to underestimate mildly (10 – 20%) the bout length for microstructures with high within-bout rates. As a result, the log-survivor method mildly underestimated the overall rate by 3 – 10%. The change-point method and log-interval method produced bout parameter estimates that were often but not always consistent with the log-survivor estimates. The change-point and log-interval method produced higher within-bout rate estimates than the log-survivor method for several microstructures, though the degree of overestimation was comparable to the log-survivor method's underestimation of the parameter. In general, estimated values of both the bout initiation rate and the probability of ending a bout were comparable across microstructures for the change-point and log-survivor methods. However, the log-interval method tended to overestimate the bout-initiation rate (25 – 40%) and overestimate the probability of terminating a bout (40 – 100%) for event records characterized by

long runs. In those microstructures, the log-interval method still produced reasonable predictions for overall rate but at the cost of mischaracterizing the temporal pattern as consisting of very short bouts with very short inter-bout pauses. For the change-point analysis, overestimating the overall rate was associated with overestimating the within-bout rate and, in some cases, probability of terminating a bout. As described in the Methods section above, the overestimation of the within-bout rate and bout termination probability may reflect a decision-criterion that was too liberal for the microstructures used in the present experiment. The estimation error was relatively small, but a more conservative decision-criterion (e.g., 2.0, corresponding to an alpha level of 0.01) may be more appropriate for high-rate data, particularly the data contain more variability than the simulated IETs used in this experiment. Taken together, these results suggest that the change-point analysis provides bout parameter estimates with accuracy comparable to the log-survivor method for an assortment of microstructures when the biexponential model's assumptions are met. The log-interval analysis also provided comparable bout structures for microstructures with short average bouts, but for long bout lengths it systematically and substantially overestimated the bout initiation rate and underestimated the bout length.

Recovery of overall response rate was generally an adequate indicator of the ability of the e individual bout parameters to model the pattern of responding correctly, especially for the change-point and log-survivor methods. However, the disparity between log-interval recovery ratios for overall rate versus bout initiation and p parameters illustrate the potential danger of basing decisions on the recovery of overall rate alone. Overall response rate is equivalent to the reciprocal of the mean IET. Although the mean IET can be viewed as a weighted composite of the means of two populations of IETs, multiple combinations of weights and means can produce the same overall mean IET. Thus, a set of parameter estimates could still adequately recapture

overall rate despite misestimating all three bout parameters if the misestimated values align just right. Outside of simulations, the true microstructure topography is not necessarily known in the absence of a bout analysis, but well established schedule-typical patterns of behavior can provide insight in free operant preparations. For example, differential reinforcement of high-rate (DRH) schedules produce behavior characterized by bursts of activity roughly equal in length to the response requirement of the schedule. For behavior maintained under a DRH schedule of reinforcement, if two partitioning methods produce the same recovery ratio for overall rate preference should be given to the method that suggests a microstructure consistent with the schedule parameters. In contrast, if two methods suggest the same microstructure but produce significantly different recovery ratios then the method whose ratio is closest to 1.0 may provide better bout parameter estimates. Thus, the overall response rate recovery ratio can be a useful metric when considered in the context of the expected temporal organization of behavior, but should not be used as the sole metric to choose between bout analysis methods.

Experiment 2

Analyses of simulated data can provide insight into model performance under idealized conditions, but evaluating the practical utility of any quantitative model of behavior requires testing the model against behavior under real world conditions. An ideal target behavior for comparing bout analysis methods should have stable response rates over experimentally useful time periods to increase the likelihood that bout structure is stationary. Reinforcement schedule is well known to affect the temporal patterning of behavior. The classic fixed-interval scallop and fixed-ratio break-and-run patterns are reliable indicators reinforcement schedule's control over the temporal organization of behavior (Ferster & Skinner, 1957). The overall rate of reinforcer delivery can affect both satiation (Sidman & Stebbins, 1954) and habituation (McSweeney &

Weatherly, 1998), both of which may impact bout structure (Tolkamp, et al., 1998). Because of the effects of reinforcement schedule, satiation, and habituation on temporal organization, preparations that rely on food (or water) reinforcers are problematic for comparisons among bout analysis techniques.

Voluntary wheel running may represent an ideal initial test case for bout analysis methods. Spontaneous running may be considered intrinsically reinforced under an unknown reinforcement schedule. Although motor fatigue and habituation may decrease the overall rate of running over extended time periods (Aoyama & McSweeney, 2001), studies have reliably shown that spontaneous running in rodents typically occurs at relatively high rates for extended periods of time (Sherwin, 1998). Further, log-survivor analysis with the biexponential model has proven useful at describing the microstructure of spontaneous running in both rats (Eikelboom & Mills, 1988) and mice (Johnson, Pesek, & Newland, 2009). Accordingly, experiment 2 compares the three bout analysis methods using spontaneous wheel running data from a sample of adult mice. In addition to accurately characterizing the temporal organization of behavior, a good bout analysis should produce bout parameters that are differentially sensitive to different classes of experimental manipulation. The log-survivor analysis produces parameter estimates that are independent and selectively sensitive to reinforcement schedule and relative reinforcement rate (e.g., Shull & Gaynor, 2003; Shull, 2004) and to the effects of drugs on behavior (Johnson, Bailey, & Newland, 2011). The parameters from log-interval analysis are similarly selective and have the same theoretical interpretations (Brackney, et al., 2011). The within-bout rate has proven consistently difficult to target experimentally due in part to the motivational impact of the most common environmental manipulations (e.g., varying reinforcement rate or increasing response effort). The neurotoxicant methylmercury causes sensorimotor deficits, including

impaired motor coordination, reduced control of distal extremities, and impaired proprioception (Takeuchi, et al., 1962). Therefore chronic methylmercury (MeHg) exposure offers a rare opportunity to selectively target the within-bout rate parameter. A useful bout analysis method should be able to identify MeHg-related impairment by showing differential sensitivity of the within-bout rate parameter in animals chronically exposed to methylmercury.

Method

Subjects

The subjects were 24 adult male BALB/c mice (purchased from Harlan laboratories, Indianapolis, IN) with no prior activity wheel experience. Mice were singly housed (two per cage) in clear polycarbonate cages divided along the diagonal with a translucent plastic barrier that permitted visual and olfactory, but not physical, contact. Animals were maintained at 24 g to 26 g body mass on a daily food ration of approximately 2.5 g and had ad libitum access to water except during experimental sessions. When not in experimental sessions, the animals were housed in an AAALAC-accredited vivarium maintained on a 12-h light-dark cycle (lights on at 7:00 AM) in a temperature and humidity controlled facility. Animal health was monitored daily by research staff and all procedures were approved by the Auburn University Institutional Animal Care and Use Committee.

Apparatus

Experimental sessions were conducted in rat operant chambers (MED-Associates, Inc., St. Albans, VT) fitted to accommodate mice and situated in sound-attenuating ventilated shells. Each chamber contained one in-chamber activity wheel (Med Associates Inc., St. Albans, VT, ENV-043A). The wheel's running surface consisted of 3.175 mm diameter stainless steel rods (6.37 cm wide) located every 6 degrees on a 17.78 cm diameter wheel. Responses were recorded

as quarter-wheel revolutions with centisecond resolution via Med-PC™ control software on a PC running Windows XP. Processing of raw MED data files, data management, and all partition analyses described below were performed using *R (*R core development team, 2013) and the arf package for animal research (Hoffman, *in preparation*).

Procedure

Animals performed in weekly running wheel sessions once per week until the end of the study, or until they reached humane endpoints criteria (described below). No experimenter-controlled stimuli were active during the sessions and no experimenter-controlled contingencies were in place for wheel running. During sessions, the front panel of the chamber was blocked with a translucent plastic panel to prevent access to the chamber's food tray or operanda. For the present analysis, only the first 60 minutes of data for each animal were analyzed to minimize any impact of within-session changes on bout structure (e.g., due to motor fatigue from extended periods of running). To ensure that data used in the present analysis represent MeHg-impaired running, the data presented are from the initial baseline running session (prior to onset of MeHg exposure) and, for the MeHg-exposed animals, the final experimental session in which the animal recorded at least 1000 interevent times; a sample size of 1000 IETs was chosen as the threshold because of the sensitivity of mixture models to low sample sizes and to be consistent with the simulations in experiment 1. Because of individual differences in the time course of MeHg impairment, control subjects were paired with MeHg-exposed subjects to create age-matched pairs to control for the potential confounding effects of age.

MeHg exposure. Prior to exposure, animals were screened using a rotarod (MED-Associates, Inc. St. Albans, VT) task to ensure there were no preexisting differences in motor coordination across groups. Then they were pseudorandomly assigned to either the control or

MeHg exposure group (N = 12 for both groups) with the constraint that the groups were similar on pre-exposure rotarod performance. Beginning at approximately 11 weeks of age, the mice were exposed chronically to either 0 or 15 ppm mercury as methylmercuric chloride via daily drinking water. Based on periodic water consumption sampling throughout the experiment, this resulted in daily MeHg doses of approximately 0 and 2.6 mg/kg/day. Brain concentrations of MeHg for animals in the present study are presented in Bailey et al., 2013.

Humane Endpoints. Research personnel performed daily health inspections on all animals throughout the study and the attending veterinarian was consulted in cases of injuries or illness. Every effort was made to treat health issues and prolong animal life as long as doing so did not prolong distress. If a mouse lost 10% of its weight, was unable to locomote, or feed itself, then it was humanely euthanized. All euthanasia criteria and methods were approved by the Auburn University Institutional Animal Care and Use Committee.

Data Analysis

All bout analysis methods were performed as described in experiment 1 above, with the exception of a different decision-criteron (2.0, corresponding to an alpha level of 0.01) for the change-point analysis. As discussed above, the change-point analysis had a tendency to slightly overestimate both the within-bout rate and the probability of ending a bout for select microstructures. Although the misestimation was small in most cases, the pattern of misestimation was consistent with a decision-criterion that was too sensitive to variations in sequential IETs. Because true bout parameter estimates are unknown for actual behavioral data, only the recovery ratio for overall response rate could be calculated in the present experiment. Differences among models for the recovery ratio as well as three bout parameters were analyzed using separate mixed factorial models including measurement time as a within-subjects factor

and MeHg exposure as a between-subjects factor.

Results

Assumptions

Empirical log-survivor distributions (top panel), empirical frequency distributions with log-transformed IETs (center panel), and cumulative records (bottom panel) are shown for 12 subjects prior to methylmercury exposure in Figure 4. The log-survivor functions for all subjects show consistent, systematic departures from a 2-state biexponential process. The initial limb begins with a plateau (segment of low, non-zero slope) before the curve accelerates to a much steeper slope. Such records are consistent with previously published log-survivor curves of high-rate behavior (e.g., Davison, 2004; Podlesnik, Jimenez-Gomez, Ward, & Shahan, 2006). The log-interval plots shown in the center panel differ from the log-interval plots of biexponentially distributed IETs in experiment 1, but are consistent with log-normally distributed IETs. There is no prominent second peak to suggest the location of the between-bout IET distribution. Based on the log-survivor plots, the between-bout IETs make up 10% or less of total IETs for all cases. The linear Y-axis therefore makes it difficult to discern the between-bout IETs for the present data (and, similarly, for any data where the proportion of within-bout IETs greatly outweighs the proportion of between-bout IETs). The same difficulty was apparent from Figure 1 of experiment 1. This appearance suggests that the IETs may not follow a biexponential distribution. The cumulative records (bottom panel) show relatively steady, high rates of responding throughout the session with some evidence of accelerating rates in the first minutes of the session (as indicated by the upward bowing seen in the first 10-15 minutes for some animals.

The bowing of a cumulative record can be quantified using the index of curvature (Fry, Kelleher, & Cook, 1960), calculated as

$$I = \frac{(n-1)R_n - 2\sum_{i=1}^{n-1} R_i}{nR_n},$$

where *I* is the index of curvature, n is the number of bins into which the session is divided, and R is the number of responses at a given bin. A steady rate of responding corresponds to $I = 0$, with negative values of *I* indicating decreases in rate and positive values indicating increases in rate; the larger the absolute value of *I*, the greater the within-session change. The lower and upper bounds of *I* can be determined by assuming that all responses fall either in the first or last bin. For the present experiment, index of curvature was used to determine whether IETs could reasonably be aggregated from the entire session to conduct the log-survivor and log-interval analyses. Each session was divided into four 15-min bins (Odum & Schaal, 200; Ward & Odum, 2006), making the range of *I* -0.75 to 0.75. The index of curvature did not differ systematically across groups (data not shown as a figure). Values of *I* were approximately 0 for both exposure groups at both measurement times: $\text{control}_{\text{Time1}} = 0.06$ (*SEM* = 0.02), $\text{control}_{\text{Time2}} = 0.05$ (*SEM* = 0.01), MeHgTime1 = 0.08 (*SEM* = 0.02), MeHgTime2 = 0.03 (*SEM* = 0.02). These results suggest that running was relatively stable within sessions and that MeHg-exposure did not markedly contribute to within-session changes in running. Accordingly, both the log-survivor and log-interval analyses were conducted using IETs aggregated from throughout the 60-min session.

Overall Response Rate

Overall response rate is presented in the top panel of Figure 5. Analysis of response rates using a mixed ANOVA revealed a significant exposure by time interaction, $F(1,22) = 12.00$, $p = 0.002$. Overall response rate was lower for MeHg-exposed animals after chronic exposure than at baseline and lower than overall rate for the control group at either time point (all *p*'s < 0.01). The overall response rate recovery ratio was calculated as described for experiment 1. The bottom

panel of Figure 5 plots the recovery ratio as a function of group and time separately for each partitioning method. In general, the change-point and log-interval analyses provided adequate estimations of overall response rate while the log-survivor analysis consistently overestimated rate by 30 – 40% at baseline and over 100% for MeHg animals at the end of exposure.

Differences among bout analysis methods on the recovery ratio and bout parameter estimates were analyzed using a 3x2x2 (method by exposure group by time) mixed model with subject included in the random error term. For the overall response rate recovery ratio, there were significant effects of partitioning method ($F(2, 88) = 107.9$, $p < 0.001$), method by exposure interaction ($F(2, 88) = 13.09$, $p < 0.001$), a significant method by time interaction ($F(2, 88) = 18.5$, $p < 0.001$), and a significant method by exposure by time interaction ($F(2,88 = 10.8$, $p < 0.001$). Post hoc testing revealed a significant effect of method at time 1, with the log-survivor estimating significantly higher overall rates than either the log-interval or change-point analysis (both p's < 0.001) and the log-interval analysis estimating significantly higher rates than the change-point analysis ($p < 0.001$). At time 2, the log-survivor recovery ratios were significantly higher than those of the log-interval and change-point analyses for both groups (p's < 0.01). The log-survivor analysis also overestimated the overall rate for MeHg animals to a greater extent than for controls ($p < 0.001$). For both groups at both time points the change-point bout parameters produced the closest estimate of the overall response rate.

Bout Analysis

For within-bout rate (Figure 6, top panel), there were significant main effects of group method ($F(1, 22) = 7.8$, $p = 0.01$), method ($F(2, 88) = 12.5$, $p < 0.001$), and a significant exposure group by time interaction ($F(1, 22) = 8.2$, $p = 0.009$). At time 1, log-survivor and log-interval analyses estimated higher within-bout rates than did the change-point analysis (both p's

< 0.001) but did not differ significantly from each other ($p = 0.28$). At baseline, the average minimum interevent time was approximately 0.3 seconds, corresponding to a maximum sprint rate of 3.33 quarter revolutions per second. Thus the log-interval method estimated an average within-bout rate only slightly lower than the animals' maximum sprinting speed, the change-point method estimated within-bout rate approximately equal to 75% of maximum sprinting speed, and the log-survivor method provided an estimate between those two extremes (approximately equal to 88% of maximum sprinting speed). At time 2, all three methods estimated significantly lower within-bout rates for MeHg animals than for controls ($p < 0.001$) with no other significant differences (all other p's > 0.10). Despite the baseline difference in estimated within-bout rates, all three methods detected a methylmercury-related decrease in within-bout rate.

