

# Exploring Individual and Sex Differences in Trace Eyeblink Conditioning (EBC) in 4- to 6-year-olds



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## Introduction

- Eyeblink Conditioning (EBC) is a form of Pavlovian conditioning that uses tone and air puff pairings to over time develop a learned association of the two stimuli within a subject.
- Trace EBC is a novel technique used to gain a better understanding of hippocampal and cerebral-dependent learning that has been previously employed in both adults and animal subjects (Takehara, Kawahara, and Kirino 2003); (Cheng et al. 2008);(Vieites et al. 2020).
- This study used Trace EBC to evaluate developmental changes in associative learning using the following metrics:
  - **Conditioned Response** (CR%) defined as the number of Eye Blinks occurring during trace period following the presentation of tone in paired trials and tone alone trials and prior to onset of unconditioned stimulus in paired trials - *Accuracy*
  - **Onset Latency** defined as onset time of blinks during paired and tone alone trials - *Precision*

## Objectives

**Aim-1:** Examine individual differences in accuracy across blocks as measured through Conditioned Response Percentage (CR%)

**Aim-2:** Examine individual differences in precision across blocks as measured through changes in SD of Onset Latency

## Methods

- **Participants:** Sixty-seven typically developing 4- to 6- years old’s completed a trace EBC task.
- **Task:** conditioned stimulus presentation (i.e., tone) followed by a 500-ms trace period of no stimulus presentation and a subsequent unconditioned stimulus (i.e., air puff to eye). Responses to stimuli (i.e., eye blinks) were measured. Stimuli presentation lasted approximately 15 minutes.
  - The EBC task consisted of 104 trials, one block of 4 practice trials to acclimate children to the stimuli and 10 blocks of test trials. Each test block consisted of 10 trials: 8- paired tone and air puff trials, 1- puff alone trial administered halfway through paired trials, and a final tone alone trial.

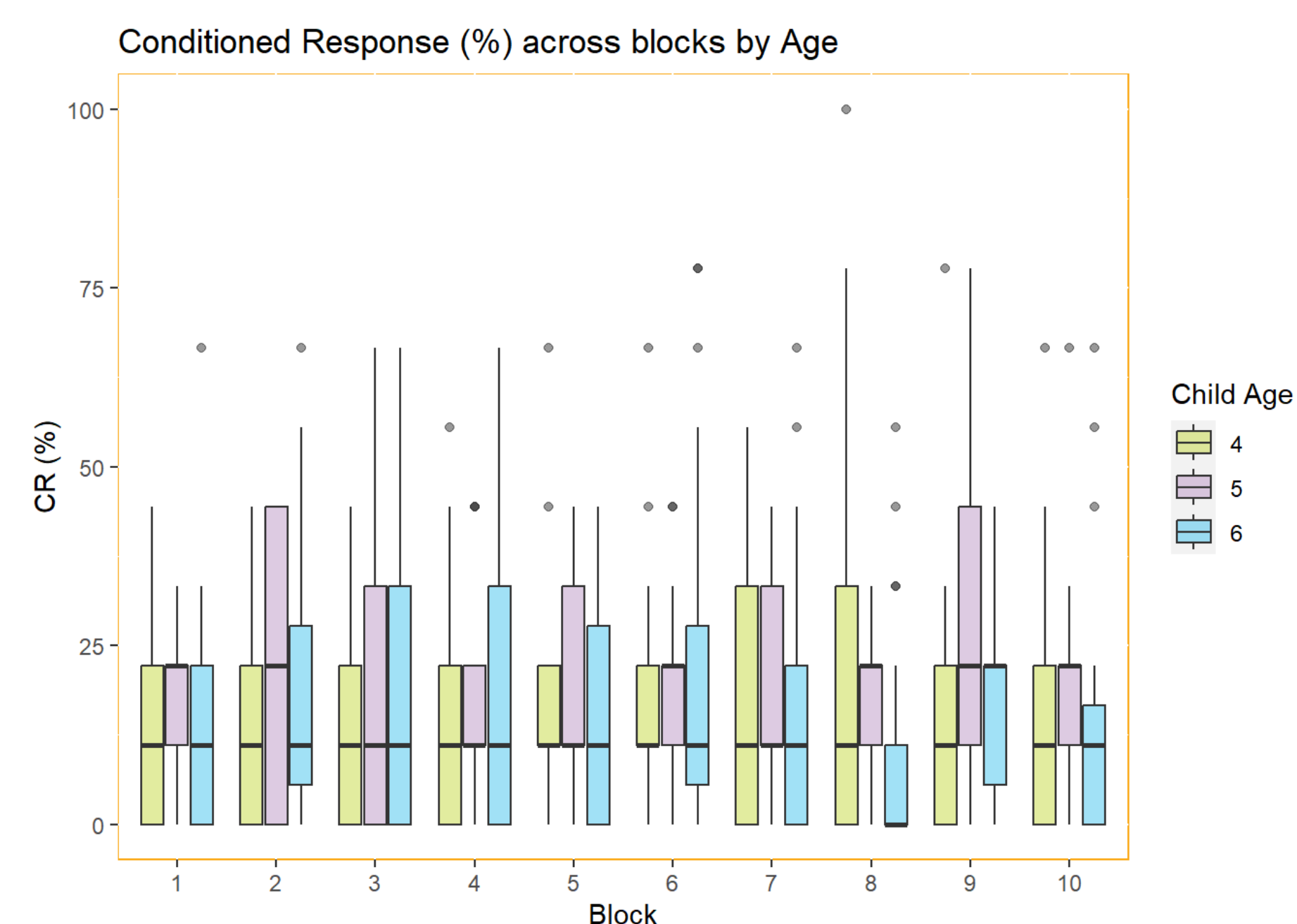
**Analysis:** Linear Mixed Effects models were implemented using *lme4* package (Bates et al. 2023)

