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Deliverable: Pilot Study Protocol
Application of Measures of Spontaneous Motor Activity for Behavioral Assessment in Human Infants

Task Number: 02-04 – Pilot Study Protocol

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Deliverable for Task 02-04 – Pilot Study Protocol

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Introduction

As a participant in planning the National Children's Study (NCS), EPA is interested in the investigation of key developmental disorders that may be associated with environmental exposures. This is particularly important in light of research that has established that prenatal and early childhood exposure to certain neurotoxicants including lead, mercury, manganese, and polychlorinated biphenyls (PCBs) can result in negative behavioral outcomes (Schettler, 2001). In some cases these outcomes are not clearly identified until children reach school age or later when the functionality of developmentally specific neurobehavioral processes is challenged. (Adams et al,, 2000; Rice & Barone, 2000). Given the plasticity of neurological systems affected by such exposures and their high level of vulnerability in the developing child, early detection is critical. The potential association between the relatively recent and large increase in the number of children diagnosed with conditions such as attention deficit-hyperactivity disorder (ADHD) and environmental factors is a public health issue of concern to the EPA.

The goal of this research is to provide, for use in a large scale field study, a method to describe and quantitatively characterize spontaneous motor activity in four cohorts: infants (4-5 months & 6-12 months) and toddlers (13-18 months & 19-24 months).

This protocol addresses two specific objectives: a) identification of sources of variance in infant's and toddler's free living daily activity levels and estimation of the number of days of actigraphy measurement necessary for a reliable measure of these activities and b) investigation of the potential correlation between activity measures (e.g., counts/epoch) averaged over long periods of time and activity measures (e.g., counts/epoch) averaged over a specific play activity.

This work continues the previous work assignment, WA 01-02, entitled "Application of Measures of Spontaneous Motor Activity for Behavioral Assessment in Human Infants and

Young Children to Predict Environmental Health and Safety Risks to Children for Use in Risk Evaluation." The current work assignment deliverable is a refinement of the previous protocol developed under WA 01-02. This refinement is a result of a review of all appropriate materials including any literature published in the previous 12 months, an update of accelerometer information including salient literature published in the previous twelve months and the identification and integration into the protocol of a non-linear movement patterns assessment appropriate for children between the ages of 0 and 24 months.

In the statistical section of the protocol we were asked to review our previous power calculations and make appropriate modifications as well as review data analysis strategies in the literature and modify the previous protocol as appropriate. We have incorporated these requested tasks into this deliverable.

We have addressed all the requirements of the work assignment, however there are two exceptions to WA 02-04 that should be noted. The WA specifically suggests four cohorts for this protocol, birth-5 months, 6-12 months, 13-18 months and 19-24 months. After a review of the literature and discussions with the WAM, it was determined that the first cohort should consist of children between the ages of 4 and 5 months. Specifically children's sleep cycles have not been established before the age of 3 months which could introduce an unaccounted form of variability in the data.

Second, after a careful review of the literature and discussions with the WAM, it was determined that the protocol would not be enhanced by the comparison of two accelerometers. Currrently the Actiwatch 64, recommended in our previous protocol, provides the sensitivity to collect data during both diurnal and nocturnal periods. This is a version of the Actical, made by Mini Mitter Co., currently used in other EPA studies.

1.0 Background and rationale

ADHD is a two dimensional disorder composed of hyperactivity/impulsivity and inattention (DSMV-IV, American Psychiatric Association, 1994). These independently measurable constructs can negatively affect cognitive, academic and social development as children mature. Because behavioral hyperactivity represents a key characteristic of this disorder, often manifested in early childhood, the measurement of spontaneous motor movement in very young children could provide a window for early detection and intervention enhancing the potential to minimize future problems.

Past ADHD research has employed several methodologies for capturing children's motor activity including real time observation, coded video-tape, questionnaires, and behavior rating scales. Porrino, Rapoport, Behar, Ismond, & Bunney (1993) were the first investigators to augment these measurement techniques with a solid-state monitoring system used to measure spontaneous motor activity. Their study reported quantified evidence that distinguished ADHD children from controls based on a consistent and significant increased rate of activity.

Since that time other researchers have noted criteria that may compromise the accuracy of spontaneous motor activity collection including artificial lab conditions as opposed to free living situations (Barkley, 1991), type of task and time of day (Dane, Schacher, & Tannock 2000). Other researchers have noted that ADHD symptomology may be dampened by novel situations (Zentall, 1985) and one on one examiner attention to the child often found in clinical or laboratory assessments. (Barkley, 1997; Barkley, 1998). Barkley also notes that ADHD assessments should begin in the child's natural environment in order to assure that measurements are true reflections of the individual child's behavior.