For bout initiation rate (Figure 6, middle panel), there were significant main effects of exposure ($F(1, 22) = 10.2$, $p < 0.01$) and partitioning method ($F(2, 88) = 102.1$, $p < 0.001$), and a significant method by exposure interaction ($F(2, 88) = 3.3$, $p = 0.04$). Post hoc analysis revealed that the log-survivor method estimated significantly higher bout initiation rates than the change-point method for both control and MeHg groups (p's < 0.001), as did the log-interval analysis both groups (p's < 0.001). Bout initiation rates from the log-survivor method suggested a microstructure characterized by brief pausing between bouts (with an average pause between bouts of roughly 0.7 seconds). In contrast, the change-point method suggested relatively longer pauses of approximately 10 seconds between bouts on average. The log-interval analysis estimated an average interbout pause of approximately 1.1 seconds, making its estimate closer to that of the log-survivor analysis than change-point analysis.

For p, the probability of ending a bout (Figure 6, bottom panel), there was a significant

main effect of exposure ($F(1\ 22) = 8.9$, $p < 0.001$, measurement time ($F(1, 22) = 15.6$, $p < 0.001$), and of partitioning method ($F(2, 88) = 71.6$, $p < 0.001$), as well as a significant two-way method by time interaction ($F(2, 88) = 7.3$, $p = 0.001$) and a significant three-way method by exposure by time interaction ($F(2, 88) = 4.2$, $p = 0.02$). For the baseline sessions, the change-point analysis estimated significantly lower values of p (corresponding to longer average bout lengths) than the log-survivor or log-interval analysis for both exposure groups (all p's < 0.001). The log-survivor analysis also estimated significantly lower values of p (longer average bout lengths) than the log-interval analysis (p's < 0.05). Toward the end of exposure, the change-point analysis ($p < 0.001$) but not the log-survivor nor log-interval analysis (both p's > 0.90) detected a significant increase in p (corresponding to a significant decrease in bout length) in MeHg animals but not controls. The relationship among methods was the same as at baseline, with the change-point analysis again estimating lower values of p than log-survivor and log-interval analysis for both groups (p's < 0.001) and the log-interval analysis estimating higher probabilities of bout termination ($p = 0.01$). The log-interval analysis's mean estimates of p ranged from approximately 0.25 to 0.35 across groups and times, corresponding to average bout lengths of 4 to 5 quarter revolutions of wheel (a distance of roughly 70 cm). In contrast, the change-point analysis estimated the probability of terminating a bout at between 0.01 and 0.05, corresponding to average bout lengths of 21 to 101 quarter revolutions (distances of approximately 3.6 to 13.9 m). The log-survivor analysis produced mean estimates of p in between the change-point and log-interval analysis, ranging from 0.04 to 0.07, corresponding to average bout lengths of 15 to 26 quarter revolutions (distances of approximately 2.1 to 3.6 m).

Discussion

Consistent with the expected sensorimotor impairment associated with methylmercury

exposure, overall response rate declined substantially from baseline for MeHg-exposed animals but did not significantly change for age-matched controls over the same time period. The three bout analysis methods were not equally successful at recapturing overall response rate. The log-interval and change-point methods produced bout parameters that resulted in an acceptable 5 – 10% prediction error across exposure groups and times. The prediction error for the change-point analysis and log-interval analysis was comparable to the degree of misestimation in experiment 1, but the two techniques produced consistently different characterizations of running's bout structure. However, the log-survivor analysis systematically overestimated overall response rate by more than 40%.

The poor performance of the log-survivor analysis on the recovery ratio relative to the other methods may be due to model misspecification. Although running within sessions appeared temporally stable based on analysis of cumulative records and the index of curvature, visual inspection of both log-survivor and log-interval plots strongly suggest that the distribution of spontaneous running IETs is not well characterized by a biexponential model. The log-survivor plots in the present experiment are similar to those seen in high-rate food-maintained behavior in pigeons (e.g., Davison, 2004; Podlesnik, et al., 2006). One explanation that has been used to explain the departure from the broken-stick pattern in log-survivor plots of high-rate pigeon behavior is that the relative reinforcement available on the target behavior is so high and the availability and quality of alternative reinforcers is so low that subjects engage in rapid responding with little to no pausing to engage in alternative activities. However, that explanation seems unlikely for the present experiment in which the maximum possible rate of behavior is roughly two orders of magnitude slower than a pigeon's fastest key-pecking and an order of magnitude slower than a rat’s fastest lever-pressing because the event, a quarter-revolution turn

of the wheel, required more time than either a pigeon's key-peck or a rat's lever-press. . Furthermore, the prevalence of IETs longer than 10 seconds suggests that the mice engaged in bouts of running separated by periods of alternative behavior. Even so, the overall temporal pattern of behavior did not appear to conform to the biexponential model. Instead, IETs appeared to be better described by a lognormal mixture. Because of the much higher rate of key-pecking than wheel running, log-survivor plots may obscure departures from exponentiality in the within-bout distribution by compressing the visual range. For example, a log-survivor plot whose x-axis extends from 0 to 10 seconds may allocate < 5% of the plot region to > 50% of the total number of IETs.

Previous research has shown that adult-onset methylmercury exposure causes deficits in spontaneous wheel running in rats (Heath, et al., 2010). The present experiment extends those findings by partitioning the reduction in running into three parameters: bout length, within-bout rate, and bout length. As in the earlier study, chronic, adult-onset methylmercury exposure also impairs spontaneous wheel running. All three bout analysis methods were sensitive to the effects of methylmercury, as evidenced by their detection of a decrease in within-bout rate after MeHg exposure. However, the change-point analysis—but not the log-survivor or log-interval analysis—also detected a significant decrease in average bout length (as measured by the increase in bout termination probability) for the MeHg group. None of the partitioning methods identified bout initiation rate as being affected by methylmercury. Thus, the ability to run long bouts was affected by chronic MeHg but the rate at which bouts were initiated, interpreted as the motivation to run (Shull & Grimes, 2003) was not affected. All three partitioning methods produced bout parameters that were sensitive to the sensorimotor-impairing effects of MeHg in a manner consistent with expectations and the known effects of MeHg on behavior. Unlike the

other partitioning methods, the change-point analysis detected significant MeHg effects on average bout length. MeHg exposure impairs motor coordination of the distal extremities and disrupts gait (Wakabayashi, et al., 1995), which may increase the difficulty of sustaining running bouts. Only the change-point analysis detected such an effect, however the initial bout length estimates from the log-survivor and, especially, the log-interval analysis may have been so low as to preclude detecting an effect on bout length.

Unlike in experiment 1, the three partitioning methods presented divergent pictures of the bout structure in experiment 2. The log-interval analysis and change-point analysis both had adequate recovery ratios of overall response rate and all three methods had similar estimates of within-bout rate. However, the change-point analysis characterized the microstructure of running as consisting of relatively long bouts (3 – 13 meters in distance) separated with long pauses (on the order of 10 – 15 seconds) whereas the log-interval analysis suggested extremely short bouts (less than 1 meter) with extremely short interbout pauses (averaging less than 1 second). The log-survivor analysis provided estimates of bout length near the low end of the change-point estimates and estimates of interbout pause times near the high end of those from the log-interval analysis. The recovery ratio alone is not enough to argue convincingly for one method over another (especially given the small differences between the recovery ratios from the log-interval and change-point methods), but the bout structure suggested by the log-interval analysis seems the least likely of the three topographies. In experiment 1, the log-interval analysis substantially overestimated both the bout initiation rate and the bout termination probability for microstructures with long average bout lengths—a finding that, in conjunction with the bout length estimates from the other methods, suggests the same may have happened in experiment 2. A previously published bout analysis of wheel running in BALB/c mice reported average

interbout pauses on the order of 10 to 12 seconds with average bout lengths of 1.5 to 2.0 meters (Johnson, Pesek, & Newland, 2009). In that study, wheel access was presented concurrently with a nosepoke and nosepoking was reinforced with food reinforcers under a ratio schedule. The concurrently available alternative may have contributed to longer interbout intervals, however the longest 1% of IETs were excluded from the bout analysis to improve fits of a 2-state biexponential model.Thus, the similarity to the estimates from the log-survivor analysis in the present experiment serves more to validate the current log-survivor fits than to suggest which of the three methods produced the best characterization of behavior. Nevertheless, together with the results of experiment 1, these results support the change-point approach as a viable bout analysis method.

Summary and General Conclusions

The present study extends the existing bout analysis by demonstrating the utility of a novel bout analysis method and by showing that all the bout analysis techniques examined herein are sensitive to the behavioral effects of neurotoxicant exposure. In experiment 1, all three bout analysis methods produced comparable and adequate estimates of bout parameters of simulated behavior when the within-bout rate was fast. The log-interval analysis in particular overestimated both the bout initiation rate and the probability of terminating a bout when the microstructure was characterized by long bouts of simulated activity. The same overestimation may have occurred in experiment 2 where the log-interval analysis estimated significantly shorter pausing between bouts and significantly shorter bout lengths than the other partitioning methods. The present experiments do not strongly support one bout analysis technique as generally better than the others, but they do support the utility of the change-point analysis as a viable alternative to distribution-based bout analysis methods. In experiment 1, the change-point analysis provided

adequate bout parameter estimates that were often comparable to those of the more established methods. When the change-point bout parameters did differ significantly from the log-survivor, they did not differ in a way that changed the character of the microstructure. In experiment 2, when the standard model assumptions were not assured, the change-point analysis did a significantly better job recapturing overall response rate than either the log-survivor or log-interval analysis. All methods provided similar estimates of the within-bout rate for exposed and control animals, and all three were sensitive to MeHg effects on within-bout rate; for the change-point analysis, the bout length was also a sensitive measure of MeHg impairment. Only the change-point analysis and the log-survivor analysis provided similar characterizations of the bout structure's topography, as the log-interval analysis suggested a microstructure dominated by extremely short bursts of activity with very short pausing between bursts. In contrast, the log-survivor and change-point bout parameters suggested the microstructure of running was made up moderate to long bursts of activity with intermediate to long pauses; of these two, the change-point bout parameters did a significantly better job of predicting the obtained overall response rate. Combined, the recovery ratio differences, similarity of bout parameter to previous running bout analysis (Eikelboom & Mills, 1988; Johnson, et al., 2009), and sensitivity of two motor-related parameters to MeHg suggest that the change-point analysis is as viable a bout analysis method as the more common methods and, perhaps, a preferable method for detecting the motoric effects of drugs and toxicants on spontaneous wheel running.

Tables

Table 1

A comparison of bout analysis features

	Log-survivor	Log-interval	Change-point
Model	$Y(t) > p_w e^{-\lambda_w t} + p_b e^{-\lambda_b t}$	$Y(t) = p_w \left(\frac{1}{\sigma_w \sqrt{2\pi}}\right) e^{-(t-\mu_w)^2/2{\sigma_w}^2}$ $+p_b \left(\frac{1}{\sigma_b \sqrt{2\pi}}\right) e^{-(t-\mu_b)^2/2{\sigma_b}^2}$	N/A
Constraints	$1 - (p_w + p_b + \cdots) = 0,$ $\{p_w, p_b, \ldots\} > 0,$ $\lambda_w > \lambda_b \geq 0$	$1 - (p_w + p_b + \cdots) = 0,$ $\{p_w, p_b, \ldots\} > 0,$ $\mu_w > \mu_b \geq 0$	$\mu_w > \mu_b \geq 0$
Distribution	Exponential Mixture	Gaussian Mixture	N/A
Form	Survivor (1 – CDF)	PDF	Cumulative Record
Shape	Monotone Decreasing, Broken-stick	Bi- or multi-modal	Slope ≥ 0 for all $x \geq 0$
Transform	Log (Y)	Log (X)	N/A
Free parameters	$2k - 1$ (k = number of components)	$2k - 1$ (k = number of components)	N/A
Assumptions	Stationary Constant initiation rate Constant termination rate	Stationary	Change-points indicate behavior transitions

Table 2

Parameter values and response rates for biexponentially simulated IETs

Structure	Within-bout Rate (events/sec) *(IET_{mean})*	Bout Initiation Rate (bouts/sec) *(IBI_{mean})*	Probability of Disengaging *(IETs per Bout)*
Fast Long Frequent	10 *(.10")*	0.5 *(2")*	0.05 *(20)*
Fast Long Infrequent	10 *(.10")*	0.25 *(4")*	0.05 *(20)*
Fast Short Frequent	10 *(.10")*	0.5 *(2")*	0.20 *(5)*
Fast Short Infrequent	10 *(.10")*	0.25 *(4")*	0.20 *(5)*
Slow Long Frequent	4 *(0.25")*	0.5 *(2")*	0.05 *(20)*
Slow Long Infrequent	4 *(0.25")*	0.25 *(4")*	0.05 *(20)*
Slow Short Frequent	4 *(0.25")*	0.5 *(2")*	0.20 *(5)*
Slow Short Infrequent	4 *(0.25")*	0.25 *(4")*	0.20 *(5)*

Figures

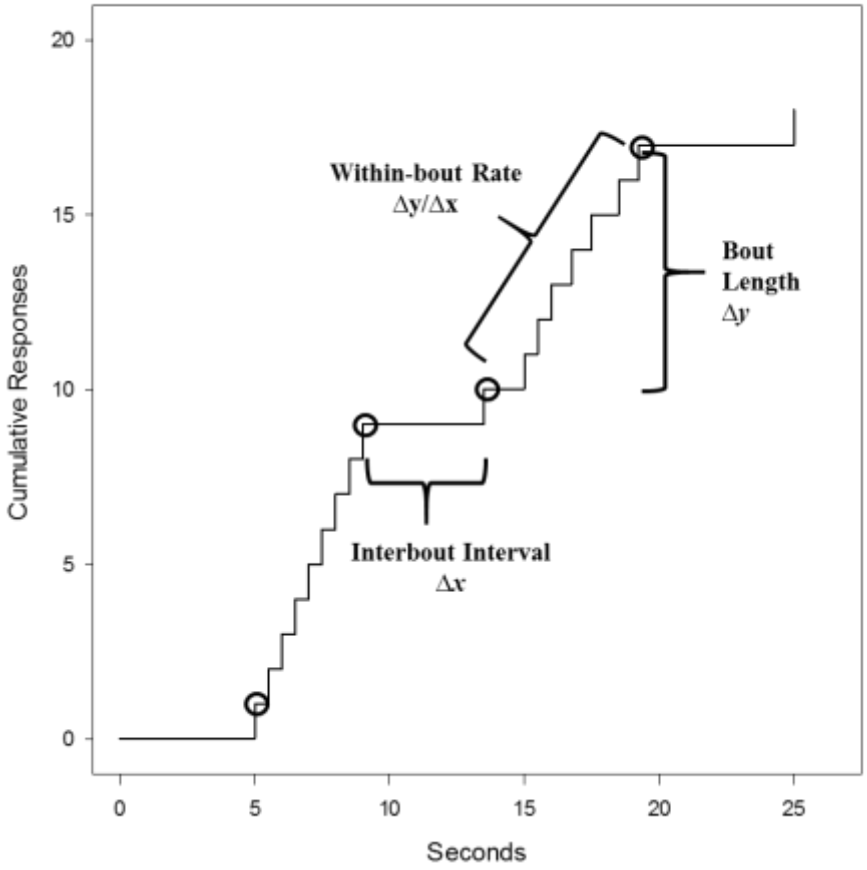

Figure 1. A simulated cumulative record showing four change-points. The change-points partition the record into successive segments that alternate between bouts and interbout intervals. For any given bout segment, the within-bout rate (speed of responding) is calculated as the slope of that segment and the bout length as the number of responses. The interbout interval is calculated as the time elapsed between the end of one bout and the beginning of the next.