## Results- Accuracy

**No age related differences in *accuracy* on Trace EBC task as measured through Conditioned Response (%) across blocks**

Type III AOV with Satterthwaite’s Method: CR (%) ~ Age \* Block

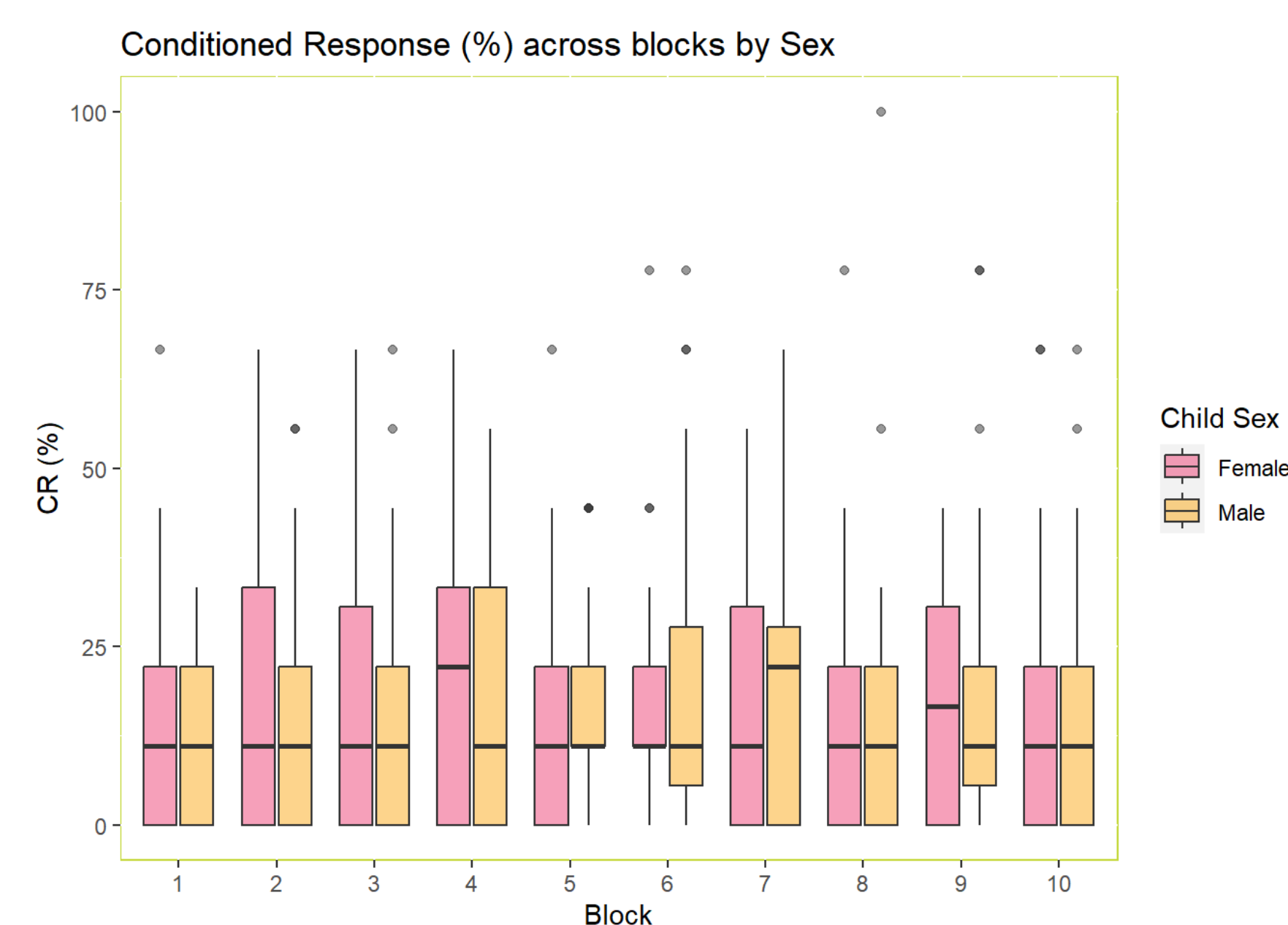
	Sum Sq	Mean Sq	DF	F Value	Pr(>F)
Block	2932.7	325.85	9	1.6538	0.09747
Child Age	66.5	66.48	1	0.3374	0.56350
Block X Child Age	3279.8	364.42	9	1.8495	0.05735



