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## Background

- Autism spectrum disorder (ASD) is characterized by early emerging deficits in social communication and restricted/repetitive behavior; in addition to these core symptoms, individuals with ASD often exhibit atypical arousal regulation.
- The locus coeruleus-norepinephrine (LC-NE) system has been linked to arousal and attention and is thought to be indexed by the pupillary light reflex (PLR); furthermore, the PLR reflex arc is acetylcholine-dependent, and the cholinergic system has been found to be altered in individuals with ASD.
- As the PLR is not under conscious control, it is a good candidate for a biomarker for infants or minimally verbal populations.
- Prior research has shown that adolescents with ASD exhibit a hyposensitive PLR; however, infants at high risk for ASD display a hypersensitive PLR.
- The developmental trajectory of the PLR, associated neural response, and relation to social function remain unclear.
- Our objectives were to investigate relationships among PLR, electrophysiological (EEG) brain correlates, and subthreshold autistic traits in typically developing adults.

## Method

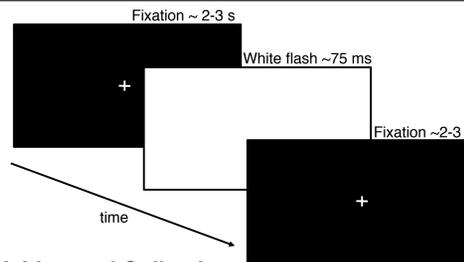
### Sample:

N	Age (med)	Max Age	Min Age
24	24	33	19

### Experimental Paradigm:

- 50 consecutive PLR trials were presented.

**Figure 1. Trial Structure.** Each trial lasted for approximately 6 s and consisted of a white central fixation on a black background that flashed white for approximately 75 ms, with a random onset, such that flashes were separated by at least 2-3 s.



### EEG and Eye Tracking (ET) Data Acquisition and Collection:

- EEG recorded at 1000 Hz with a 128-channel Hydrocel Geodesic Sensor net.
- ET data collected using an Eyelink-1000 remote camera system at 500 and 1000 Hz.

### EEG Preprocessing:

- Data were cleaned utilizing PREP pipeline with line noise removal, a high-pass filter, and then re-referenced to average reference.
- Data were filtered from 0.1-100 Hz.

### ERP Analysis:

- Data were segmented from -100 ms before the flash and 300 ms after, baseline corrected, and artifact detected.
- P100 (30-100ms) and N100 (100-200ms) were extracted from frontal central electrodes (see Figure 2).
- Index of amplified neural response was created by taking the difference between N1 minimum amplitude and P1 maximum amplitude to the flash (see Figure 3).

### PLR Analysis:

- Trials were hand coded to identify maximum pupil constriction after the flash, and PLR latency and relative pupil constriction were calculated.
- Trials where pupil was obscured due to blinking, such that dependent variables were not identifiable, were discarded.

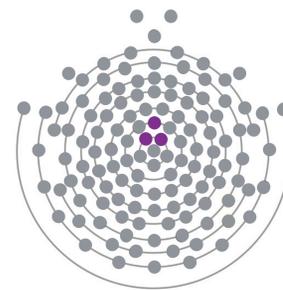
### Behavioral Data:

- Autistic traits were collected using the Autism Quotient (AQ) and the Social Responsiveness Scale (SRS) self-report questionnaires with a focus on the Restricted and Repetitive Behaviors (RBB) and Social Awareness (SA) subscale scores.

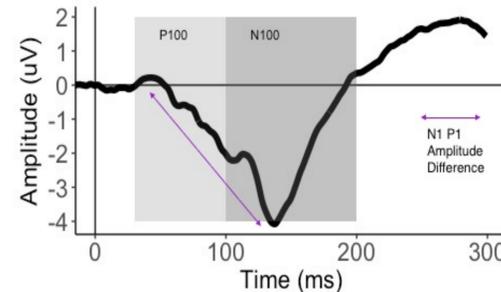
### Statistical Analyses:

- Correlations were run between variables of pupillary light reflex, neural response to the flash, and autistic traits.
- A structural equation model (SEM) was constructed to investigate the mediating effects of pupillary light reflex latency on the relationship between neural response and autistic traits.