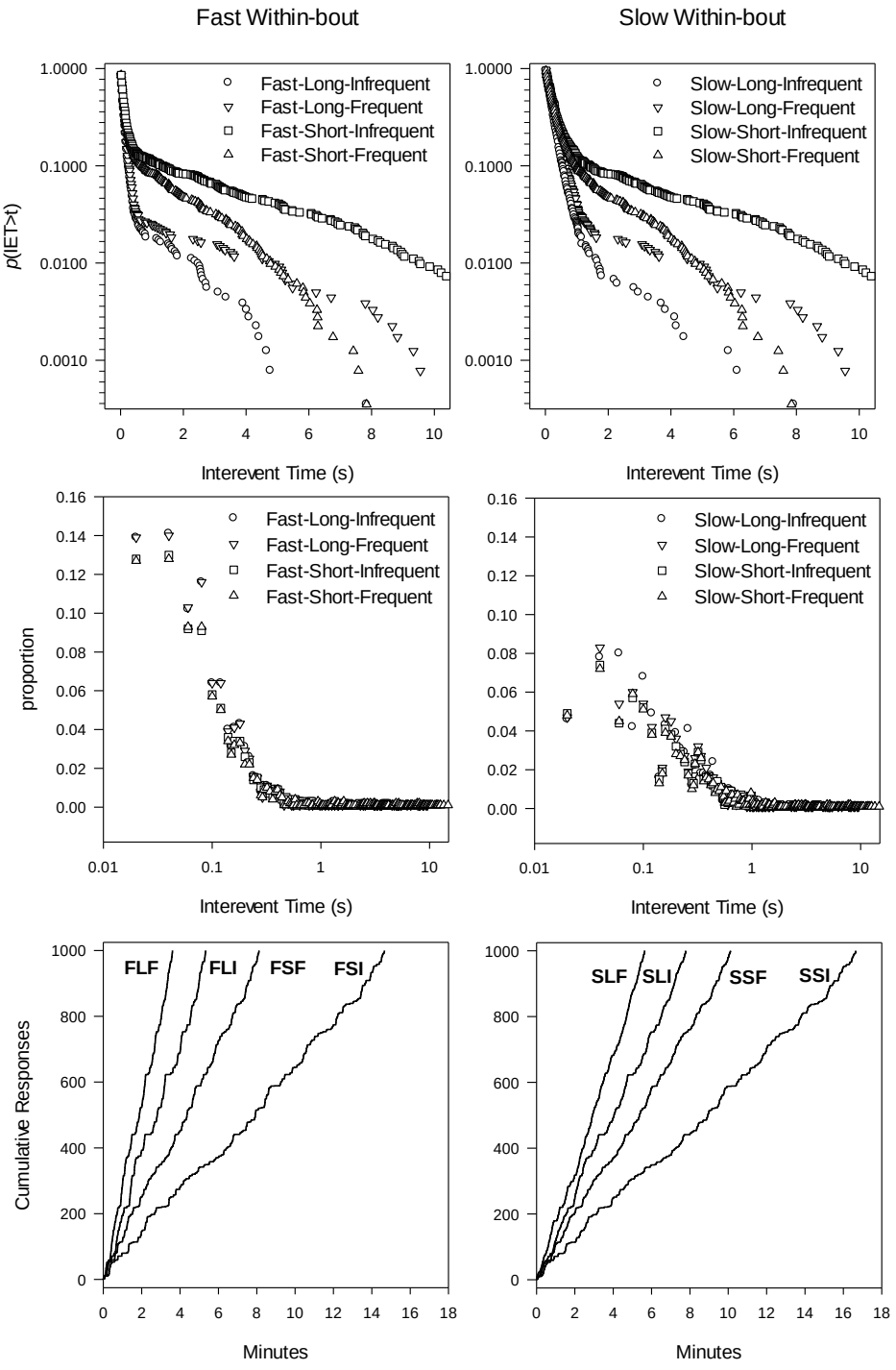

Figure 2. Log-survivor functions (top), log-interval plots (middle), and cumulative records (bottom) of a representative sample from each of the eight simulated microstructures separated by fast (left column) and slow (right column) within-bout rate.

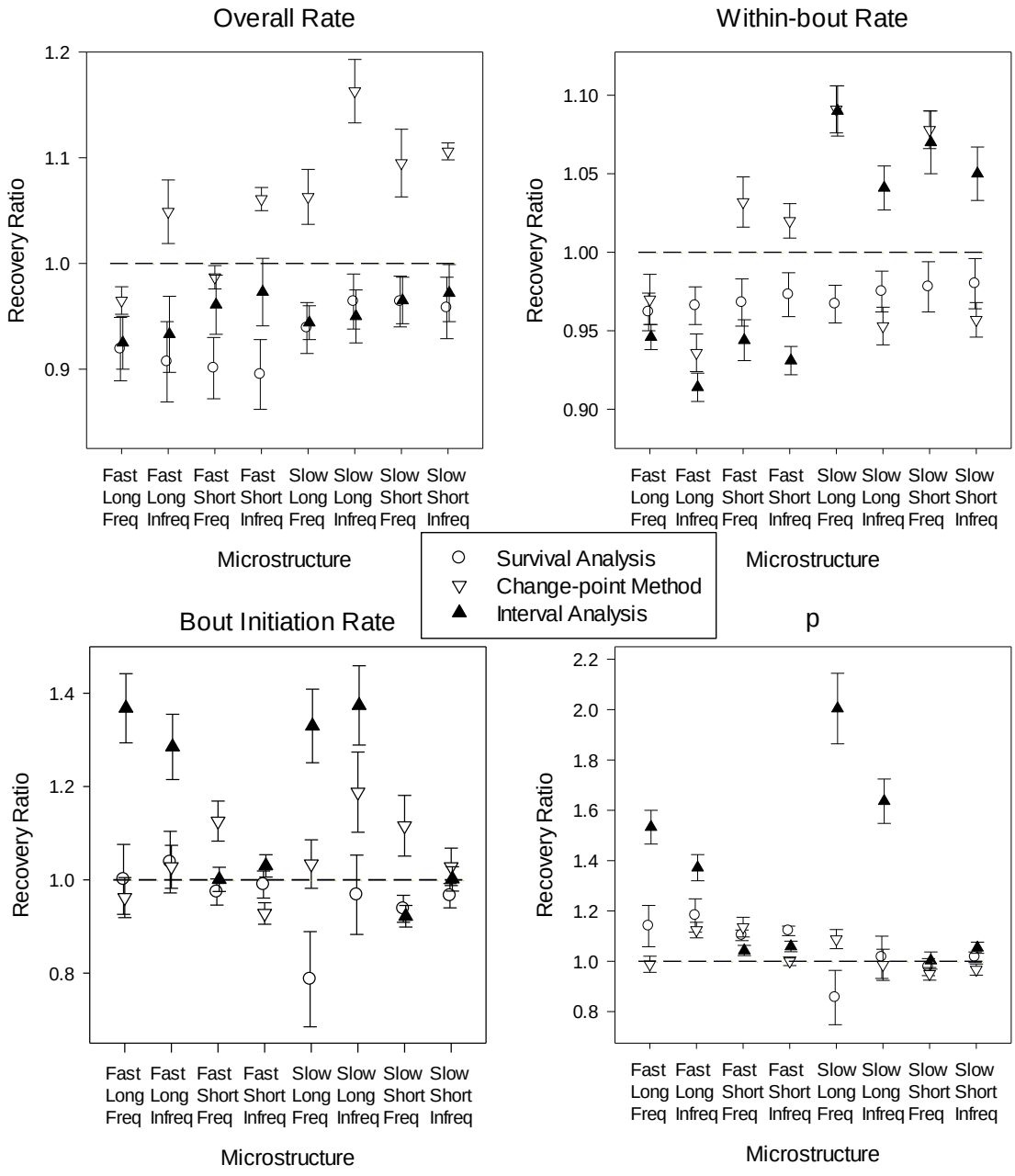

Figure 3. Mean (+/- SEM) recovery ratios for each of the eight simulated microstructures across partitioning method. Top left: Overall response rate. Top right: Within-bout rate. Bottom left: bout initiation rate. Bottom right: the probability of ending a bout. The dashed reference line in each panel corresponds to a recovery ratio of 1.0, which would signify 0% estimation error.

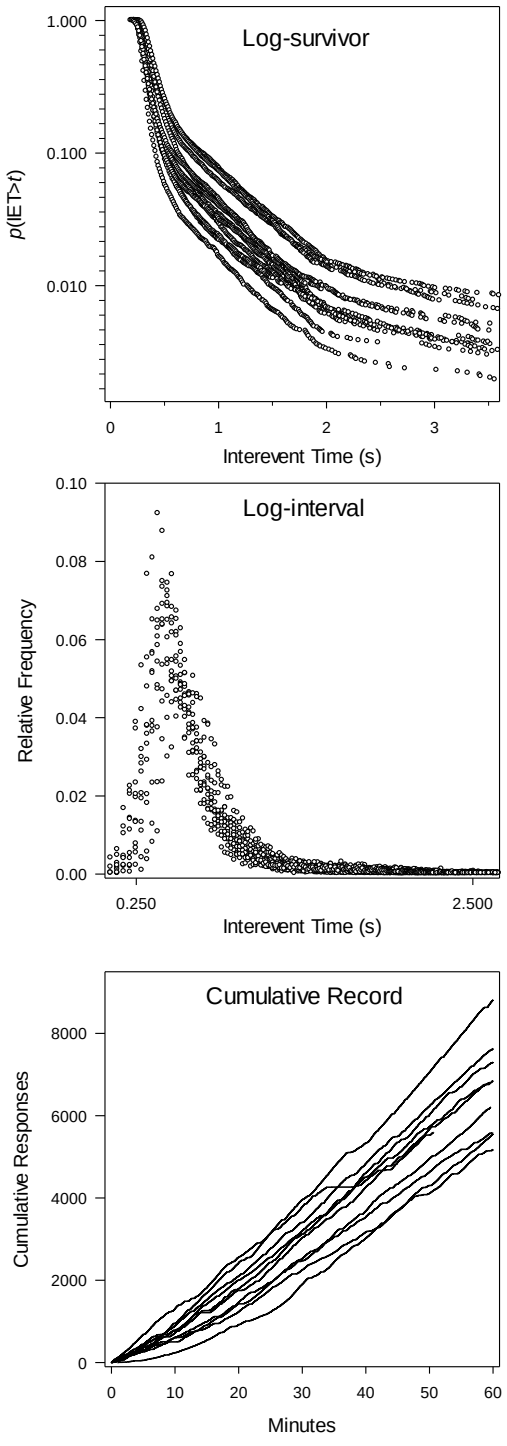

Figure 4. Log-survivor functions (top), log-interval frequency distributions (center), and cumulative records (bottom) for spontaneous running of individual subjects during the baseline session of experiment 2.

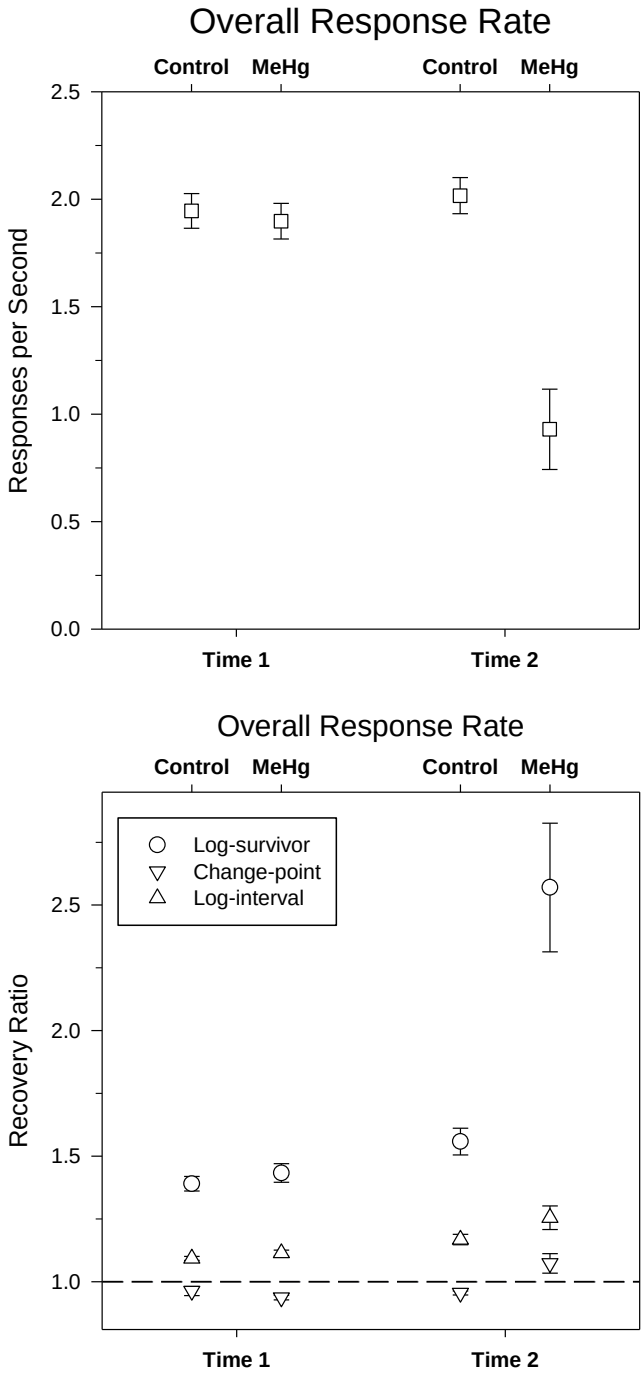

Figure 5. TOP: Mean (+/- SEM) of the overall response rate for each exposure group at the two measurement times. Bottom: Mean (+/- SEM) recovery ratio for the overall response rate for each of the three partitioning methods. The dashed horizontal line plots a recovery ratio of 1.0, which corresponds to 0% prediction error.

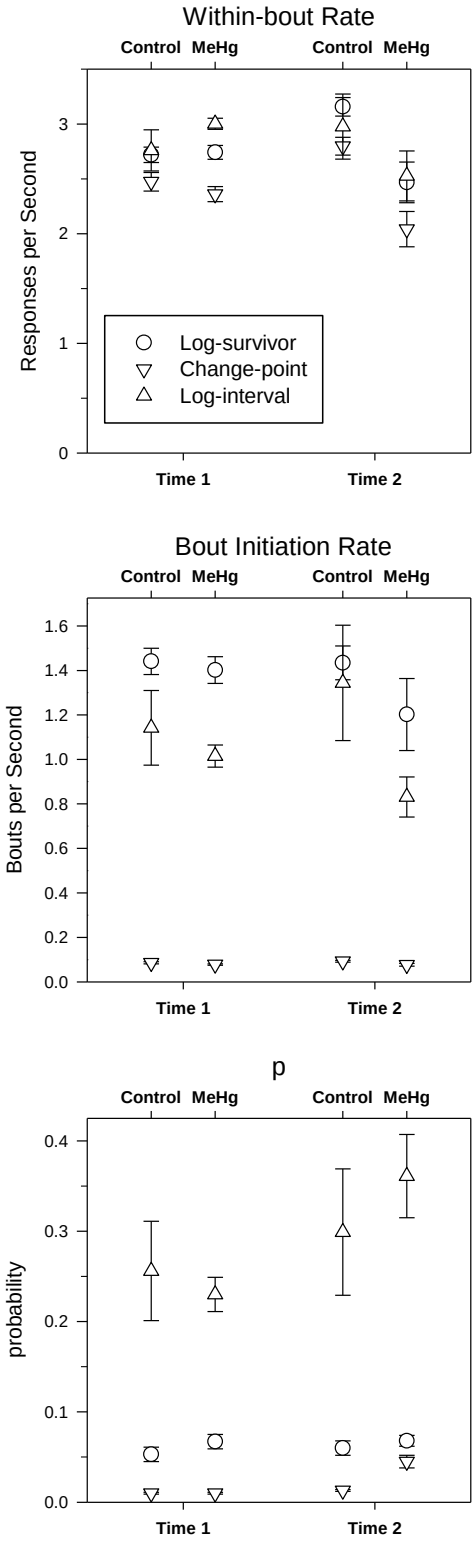

Figure 6. Mean (+/- SEM) of the estimated within-bout rate (top), bout-initiation rate (middle), and probability of ending a bout (bottom) for each of the three partitioning methods.

