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# Between Site Reliability of Startle Prepulse Inhibition Across Two Early Psychosis Consortia

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## Abstract

Prepulse inhibition (PPI) and reactivity of the acoustic startle response are widely used biobehavioral markers in psychopathology research. Previous studies have demonstrated that PPI and startle reactivity exhibit substantial within-site stability; between-site stability, however, has not been established. In two separate consortia investigating biomarkers of early psychosis, traveling subjects studies were performed as part of quality assurance procedures in order to assess the fidelity of data across sites. In the North American Prodromal Longitudinal Studies (NAPLS) Consortium, 8 normal subjects traveled to each of the 8 NAPLS sites and were tested twice at each

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site on the startle PPI paradigm. In preparation for a binational study, 10 healthy subjects were assessed twice in both San Diego and Mexico City. Intraclass correlations between and within sites were significant for PPI and startle response parameters, confirming the reliability of startle measures across sites in both consortia. There were between site differences in startle magnitude in the NAPLS study that did not appear to be related to methods or equipment. In planning multi-site studies, it is essential to institute quality assurance procedures early and establish between site reliability to assure comparable data across sites.

**Keywords**

Endophenotype; Reliability; Startle; Prepulse Inhibition

## Introduction

Prepulse inhibition (PPI) and reactivity of the acoustic startle response are widely used translational biomarkers in psychopathological research. PPI is an index of sensorimotor gating and is used in animal and human studies to understand brain disorders such as schizophrenia and Tourette's Disorder that are characterized by gating impairments in the neural substrates that underlie sensory information processing [1]. In the PPI paradigm, weak lead stimuli inhibit the startle response to intense, abrupt stimuli (acoustic, visual, tactile) [2]. PPI is typically reduced in individuals with schizophrenia [3], stable with repeated within site testing [4-10], heritable [11,12], and associated with genes of relevance to psychosis[13,14], suggesting its utility as an endophenotype and as a vulnerability marker for psychosis risk [15].

An increasing emphasis in schizophrenia research has been in the area of early detection and intervention. The use of biobehavioral markers such as PPI in the study of the prodromal phase of psychosis provides a means of not only identifying individuals at greatest risk for psychosis but also understanding neurodevelopmental abnormalities early in the course of illness that can contribute to better informed treatment [16]. Although it is possible to use empirically derived criteria for a prodromal psychosis syndrome [17] to identify individuals at increased risk of psychotic illness, the 2 year psychotic conversion rate is between 15-35% [18], making it difficult to recruit a sufficient number of subjects at any one site. Therefore, multisite studies are essential to attain sufficient statistical power to investigate the prodromal phase of illness.

For biomarkers such as PPI to be useful in multisite studies that are needed to increase statistical power, facilitate the identification of disease risk, increase the odds of finding uncommon genetic variation or identification of relevant subgroups however, the measures need to be stable with repeated assessment and reliable across sites [19]. Because differences in testing conditions and procedures across sites can introduce uncontrolled variance in experimental measures, it is essential to understand potential site differences and control variation across sites as much as possible. Although multisite studies have investigated PPI [19], to our knowledge, there are no published reports of between site reliability of startle measures using normal subjects traveling between sites. This study investigated the within-

and between-site reliability of PPI and startle reactivity in two consortia designed to identify vulnerability markers in early psychosis: The NAPLS (North American Prodromal Longitudinal Studies) Consortium and a UCMEXUS (University of California Institute for Mexico and the United States).

## Materials and Methods

### Participants

Participants included: 1) 8 healthy subjects recruited from each of the 8 NAPLS sites (Emory, Harvard, University of Calgary, UCLA, UCSD, UNC, Yale, Zucker Hillside) (age 19-30, 4 males and 4 females) and 2) 10 healthy subjects (ages 28-38, 4 males and 6 females), recruited from UCSD and the National Institute of Neurology and Neurosurgery (INNN) in Mexico City. All 9 institutions received approval from their individual ethics committees for the study. Subjects provided written informed consent after the procedures were fully explained. Subjects were excluded if they had the following: any concomitant medical or neurological illness, current substance abuse or dependence (excluding nicotine), any Axis I disorders (per Structured Interview for DSM-IV) or positive family history of psychosis.