**No sex related differences in *accuracy* on Trace EBC task as measured through Conditioned Response (%) across blocks**

Type III AOV with Satterthwaite’s Method: CR (%) ~ Sex \* Block

	Sum Sq	Mean Sq	DF	F Value	Pr(>F)
Block	1354.60	150.511	9	0.7455	0.6671
Child Sex	12.23	12.235	1	0.0606	0.8064
Block X Child Sex	872.46	96.940	9	0.4802	0.8882

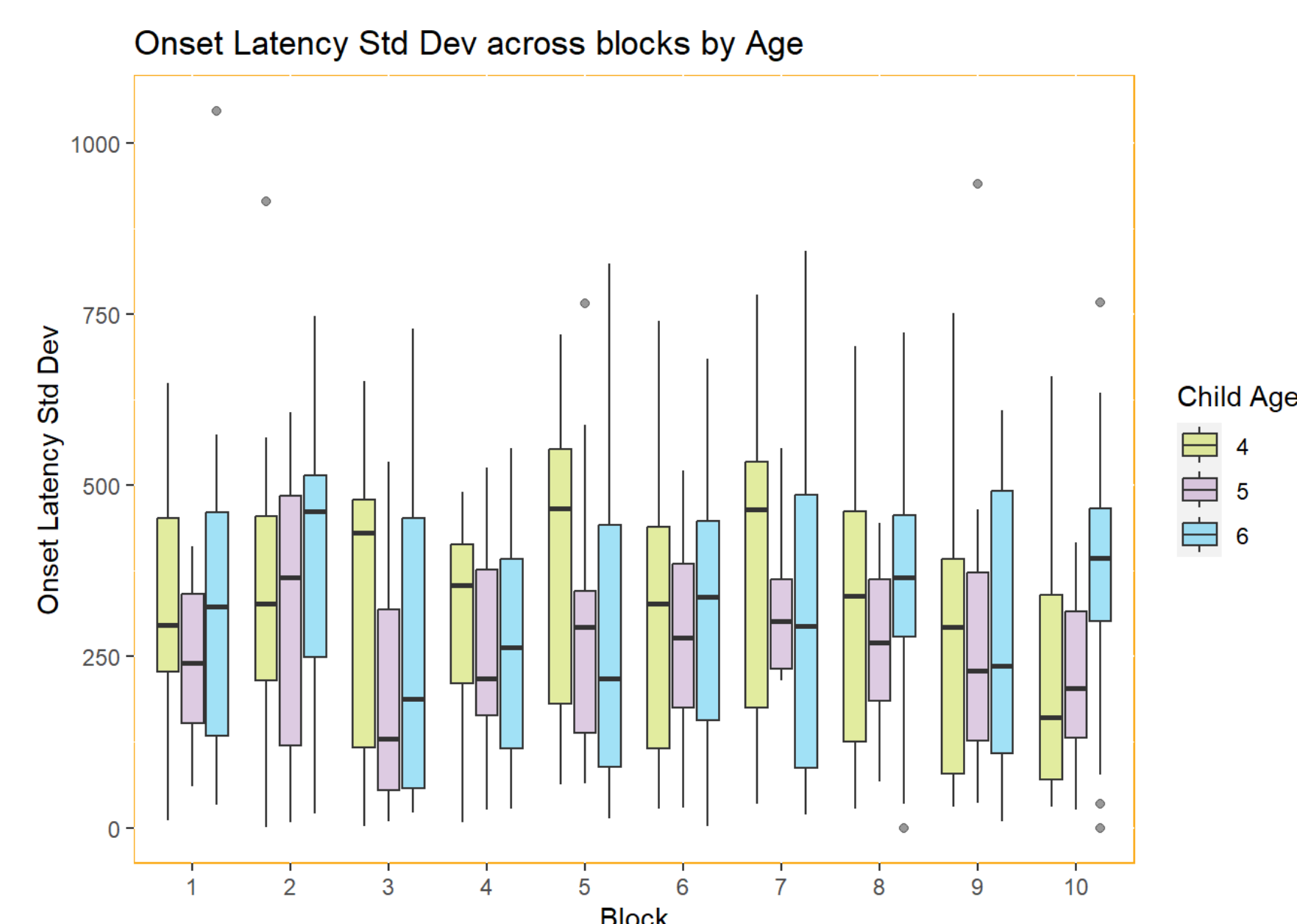