References

Aoyama, K., & McSweeney, F. K. (2001). Habituation contributes to within-session changes in free wheel running. *Journal of the Experimental Analysis of Behavior, 76*(3), 289–302.

Bailey, J. M., Hutsell, B. A., & Newland, M. C. (2013). Dietary nimodipine delays the onset of methylmercury neurotoxicity in mice. *Neurotoxicology, 37*, 108–117.

Benaglia, T., Chauveau, D., Hunter, D. R., & Young, D. S. (2009). mixtools: An R package for analyzing finite mixture models. *Journal of Statistical Software*, 1–29.

Brackney, R. J., Cheung, T. H., Neisewander, J. L., & Sanabria, F. (2011). The isolation of motivational, motoric, and schedule effects on operant performance: a modeling approach. *Journal of the Experimental Analysis of Behavior, 96*(1), 17–38.

Cheung, T. H., Neisewander, J. L., & Sanabria, F. (2012). Extinction under a behavioral microscope: isolating the sources of decline in operant response rate. *Behavioural Processes, 90*(1), 111–123.

Davison, M. (2004). Interresponse times and the structure of choice. *Behavioural Processes*, *66*(3), 173–187. doi:10.1016/j.beproc.2004.03.003

Eikelboom, R., & Mills, R. (1988). A microanalysis of wheel running in male and female rats. *Physiology & Behavior*, *43*(5), 625–630.

Ferster, C. B., Skinner, B. F., Harvard University, United States, & Office of Naval Research. (1957). *Schedules of reinforcement, by C.B. Ferster and B.F. Skinner.* New York: Appleton-Century-Crofts.

Fry, W., Kelleher, R. T., & Cook, L. (1960). A mathematical index of performance on fixed-interval schedules of reinforcement. *Journal of the Experimental Analysis of Behavior, 3*(3), 193–199.

Gallistel, C. R., Fairhurst, S., & Balsam, P. (2004). The learning curve: implications of a quantitative analysis. *Proceedings of the National Academy of Sciences of the United States of America*, *101*(36), 13124–13131.

Gallistel, C. R., Mark, T. A., King, A. P., & Latham, P. E. (2001). The rat approximates an ideal detector of changes in rates of reward: implications for the law of effect. *Journal of Experimental Psychology: Animal Behavior Processes*, *27*(4), 354.

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*(2), 169–179.

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 strains. *Pharmacology Biochemistry and Behavior*, *97*(3), 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*(2), 309–315.

Kessel, R., & Lucke, R. L. (2008). An analytic form for the interresponse time analysis of Shull, Gaynor, and Grimes with applications and extensions. *Journal of the Experimental Analysis of Behavior*, *90*(3), 363–386.

Machlis, L. (1977). An analysis of the temporal patterning of pecking in chicks. *Behaviour*, 1–70.

McSweeney, F. K., & Weatherly, J. N. (1998). Habituation to the reinforcer may contribute to multiple-schedule behavioral contrast. *Journal of the Experimental Analysis of Behavior*, *69*(2), 199–220.

Odum, A. L., & Schaal, D. W. (2000). The effects of morphine on fixed-interval patterning and temporal discrimination. *Journal of the Experimental Analysis of Behavior*, *74*(2), 229–243.

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*(3), 329–347. doi:10.1901/jeab.2006.47-05

R Core Team (2013). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/.

Shull, R. L. (2004). Bouts of responding on variable-interval schedules: effects of deprivation level. *Journal of the Experimental Analysis of Behavior*, *81*(2), 155–167.

Shull, R. L. (2011). Bouts, changeovers, and units of operant behavior. *Eur J Behav Anal*, *12*, 49–72.

Shull, R. L., Gaynor, S. T., & Grimes, J. A. (2001). Response rate viewed as engagement bouts: Effects of relative reinforcement and schedule type. *Journal of the Experimental Analysis of Behavior*, *75*(3), 247–274.

Shull, R. L., & Grimes, J. A. (2003). Bouts of responding from variable-interval reinforcement of lever pressing by rats. *Journal of the Experimental Analysis of Behavior*, *80*(2), 159–171.

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*(1), 65–83.

Sibly, R. M., Nott, H. M. R., & Fletcher, D. J. (1990). Splitting behaviour into bouts. *Animal Behaviour*, *39*(1), 63–69. doi:10.1016/S0003-3472(05)80726-2

Sidman, M., & Stebbins, W. C. (1954). Satiation effects under fixed-ratio schedules of reinforcement. *Journal of Comparative and Physiological Psychology*, *47*(2), 114.

Slater, P. J. B. (1974). The temporal pattern of feeding in the zebra finch. *Animal Behaviour*, *22*(2), 506–515.

Slater, P. J. B., & Lester, N. P. (1982). Minimising errors in splitting behaviour into bouts. *Behaviour*, 153–161.

Takeuchi, T., Morikawa, N., Matsumoto, H., & Shiraishi, Y. (1962). A pathological study of Minamata disease in Japan. *Acta Neuropathologica*, *2*(1), 40–57.

Team, R. C., & others. (2012). R: A language and environment for statistical computing. Retrieved from http://cran.case.edu/web/packages/dplR/vignettes/timeseries-dplR.pdf

Tolkamp, B. J., Allcroft, D. J., Austin, E. J., Nielsen, B. L., & Kyriazakis, I. (1998). Satiety Splits Feeding Behaviour into Bouts. *Journal of Theoretical Biology*, *194*(2), 235–250. doi:10.1006/jtbi.1998.0759

Tolkamp, B. J., & Kyriazakis, I. (1999). To split behaviour into bouts, log-transform the intervals. *Animal Behaviour*, *57*(4), 807–817.

Wakabayashi, K., Kakita, A., Sakamoto, M., Su, M., Iwanaga, K., & Ikuta, F. (1995). Variability of brain lesions in rats administered methylmercury at various postnatal development phases. *Brain Research*, *705*(1–2), 267–272. doi:10.1016/0006-8993(95)01208-7

Ward, R. D., & Odum, A. L. (2006). Effects of prefeeding, intercomponent-interval food, and extinction on termporal discrimination and pacemaker rate. *Behavioural Processes, 71*, 293-306.

Yeates, M. P., Tolkamp, B. J., Allcroft, D. J., & Kyriazakis, I. (2001). The use of mixed distribution models to determine bout criteria for analysis of animal behaviour. *Journal of Theoretical Biology*, *213*(3), 413–425.

Chapter 3: Chronic Methylmercury Exposure Decreases the Ability, But Not The Motivation to Run: A Microstructural Analysis and Protection by Nimodipine.

Abstract

Adult-onset methylmercury (MeHg) exposure produces sensorimotor impairment and related changes in behavior. The present study investigated MeHg effects on the microstructure of spontaneous wheel running in adult BALB/c mice chronically exposed to 0 ppm or 15 ppm MeHg via daily drinking water. Additionally, we examined the role of calcium levels in MeHg-induced neurotoxicity by treating mice in each MeHg exposure group to 0, 20, or 200 ppm of the L-type calcium-channel blocker nimodipine via their daily chow diet. To examine MeHg-related changes in the microstructure of running, we partitioned spontaneous wheel running into activity epochs using a change-point algorithm and derived estimates of the rate of within-bout responding (primarily a measure of motor effects), the duration of pausing between response bouts (primarily a measure of motivational effects), and the length of response bouts from those epochs. Mice also performed regular rotarod sessions to provide a secondary measure of motor coordination. MeHg impaired rotarod performance and nimodipine dose-dependently mitigated that effect. MeHg decreased the distance run and within-bout running rate, especially during the final weeks of exposure and nimodipine protected against that impairment. The interbout interval, thought to reflect motivation to run, was slightly but significantly increased by MeHg with no evidence of decline at the end of exposure and no influence of nimodipine. These findings suggest that the microstructure of behavior provides sensitive and interpretable measures of MeHg effects, support the utility of bout analysis for examining the behavior effects of drug and toxicant exposure, and show selective neuroprotection by nimodipine.

Introduction

Methylmercury (MeHg) is a potent neurotoxicant that crosses the placental and blood-brain barriers, resulting in sensorimotor deficits in individuals exposed as adults and additional cognitive deficits associated with gestational exposure (Harada, 1995; Takeuchi et al., 1962; Newland, et al., 2004). Because methylmercury bioaccumulates, humans are at risk of exposure throughout the lifespan from methylmercury-containing foods such as long-lived predator fish species and methylmercury-contaminated grain products, most notably rice (Barrett, 2010). The behavioral consequences of chronic, adult-onset exposure are less well characterized than the effects of severe, acute exposure. Chronic low-level exposure produces subtle effects that can be difficult to detect. Effects of adult-onset exposure also do not occur immediately, but instead appear after a latent period (Weiss et al., 2002). Detecting the effects of low-level exposure is therefore challenging; this is especially true of effects during the early stages of impairment when an organism may exhibit no discernible overt signs of exposure. However, operant reinforcement schedules that generate high rates of behavior have provided a number of preparations sensitive to these subtle effects (Newland, 1995).

Chronic, adult-onset MeHg exposure causes decrements in global measures of running, such as total distance run, in rodent models, effects that are ameliorated by concurrent selenium exposure (Heath, et al., 2010). The temporal pattern of unconstrained behavior, like running, is commonly organized into bouts separated by periods of inactivity (i.e., periods of alternative behavior) (Machlis, 1977; Shull, 2011). The rate at which bouts are initiated is sensitive to motivational variables (Shull et al, 2001; Shull, 2004) and the rate of responding within bouts is sensitive to motoric variables (Johnson, Bailey, & Newland, 2011; Brackney, Cheung, & Sanabria, 2011). Analyses of the bout structure of running could reveal how MeHg reduces

running and whether any protection by another substance applies to all aspects of running.

The present study was designed to examine changes in the microstructure of spontaneous running, as revealed in the structure of response bouts, associated with adult-onset, chronic exposure to MeHg. Given the sensorimotor effects (e.g., impaired coordination, control of distal extremities, impaired proprioception) associated with adult-onset MeHg exposure, a reasonable prediction is that chronic MeHg exposure will adversely impact the rate of running within bouts. MeHg exposure also may alter the duration or length of running bouts, but the direction of the effect is more difficult to predict than with within-bout rate. Intuitively, MeHg exposure may decrease bout length directly as a result of sensorimotor impairment and loss of coordination and associated decrement in the rate of intrinsic reinforcement. Alternately, adult-onset MeHg exposure may decrease the rate of switching between competing alternative behaviors as developmental exposure has been shown to do (Newland, Hoffman, Heath, & Donlin, 2013). Such an effect would result in longer bouts of running that occur less frequently. Longer bouts may increase the rate at which fatigue or habituation develop and, in conjunction with longer between-bout pauses, result in a global decrease in running distance consistent with reported effects of MeHg on spontaneous running (Heath et al., 2010).

Another aim of the present study is to investigate protection by the L-type calcium channel blocker nimodipine's using a preparation that is heavily dependent on motor function. Nimodipine is protective against MeHg-induced behavioral deficits in a repeated acquisition procedure (Bailey, Hutsell, & Newland, 2013) and in in vitro preparations (Limke, et al., 2004). Nimodipine may confer global protection against MeHg-related neurotoxicity or, alternately, it may prove selectively protective, for example by mitigating MeHg effects on only a subset of bout properties. In either case, the result will provide additional information on the nature of

MeHg-induced motor impairment and, further, provide increased evidence of the applicability of bout analysis methods to behavioral pharmacology and toxicology.

Running deficits were examined in two ways. First, to examine the latency to the appearance of a deficit and neuroprotection by nimodipine on total distance run was examined as a function of exposure week. Second, to examine the relative sensitivity of different bout parameters and characterize the pattern of functional deterioration, an analysis that emphasized the sequence in which these parameters changed was undertaken. Since there was much inter-subject variability in the latency to deficits, this latter analysis was anchored to the termination of the experiment for an individual mouse. That is, analyses were anchored at the day of euthanasia, where applicable, or the end of the study, and days were counted backward from this point. This approach decreased variability and emphasized the progression of deficits.

Mice exposed chronically to MeHg with or without nimodipine were provided opportunities to run in weekly three-hour sessions. Running was monitored continuously with 0.01” resolution by recording the time required to complete quarter-revolutions of the running wheel. The time between two quarter-revolutions is an interevent time (IET). The microstructure of spontaneous wheel running microstructure was examined by partitioning the time series of IETs into bouts of running separated by interbout intervals. Bouts were identified statistically by identifying runs of short IETs. The time separating bouts is reflected in the long IETs. For comparison, MeHg’s effects on the mice’s sensorimotor function was examined rotarod performance.

Method

Subjects

The subjects were adult male BALB/c mice purchased from Harlan laboratories,

Indianapolis, IN with no prior activity wheel experience. Mice were singly housed in clear polycarbonate cages divided along the diagonal with a clear plastic barrier that permitted visual and olfactory, but not physical, contact. Animals were maintained at 24 g to 26 g body mass on a daily food ration of approximately 2.5 g and had ad libitum access to water except during data collection and health checks. The vivarium and procedure rooms were maintained on a 12-h light-dark cycle (lights on at 7:00 AM) in a temperature and humidity controlled AAALAC-accredited facility.

Nimodipine and MeHg Exposure

Prior to exposure, animals were screened using a rotarod (MED-Associates, Inc. St. Albans, VT) task to ensure there were no preexisting differences in motor coordination across groups. Then they were randomly assigned to exposure groups with the constraint that the groups were similar on pre-exposure rotarod performance. Beginning at approximately 11 weeks of age, the mice were exposed chronically to 0 or 15 ppm mercury as methylmercuric chloride via daily drinking water and 0, 20, or 200 ppm nimodipine mixed with their daily chow diet according to a 2 x 3 factorial design (MeHg by nimodipine). The nimodipine diet was based on the Prolab RMH 1800 rodent diet (5LL2; manufactured by Purina LabDiet, St. Louis., Missouri). The daily food ration described above (2.5 g +/- 0.5 g chow) resulted in nimodipine doses of about 0, 2, and 20 mg/kg/day. Based on periodic water consumption sampling throughout the experiment, the drinking water MeHg concentrations resulted in daily MeHg doses of approximately 0 and 2.6 mg/kg/day.

Apparatus

Motor coordination tests were conducted using a 5-station standalone accelerating rota-rod for mice (MED Associates, ENV-577M). The rotating cylinder had a circumference of 10 cm

with a lane width of 5.7 cm. Falls from the rod were recorded by infrared beam breaks located 16.5 cm below the cylinder. Spontaneous wheel running sessions were conducted in rat operant chambers (MED-Associates, Inc., St. Albans, VT) fitted to accommodate mice and situated in sound-attenuating ventilated shells. Each chamber contained one in-chamber activity wheel (Med Associates Inc., St. Albans, VT, ENV-043A). The wheel's running surface consisted of 3.175 mm diameter stainless steel rods (6.37 cm wide) located every 6 degrees on a 17.78 cm diameter wheel. Responses were recorded as quarter-wheel revolutions with centisecond resolution via Med-PC™ control software on a PC running Windows XP. Processing of raw MED data files, data management, and the partition analysis described below were performed in *R (*R core development team, 2013) using the arf package for animal research (Hoffman, *in preparation*).

Procedure

Rota-rod sessions were conducted on alternate weeks at the beginning of exposure, changing to weekly measurements beginning at the 8th week of exposure. Mice ran one rota-rod trial per measurement session. During each trial, the rod accelerated from 4.0 to a maximum 40.0 rpm with a constant acceleration of 0.12 revolutions/sec. An individual subject's trial ended upon falling from the rod and breaking the infrared beam below. All trials were conducted such that the rod's direction of rotation was counter-clockwise relative to the subject's body. Wheel running sessions were conducted as 3-hr extended access sessions once per week throughout the study. No experimenter-controlled stimuli were active during the session and no experimenter-controlled contingencies were in place for wheel running. On the other days of the week, animals performed an incremental repeated acquisition procedure; these data are described elsewhere (Bailey, Hutsell, & Newland, 2013). During wheel sessions, the front panel of the chamber,

containing nosepoke devices and the pellet delivery trays, was blocked with a clear plastic divider to prevent access to the chamber's food tray or other response operanda.