## Method



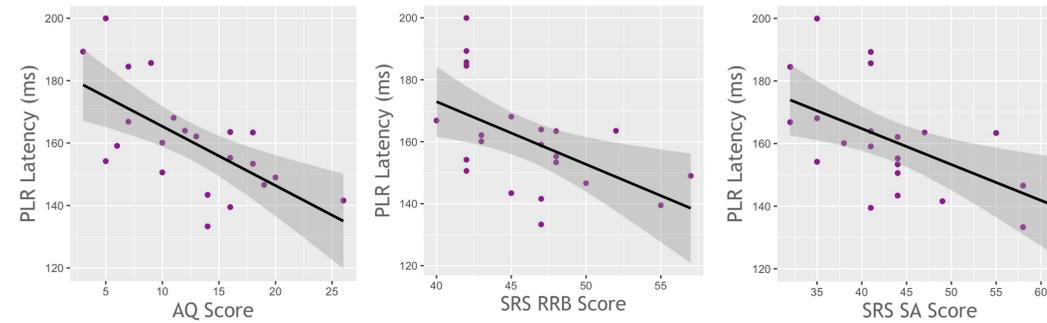
**Figure 2. Recording sites.** Frontal central P1 and N1 were extracted from electrode cluster depicted.



**Figure 3. ERP.** Grand average of P1 and N1 response to flash at frontal central electrodes.

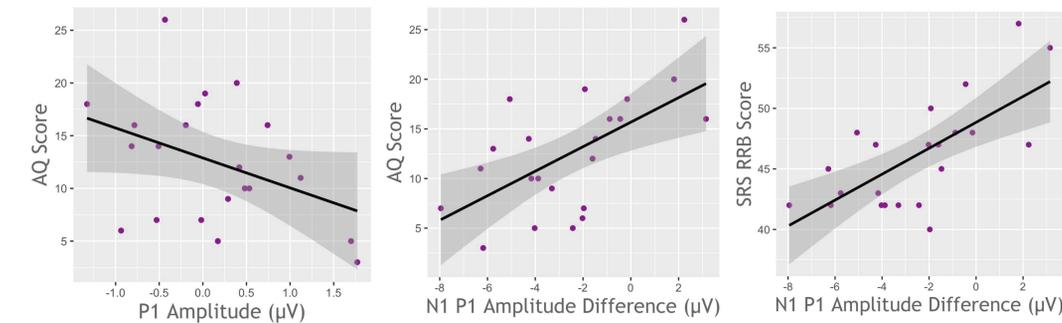
## Preliminary Results

### Pupillary Light Reflex and Behavior



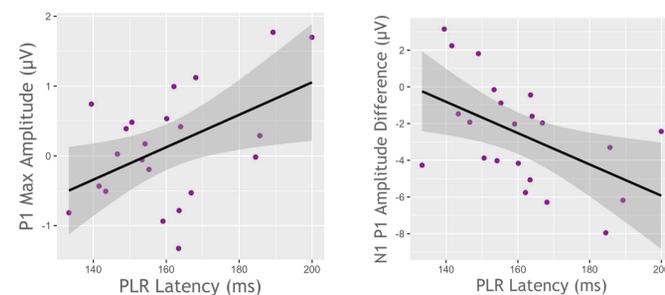
**Figure 4. Relations between pupillary response and autistic traits.** A faster pupillary light reflex was positively correlated with greater autistic traits as measured by the AQ ( $r=-.65, p<.001$ ) and more severe traits of RRB ( $r=-.53, p<.01$ ) and SA ( $r=-.56, p<.01$ ).

### Behavior and Neural Response



**Figure 5. Relations between autistic traits and neural response to flash.** Greater autistic traits as measured by the AQ associated with decreased P1 amplitude ( $r=-.547, p<.01$ ) to the flash, as well as a greater difference between amplitude of the N1 and P1 to the flash ( $r=.55, p<.01$ ). More severe RRB as measured by the SRS associated with a larger difference between N1 and P1 amplitude to the flash ( $r=.65, p<.001$ ).