## Results- Precision

**No age related differences in *precision* on Trace EBC task as measured through Onset Latency (SD) across blocks**

Type III AOV with Satterthwaite’s Method: Onset Latency ~ Age \* Block

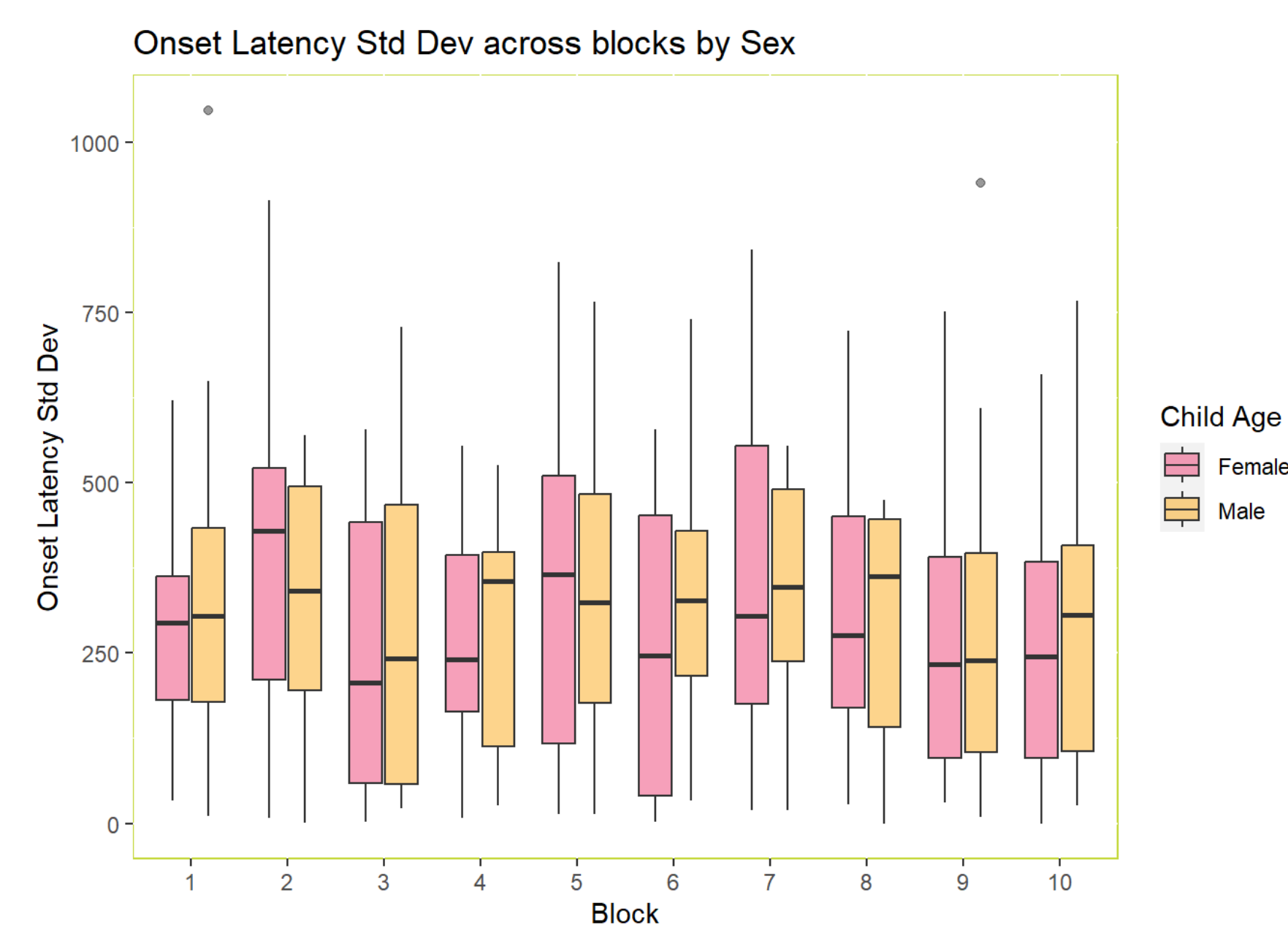
	Sum Sq	Mean Sq	DF	F Value	Pr(>F)
Block	315785	35087	9	1.7857	0.06943
Child Age	2663	2663	1	0.1355	0.71425
Block X Child Age	298441	33160	9	1.6876	0.09025



