Maturation of Temporal Saccade Prediction from Childhood to Adulthood: Predictive Saccades, Reduced Pupil Size, and Blink Synchronization

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When presented with a periodic stimulus, humans spontaneously adjust their movements from reacting to predicting the timing of its arrival, but little is known about how this sensorimotor adaptation changes across development. To investigate this, we analyzed saccade behavior in 114 healthy humans (ages 6–24 years) performing the visual metronome task, who were instructed to move their eyes in time with a visual target that alternated between two known locations at a fixed rate, and we compared their behavior to performance in a random task, where target onsets were randomized across five interstimulus intervals (ISIs) and thus the timing of appearance was unknown. Saccades initiated before registration of the visual target, thus in anticipation of its appearance, were labeled predictive [saccade reaction time (SRT) < 90 ms] and saccades that were made in reaction to its appearance were labeled reactive (SRT > 90 ms). Eye-tracking behavior including saccadic metrics (e.g., peak velocity, amplitude), pupil size following saccade to target, and blink behavior all varied as a function of predicting or reacting to periodic targets. Compared with reactive saccades, predictive saccades had a lower peak velocity, a hypometric amplitude, smaller pupil size, and a reduced probability of blink occurrence before target appearance. The percentage of predictive and reactive saccades changed inversely from ages 8–16, at which they reached adult-levels of behavior. Differences in predictive saccades for fast and slow target rates are interpreted by differential maturation of cerebellar-thalamic-striatal pathways.

Key words: blink rate; development; eye movements; pupil diameter; rhythm; timing

Significance Statement

From the first moments of life, humans are exposed to rhythm (i.e., mother’s heartbeat in utero), but the timeline of brain development to promote the identification and anticipation of a rhythmic stimulus, known as temporal prediction, remains unknown. Here, we used saccade reaction time (SRT) in the visual metronome task to differentiate between temporally predictive and reactive responses to a target that alternated at a fixed rate in humans aged 6–24. Periods of age-related change varied little by target rate, with matured predictive performance evident by mid-adolescence for fast and slow rates. A strong correlation among saccade, pupil, and blink responses during target prediction provides evidence of oculomotor coordination and dampened noradrenergic neuronal activity when generating rhythmic motor responses.

Introduction

From an early age, we are exposed to rhythms in our environment. In the womb, we listen to our mother’s heartbeat, and as an infant, we find comfort in the swaying of a rocking chair. Attending to stimuli with periodicity has been shown to induce physiological relaxation (Johnson and Trawick, 1938; Brauchli et al., 1995). Identifying rhythmicity and shifting motor behavior from reacting to predicting an upcoming stimulus, known as temporal prediction, occurs spontaneously and is fundamental for adaptive sensorimotor behavior (Fitch, 2013; van der Steen and Keller, 2013). Neural maintenance of an interstimulus...
interval (ISI) and coordination of endogenously driven motor commands to anticipate its arrival are required to predict rhythmic stimuli (Repp and Su, 2013). Brain regions important for timing-related signals include the frontal cortex (Maimon and Assad, 2006; Jazayeri and Shadlen, 2015), basal ganglia (Lee and Assad, 2003; Turner and Anderson, 2005), cerebellum (Ashtore and Sommer, 2013), and thalamus (Matsuoka and Tanaka, 2021). Neuronal firing in these areas correlates with the ISI of a periodic target, and pharmacological silencing impairs predictive movements (Buhusi and Meck, 2005; Merchant et al., 2013; Matsuoka and Tanaka, 2021; Tanaka et al., 2021). Additionally, an impaired ability to anticipate rhythmic stimuli to respond predictively is evident in multiple brain-related disorders, including developmental coordination disorder (Debrabant et al., 2013), developmental dyslexia (Lukasova et al., 2016), Huntington’s disease (Vaca-Palomares et al., 2019), and spinocerebellar atrophy (Bares et al., 2007). Despite the important role temporal prediction plays in essential sensorimotor learning, and its clinical relevance to various neurologic conditions, its developmental timeline in normal controls remains unknown.