Advanced accelerometry allows researchers to collect more refined quantitative measurements of a specific component of ADHD, spontaneous motor movement, in a naturalistic setting over extended periods of time. With modest burden to the subject, the reusable actigraph is an objective and non-reactive measurement tool. It has been used extensively in physical activity assessment research with adults and adolescents (Sirard & Pate, 2001; Trost, 2001) and

has a well documented and standardized methodology used in sleep/wake studies with numerous age groups (Acebo et al., 1999; Thoman, 2001; Welk, Corbin, & Dale, 2000; Littner et al., 2003). Recently, actigraphy has been used extensively with children between the ages of 3 and 6 in an effort to understand childhood obesity by measurement of both motor movement activity and inactivity (Jackson et al., 2003; Janz et al., 2002; Reilly et al., 2003; Trost, Sirard, Dowda, Pfeiffer, & Pate, 2003). While obesity studies have measured activity movement with children as young as 3, little research has implemented this technology with infants and toddlers. Several studies using actigraphy to measure sleep/wake cycles in infants (Korte, Hoehn, & Siegmund, 2004; Korte, Wulff, Oppe, & Siegmund, 2001; Mennella & Gerrish, 1998) have shown that this form of measurement is possible.

It is clear that accelerometry provides an opportunity to quantitatively measure motor activity in infants and young children thus increasing the accuracy and understanding of this component of development and its role as a marker of future pathology.

In order to measure children's spontaneous movement in a large-scale study such as the National Children's Study (NCS), methodological considerations must take into account advances in the measurement field, such as the availability of new technologies and their use in combination with other developmentally appropriate instruments in order to gather the most comprehensive picture of children's movement over extended periods of time. This type of study does, however, present both methodological and pragmatic challenges. Features in this protocol design specifically address these issues.

2.0 Study design

2.1 Objectives

Objectives for the pilot study include:

- Objective 1: Identifying the sources of variance in children's daily activity levels and estimating the number of days of actigraphy measurements necessary for a reliable measure of activities.
- Objective 2: Investigating the correlation between activity count/min averaged over long period ("long term average activity") and activity count/min averaged over a specific play activity ("specific play average activity").

2.2 Design background methodology

To measure children's spontaneous movement in a large-scale study the measurement method requires the following features:

- Ability to provide both a reliable measurement and distinguish more active children from normal ones, under the age of 24 months.
- Relative ease for caregivers to collect the information with little or no field assistance.
- Maximum flexibility to formulate different indices to characterize "spontaneous movement."

In order to address reliability concerns, it is important to understand the complexity and variety of motor movements over time. It is known that human physical activity varies from day to day, perhaps more so in children. Individual differences as a function of genetic traits and unique experiences also contribute to substantial within-subject variability. In particular, researchers have cited the influence of temperament on motor movement (Teicher, 1995; Thomas and Chess, 1977). Children also experience a steep developmental motor trajectory within the first few years of life, resulting in multiple developmental motor milestones between the ages of 0 and 24 months. (See Appendix A.)

Thus, when establishing a reliable measurement of spontaneous motor activity, it is necessary to consider motor maturation level and personality characteristics as well as external events that may have a differential impact on children's activities.

A key question for using any device to measure spontaneous motor activity is the determination of how many days of a certain level of consistent activity are necessary to provide a reliable measure of spontaneous motor activity. An abnormally active child may have a day not characterized by abnormal motor movement whereas a child without ADHD may exhibit abnormally active days on occasion. A reliable measurement device must account for this variation.

Reliability, defined as the proportion of total variance accounted for by differences between subjects (Snedecor & Cochran, 1989), is a measurement that has been used in many published studies on accelerometers (Acebo et al., 1999; Matthews et al., 2002). The general acceptable reliability appeared to be 70-80% or above. Using this criterion, Acebo et al. (1999), a well known sleep patterns researcher, concluded 5 or more nights of usable activity recording are required to obtain reliable actigraphy measures of sleep for children and adolescents. Trost et al. (2000) found a single-day reliability of moderate-to-vigorous physical activity of 0.46 to 0.49 among children in grades 1 to 6. He estimated that between 4 and 5 days of monitoring would be necessary to achieve a reliability of 0.80 in children and between 8 and 9 days to achieve a reliability of 0.80 in adolescents. Matthews et al. (2002) concluded at least 3 to 4 days of monitoring are needed to obtain a reliable measure of physical activity in adults with additional days of monitoring needed to assess patterns of inactivity.

No data are available to determine how many days will be needed to obtain reliable measurements in children younger than 2 years old using an accelerometer. We plan to collect data in this pilot study to make this determination and evaluate issues of reliability so that future studies can build on our findings.

Any field data collection will be facilitated by a methodology that is both user friendly and establishes a modest subject burden, but is also able to assess reliable measurement. In addition it must distinguish more active children from children exhibiting normal ranges of activity while accounting for the potential impact of individual environmental variables.

While collecting data only in a laboratory environment limits the length of the assessment and burden, there are concerns that children feel more inhibited within a lab setting and will not express themselves as they would in their own environment. In addition, Dane et al. (2000) report that time of day and type of task may skew motor results.

To address this we will be monitoring children for a longer period of time with recorded details of both a brief lab activity and more general home activities through the use of a time activity diary to facilitate an optimal data collection methodology.