**No sex related differences in *precision* on Trace EBC task as measured through Onset Latency (SD) across blocks**

Type III AOV with Satterthwaite’s Method: Onset Latency ~ Sex \* Block

	Sum Sq	Mean Sq	DF	F Value	Pr(>F)
Block	358550	39839	9	1.95750	0.04112*
Child Sex	146	146	1	0.0072	0.93259
Block X Child Sex	107262	11918	9	0.5908	0.80470



## Discussion

- The findings suggest no age or sex related differences in both precision and accuracy in a Trace Eyeblink Conditioning task as measured through conditioned responses (%) and blink onset latency.
- As seen through Figure 1 and Figure 2, children in this age range were performing below chance in CR(%) suggesting that they were not learning the association across trial blocks.
- The lack of changes in standard deviation across trial blocks indicates children were also not getting more precise with their blinks across trial blocks.
- Future directions: Are there individual differences in Delay EBC compared to Trace EBC? At what age do children develop the association?

## References

- Bates, Douglas, Martin Maechler, Ben Bolker [aut, cre, Steven Walker, Rune Haubo Bojesen Christensen, Henrik Singmann, et al. 2023. “lme4: Linear Mixed-Effects Models Using ‘Eigen’ and S4.” <https://CRAN.R-project.org/package=lme4>.
- Cheng, Dominic T., John F. Disterhoft, John M. Power, Deborah A. Ellis, and John E. Desmond. 2008. “Neural Substrates Underlying Human Delay and Trace Eyeblink Conditioning.” *Proceedings of the National Academy of Sciences of the United States of America* 105 (23): 8108–13. <https://doi.org/10.1073/pnas.0800374105>.
- Takehara, Kaori, Shigenori Kawahara, and Yutaka Kirino. 2003. “Time-Dependent Reorganization of the Brain Components Underlying Memory Retention in Trace Eyeblink Conditioning.” *The Journal of Neuroscience* 23 (30): 9897–9905. <https://doi.org/10.1523/JNEUROSCI.23-30-09897.2003>.
- Vieites, Vanessa, Shannon M. Pruden, Anna Shusterman, and Bethany C. Reeb-Sutherland. 2020. “Using Hippocampal-Dependent Eyeblink Conditioning to Predict Individual Differences in Spatial Reorientation Strategies in 3- to 6-Year-Olds.” *Developmental Science* 23 (1): e12867. <https://doi.org/10.1111/desc.12867>.

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