Humane Endpoints

Research personnel performed daily health inspections on all animals throughout the study and the attending veterinarian was consulted in cases of injuries or illness. Every effort was made to treat health issues and prolong animal life as long as doing so did not prolong distress. If a mouse abruptly lost 10% of its weight, was unable to locomote, or unable to feed itself, then it was humanely euthanized. All euthanasia criteria and methods were approved by the Auburn University Institutional Animal Care and Use Committee.

Bout analysis

The microstructure of spontaneous running was examined using a partition analysis based on change-point detection (Figure 1), an approach taken because of its ability to adequately describe the bout structure of spontaneous wheel running and to detect methylmercury-related changes in bout structure (Hoffman & Newland, *manuscript in preparation*). To partition a behavioral record into epochs, the algorithm begins at the origin (i.e., at time = 0 and cumulative responses = 0) and sweeps through the cumulative record of IETs, inserting each new IET into a growing vector of sequential IETs. At each new recorded event, the algorithm evaluates the log-odds that the associated IET differs from what would be predicted based on the slope of the current IET vector more than would be expected by chance alone. This decision is based on an *a priori* decision criterion analogous to the alpha used in null hypothesis significance testing. If the new point was unlikely to have occurred by chance then that is identified as a change point (the point at which the slope changes). When a significant change-point is identified, the IET vector is emptied and the most recent change-point is treated as the

new origin (time = 0, cumulative responses = 0). The bout length, within-bout rate, and bout initiation rate are stored and the algorithm then begins another sweep through the behavioral record from the current (new) origin. This continues to the end of the record and the process repeats until no significant change-points are identified.

Any given epoch is either a period of engagement (i.e., a bout of behavior) or a period of disengagement (i.e., an inter-bout interval). Thus, the CPM partitions the cumulative record into activity epochs. These activity epochs provide information comparable to conventional IET distribution-based bout analysis: an estimate of response speed within bouts, an estimate of pausing between bouts, and an estimate of bout length or duration that are used to derive the bout parameters of interest. Because the epochs, representing bouts and interbout pauses, are identified directly, the CPM also requires no assumptions about the shape or number of IET distributions. Because each putative change-point is evaluated relative to a local, sliding window of events, the CPM also does not assume stationary bout processes. Multimodality or changes over time in either epoch distribution can be examined directly when needed.

Statistical analysis

Some statistical analyses were anchored at the beginning of exposure to examine the latency to a deficit and others were anchored at the termination of the study or an individual mouse's death to examine the sequence in which the different measures began to show deficits. The latter individualized approach was done because some mice died before the end of the study and performance deteriorated prior to death, causing the mean and variability of group data to change during this deterioration and then restabilize upon the individual animal's death. This resulted in a time series that was unstable when anchored at the beginning of the study, making it difficult to detect statistically significant effects.

To address our interest in determining the protection by nimodipine against MeHg neurotoxicity we conducted a traditional survival analysis by quantifying the latency to death for each of the six experimental groups and examined the latency to reach 20% and 50% of control value in total distance run graphically and as a survival analysis. To address our interest in characterizing which specific aspects of behavior (e.g., motor coordination, motivation to run) were most sensitive we anchored analyses of those endpoints at death or the end of the study for analysis.

Effects of MeHg and nimodipine on rotarod, total distance run and the separate bout parameters were analyzed using hierarchical mixed-effects models. Statistical analyses were conducted in R with the lme4 package for linear mixed effects (Bates, Maechler, Bolker, & Walker, 2014); figures were produced in SigmaPlot Version 12.0 (Systat Software Inc., San Jose, CA, USA). A set of nested models was examined for each measure. To account for potential individual differences in both running and sensitivity to MeHg exposure, all models were fit with a random intercept (random effect of subject) and a random slope term (random effect of time). Models also were evaluated using a quadratic term because of the bowing evident in the plotted functions (see graphs below).

Along with the full model (containing two- and three-way interactions) and to determine whether the full model was necessary, a series of restricted models was also examined. The first restricted model used as the initial basis for comparison included fixed effects for MeHg and nimodipine, along with the random intercept term and a random slope term. The remaining models entered new predictors one at a time: quadratic term for exposure time, two-way MeHg by time interaction, two-way MeHg by nimodipine interaction, and three-way MeHg X nimodipine X time interaction term. Models were compared using Chi-squared (likelihood-ratio)

tests. Because only one term was added in each model, the likelihood-ratio test can be considered a test of the single term added at that stage. If the most recently added predictor did not significantly improve model fit that predictor was excluded from all subsequent models. If the most recently added predictor did significantly improve model fit that model served as the reference for the next model. Beta coefficients for specific effects are presented below with their associated t-values. The beta coefficient in mixed-effects models is interpreted the same as the beta coefficient in traditional ordinary least squares (OLS) regression. For main effects, beta is the slope. For interaction terms, beta indicates the amount of change in a variable's influence as a function of different levels of a second, moderating variable. For example, in a significant methylmercury by nimodipine interaction, different beta values for MeHg-exposed mice at the three nimodipine levels would suggest that nimodipine dose-dependently altered methylmercury's effects.

As with traditional ordinary least squares (OLS) regression methods, the *t*-score associated with each effect's parameter estimate is calculated as the square root of an *F* ratio with numerator degrees of freedom equal to 1. However, unlike OLS regression and traditional ANOVA designs, the *F* statistic in a linear mixed-effects design is not calculated as a ratio of observed to expected error terms (Pinheiro & Bates, 2000). Instead, in mixed-effects algorithms in R (e.g., lme4), S, and SAS, the *F* ratio is calculated using either maximum likelihood (MLE) or residual maximum likelihood estimates REML). Because the *F* ratio is not based directly on error strata, the appropriate adjustment to the denominator degrees of freedom for each term is unclear. Further, because the *F* ratio is not based on error strata there is no agreement in the statistical literature as to the appropriate null reference distribution (Bates, 2010). Some routines, for example SAS's PROC MIXED, provide p-values using uncorrected denominator degrees of

freedom and assuming a central *F* distribution. However, the uncertainty about the appropriate reference distribution and the lack of correction to the denominator degrees of freedom make the conventional Fisherian interpretation of those *p*-values problematic. Accordingly, this manuscript does not present *p*-values of parameter estimates to avoid potential confusion; however, p-values are included for likelihood ratio tests as well as other inferential statistics.

Results

Survival Analysis

A Kaplan-Meier survival analysis was conducted to examine differences in mortality across experimental groups. Mice were allowed to survive for an additional month after the end of running wheel sessions so the time base for survival differed from that for other measures. Figure 2 shows survival curves for the different groups. A log-rank test indicated group differences in survival, χ^2 (5) = 708.7, $p < 0.001$. Multiple pairwise comparisons revealed that the 15 ppm MeHg 0 ppm nimodipine group had significantly greater mortality than those not exposed to MeHg (all *p*'s < 0.0001), and greater mortality than both groups of MeHg-exposed mice treated with nimodipine (*p*'s < 0.0001). Nimodipine dose-dependently increased survival in MeHg-exposed mice ($p < 0.0001$ for 20 ppm nimodipine relative to controls; $p < 0.0001$ and for 200 ppm nimodipine relative to 20 ppm nimodipine). Nimodipine also dose-dependently increased survival for mice not exposed to MeHg ($p = 0.001$ for 20 ppm nimodipine relative to 0 ppm nimodipine; $p < 0.0001$ for 200 ppm nimodipine relative to 20 ppm nimodipine). The survival rate for the 15 ppm MeHg/200 ppm nimodipine was indistinguishable from the control mice exposed to neither nimodipine nor MeHg. MeHg exposure was associated with increased mortality and nimodipine dose-dependently reduced mortality in both MeHg-exposed and unexposed mice.

Rotarod Performance

Motor coordination was examined by accelerating a rotarod from 4 RPM gradually until the mouse fell off; the maximum rotation speed (in revolutions per minute) reached on a rotarod trial was the dependent measure. The mean (with standard error) maximum speed anchored by final session are plotted for all exposure groups in Figure 3. Maximum speed was analyzed via a set of nested hierarchical mixed-effects models as described above in the statistical analysis section. The quadratic term significantly improved model fit (χ^2 (1) = 5.34, p = 0.02) and was therefore included in all other models of rotarod performance. The MeHg by nimodipine interaction (χ^2 (2) = 6.88, p = 0.03), but not the MeHg by time interaction (p > 0.90), significantly improved model fit. The three-way MeHg by nimodipine by time interaction also improved model fit (χ^2 (5) = 20.59, p < 0.001). The 15 ppm MeHg 0 ppm nimodipine mice showed evidence of a downward trend in maximum speed attained (corresponding to shorter durations of time on the rotarod) over their final weeks (β = 0.63, SE = 0.20, t = 3.12). That pattern was present but weaker in the 15 ppm MeHg 20 ppm nimodipine group (β = 0.35, SE = 0.20, t = 1.74) and absent in the 15 ppm MeHg 200 ppm nimodipine group (β = -0.06, SE = 0.19, t = -0.29). Thus methylmercury exposure was associated with diminished rotarod performance in the weeks preceding termination and nimodipine dose-dependently attenuated that effect. Impairment began to appear three to four weeks before death or the end of the study. There was no evidence of nimodipine related effects for MeHg unexposed mice (for all t's, -1.0 < t < 1.0)

Total Distance Run

Distance run did not differ across groups during baseline as a function of subsequent methylmercury *(*F (1, 67) = 0.43, p = 0.51), or nimodipine (F (2, 67) = 0.47, p = 0.63), or MeHg X nimodipine interaction (F (2, 67) = 0.881, p = 0.42) so the groups were evenly matched on this

measure. Figure 4 shows the total weekly distance run plotted as the proportion of control for individual subjects factored by experimental group. The control is an individual mouse's distance run at the beginning of the study. Dashed horizontal lines mark 75% and 50% of baseline, corresponding to a 25% and 50% reduction in distance run. For a majority of subjects in all conditions the total weekly distance run increased over the first several weeks of the study. Only MeHg-exposed animals showed stable reductions greater than 25%. Separate Kaplan-Meier survival analyses (described above) were conducted on the latency to reach a persistent (defined as more than 2 successive weeks) decline of 25% and 50% in running. There were significant group differences in latency to 25% impairment (χ^2 (5) = 71.4, $p < 0.01$) and to 50% impairment (χ^2 (5) = 92.9, $p < 0.001$). The latency to both 25% and 50% impairment was shorter for the mice exposed to MeHg without nimodipine (top right panel) than all other groups ($p < 0.001$ for all pairwise comparisons), indicating that nimodipine significantly delayed or prevented the onset MeHg-related running impairment.

Figure 5 shows the total distance run by exposure group for the 0 ppm MeHg (top panel) and 15 ppm MeHg groups anchored at each subject's last session in the experiment, rather than at time since onset of exposure to facilitate the comparison of early indicators of MeHg toxicity. The 0^{th} Week to Termination therefore represents an animal's final running session, either because the subject reached humane endpoints criteria, usually reflecting advanced MeHg neurotoxicity, or because it was the end of the study. Running wheel sessions were no longer conducted after only two animals remained in the 15 ppm MeHg 0 ppm nimodipine group. As can be seen in Figure 5, average running distance generally increased slightly or remained steady for all groups at a level of just over 3000 m per session (~1 km/hr); the increase at the group level is consistent with the individual effects shown in Figure 4. The mercury-exposed, 0 ppm

nimodipine group appeared to begin diminished running relative to nimodipine-treated mice beginning at approximately 14 weeks prior to their demise and showed a clear downward trend over their final 2 weeks.

Distance run was analyzed using a set of nested hierarchical mixed-effects models as described above. The first included only fixed effects of mercury and nimodipine, along with random effects for subject and session. Adding the quadratic term for session significantly improved model fit (χ^2 (1) = 103.51, $p < 0.001$); all other models of total distance therefore included a quadratic term. The two-way MeHg X nimodipine interaction did not significantly improve the model (χ^2 (2) = 3.37, $p = 0.18$), but the two-way MeHg by time interaction did ($p < 0.002$). The three-way MeHg by nimodipine by time interaction significantly improved model fit (χ^2 (6) = 12.9, $p = 0.04$), supporting a significant three-way interaction. The parameter estimates for the MeHg X nimodipine X time levels suggest total running decreased as the end of study approached for mice in the 15 ppm MeHg 0 ppm nimodipine group ($\beta = 69.3$, SE = 20.3, $t = 3.41$). That trend was smaller or absent for mice in the 15 ppm MeHg 20 ppm nimodipine ($\beta = 22.4$, SE = 19.5, $t = 1.15$) and 15 ppm MeHg 200 ppm nimodipine ($\beta = 24.8$, SE = 19.1, $t = 1.30$) groups.

Two-way factorial ANOVAs were conducted on total distance at two time points, the final week of the study for an individual mouse and one month prior. At one month prior to the study's end, there was no significant effect of MeHg (F (1, 66) = 0.26, $p = 0.61$), no significant effect of nimodipine (F (2, 66) = 0.75, p = 0.48) and no significant MeHg X nimodipine interaction (F (2, 66) = 0.70, p = 0.50). At the final week, there was a significant MeHg X nimodipine interaction, F (2, 66) = 5.56, $p = 0.005$, as well as significant main effects of MeHg (F (1, 66) = 15.48, $p < 0.001$) and nimodipine (F (2, 66) = 11.53, p < 0.001). Post hoc testing

revealed the 15 ppm MeHg 0 ppm nimodipine mice ran significantly less compared to all other groups (for 0 ppm MeHg 0 ppm nimodipine, mean difference = 1589 meters, $p < 0.001$; for 0 ppm MeHg 20 ppm nimodipine, mean difference = 1643, $p < 0.001$; for 0 ppm MeHg 200 ppm nimodipine, mean difference = 2119 meters, $p < 0.001$; for 15 ppm MeHg 20 ppm nimodipine, mean difference = 1528 meters, $p = 0.04$; for 15 ppm MeHg 200 ppm nimodipine, mean difference = 1536 meters, $p < 0.001$). There were no other significant pairwise differences (all p's > 0.45). Thus, the mice exposed to MeHg and nimodipine were indistinguishable from controls.

Within-session Changes

On a cumulative record, a constant rate of running appears as a straight line and systematic within-session changes in rate appear as bowing of the curve. If rate declines over the course of a session, the cumulative record will appear convex; if rate increases, the record will appear concave. To avoid subjectivity inherent in visual analysis, the cumulative record's bowing can be quantified using an index of curvature (Fry, Kelleher, & Cook, 1960), *I*, calculated as

$$I = \frac{(n-1)R_n - 2\sum_{i=1}^{n-1} R_i}{nR_n},$$

where *I* is the index of curvature, n is the number of bins into which the session is divided, and R is the number of responses at a given bin. A steady rate of responding corresponds to $I = 0$, with negative values of *I* indicating decreases in rate and positive values indicating increases in rate. The lower and upper bounds of *I* can be determined by assuming that all responses fall either in the first or last bin. For the present analysis, each session was divided into six 30-min bins, making the range of *I* -0.83 to 0.83. The index of curvature did not differ systematically across groups or across weeks within groups (data not shown as a figure). Values of *I* were approximately 0 for all exposure groups (M1 = -0.02, range = -0.10, 0.03, M2 = 0.00, range = -

0.05, 0.04, M3 = 0.00, range = -0.08, 0.03, M4 = 0.00, range = -0.12, 0.06, M5 = 0.00, range = -0.08, 0.03, M6 = 0.00, range = -0.09, 0.02) across weeks with no apparent trends. This suggests that running was stable within sessions.

Microstructure of Spontaneous Running

A change-point bout analysis was conducted in the manner described above using a decision criterion of 2.0, corresponding to a conventional alpha level of 0.01 (the criterion is the negative power to which 10 is raised to achieve the alpha level). The change-points partitioned individual cumulative records into successive segments corresponding to bouts of wheel running and periods of non-running. Because running was stable through the course of a session, the entire session's data were used for this analysis, so bout and non-bout segments were separately aggregated from across the session to produce session-wide bout parameter estimates for individual subjects. As with total running, all bout measures were anchored from the termination of exposure rather than the onset.