We will assess children first in the lab using both a structured motor development task and an unstructured play task. The purpose for this lab visit is twofold. During this time, the child will be tested for normal motor development to assure that he/she is eligible to participate in the study by scoring within the normal range on the Peabody Developmental Motor Scale – 2nd edition (PDMS -2). The second purpose is to familiarize both the caregiver and the child with the device and the diary/questionnaire.

This lab visit will allow investigators to obtain a baseline actigraphy measure for a structured task (PDMS-2). At the completion of the PDMS-2 the parent/caregiver and the child will participate in a brief unstructured play activity with an age appropriate toy. The child will continue to wear the actigraphy monitor during the unstructured lab play task to capture a lab measurement of spontaneous motor movement during an undirected activity.

To capture temperament information, a standardized questionnaire will be administered to the parent/caregiver during the lab visit. If the child is less than 12 months of age, parents/caregivers will complete Rothbart's Infant Behavior Questionnaire (IBQ). For children older than 12 months, the caretaker will be asked to complete Carter's ITSEA (The Infant-

Toddler Social and Emotional Assessment (ITSEA). These measures are discussed in greater detail in Section 6.1.

In addition to the lab visit, the child will wear the actigraphy monitor for a 7-day period of time in his/her home environment. This will be done in combination with a completed activity diary from the parent/caregiver regarding general activity categories such as mealtimes or interactive play. By obtaining measurements under multiple circumstances, investigators will be able to determine whether a short lab task is a proxy for a longer home measurement to accurately determine the most advantageous route of measurement that reflects the minimum subject burden.

Finally, we will examine different measurement epochs of activity in the study. Although the most commonly used index for spontaneous movement measured by accelerometer is count/minute, it is possible that count/minute does not capture the most important component of the spontaneous movement that is specifically predictive of neurobehavioral symptoms. It is also possible that the magnitude of the movement or count of activities with magnitude larger than the mean is correlated to neurobehavioral symptoms.

3.0 Sample characteristics

Because development in children is particularly steep between the ages of 4 and 24 months, it is important to carefully account for age and expected developmental stage in establishing a measurement protocol.

This study will consist of four specific cohorts, infants (4-5 months & 6-12 months) and toddlers (13-18 months & 19-24 months). Between 4 and 24 months of age, children experience a steep motor development trajectory with many gross and fine motor milestones. In the first cohort, children will be tested at approximately 4-5 months of age. Because children are establishing their sleep/wake patterns through the first three months of life (Nishihara, Horiuchi, Eto & Uchida, 2002) data will not be collected on children younger than 4 months to avoid potentially noisy data that may be difficult to interpret. Because children will wear the actigraphy

monitor during both diurnal and nocturnal times, we do not want to collect information that reflects a lack of development as opposed to a true variance in behavior. Children in the second, third and fourth cohorts will represent a wider age range; 6-12 months, 13-18 months and 19-24 months respectively. These ranges allow the collection of data at multiple developmental points including children who are crawling, walking and running. All four cohorts will also include children whose movements are both parent-directed and self-directed.

Because some physical activity assessment studies have noted a difference in activity levels between boys and girls at older ages (Jackson et al., 2003) the ratio of boys to girls will be approximately 1:1 in order to have equal representation so that gender differences can be assessed.

4.0 Sample size and power calculations

To determine the number of subjects required in each age cohort, the literature was reviewed to ascertain a reasonable mean level of activity, derived using accelerometry (see Appendix B). Gender differences, attrition concerns and extended lengths of data collection were also taken into consideration.

4.1 Number of infants required

Miller and Kraft (1994) provided a mean activity of 215 +/- 15 movements per minute while awake and 21 +/- 5 movements per minute while asleep for non-ADHD children but cautioned that the data were based on a small number of subjects. Paavonen et al. (2002) reported mean activity levels for diurnal activity of 235.89 +/- 14.22 and 214.61 +/- 15.18 among school-aged children wearing monitors at the wrist and waist, respectively. In a small study of infants aged 1.5 to 5.6 months, Mennella and Gerrish (1998) reported mean activity counts during wakefulness of 211.9 +/- 6.6. Finn et al. (2002) reported similar findings in a larger study involving 3 to 5 year old children.

Based on these figures, we calculated the number of subjects required to estimate mean movements per minute for a range of confidence interval widths. Table I presents the results.

Table I. Determining Number of Subjects

	Mean = 215 SD = 15			Mean = 21 SD = 5		
Distance from mean to limit	10	7.5	5	3	2.5	2
Number of infants	14	22	44	17	22	32

* Based on a 2-sided confidence interval with confidence level 95%.

From this, we conclude that, if the population mean is 215 movements per minute while awake and the population standard deviation is 15, a sample size of 22 subjects is needed to estimate the mean to within 7.5 movements per minute (one-half of a standard deviation). This corresponds to being able to detect a difference of 15 movements per minute in the value of two means (the mean number of movements per minute for boys and the mean number of movements per minute for girls, for example) with a power of 89%. As suggested by Table I, comparable results are obtained if sample size estimation is based on movements per minute while sleeping.