### Acoustic startle paradigm

Equipment and procedures were identical at the 8 NAPLS sites as well as between UCSD and INNN. Manuals with equipment setup, testing procedures and instructions to subjects were developed in English and Spanish (for INNN). A meeting was held in Boston October 2009 to train all NAPLS sites; UCSD staff visited the Mexico site in September 2009 to train INNN using the same procedures [20].

Subjects were screened for hearing impairment (>45 dB 1000 Hz). Smokers were allowed to smoke up to 30 min prior to startle testing to avoid nicotine withdrawal or intoxication. A customized Startle-stimulus generating system (Grace Design Model m902 Amplifier and Neurobehavioral Systems Presentation software) developed by the UCSD site was used for all sites. The sound was calibrated at all sites using a Quest 210 Sound Level Meter and a custom-made PPI calibration session to ensure 70dB for background noise and 115dB for extended length startle bursts at each of the sites. Neurophysiologic recordings at NAPLS sites were performed using identical Biosemi systems and recording software (Biosemi, Amsterdam, Netherlands). For the UCMEXUS study, data were recorded using NeuroScan equipment and software (NuAmps Digital EEG Amplifier, Neuro Scan Labs, Sterling, VA). Electrodes (Ag/AgCl) were placed below and at the outer canthus of the right eye with resistances less than 10 kΩ [20]. Startle stimuli were presented binaurally through identical headphones (TDH-39P) at all sites. A 70 dB [A] broadband background noise was used with a pulse (115 dB [A], 40 ms noise burst) presented either alone or following (30, 60 or 120 ms interstimulus interval; ISI) a prepulse (86 dB [A], 20 ms noise burst). The paradigm began with a 5-minute acclimation period, then five pulse alone stimuli followed by 30 trials consisting six trials each of the three prepulse conditions and 12 pulse alone stimuli presented in a fixed, pseudorandom order. The paradigm ended with five more pulse alone stimuli for a total of 40 trials. EMG activity for both consortia was analyzed at UCSD using

Brain Vision Analyzer (Brain Vision LLC, Morrisville, NC) and high-pass filtered at 28Hz at 12dB/Oct. Waveforms were smoothed using a 40Hz 24dB/Oct low-pass filter. All trials were manually inspected for artifacts. Startle data were analyzed using wave-form averaging for each of the four different trial types within each block, after applying baseline correction and rectification of the data. The magnitude of the peak startle response (highest point relative to baseline between 30 and 120 ms after onset of startle stimulus) was determined. All subjects demonstrated a robust startle response to the first block of startle stimuli but subjects who demonstrated a relative lack of startle stimulus elicited eye blink to the second block of startle stimuli in any test session were excluded per established methods [20]. The following startle measures were examined: 1) reactivity, or the mean magnitude of response to pulse alone stimuli, and 2) prepulse inhibition (PPI), the percentage of change in startle magnitude to prepulse +pulse versus pulse-alone trials ((pulse –prepulse + pulse)/ pulse)*100). The stability of the startle measures between and within sites was assessed using intraclass correlations (ICC; Random, Consistency Model) and repeated measures analysis of variance (ANOVA) design. All subjects were tested twice at each site and traveled to the other sites within 3 months for NAPLS and within 1 year (Mean 5.5 months) for UCMEXUS. The order of testing was balanced across sites in both studies with a specified order for NAPLS subjects starting with the home site and within the 9 subjects who were included in the UCMEXUS study (5 at UCSD first, 4 at INNN first).