To evaluate the bout analysis' accuracy, bout estimates were used to predict overall response rate and a recovery ratio was calculated by dividing the predicted value by the obtained overall response rate for each session. Perfect response rate estimation corresponds to a recovery ratio of 1.0, with values less than 1.0 representing underestimation of rate and values greater than 1.0 representing overestimation. The recovery ratio did not differ systematically across groups or across weeks within groups (data not shown as a figure). Mean values of the recovery ratio across exposure groups were approximately 0.95, ranging from 0.92 to 1.03. Individual recovery ratios ranged from 0.81 to 1.31, with the extreme values occurring in sessions where running was very low and few bouts were detected. This suggests that even though behavior can be partitioned with only a few interevent times, accurate estimation requires more than a few bouts.

As can be seen in Figure 6, within-bout running rate increased across the study for all groups, from an initial low of approximately 0.32 m/s to a stable high of approximately 0.40 m/s. There was no apparent difference across groups until the end of the study. As with total distance, the MeHg, no-nimodipine group showed visual evidence of a downward trend in within-bout rate over the three to four weeks prior to euthanasia. Hierarchical mixed-effects model comparisons were carried out in the manner described above for rotarod and total distance run. Model comparisons revealed a significant 3-way interaction (MeHg by nimodipine by time) effect on within-bout rate, (χ^2 (7) = 17.28, p = 0.016). Within-bout running rate decreased as the end of the study approached for mice in the 15 ppm MeHg 0 ppm nimodipine group (β = 0.03, SE = 0.01, t = 2.86). That pattern was smaller or absent for mice in the 15 ppm MeHg 20 ppm nimodipine (β = 0.02, SE = 0.01, t = 2.01) and 15 ppm MeHg 200 ppm nimodipine (β = 0.01, SE = 0.01, t = 1.25) groups. Two-way factorial ANOVAs were conducted on within-bout rate at two time points, the final week of exposure and one month prior. At one month prior to the study's end, there was no significant effect of MeHg (F(1, 66) = 2.61, p = 0.11), no significant effect of nimodipine (F(2, 66) = 0.57, p = 0.57) and no significant MeHg X nimodipine interaction (F(2, 66) = 0.22, p = 0.80). At the final week, there was a significant MeHg X nimodipine interaction, F(2, 66) = 3.9, p = 0.02, as well as significant main effects of MeHg (F(1, 66) = 22.2, p < 0.001) and nimodipine (F(2, 66) = 3.53, p = 0.04). Post hoc testing revealed significantly slower within-bout rate for the 15 ppm MeHg mice relative to all other groups (for 0 ppm MeHg 0 ppm nimodipine, mean difference = 0.86 m/s, p < 0.001; for 0 ppm MeHg 20 ppm nimodipine, mean difference = 0.94 m/s, p < 0.001; for 0 ppm MeHg 200 ppm nimodipine, mean difference = 0.79 m/s, p < 0.001; for 15 ppm MeHg 20 ppm nimodipine, mean difference = 0.52 m/s, p = 0.04; for 15 ppm MeHg 200 ppm nimodipine, mean difference = 0.61 m/s, p < 0.001).

Figure 7 shows the mean inter-bout interval (with standard error) for the 0 ppm MeHg (top panel) and 15 ppm MeHg (bottom panel) groups. A minority of subjects (2 of 12) in the 15 ppm MeHg 0 ppm nimodipine group showed large increases in mean inter-bout interval during their final week. Those two subjects' data were assessed for outlier status using a criterion of +/- 3*IQR* outside the midspread; relative to outlier criteria based on standard deviation units, the IQR-based criterion is robust to the effects of potential outliers on the estimate of central tendency, avoiding a potential confound in outlier classification. The affected subjects had inter-bout intervals that were 4.3 *IQR* and 58.5 *IQR* above the 75th percentile of the group's IBI distribution and are shown as separate symbols in Figure 7. When these data were excluded from statistical analysis,the model including the quadratic term outperformed the model containing main effects only(χ^2 (1) = 150.27, $p < 0.0001$). The two-way MeHg X time interaction term significantly improved model fit (χ^2 (1) = 6.11, $p < 0.02$), but neither the two-way MeHg X nimodipine nor the three-way MeHg X nimodipine X time interaction improved the model (χ^2 (2) = 0.05, $p = 0.98$ and χ^2 (7) = 3.49, $p = 0.84$). When the analysis was re-conducted with the outliers included, the outcome of the model comparisons was unaffected: the quadratic term remained significant, χ^2 (1) = 118.05, $p < 0.0001$, but neither the two-way MeHg X nimodipine nor the three-way MeHg X nimodipine X time interaction improved model fit (χ^2 (2) ≈ 0, $p \approx 1$ and χ^2 (7) = 0.71, $p = 0.99$, respectively).

The interbout interval decreased over the course of the study (β = -0.69, SE = 0.06, t = -11.34) for all groups, but the magnitude of that decrease was attenuated in MeHg-exposed animals (β = -0.25, SE = 0.10, t = -2.49).The mean IBI was slightly higher (1 – 1.5 seconds) for in MeHg-exposed mice compared to unexposed mice, corresponding to slightly lower rates of bout initiation.There was no evident effect of nimodipine on bout initiation (-1.0 < t < 1.0 for

remaining *t*'s). Two-way factorial ANOVAs were conducted on IBI at two time points, the final week of exposure and one month prior. At one month prior to the study's end, there was a significant effect of MeHg, $F(1, 66) = 8.10$, $p < 0.01$, but no effect of nimodipine ($F(2, 66) = 0.21$, $p = 0.81$) and no significant MeHg X nimodipine interaction ($F(2, 66) = 0.72$, $p = 0.49$). Post hoc testing revealed that MeHg-exposed mice paused an average of 1.5 seconds longer between bouts than unexposed mice ($p = 0.006$), corresponding to a difference of approximately 5% – 7% in bout initiation rate. At the final week, there was a significant MeHg effect, $F(1, 65) = 12.87$, $p < 0.001$, but no effect of nimodipine ($F(2, 65) = 0.77$, $p = 0.47$) and no significant MeHg X nimodipine interaction ($F(2, 65) = 1.79$, $p = 0.17$). Post hoc testing revealed that MeHg-exposed mice paused an average of 3.09 seconds longer between bouts than unexposed mice ($p < 0.001$).

Bout lengths were highly variable (week-to-week and subject-to-subject) for MeHg exposed than control subjects so bout length (in quarter revolutions) was reciprocal transformed to calculate the probability, 1/BL*,* that a given response transitioned out of a running bout. Figure 8 plots the probability of transitioning out of a bout on the primary y-axis with the bout length in meters plotted on the secondary y-axis. After the first few running sessions, average bout length declined (as p increased) from around 15 meters per bout to between 10 and 12 meters per bout for MeHg-unexposed mice and remained relatively stable for the remainder of the study. That early change in bout length was smaller for MeHg-exposed groups, which continued to run 12 to15 meters per bout until, for the 15 ppm MeHg 0 ppm nimodipine and 15 ppm MeHg 20 ppm nimodipine groups, a downward trend in bout length (upward trend in p) appeared in the three to four weeks leading up to termination. Mixed-effects model comparison were conducted as described above using 1/BL as the dependent measure. The full model containing the three-way

MeHg X nimodipine X time interaction fit the data best χ^2 (7) = 22.82, p = 0.002. The probability of leaving a bout increased for mice in the 15 ppm MeHg 0 ppm nimodipine group as they approached the end of exposure (β = -0.0016, SE = 0.0004, t = -3.75), but not for 15 ppm MeHg mice treated with 20 or 200 ppm nimodipine (β = -0.00032, SE = 0.0004, t = -0.78 and β = -0.00017, SE = 0.0004, t = -0.42, respectively). Both doses of nimodipine protected against MeHg effects on the probability of terminating a running bout. Two-way factorial ANOVAs were conducted on 1/BL at two time points, the final week of exposure and one month prior. At one month prior to the study's end, there were no significant interactions $F(2, 66) = 0.49$, $p = 0.61$ or main effects ($F(1, 66) = 2.22$, p = 0.14 for MeHg and $F(2, 66) = 0.60$, $p = 0.55$ for nimodipine). At the final week, there was a significant MeHg X nimodipine interaction, $F(2, 66) = 8.0$, $p < 0.001$. Post hoc testing revealed that the 15 ppm MeHg 0 ppm nimodipine group was significantly more likely to terminate a running bout than all other groups (all p's < 0.001) and no other groups differed significantly (all p's > 0.85)

Discussion

Chronic methylmercury increased mortality in exposed mice. Both latency to onset and total survival were lower for mice exposed to methylmercury without nimodipine than for nimodipine-treated groups. Nimodipine dose-dependently improved survival rates in methylmercury-exposed mice and even increased survival for mice not exposed to methylmercury. This dose-dependent protection occurred even though brain concentrations of MeHg were not statistically different for the 15 ppm MeHg 0 ppm nimodipine and 15 ppm MeHg 20 ppm nimodipine groups (brain concentrations for animals in the present study are presented in Bailey et al., 2013). Brain MeHg concentration for the 15 ppm MeHg and 200 ppm nimodipine groups was slightly, but significantly, lower than for the other two groups.

Consistent with well-documented motoric effects of MeHg, animals chronically exposed to methylmercury exhibited deficits on the rotarod during chronic exposure. This decrease appeared over the final two to three weeks before termination for mice in the 0 ppm and 20 ppm nimodipine groups, but was absent for the 200 ppm nimodipine mice. That is, 200 ppm nimodipine completely prevented a decline in rotarod performance in MeHg-exposed mice. A similar pattern of effects was evident in the total distance run. Both doses of nimodipine protected against behavioral impairment in nimodipine-treated subjects, supporting the role of calcium in MeHg-related behavioral toxicity. The neuroprotective effect is consistent with protection demonstrated in the incremental repeated acquisition of a response chain (Bailey, Hutsell, & Newland, 2013) and body weight (Sakamoto, Ikegami, & Nakano, 1996), as well as with in vitro findings that calcium channel blockers mitigate MeHg-induced neural cell death (Hare & Atchison, 1995).

Earlier research on methylmercury-impaired spontaneous running in rats showed that 5 ppm of MeHg was associated with attenuated increases in running activity and that 15 ppm produced rapid and substantial decreases in running (Heath, et al., 2010). In that study, neuroprotection was conferred by co-exposure to dietary selenium. Heath et al. used exposures of 0.5, 5, and 15 ppm MeHg and found that the latency to behavioral toxicity and rapidity of progression were dose-related. The latency to the onset of running deficits for rats exposed to 15 ppm MeHg with low selenium (0.06 ppm) in that study was comparable to the latency to show 25 and 50% decreases in running (approximately 8 to 10 weeks) as proportion of control for mice in the present study, however rats survived exposure longer on average (approximately 36 weeks of chronic exposure for rats versus approximately 13 weeks for mice). Thus, although the latency to onset of impairment was comparable between rats and mice, the transition from the

onset of behavioral impairment to severe declines in overall health occurred more rapidly for mice than rats. However, that difference may be attributable to a difference in daily MeHg intake rather than species-related differences in the progression of MeHg toxicity. Although both studies used 15 ppm MeHg, the daily intake in the present study was 2.6 mg/kg/day for the mice compared to 1.2 mg/kg/day for the rats in the Heath et al. study.

In the present study, changes in the microstructure of running were quantified using a bout analysis (see Shull, 2011, for a bout analysis review). Previous studies (Shull, Gaynor, & Grimes, 2001; Johnson, Pesek, & Newland, 2009) have shown that bout parameters are differentially sensitive to motoric and motivational effects of drugs and environmental contingencies. Bout analysis also provides a means for quantifying the effects of MeHg on high-rate operant behavior (Newland, Hoffman, Heath, & Donlin, 2013). Typically, bout analysis methods employ mixture models of interevent time (IET distributions) to estimate average bout properties (Shull, 2011). IET mixture models commonly assume that there are two populations of IETs, within-bout and between-bout, and that the bout parameters are stationary. For spontaneous wheel running, a small percentage of the longest IETs are typically excluded from analysis to improve the fit of a two-state (one for each of two IET populations) model (Johnson, Pesek, & Newland, 2009). Excluding the longest IETs from analysis to fit a two-state mixture model may be justified if there are more than two IET populations but only the first two are of research interest, for example if bouts of responding occur in clusters separated by long periods of extended disengagement (Eikelboom & Mills, 1988). Independent of the two-state assumption, the rate of wheel running may change within experimental sessions due to motor fatigue or habituation (Aoyama & McSweeney, 2001). Together these factors suggest that conventional

bout analysis techniques may not be ideally suited to analyze the microstructure of spontaneous running.

Accordingly, we partitioned wheel running into activity bouts using an alternative approach based on a change-point detection algorithm (as described in Gallistel, et al., 2001 and Gallistel, Fairhurst, & Balsam, 2004). Unlike most bout analysis methods, which indirectly estimate bout properties by fitting a mixture model to an aggregate interevent time distribution, the change-point method (CPM) partitions a behavioral record into activity epochs directly using on sequential interevent times (IETs). The change-point bout analysis makes no assumptions about the number of IET distributions comprising the mixture or their shape because aggregate IET distributions are not used for the analysis. Instead, the CPM assumes that for a target behavior, *X*, there are only two behavioral states: engaging in *X* and not engaging in *X*.

The change-point bout analysis provided bout estimates that were sensitive to MeHg-induced behavioral impairment. Methylmercury decreased within-bout running rate in exposed animals and nimodipine prevented that decrement. The pattern of effects on within-bout rate is qualitatively consistent with that seen on rotarod performance, suggesting that methylmercury impaired motor coordination and that the effect was mitigated by nimodipine. However, 20 ppm of nimodipine protected against running deficits but less so against rotarod deficits. Methylmercury impairment was also associated with a downward trend in average bout length (equivalently, an upward trend in the probability to disengage from a running bout) over the final month of exposure, consistent with impaired motor coordination. Nimodipine prevented the reduction in bout length in mice exposed to methylmercury. However, throughout most of exposure, MeHg was associated with longer average bout lengths (and, equivalently, lower average probabilities of disengaging from running bouts). Running for longer distances per bout

of running does not necessarily reflect impaired motor ability, but longer bout lengths are consistent with a decreased transition rate from running to other behavior (e.g., grooming). Although persistent behavior is not necessarily maladaptive, the clear differences between MeHg-exposed mice and controls suggests that the decrease transition rate is a form of behavioral toxicity. A similar effect on behavior under shifting reinforcement schedules has been reported on with gestational exposure to methylmercury (Newland et al., 2013).

All three MeHg groups also showed a higher average interbout interval, indicating a lower probability of transitioning into a bout of running from a period of alternative behavior, and there was no influence of nimodipine on this measure. This provides additional support for the transition rate interpretation of bout length and suggests a possible effect of chronic methylmercury exposure on reinforcement processes. Moreover, interbout intervals did not show an upward trend prior to euthanasia. Interbout interval, which is the inverse of the bout initiation rate, reflects motivational effects (Shull, 2011) and therefore is a marker of the motivation to run. Thus, even though there was significant sensorimotor impairment, as indicated by rotarod performance and significant decreases in within-bout response rate, the motivation to run was relatively unaffected in these mice even while they were displaying significant motor impairment.