As depicted in Appendix B, review of the literature revealed that the means and standard deviations reported for movements per minute varied considerably according to the device used, the placement of the device, the population tested, and the activities performed during the measurement period. For instance, Jackson et al. (2003), Reilly et al. (2004), and Matthews et al. (2002) each presented the findings of studies collecting data using Computer Science and Applications (CSA) model 7164 accelerometers. In children aged 3 to 4, Jackson et al. (2003) found a mean total activity of 777 +/- 207 counts per minute for boys and 657 +/- 172 counts per minute for girls. Reilly et al. (2004) reported a mean activity level of 692 +/- 176 counts per minute among 3 year old children and 818 +/- 185 counts per minute among 5 year old children. Matthews et al. (2002) reported an average count per minute of 330.5 +/- 141.7 for male adults and 300.4 +/- 131.7 for female adults.

While these figures are greater in magnitude and somewhat more variable than those on which our sample size calculations are based (possibly due to the use of uni-axial

accelerometers), a sample size of 22 remains adequate to detect a difference of 1 standard deviation from the mean with a power of 89%. Thus, if the population mean is actually 777 movements per minute with a population standard deviation of 207, a sample size of 22 infants will allow us to estimate the mean to within 103.5 movements per minute. As before, this corresponds to being able to detect a difference of 207 movements per minute in the value of two means with a power of 89%.

Clearly, issues of attrition must be considered when determining the number of subjects required. Subjects may fall outside of the normal range of motor development on the PDMS-2, may become ill during data collection, or may have data that are unusable due to extreme irresolvable discrepancies between the activity diary and accelerometer. Though Table I does not adjust for these types of issues, such an adjustment is easily made. For example, if attrition is assumed to be 10%, the required number of subjects from Table I (22, for example) would need to be inflated by the same percentage, i.e., the new required sample size would be 22 * 110/100 = 24.2 ≈ 25.

To allow for gender comparisons within an age cohort, the attrition-adjusted number of subjects would be needed in each gender group within that cohort. For example, in a study design cohort consisting of infants 6 to 12 months of age and having a male to female ratio of 1:1, the total number of infants necessary to accurately measure mean movements per minute would be 50: 25 boys and 25 girls. With this sample size, and population mean and standard deviation as indicated in Table I, we would have a power of 89% to detect a difference of size 15 in the mean activity level for 6 to 12 month old boys and the mean activity level for 6 to 12 month old girls.

In order to fully implement this study a total of 200 subjects will be necessary: 50 in each age cohort. Thus, the birth to 5 month old cohort will include 25 boys and 25 girls, the 6 to 12 month old cohort will include 25 boys and 25 girls, the 13 to 18 month cohort will include 25 boys and 25 girls, and the 19 to 24 month cohort will include 25 boys and 25 girls.

4.2 Number of measurements required per infant

Acebo et al. (1999) reported that although aggregate data collected over a 5-night period provided adequate reliability, almost 28% of these data could be considered lost to patient non-compliance, technical error and unexpected complications including child illness. With that in mind, Acebo recommends that at least 7 nights of data collection is necessary to compensate for predicted data loss.

We plan to collect data continuously for a 7-day period with activity level recorded every 30 seconds. This affords us a large number of measurements, more than adequate to estimate reliability with a high degree of precision (see Appendix C for calculations regarding the number of 30-second measurements required for each infant).

5.0 Accelerometer selection and placement

After careful review of current research, manufacturers' specifications and key factors affecting the success of this study we have chosen the Actiwatch AW 64 (Mini Mitter Co., Inc. Bend, Oregon) for use in this study because of its waterproof characteristics, length of battery time, size and cost.

This device is omnidirectional and has sensitivity to movements between 0.05 G and 10 Gs (force of gravity). It also has a reporting duration (epoch length) of between 15 seconds and 15 minutes. According to the manufacturer, the average movement for each second is recorded and the total of all of the movements over the minimum 15 s epoch duration is reported. Table II outlines the characteristics of the Actiwatch 64.

This accelerometer has been used successfully in numerous studies (Finn & Specker 2000; Finn et al., 2002 and Puyau et al., 2002), providing the sophistication and sensitivity to measure both awake and asleep activity.

Littner's 2003 update of actigraphy parameters notes that the optimal placement on different parts of the body is not currently established. As shown in Appendix B, researchers have placed actigraphy monitors on the leg, wrist and waist. For this study the accelerometer will be placed on the waist or ankle using an unobtrusive hook and loop strap

For children between the ages of 6 months and 24 months monitors will be placed on the waist. This placement allows the capture of most movement and has been shown to be successful in other studies. For children in the first cohort, 4-5 months, the monitor will be placed on the ankle. In children this age, waist placement is not optimal for several reasons. First, their torso movement is compromised by the fact that they are not crawling or walking. Second, a waist placement could potentially reflect respiration movement as opposed to spontaneous motor movement.