One commonly used method of measuring temporal prediction is the visual metronome task (Stark et al., 1962), where participants are asked to move their eyes in time with a square-wave target that alternates at a fixed rate between two known locations. Saccades initiated before neural registration of the visual target, in anticipation of its upcoming temporal appearance, are categorized as predictive (saccade reaction time (SRT) < 90 ms; Fischer et al., 1997; Munoz et al., 1998; Lee et al., 2016). Saccades initiated in response to the visual target are considered reactive (SRT > 90 ms). Analysis of saccade behavior in this task is particularly useful for estimation of brain functioning of areas relevant to temporal prediction given that they overlap with brain regions required for saccade initiation (i.e., brainstem, cerebellum, superior colliculus (SC), thalamus, basal ganglia and frontal cortex). Based on a handful of studies using the visual metronome task in children [2–53 child participants per study (age range: 4–15 years)], it is clear that compared with adults, children make fewer predictive saccades and on average, have SRTs that are on average, 100–150 ms slower (Kowler and Martins, 1982; Ross and Ross, 1987; Ross et al., 1994; Lukasova et al., 2018). However, given the small sample sizes of these studies and paucity of adolescent participant data, normal predictive saccade performance from early childhood to young adulthood has yet to be characterized.

To investigate temporal predictive performance across developmental ages, we measured predictive saccades in 114 healthy participants, aged 6–24 years, performing the metronome task at five different target rates. Because pupil size has reliably been shown to correlate with noradrenergic modulation by the locus coeruleus norepinephrine (LC-NE) system (Almas et al., 2014; Murphy et al., 2014), we sought to test whether previous reports of autonomic relaxation while attending to rhythm (Johnson and Trawick, 1938; Brauchli et al., 1995) could be replicated based on evidence of a decreased pupil size when participants predict versus react to targets, indicative of dampened LC-NE signaling. Finally, we tested whether eyeblink timing varied based on participant prediction or reaction to targets. To ensure that age-related changes in performance were not driven by improvements in processing speed or oculomotor kinematics, participants also performed a random task, which is identical to the metronome task, except the timing of the target is randomly selected for each target step, making the target steps unpredictable.

Materials and Methods

Study participants

The research protocol was approved by Queen’s University Faculty of Health Sciences (protocol ID: PHYS-007-97). A total of 118 participants completed the study (mean age: 14.9 ± 3.8 years; 55.1% female). Adults aged ≥18 years provided their written consent. Children aged <18 years gave their verbal assent and parents/legal guardians provided written informed consent. All participants were free of neurologic, psychiatric or ocular diagnoses and were not taking psychotropic medications, and participants had normal or corrected-to-normal vision.

Recording of eye movements

Participants were seated 60 cm away from a computer screen with their head position stabilized by a fixed head mount and chin rest at a viewing angle of 32 × 26°. Experimental stimuli were presented as 0.5° diameter sized circular red targets (luminance measured 44 cd/m² with an optometer for LCD monitors) on a 17-inch LCD iiYama Proline monitor at a screen resolution of 1280 × 1024 pixels with a 60-Hz refresh rate. Experiments were completed in the dark with the only light source being the experimentally controlled stimuli. Monocular eye tracking was conducted (right eye position was measured) using the video-based eye tracker EyeLink 1000 Plus in 83 participants and the EyeLink II in 35 participants (unpublished data previously collected in the lab that used the same experimental task; SR-Research Ltd.). Both cameras of the EyeLink systems had a sampling rate of 500 Hz and a mean eye position accuracy of ≤0.5°. Eye movements to a nine-point calibration grid were performed by each subject before each experiment, with eye position accuracy within 1° of the visual target considered acceptable. Eye position was reassessed for drift after every five trials of target presentation and calibration was performed again if needed.

Experimental design

Participants were cued with a red central fixation point (FP) on a blank screen that had a random offset (i.e., the FP disappeared) between 1 and 1.5 s. Coincident with the disappearance of the FP, a peripheral target appeared 10° right or left from the central FP, on the horizontal axis. The target alternated between the two fixed locations on the horizontal plane for a total of 12 target steps (i.e., six in each direction; see Fig. 1A for task design). In the visual metronome task, a square-wave target alternated consistently at one of the five target rates (0.66, 0.8, 1, 1.33, and 2 Hz). These five target rates correspond to the following ISIs: 1500, 1250, 1000, 750, and 500 ms, respectively. Participants were instructed to move their eyes in time with the jumping target given both the target location and timing of the next target were predictable after the first presentation. In the random task, one of the five ISIs used in the metronome task was randomly used for each target step, such that the target location was predictable, but the timing of the next target appearance was unpredictable. The randomization was set so that the same ISI could not appear more than three times in a row. Thus, the only difference between the metronome and random task was the temporal predictability of target appearance. Participants were offered a break in between the two saccade tasks to assure alertness. The order of the metronome and random tasks was counterbalanced across participants. All stimulus timings were verified independently with a photosensor.