A common data-analysis issue in studies of chronic exposure is attrition due to subject mortality, and the complications that this introduces can be exacerbated by individual differences to a neurotoxicant compound such as methylmercury. Because MeHg toxicity is progressive, behaviorally impaired animals are likely to contribute data for a limited time during the study before meeting humane endpoints criteria. Animals will show initial signs at different latencies and degeneration progresses at different rates. Ironically, this makes the detection of statistically

significant treatment effects more difficult because of the variability introduced by these impaired subjects. The present study used two statistical techniques to circumvent this issue. First, data were anchored in time from the end of an animal's time in the study rather than from the onset of exposure. That anchor point corresponds to the point of maximal impairment for the animals that met humane endpoints criteria during the study. For all other subjects, running ceased when the number of surviving animals in the MeHg exposed group reached two mice (but survivor analyses continued for an additional four weeks). Anchoring the data from the end of exposure increases statistical power during the points in exposure where power is most important for detecting the pattern of deterioration. An added benefit of this approach is that it facilitates comparisons in the relative sensitivity among behavioral measures, with the within-bout rate and bout length appearing particularly sensitive to chronic MeHg exposure. Second, the mixed-effects models were fit with random slope and intercept terms to allow for and capture individual differences in susceptibility to MeHg toxicity. These two departures from convention in the neurotoxicology literature appear to have practical utility in evaluating the behavioral effects of chemical exposure.

In the present study, adult-onset methylmercury exposure increased lethality, impaired rotarod performance, and decreased spontaneous running. Nimodipine prevented those deficits, providing support from behaving animals for the role of calcium dysregulation as a neurophysiological mechanism for MeHg's neurotoxicity. MeHg-impaired mice ran more slowly and for shorter distances within bouts and nimodipine co-treatment attenuated both of those effects. Nimodipine was not associated with changes in running in mice not exposed to MeHg. The change-point bout analysis provided estimates of the microstructure of responding that were sensitive to MeHg and nimodipine effects, providing evidence for the utility of this bout analysis

approach to studying drug and toxicant effects.

Figures

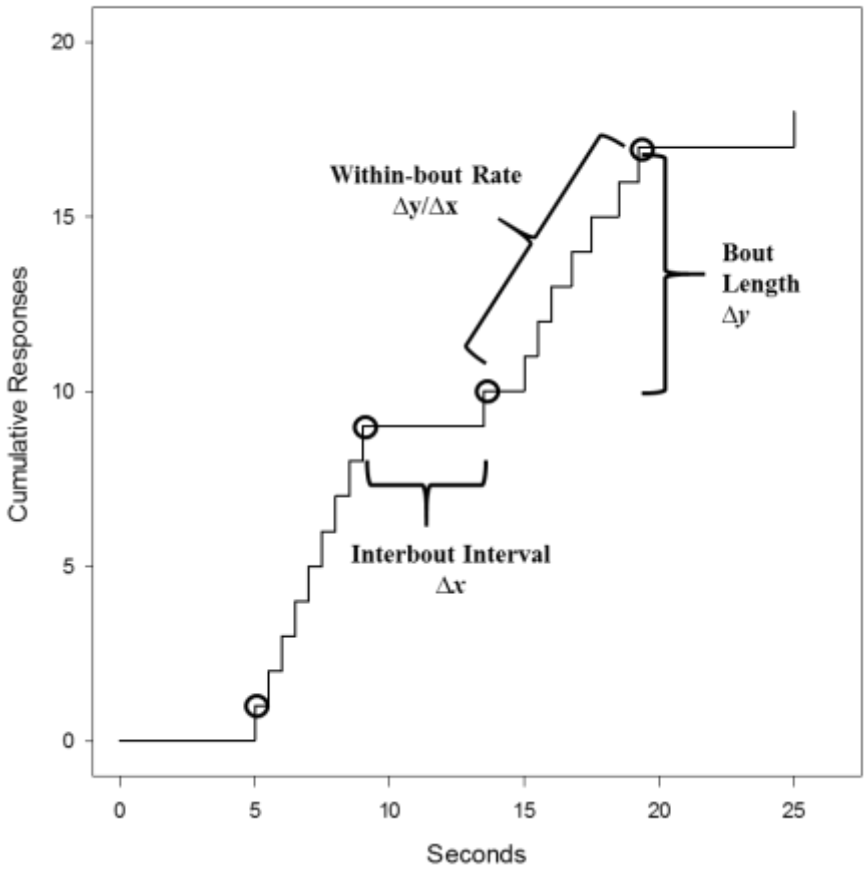

Figure 1. A simulated cumulative record showing four change-points. The change-points partition the record into successive segments that alternate between bouts and interbout intervals. For any given bout segment, the within-bout rate (speed of responding) is calculated as the slope of that segment and the bout length as the number of responses. The interbout interval is calculated as the time elapsed between the end of one bout and the beginning of the next.

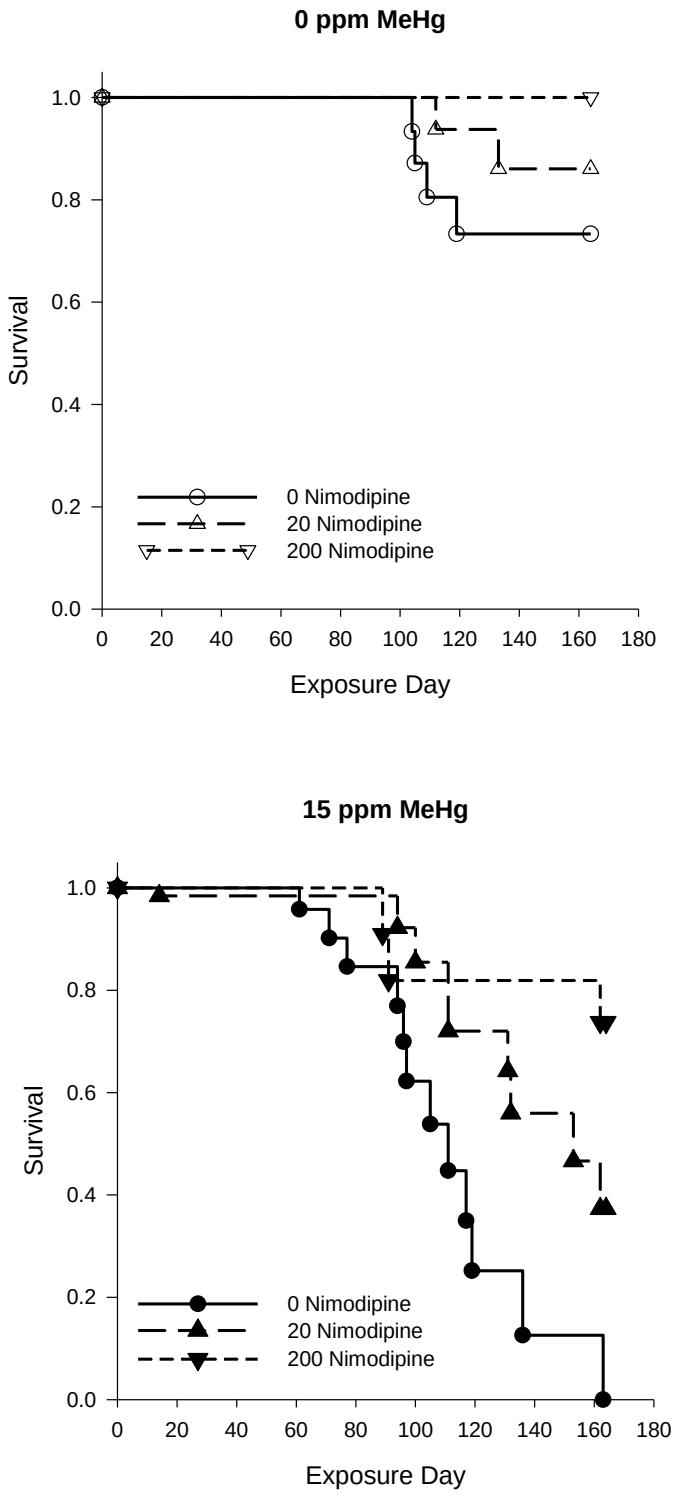

Figure 2. Kaplan-Meier survival functions plotted by nimodipine exposure for 0 ppm MeHg (top panel) and 15 ppm MeHg (bottom panel).

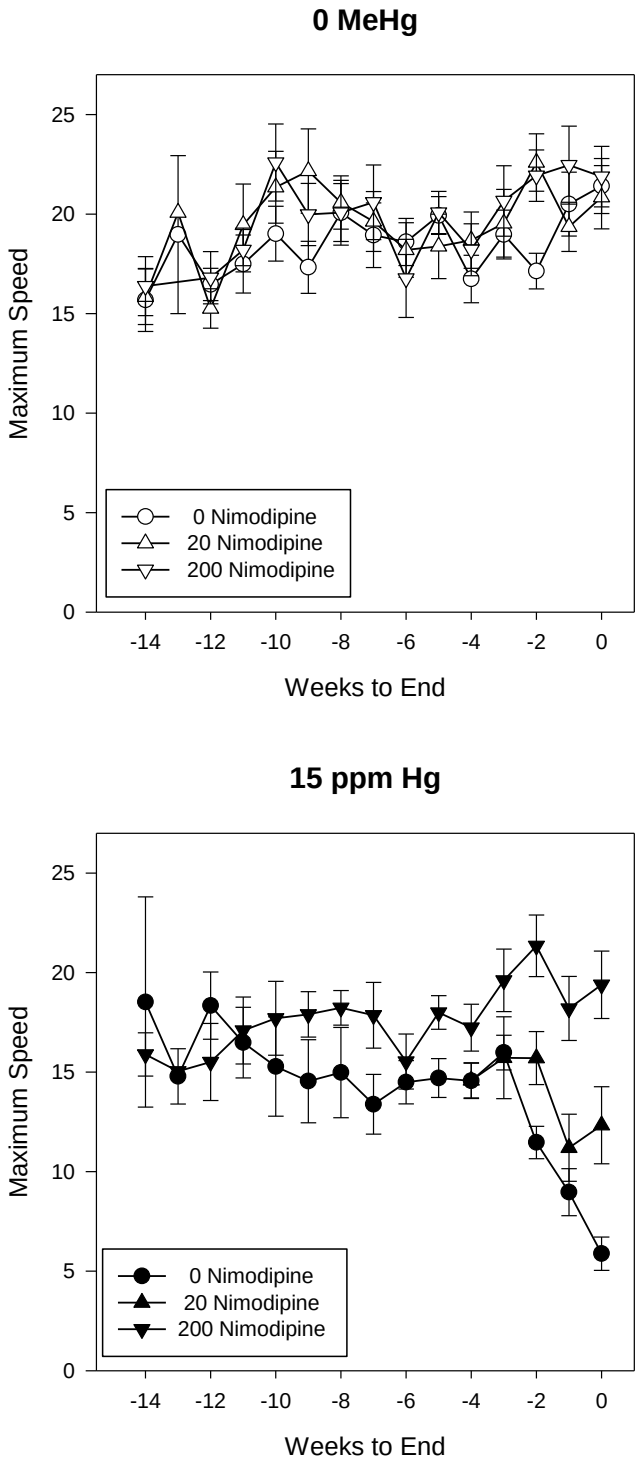

Figure 3. Mean (+/- SEM) maximum rotarod speed plotted against weeks remaining in the study so that week 0 indicates the last data collected for a subject.

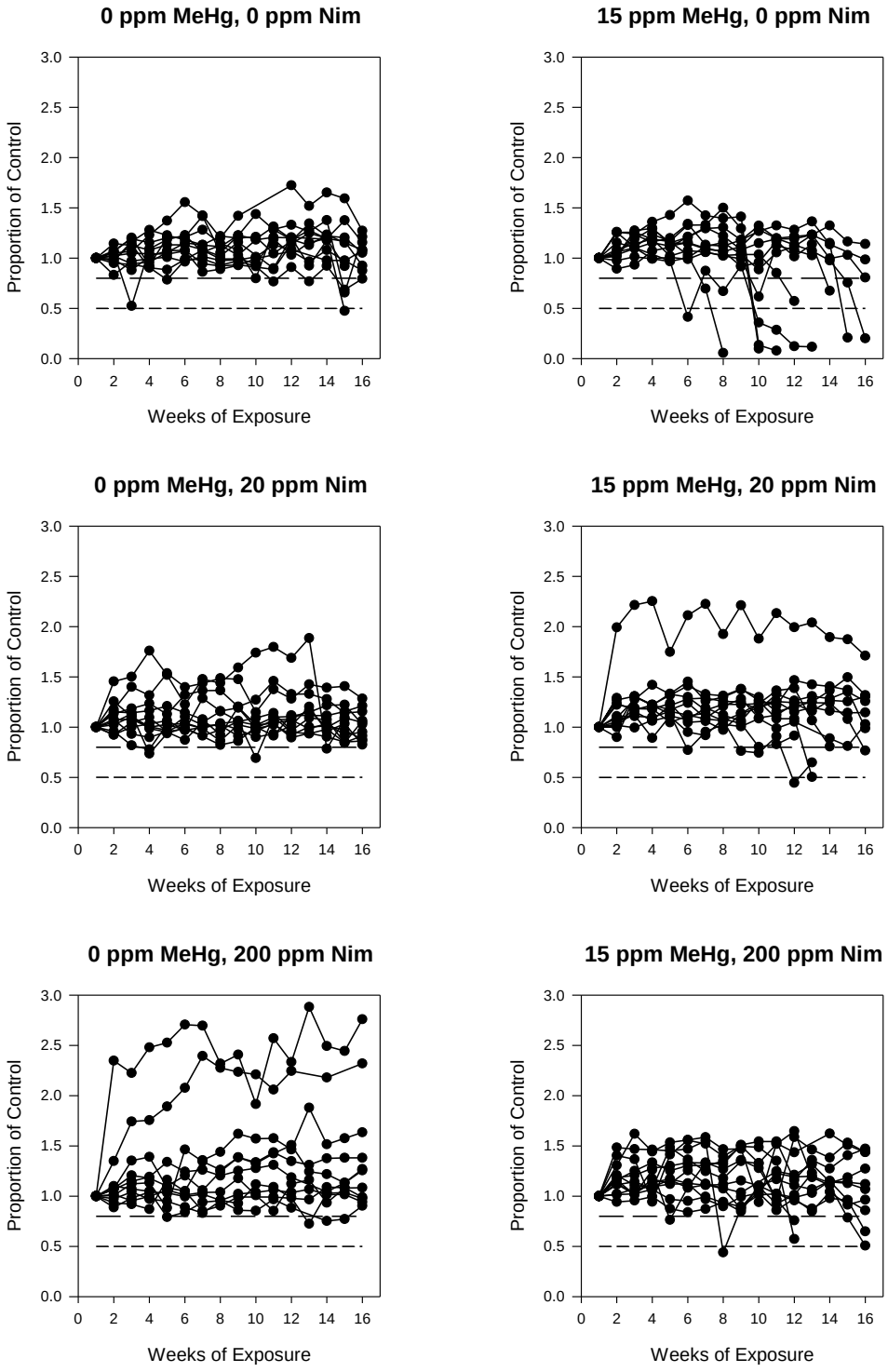

Figure 4. Distance run as proportion of control for individual subjects. The dashed reference lines correspond to 0.80 and 0.50 proportion of control. Individuals in all groups show increases in running across early weeks of the study relative to baseline, but that effect is muted for animals in the 15 ppm MeHg 0 ppm nimodipine group. Several individual subjects show 1-week dips in running on the order of 10-20%, but only the MeHg-control group shows sustained reductions consistent with motor impairment.