Table II

Actiwatch AW 64
Minimitter, www.minimitter.com

FEATURES	DESCRIPTION DETAILS	
Specifications Omnidirectional	Size Dimensions:	• 16.5 g (0.6 oz.) • 2.8 x 2.7 x 1.0 cm
	Byte space:	• 64KB
	Battery life:	• 180 days (remains constant).
	Body placement:	• Wrist, ankle, waist.
	Waterproof:	• Yes. • Can be worn during bathing, and swimming. • Is shower and bathtub safe.
	Recording capacity:	• 44 days at 1 min epoch. • 22 days at 30 sec epoch.
	Sensitivity Level	• 0.05 – 10 Gs
	Ceiling on linear range	• 32,000 counts
Data Output/Software	• Actiware software is compatible with Windows '95*, '98, ME, NT4, 2000, XP operating systems. • Actiware Rhythm allows you to program and download any Actiwatch model. ○ This software allows for basic reporting of activity data and examination of Circadian patterns using an Actogram display. • Actiware Sleep software contains all the features of Actiware Rhythm ○ Activity data may be scored for sleep or wake on an epoch by epoch basis. ○ Analysis of the activity data for naps may also be performed.	
Time Increment Flexibility	• 15 sec to 15 min programmable epoch lengths.	
Sampling Rate	• 32 Hz	
Data Acquisition Method	• Peak value on 1 second interval	
Data Recording Method	• Accumulate number of 1 second values in each epoch	
Cost	• Unit…… $ 985.00 • ActiReader…….. $ 725.00 • ActiWare Sleep software…… $1200.00 • Actiwatch-64 software……$ 500.00 • Battery service kit……$ 75.00 • Additional wrist bands or Velcro… $ 25.00 – $120.00 for a pack of 10	
Advantages	• Waterproof. • Higher sensitivity to subtle movements than Actical.	
Contact	John Breeden @ 1-800-685-2999; johnb@minimitter.com	

6.0 Assessment strategy/data collection protocol

We recommend a strategy that assesses each child's initial motor capabilities and temperament while providing a modest amount of data during a structured and unstructured task in a lab setting. This assures that children can be tested to determine if they fall within a normal-for-age range of motor development and also provides baseline data while giving the child and caregiver adequate time to understand how to use the accelerometer.

As noted earlier, personality characteristics such as temperament play a role in the level of motor movement expressed by an individual. Rothbart (1981) has defined temperament as individual differences in reactivity and self-regulation. This personality characteristic is assumed to have a constitutional basis, with "constitutional" defined as the relatively enduring biological makeup of the individual, influenced over time by the interaction of heredity, life experience, and maturation. The concept of temperament provides an integrative approach to the study of the development of individual differences.

Researchers have noted that in the study of temperament, one of the most consistent dimensions across time was that of activity level (Thomas & Chess, 1977). Teicher's review (1995) of actigraphy and motion analysis research with respect to psychiatric conditions cites temperament as a variable of note and an indicator of movement frequency.

In addition to these assessments, the collection of data through the use of a time activity diary will allow the study to capture information about the types of movement contexts and specific activities that the child participates in both sporadically and habitually.

6.1 Laboratory assessments

6.1.1 Motor assessment

The Peabody Developmental Motor Scale, 2nd Edition (PDMS-2) will be administered to each child to assure that they fall into the normal range of development for both gross and fine motor skills. This assessment is an early childhood motor development test divided into two scales (gross and fine motor) and comprised of six subsets that measure the interrelated motor abilities developed early in life (from birth through 5 years of age). The PDMS-2 tests the domains shown in Appendix D. The second edition has been normed on a representative sample of 2,000 children in the United States and is a criterion-referenced exam with both gross motor and fine motor scales and high test-retest and inter-rater reliability establishing it as a gold standard measure.

The assessment takes approximately 45 minutes to complete and measures motor maturity in the domains of reflexes, stationary control and equilibrium, locomotion, object manipulation, grasping and visual motor integration. Any child not falling into the normal range will not continue with the study.

This exam will serve as both a screening mechanism and as a structured task for the child during the lab visit. The child will wear the accelerometer during this task.

6.1.2 Temperament assessments

Unfortunately, an adequately tested measure of temperament that spans the ages of 4-24 months is not available, however two measures are discussed below that capture the characteristics of temperament important to this study. The Infant Behavioral Questionnaire (IBQ) would be used with children under the age of 12 months. The Infant Toddler Social and Emotional Assessment (ITSEA) is appropriate for older children.

The IBQ, developed by Rothbart, is a 90-item parent report questionnaire used to measure temperament for children between the ages of 0 and 12 months of age. Numerous behavioral events are identified by an initial yes/no query regarding whether the event happened in a particular time frame. For those where the informant responds yes, specific queries about infant behavior follow. Summary scores are available for activity level, smiling and laughter, distress and latency to approach sudden or novel stimuli, distress to limitations, soothability, and duration of orienting. Administration time is 15 minutes.

Carter's ITSEA is a comprehensive, multi-dimensional instrument developed to assess social-emotional problems and competencies in 12 to 48 month old children in clinical and research settings. It was designed to be administered as a self-report questionnaire or structured interview to parents or child care providers consisting of 166 items assessing the behaviors of externalizing, internalizing, dysregulation, competencies, maladaptive, atypical behavior, and social relatedness. Appendix E summarizes the domains, scales and indices contained in the ITSEA.