Eye-tracking analysis

Offline analyses of eye-tracking data were performed using MATLAB version R2019b (MathWorks). Saccades were identified based on their instantaneous velocity which was calculated on a three-point moving average of x and y eye positions in degrees. When the instantaneous velocity exceeded 2 SD above the mean fixation velocity (defined as <50%/s) for at least five continuous points, a saccade was labeled.

SRT

SRT was computed by subtracting the time of peripheral target appearance from the time of primary saccade onset. Saccades were labeled as predictive, express, and regular based on their SRTs, being <90, 90–120, and >120 ms, respectively (Fig. 1B,C; Fischer and Ramsperger, 1984;
Fischer and Weber, 1993; Dorris and Munoz, 1998; Munoz et al., 1998). The 90-ms cutoff for predictive saccades was based on data by Munoz et al. (1998) which demonstrated that pro-saccades to one of two potential target locations were correct only 50% of the time when initiated before 90 ms after target appearance, whereas those with an SRT > 90 ms were correct 95% of the time. Therefore, 90 ms is the lower limit of time to allow visual processing and motor reaction to a peripheral external target, and saccades with SRTs below this cutoff are internally generated. Express and regular saccades are both reactive (e.g., made in response to a visual target), but have unique SRT distributions. Express saccades are the fastest visually evoked eye-movements and are generated when the incoming visual transient signal to the SC is transformed directly into a saccade motor command (Edelman and Keller, 1996; Dorris et al., 1997). Previous research supports that the express saccade epoch varies based on the specific parameters of an eye-tracking task, for example variations to target luminance can alter the timing of visual response latencies in the SC (Marino et al., 2015). Moreover, it is known that variables including target eccentricity, target predictability, previous training, and the presence or absence of a gap between fixation and target appearance all influence the probability of express saccades in a given behavioral task (Weber et al., 1992; Fischer and Weber, 1993; Paré and Munoz, 1996; Dorris and Munoz, 1998). Thereby we sought to quantify the SRT range of express saccades in the metronome and random task, respectively, and later assess the effect of target properties (e.g., rate) on their occurrence. We plotted the distribution of saccades with a SRT > 90 ms in the metronome and random task is bimodal, with an express saccade epoch from 90 to 120 ms (solid light blue line) and regular saccade SRTs being > 120 ms in both task conditions. The BCs for the distribution of SRTs > 90 ms in the metronome and random task were 0.63 (skewness = 4.7; kurtosis = 33.5) and 0.54 (skewness = 4.3; kurtosis = 33.8), respectively. A, In the metronome task (first developed by Stark et al., 1962), participants were cued with a central FP with a random interval offset (1000–1500 ms) followed by 12 targets that alternate 10° right (R) and left (L) from center at a fixed target rate. Five target rates (0.66, 0.8, 1.0, 1.33, 2.0 Hz) were delivered over five blocks of trials with 12 targets each (60 targets per stimulus rate), with a pseudorandom trial order. These target rates correspond to an ISI of 1500, 1250, 1000, 750, and 500 ms, respectively. Participants were instructed to move their eyes in time with the targets. In the random task, target rates were randomized such that the participant could not anticipate the timing of the upcoming target while all other aspects of the task were held constant. B, Schematic of the characterization of saccades by SRT: predictive (SRT < 90 ms) in dark blue, express (SRT: 90–120 ms) in light blue, and regular (SRT > 120 ms) in red. C, Example eye position data collected from a single subject over a single trial. Colored bars correspond with the type of saccade generated according to its SRT toward the alternating target. D, The distribution of saccades with a SRT > 90 ms in the metronome and random task is bimodal, with an express saccade epoch from 90 to 120 ms (solid light blue line) and regular saccade SRTs being > 120 ms in both task conditions. The BCs for the distribution of SRTs > 90 ms in the metronome and random task were 0.63 (skewness = 4.7; kurtosis = 33.5) and 0.54 (skewness = 4.3; kurtosis = 33.8), respectively. E, Histogram of participants’ ages in the metronome and random task that were included for experimental analysis (bin width = one year). White boxes represent female participants and dark blue boxes represent males.
Saccade metrics
Saccadic amplitude and peak velocity were analyzed for predictive, express, and regular saccades to all target steps made in the metronome task. To ensure quality of saccade data, we removed saccades with a maximum peak velocity value above 1000°/s (N = 81/34,497; 0.23% of saccades), while still including any remaining saccades in the trial if they had a peak velocity below 1000°/s. To compare the main sequence for predictive, express, and regular saccades, peak velocity versus amplitude were plotted for each saccade type. A square root model (y = V / x) was used to fit the main sequence data, based on the results of a recent paper by Gibaldi and Sabatini (2021), which demonstrated that this model is highly robust for characterizing the main sequence of saccades with eccentricities between 5° and 20°. Metronome main sequence fits were derived at a group level (e.g., all saccades in the metronome task combined and categorized by saccade type) and at an individual subject level (e.g., an individual subject’s model coefficients for predictive, express, and regular saccades generated in the metronome task). Square root model coefficients were then analyzed by saccade type and across participants’ age to test whether (1) the model fit for the main sequence varied by saccade type, and (2) whether the main sequence for each saccade type varied with participant age.