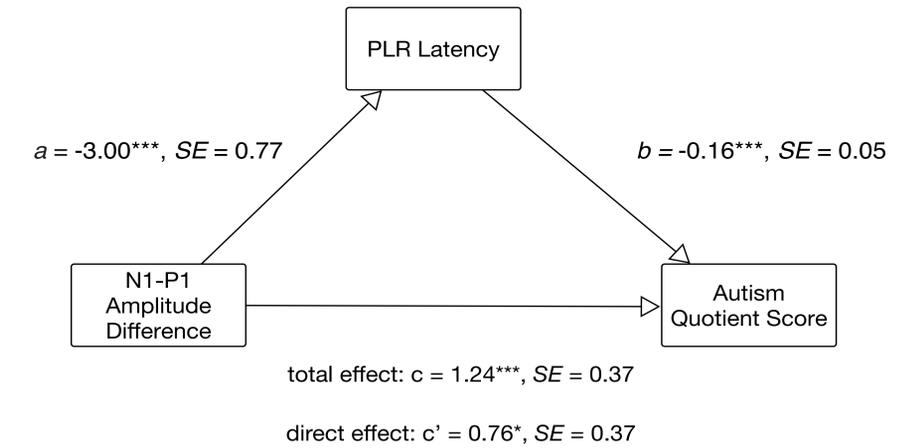
### Neural Response and Pupillary Light Reflex



**Figure 6. Relations between neural response to flash and pupillary light reflex.** Faster pupillary light reflex was associated with larger P1 amplitude to flash ( $r=.49, p<.05$ ), as well as greater difference in N1-P1 response ( $r=-.51, p<.05$ ).

## Preliminary Results

### Pupillary Light Reflex, Neural Response, and Behavior



**Figure 7. Mediation model.** Pathway of PLR latency mediating difference in N1-P1 response to flash and autistic traits, including standardized regression coefficients ( $*p<.05, **p<.01, ***p<.001$ ).

		Estimate	P-Value	Lower CI	Upper CI
Indirect effect	$a*b$	0.47	0.02	0.16	1.02
Direct effect	$c'$	0.76	0.04	0.05	1.54
Total effect	$c'+(a*b)$	1.24	0.00	0.55	2.00

**Figure 8. Table of standardized coefficients.** Standardized coefficients were obtained using a bootstrap estimation of 10,000 resamples at the 2.5<sup>th</sup> and 97.5<sup>th</sup> percentiles.

As illustrated in Figure 6, the structural equation model showed acceptable fit on multiple SEM fit statistics and indices: Comparative fit index=1.00; Root mean square error of approximation=0.00; Tucker-Lewis index=1.00. Approximately 53% of variance in autism quotient score was accounted for by the model ( $R^2 = 0.53$ ). The indirect effect accounted for 61% of the direct effect, suggesting a strong partial mediation.

## Conclusions

- This was the first study to investigate the neural correlates of the pupillary light reflex in relation to autistic traits in typically developing adults.
- Preliminary results reveal that a hypersensitive PLR is associated with more severe autistic traits.
- Greater restricted repetitive behaviors and AQ scores were associated with a larger difference in N1 and P1 amplitude, suggesting that amplified neural response of low level sensory processing is associated with greater autistic traits.
- Amplified neural response as indexed by a greater difference in N1 and P1 amplitude to the flash was associated with a hypersensitive PLR.
- Further investigation using SEM showed that amplified neural response contributes to autistic traits, but having a hypersensitive PLR significantly moderates this relationship, such that amplified neural response is associated with a faster PLR, which then contributes to more severe autistic traits; thus, the LC-NE system may be providing rapid input to low level sensory processing that is significantly contributing to the presentation of autistic traits in the broader autism phenotype.
- The relationship between PLR, early sensory components, and autistic traits may reflect brainstem modulated cortical gain. This suggests that there may be overlapping cortical and subcortical networks that modulate variability in autistic traits.
- In line with research showing that infants at risk for autism have a hypersensitive PLR, our findings suggest that a faster PLR is associated with greater autistic traits and mediates the relationship between specific brain response to the PLR and autistic traits.