The ITSEA has a reading level between 4th and 6th grade and requires approximately 20-30 minutes to complete if self-administered. Items are rated on a 3-point scale of not true/rarely, somewhat true/sometimes, and very true/often. A "No opportunity" response option allows parents to indicate that they have not had the opportunity to observe certain behaviors.

6.1.3 Time activity diary

In a recent study of 3 and 4 year olds, when using an average activity/count over 3 days as a measure for activity without knowing details of the activity (no activity diary was used), boys were shown to have a higher activity level than girls (Jackson et al., 2003). Although this study showed the possibility of collecting data without an activity diary, this lack of detail leaves many questions unanswered that may ultimately compromise the results especially in light of research that report the impact of physical location, age, gender, ethnicity and time of year on motor activity (Baranowski, Thompson, Durant, Baranowski & Puhl, 1993). This data will be crucial information in the editing, cleaning and analysis of actigraphy data.

Parents will be provided with a checklist for each day of the 7-day monitoring period. Understanding that a complicated form of recording activity is likely to cause increased noncompliance, the checklist will contain general categories of activities that the child may participate in. These will include the following:

- Meals
- Sleeping (night time and nap time)
- Crying/Tantrums (for the older age group)
- Bath Time
- Quiet Alert (child is not interacting with toys, but is observing surroundings)
- Solitary Play (may include sitting in an excersaucer, swing, bouncy seat or a pack-n-play and entertaining themselves)
- Interactive Play (with another person -- sibling, parent, etc.)
- Watching TV (including videos or DVDs)
- Travel Time
- Shopping
- Preschool/Daycare
- Outdoor Play (swinging, swimming, running outside, and other activities that cannot take place indoors)

Parents/caregivers will note on the diary the beginning and ending time of each activity.

The parent/caregiver's checklist is important for two reasons. First, it provides an opportunity to examine the data for patterns that may specifically reflect particular activities in the child's life. Second, it serves as an edit check against the data. If there are particular peaks or valleys that remain unexplained, the examiner can immediately contact the parent/caregiver in order to review the checklist.

The parent/caregiver will use the list of codified responses to mark categorical motor events. This approach will be easier and more accurate than a free form diary, but will still allow the collection of events that the child experiences.

The complete Time Activity Diary is shown in Appendix F.

6.2 Laboratory/home visit procedures

Each mother-child dyad selected to be part of the study will be invited to the laboratory for an initial visit with the study examiner. At that time mother/caregiver will be instructed on how to use the accelerometer, provided with a list of Frequently Asked Questions (FAQs) about the equipment, and instructed on how to fill out the daily checklist. She will also complete the appropriate temperament questionnaire and participate in an age appropriate play activity with the child. The study examiner will administer the PDMS-2 to the child.

Dyads will also be seen in the home environment to collect actigraphy data. Examiners will make additional home visits as necessary if problems arise and parents/caregivers need assistance throughout the data collection period.

In order to assure consistency across subjects the following steps will be followed by study examiners for each child seen in the study.

- Scheduling of appointments and preparation of assessment location
- Preparation of accelerometer
- Introduction of study to parent
- Consent
- Placement and activation of accelerometer
- Child assessment
- Parental instruction on use of accelerometer
- Parental instruction on use of time activity diary and questionnaire
- Play activity
- Distribution of materials and incentive for lab assessment
- Follow-up contact during home assessment
- Collection of accelerometer and time activity diary
- Downloading accelerometer data
- Distribution of incentive for home assessment
- Follow-up contact after home assessment (if necessary)

Detailed descriptions of each step are shown in Appendix G.

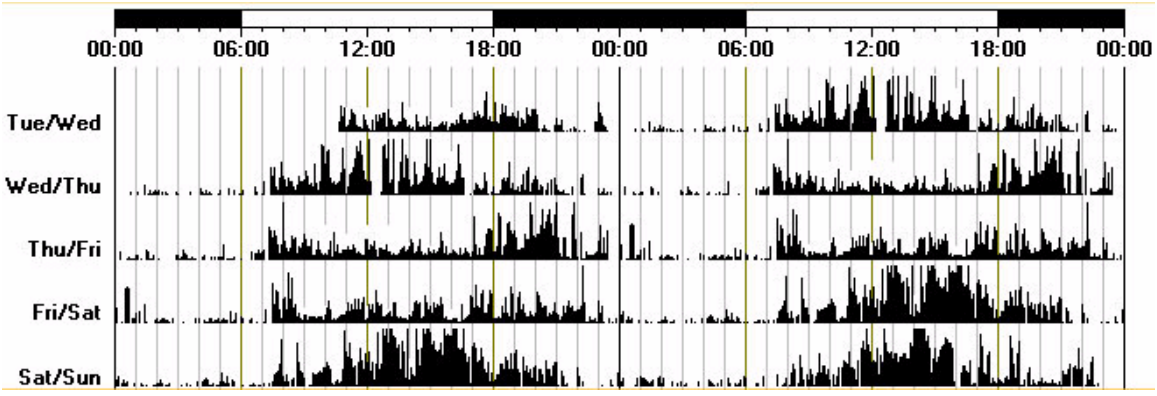
7.0 Statistical analysis

7.1 Exploratory data analysis

Prior to beginning formal statistical analyses, a thorough exploratory data analysis will be conducted. Through this exploratory analysis, we will 1) visually inspect the data, 2) identify and resolve any outlying observations, and 3) explore various summary measures, which may offer alternatives to the mean.