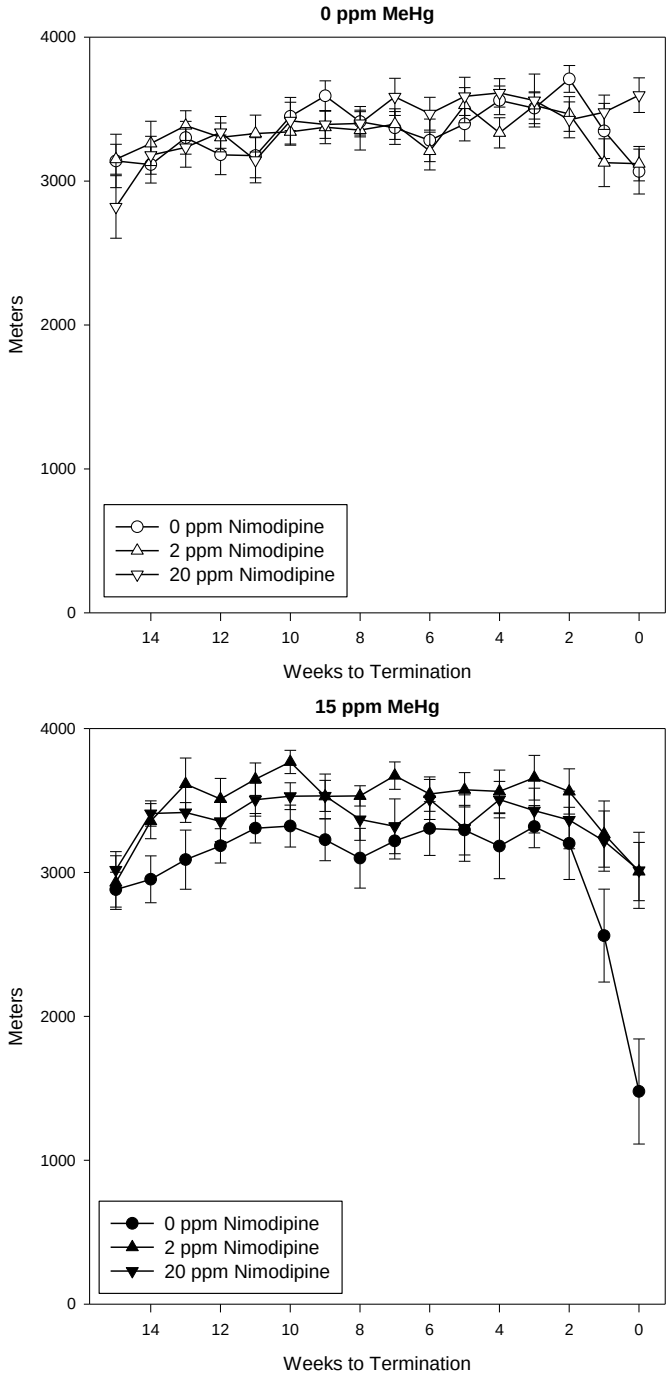

Figure 5. Mean (+/- SEM) total distance run plotted against weeks remaining in the study so that week 0 indicates the last data collected for a subject.

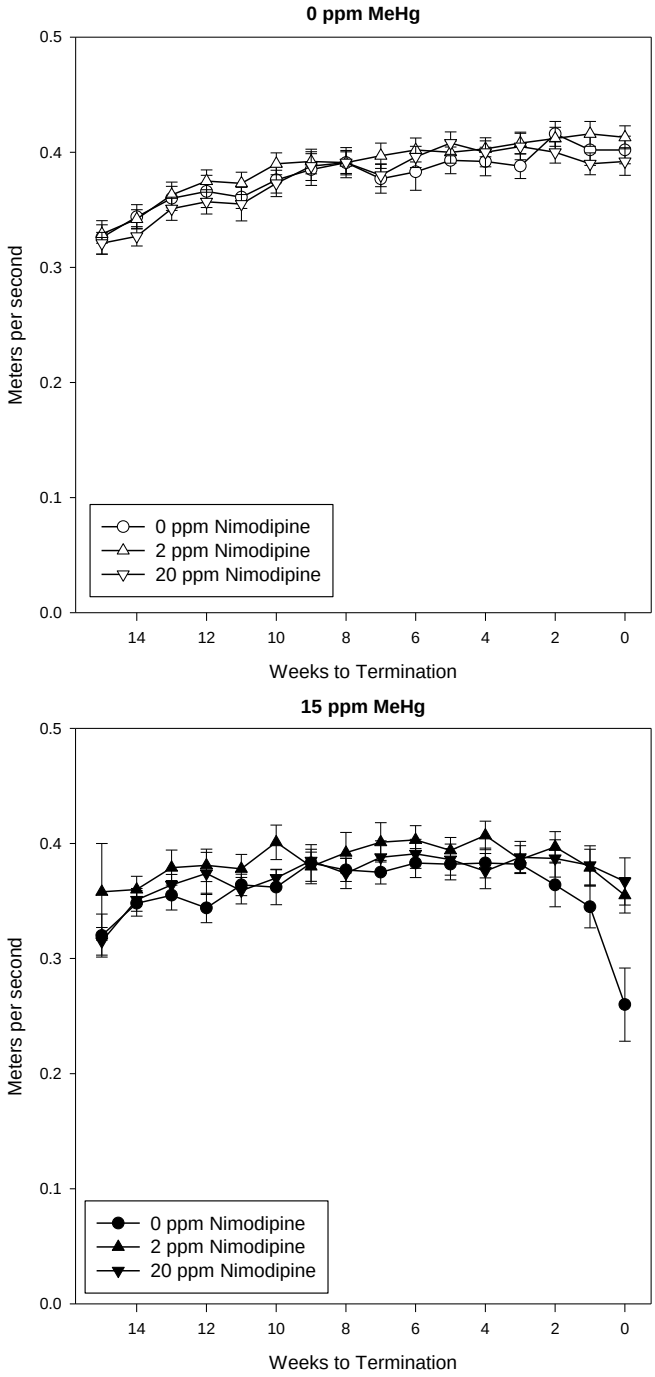

Figure 6. Mean (+/- SEM) of the within-bout rate for control (top panel) and MeHg exposed (bottom panel) animals.

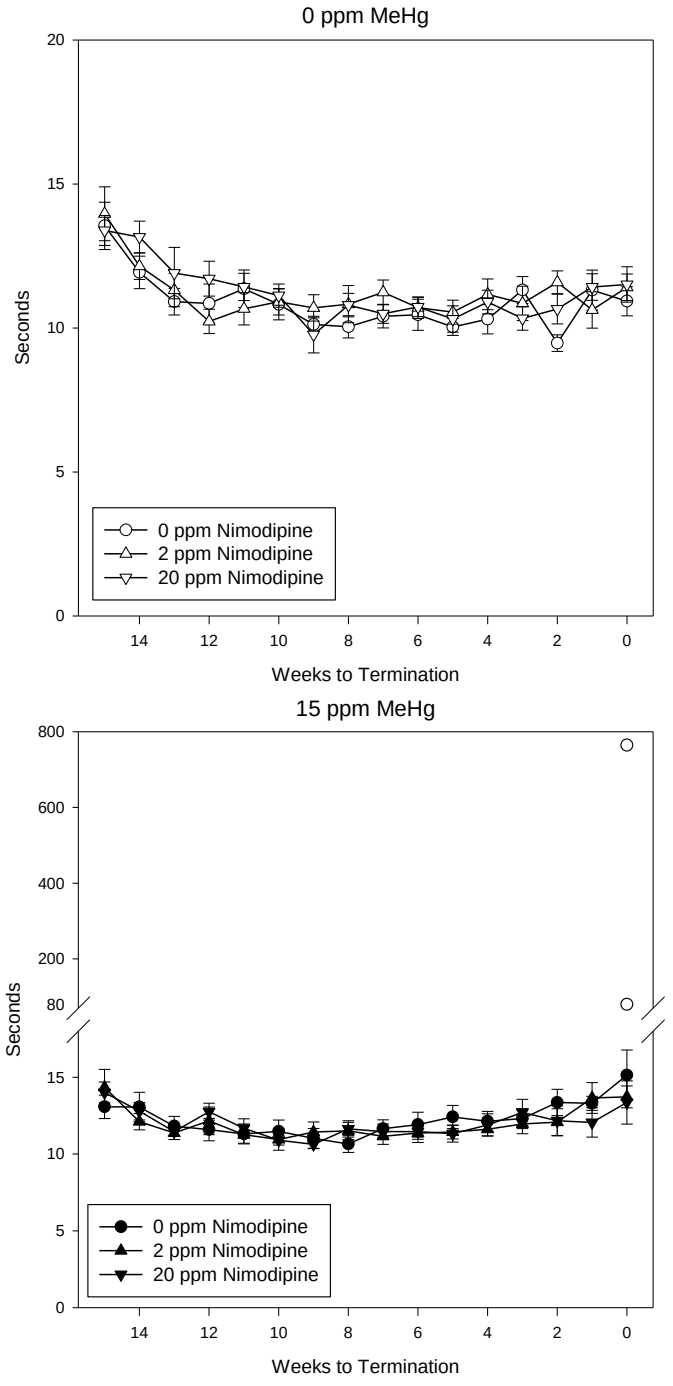

Figure 7. Mean (+/- SEM) of the interbout interval for control (top panel) and MeHg exposed (bottom panel) animals. Note that for the 15 ppm MeHg 0 ppm nimodipine group two data points have been identified as outliers and removed from the group function for their final experimental sessions. These points (corresponding to y-values of 79 and 760 seconds, respectively) are shown on the graph as individual data points.

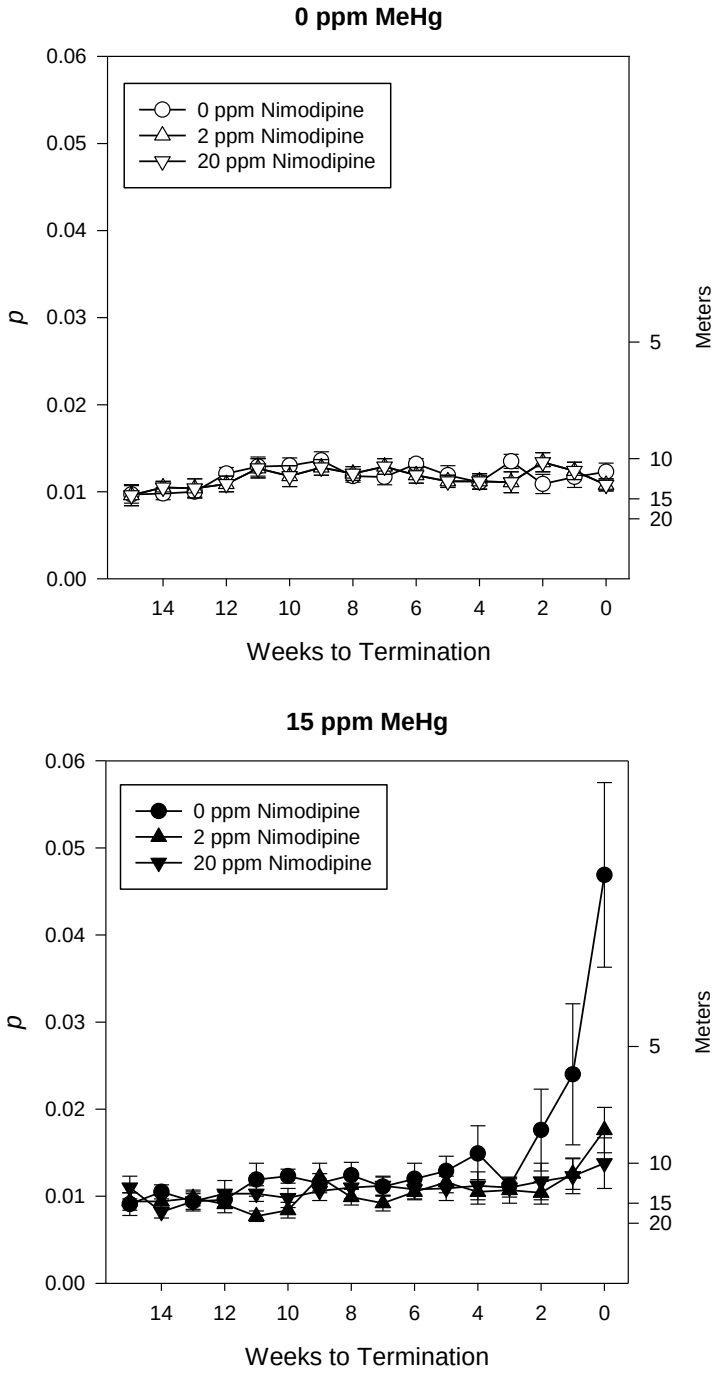

Figure 8. Mean (+/- SEM) of (1/bout length) for control (top panel) and MeHg exposed (bottom panel) animals. The second y-axis plots bout distance in meters.

References

Aoyama, K., & McSweeney, F. K. (2001). Habituation contributes to within-session changes in free wheel running. *Journal of the Experimental Analysis of Behavior*, *76*(3), 289–302.

Bailey, J. M., Hutsell, B. A., & Newland, M. C. (2013). Dietary nimodipine delays the onset of methylmercury neurotoxicity in mice. *Neurotoxicology*, *37*, 108–117.

Barrett, J. R. (2010). Rice is a significant source of methylmercury: research in China assesses exposures. *Environmental Health Perspectives*, *118*(9), A398.

Bates, D. M. (2010). lme4: Mixed-effects modeling with R. *URL http://lme4. R-Forge. R-Project. Org/book*.

Bates, D., Maechler, M., Bolker, B. and Walker, S. (2014). *lme4: Linear mixed-effects models using Eigen and S4*. R package version 1.1-7, http://CRAN.R-project.org/package=lme4.

Brackney, R. J., Cheung, T. H., Neisewander, J. L., & Sanabria, F. (2011). The isolation of motivational, motoric, and schedule effects on operant performance: a modeling approach. *Journal of the Experimental Analysis of Behavior*, *96*(1), 17–38.

Eikelboom, R., & Mills, R. (1988). A microanalysis of wheel running in male and female rats. *Physiology & Behavior*, *43*(5), 625–630.

Fry, W., Kelleher, R. T., & Cook, L. (1960). A mathematical index of performance on fixed-interval schedules of reinforcement. *Journal of the Experimental Analysis of Behavior*, *3*(3), 193–199.

Gallistel, C. R., Fairhurst, S., & Balsam, P. (2004). The learning curve: implications of a quantitative analysis. *Proceedings of the National Academy of Sciences of the United States of America*, *101*(36), 13124–13131.

Gallistel, C. R., Mark, T. A., King, A. P., & Latham, P. E. (2001). The rat approximates an ideal

detector of changes in rates of reward: implications for the law of effect. *Journal of Experimental Psychology: Animal Behavior Processes*, *27*(4), 354.

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

Hare, M. F., & Atchison, W. D. (1995). Nifedipine and tetrodotoxin delay the onset of methylmercury-induced increase in [Ca2+] i in NG108-15 cells. *Toxicology and Applied Pharmacology*, *135*(2), 299–307.

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*(2), 169–179.

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 strains. *Pharmacology Biochemistry and Behavior*, *97*(3), 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*(2), 309–315.

Limke, T. L., Heidemann, S. R., & Atchison, W. D. (2004). Disruption of intraneuronal divalent cation regulation by methylmercury: are specific targets involved in altered neuronal development and cytotoxicity in methylmercury poisoning? *Neurotoxicology*, *25*(5), 741–760.

Machlis, L. (1977). An analysis of the temporal patterning of pecking in chicks. *Behaviour*, 1–70.

Newland, M. C. (1995). Motor function and the physical properties of the operant: applications to screening and advanced techniques. *Neurotoxicology: Approaches and Methods*, 265–

299.

Newland, M. C., Hoffman, D. J., Heath, J. C., & Donlin, W. D. (2013). Response inhibition is impaired by developmental methylmercury exposure: Acquisition of low-rate lever-pressing. *Behavioural Brain Research*, *253*, 196–205.

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*(2), 179–194.

Pinheiro, J. C., & Bates, D. M. (2000). *Mixed-effects models in S and S-PLUS*. Springer.

R Core Team (2013). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/.

Shull, R. L. (2011). Bouts, changeovers, and units of operant behavior. *Eur J Behav Anal*, *12*, 49–72.

Shull, R. L., Gaynor, S. T., & Grimes, J. A. (2001). Response rate viewed as engagement bouts: Effects of relative reinforcement and schedule type. *Journal of the Experimental Analysis of Behavior*, *75*(3), 247–274.

Takeuchi, T., Morikawa, N., Matsumoto, H., & Shiraishi, Y. (1962). A pathological study of Minamata disease in Japan. *Acta Neuropathologica*, *2*(1), 40–57.

Weiss, B., Clarkson, T. W., & Simon, W. (2002). Silent latency periods in methylmercury poisoning and in neurodegenerative disease. *Environmental Health Perspectives*, *110* (Suppl 5), 851.