## Results

As shown in Table 1, within site ICCs of startle and PPI variables were significant across all reactivity and PPI conditions in both NAPLS and UCMEXUS (Table 1). Between-site analyses were similarly performed comparing time 1 to time 1 and time 2 to time 2 across sites. All but the 30 ms PPI ($p=0.056$) condition for UCMEXUS were significant. Finally, within- and between-site ICCs were calculated using both sessions at each of the sites and all were significant. In repeated measures ANOVA of PPI (NAPLS: 8 Sites X 2 sessions X 3 ISIs; UCMEXUS: 2 Sites X 2 sessions X 3 ISIs) there were no statistical site (NAPLS: $F[7,108]=0.45$, ns; UCMEXUS: $F[1,9]=0.51$, ns) or session (NAPLS: $F[1,108]=0.72$, ns; UCMEXUS: $F[1,9]=0.83$, ns) main or interaction effects supporting the within- and between-site reliability. In contrast, a repeated measures ANOVA of startle reactivity (NAPLS: 8 sites X 2 times X 3 blocks; UCMEXUS: 2 sites X 2 times X 3 blocks) revealed a significant site effect ($F[7,28]=2.46$, $p<0.05$) for the NAPLS study due to one site having greater startle amplitude relative to the other sites (see Figure 1), but no session or interaction effects. When NAPLS site 4 was removed from the analysis, the significant site effect was no longer present ($F[6,30]=1.97$, ns). The site main effect for UCMEXUS ($F[1,9]=0.48$, ns) was non-significant as were session and interaction effects.

## Discussion

This is the first report of between-site reliability of PPI and startle reactivity measured with traveling subjects. The present findings replicate previous studies that demonstrate within-site stability of startle measures in normal and schizophrenia spectrum subjects [4-10] and extend these findings to demonstrate measurement comparability across laboratories in two separate multisite studies using 2 different types of equipment for neurophysiologic

recording. It is likely that the standardization of equipment, protocols, training, analysis and quality assurance procedures across sites contributed to the observed consistency of startle data.

Although startle reactivity was stable both within- and between-sites, significant site differences were observed in the NAPLS study driven by larger startle amplitude at one of the sites, prompting a review of equipment settings, stimulus calibration, ambient acoustic noise, electrical noise, placement of electrodes, subject instructions, and testing environment across sites. A decibel meter from UCSD was mailed to the site in question (site 4) to assure the loudness of the startle stimuli was accurate and consistent across sites. No methodological or equipment differences were identified. Individual subject data revealed that three subjects had larger startle responses at site 4 (Figure 2), accounting for the site differences. One subject with a large startle response was first exposed to the startle stimuli at site 4, perhaps accounting for the larger response. Since each subject began their travels at their home site, it is unlikely that order effects account for the observed differences. Thus, despite institution of careful quality assurance procedures, identical subjects, methodology and equipment, site differences still occur and need to be examined and controlled for in biomarker studies. Future analyses of NAPLS consortium data will continue to examine site differences in reactivity and site will be used as a between subjects factor.

A limitation of the 2 studies is the relatively small sample size in each (NAPLS included 8 subjects tested at 8 sites and UCMEXUS included 10 subjects tested at 2 sites). The sample sizes, however, are consistent with the few traveling subjects studies performed to establish reliability of neuroimaging measures across sites [22,23]. Although future between-site biomarker reliability studies should ideally use more subjects, sending multiple subjects to different cities and countries for multiple testing sessions obviously presents financial and logistical challenges.

## Conclusion

In planning multi-site biomarker studies, it is essential to institute standardized quality assurance procedures prior to data collection of targeted research samples. The use of identical equipment, training and similar testing environments appears to be useful to minimize sources of cross-site variance in electrophysiological studies. The observed reliability of startle measures across laboratories provides support for the utility of these measures as biomarkers and endophenotypes in large multisite studies. Investigation and statistical control of potential site differences is essential in any multisite biomarker study.