Exploratory data analysis will begin with visual inspection of the data. Such an inspection will allow us to gain a better understanding of the data and to identify any patterns which may exist across subjects (noticeable differences in activity level due to day of the week or time of day, for example). Software provided with the Actigraph monitors will be utilized to generate actograms, which plot a subject's activity levels for each epoch across a specified period of time. Figure 1, downloaded from the Mini Mitter website (www.minimitter.com), provides an example of a 2-day actogram.

Figure 1. 2 Day Actogram



Each caregiver will be asked to keep a record of the activities of his or her child during the data collection period. This checklist will be used to assess any outlying observations which are identified during the exploratory data analysis. Obvious outliers, such as long periods with

zero activity or abnormally high activity at unexpected times, will be rejected if irresolvable (Littner, 2003).

In collaboration with study investigators, a set of measurements will be developed to characterize children's activity. These measures will include the following, which are commonly reported in the literature:

- Mean count per minute
- Mean count per minute while awake
- Mean count per minute while asleep
- Mean count per minute for specific activities (eating, crying/tantrums, bath time, etc.)

Summary measures, other than mean counts per minute, may also be of interest. In looking at activity levels in children with ADHD, Teicher (1995) found that the percentage of time a child spent at low, medium, and high activity levels provided a better index of altered activity than mean activity levels. Constructs incorporating temperament may also be of interest.

Insight gained through visual inspection of the data will be of use in determining the most appropriate summary measures for the data collected. As part of the exploratory data process, a determination of whether to report counts per 30-second interval, as collected, or per 1-minute interval, as more commonly seen, will also be made.

7.2 Descriptive analyses

Data will be collected for a 7-day period. Summary measures, as described above, will be calculated for each age cohort, for the entire data collection period and for each day within the period. We expect that summaries for shorter periods of time (hourly, etc.) and gender-specific summaries may also be of interest.

Puyau et al. (2002) provide thresholds for categorizing physical activity levels based on Mini Mitter Actiwatch counts. These threshold counts were derived based on energy

expenditure and resting metabolic rate in a study of 6 to 16 year old children. According to these thresholds and placement of the accelerometer in a given age cohort (threshold counts are provided for hip placement as well as for leg placement), we will categorize activities as sedentary, light, moderate, and vigorous. The amount of time spent at each activity level will be determined.

7.3 Objective 1

This study has as its first objective to identify sources of variance in children's daily activity levels and to estimate the number of days necessary to obtain a reliable measure of activity.

To accomplish this, we will first estimate within- and between- subject variance using a one-way nested random-effects analysis of variance (ANOVA) model. A one-way nested random-effects ANOVA model accounts for correlations which result when repeated measurements are taken on the same subject, and takes the following form:

$$Y_{ij} = \mu_y + \beta_i + \varepsilon_{ij} \text{ for } (i=1,2\ldots k) \text{ and } (j=1,2,\ldots n_j)$$

Here, Y_{ij}= mean count per minute of the i^{th} child on the j^{th} day

μ_y= overall mean of Y_{ij}

β_i= deviation from the overall mean for the i^{th} child

ε_{ij}= deviation from the overall mean for the i^{th} child on the j^{th} day

Using this model, the between-subject variance (reflecting true differences between subjects) and within-subject variance (reflecting day to day variation within subjects) can be calculated and compared. Similarly, by using gender as a factor in the ANOVA design, differences in variances between boys and girls may also be explored.

Estimates of between-subject variation and within-subject variation obtained from the above random-effects ANOVA model will be used to calculate reliability for 1, 2, 3, …, 7 day

periods using the Spearman-Brown prophecy formula (Winer, 1971). Here, our approach will mirror that taken by Acebo et al. (1999) to recommend the number of nights of data collection needed to reliably estimate sleep measures such as sleep minutes and sleep efficiency. Based on estimated reliability, an appropriate length of data collection will be recommended for each of the measures of interest. As we would expect the appropriate length of data collection to vary depending upon age, recommendations will be made for each age cohort.

7.4 Objective 2

A second objective of the pilot study is to investigate the association between activity counts averaged over a long period of time (a "long term activity average") and average counts for specific play activities ("specific play activity averages"). Here we wish to assess how activity levels measured in a subject's natural environment are associated with activity levels measured in a more structured setting (i.e., during the lab assessment described in Section 6).

Correlation techniques, which measure the strength of the linear association between two variables, offer one approach for doing this. A high correlation between average counts for specific activities and average counts for longer periods of time, if found, could have implications for future data collection. Such a correlation would tend to suggest that information obtained from data collected during specific activities is "as good as" information obtained from data collected during longer periods of time, in terms of representing spontaneous movement.

One frequently used estimator of correlation is the Pearson product-moment correlation coefficient (Pearson, 1896). If a linear association is suspected (and confirmed graphically during exploratory data analysis), and necessary assumptions regarding the distribution of the data are met, the Pearson correlation coefficient will be used to assess correlation. If distributional assumptions are not met (i.e., if the data are non-normal), the Spearman rank correlation coefficient (Spearman, 1904) is appropriate and will be used. If the association is non-linear, transformations may be used to linearize the relationship. In this case, a measure of correlation will be determined using linear regression.

7.5 Gender comparisons

Although not a primary objective of the study, it may also be desired to determine whether there are differences in mean activity level due to gender. Tests based on the t-distribution (Gosset, 1908) are commonly used to determine whether the mean value of a continuous outcome, such as activity count per minute, in one group differs significantly from the mean value of the outcome in another group. T-tests rely on the assumptions that (1) the distribution of outcome in the two groups is approximately normal and (2) the variance is the same in both groups. If both of these assumptions are met in our data, we will use t-tests to compare mean counts for boys and girls. If the assumption of equal variances is not met, an adjusted t-test is available. If it is determined that the data are not normally distributed, non-parametric tests that do not depend upon a particular distribution, such as the Wilcoxin signed-rank test (Wilcoxin, 1945), may be used. If more sophisticated comparisons are required (to compare measures other than means, for example), a random-effects ANOVA model, like that described in Section 7.3, may be used.

8.0 Recruitment

Parent/child dyads will be recruited using EPA's recruitment center. We envision implementing two recruitment strategies in order to capture data from children in multiple contexts. It has been reported by Sirard and Pate (2001) that the contextualization of movement is an important consideration in any free-living measurement situation. With this consideration in mind our recruitment strategy includes children both in and out of daycare.

The advantage of daycare recruitment is the ability to test an optimal number of children in the least amount of time. Daycare facilities have children grouped by general age categories that will be scattered across 24 months. This will allow us to present information to parents at one point in time and enroll children as they approach their birthday. The disadvantage is that all children in the sample will represent a population who spend a majority of their time outside of the home in a potentially more structured situation, not necessarily representative of all children in the general population.

To address this we will also recruit through advertising and/or contacts within the community in an effort to recruit children who are with at-home parents/guardians on a full-time or part-time basis. It should be noted that this recruitment is likely to take a long time to complete. Children will need to be recruited on a rolling basis to assure that their birthdays coincide with the window designated by the study parameters.

9.0 Eligibility/exclusion criteria

All children must fall into the appropriate age range for a particular cohort. There must be an equal number of boys and girls for each cohort and each child must have apparently normal motor development. Any child with a diagnosed developmental delay or medical problem that would interfere with normal motor development such as Cerebral Palsy (CP) or Muscular Dystrophy will not be eligible for the study. All children must also score in the normal range on the PMDS-2 for their age.

10.0 Consent forms/invitation to parents

All parents/caregivers will receive an explanatory invitation to participate and will be required to sign consent forms (Appendix H) at the beginning of the first contact (home or lab visit). These forms will have been approved by the appropriate IRB's before the start of the study. Also, all appropriate federal and institutional guidelines will be followed concerning data confidentiality.

11.0 Incentives

We believe that providing incentives to respondents will have an important impact on compliance rates. Many studies have demonstrated their positive effect, particularly for diary-keeping studies. The following schedule of incentives is suggested.

Age Group	Testing Type	Cash Payment to Parent/Caregiver	Gift Certificate for Child	Total Payment Value Per family
All ages	Lab Visit	$20	$20	$40
	Home Assessment	$100		$100

Families will receive a modest cash payment after the initial lab visit and a gift certificate for the child. When the accelerometer and parent log have been retrieved and data have been downloaded, the parent/caretaker will receive the remaining cash payment assuming that the child has worn the accelerometer for at least 5 days.

12.0 Training, data management, quality control and corrective actions

Study examiners will be trained and certified by an experienced examiner to administer the Peabody Developmental Motor Scales. In addition, a complete training manual will be prepared for each study examiner that outlines how the lab visit will be handled. See Appendix G for outline of proposed manual. The manual will also include a script for providing caregivers with information about the study procedures, directions on how to interact with the infants in a standardized manner, and instructions for use of the accelerometer. In addition, the study examiner will receive instructions on how the accelerometer is to be retrieved and data is to be downloaded using vendor provided software. Appropriate quality assurance/quality control measures will be implemented in accordance with EPA requirements.

EPA will receive both raw data sets and data analysis sets depending on the types of analysis that are selected. After each actigraphy data set is retrieved, it will be compared to the diary filled out by the parent/caretaker. If the data appear to have inconsistencies, the parent will be called and the diary will be reviewed in order to provide a clean data set.

Because each study examiner will have contact with the study dyad every 48 hours and data will be cleaned within 72 hours of retrieval, problems will be ascertained and corrected in a timely manner.

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