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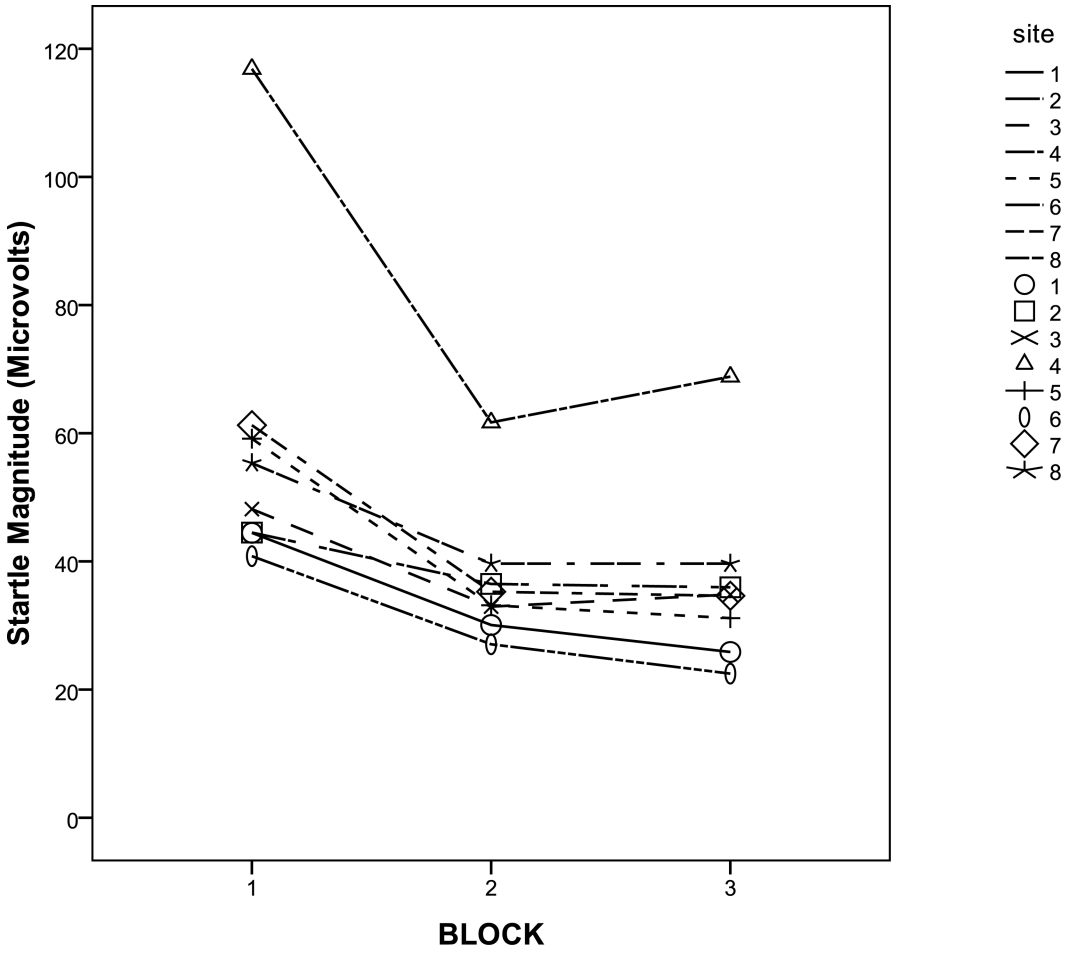


**Figure 1.**

Site differences in startle reactivity were evident in the NAPLS consortium. Data represents estimated marginal means that collapsed two test sessions at each site.

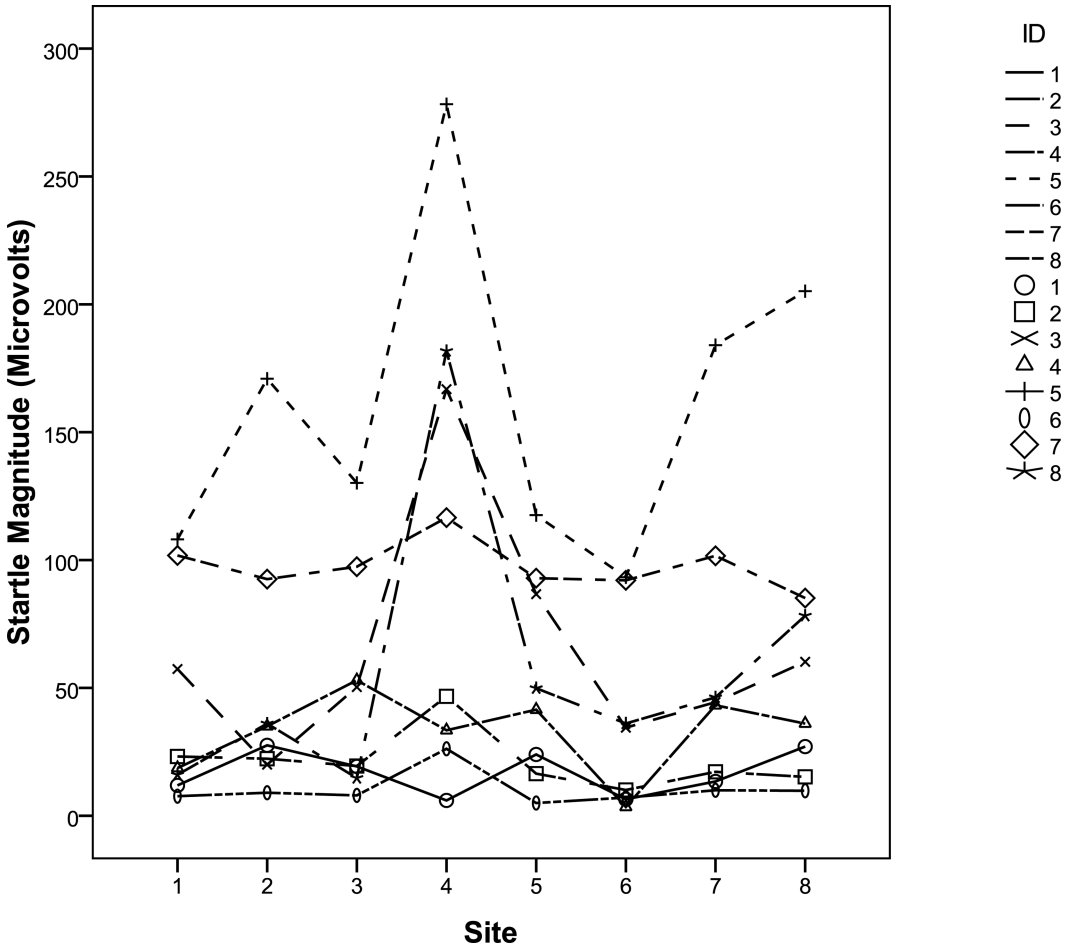


**Figure 2.**

Individual traveling subjects data from the NAPLS consortium demonstrates that 3 subjects accounted for the observed site differences. Data represents estimated marginal means that collapse both test sessions and 3 blocks of startle magnitude in response to pulse alone stimuli.

## Table 1

Intraclass Correlations (ICC – Random/Consistency Model) of startle reactivity and Prepulse Inhibition (PPI) within and between sites in the NAPLS and UCMEXUS studies.

| | NAPLS Within Site ICC | NAPLS Between Sites ICC | NAPLS Within and Between Site ICC | UCMEXUS Within Site ICC | UCMEXUS Between Sites ICC | UCMEXUS Within and Between Site ICC |
|---|---|---|---|---|---|---|
| **Startle Reactivity** | | | | | | |
| **Block 1** | 0.43*** | 0.46*** | 0.51*** | 0.85*** | 0.43* | 0.60*** |
| **Block 2** | 0.80*** | 0.76*** | 0.79*** | 0.88*** | 0.52** | 0.65*** |
| **%PPI** | | | | | | |
| **PPI 30 ms** | 0.67*** | 0.50*** | 0.57*** | 0.63* | 0.31 | 0.38** |
| **PPI 60 ms** | 0.60*** | 0.57*** | 0.48*** | 0.67** | 0.80*** | 0.52*** |
| **PPI 120 ms** | 0.68*** | 0.73*** | 0.78*** | 0.59** | 0.80*** | 0.69*** |

*** **p < 0.001**

** **p<0.01**

* **p<0.05**