Pupil size
To evaluate whether physiological arousal varied based on the reaction time to the alternating target, we analyzed pupil size in the metronome task. Pupil size, recorded every 2 ms, was averaged over a 200 ms epoch following the end of a saccade, provided the participant remained fixated on that location for a minimum of 200 ms. This window length was selected to avoid any pupil size changes induced by the pupillary light reflex if the target appeared post-saccade (i.e., a source of light stimulates pupil constriction ~300 ms following its appearance on a dark background; Ellis, 1981; Wang et al., 2018). Pupil trials that met the minimum fixation length criterion were excluded from analysis if a blink or saccade occurred during peripheral target fixation, if the timing of fixation onset exceeded the minimum gap (100 ms) between fixation and next target appearance, and if pupil velocity fell outside the range of −5000°/s to 5000°/s. This left 19175 viable pupil trials in the metronome task to analyze (2 Hz: 4093 trials; 1.33 Hz: 3904; 1 Hz: 3845 trials; 0.8 Hz: 3794 trials; and 0.66 Hz: 3539 trials), and these trials were divided into three categories based on SRT: predictive (6094 trials), express (2384 trials), and regular (10697 trials). To query whether pupil size varied based on prediction or reaction to the rhythmic target, two analyses were performed. First, a one-way ANOVA was computed to test for a main effect of saccade type (predictive, express, and regular) on pupil size. Second, saccade types were combined to test whether SRT correlated with pupil size using a Spearman rank correlation. To test whether pupil size varied across the developmental age range, baseline pupil size for predictive, express, and regular saccades generated in the metronome task. Square root model coefficients were then analyzed by saccade type and across participants’ age to test whether (1) the model fit for the main sequence varied by saccade type, and (2) whether the main sequence for each saccade type varied with participant age.

Blink metrics
Inspection of loss of eye-tracking revealed a consistent profile of blinks across the developmental age range, baseline pupil size for predictive, express, and regular saccades generated in the metronome task. Square root model coefficients were then analyzed by saccade type and across participants’ age to test whether (1) the model fit for the main sequence varied by saccade type, and (2) whether the main sequence for each saccade type varied with participant age.

SRT, amplitude and peak velocity (F(18,944) = 1.19, Wilk’s Λ = 0.815, p = 0.288), participant mean pupil size for predictive, express, and regular saccades by system (F(4,108) = 0.90, Wilk’s Λ = 0.975, p = 0.442), and mean blink rate for the five target rate conditions by system (F(4,108) = 1.50, Wilk’s Λ = 0.935, p = 0.195)). Therefore, eye-tracking data were collapsed for analysis. Generalized additive models (GAMs; Wood, 2017) were used to estimate the effect of age on eye-tracking parameters. GAMs were selected for their semiparametric nature, robustness to overfitting, and ability to query age-related effects across adolescence without assuming the shape of the developmental trajectory (Wierenga et al., 2019; Luna et al., 2021). Age was entered as a smooth function in the GAM model, y_i = β0 + S_i(Age_i) + error, with β0 denoting the random intercepts, β_i as the smoothness parameter of the Age of the individual i and individual error as error_i. Smoothing parameter estimation was performed with the restricted maximum likelihood method (REML), as it is less prone to undersmoothing than other criteria (e.g., GCV, AIC, and UBRE; Wood, 2011). From a Bayesian perspective, GAM’s computed smoothing penalty A (k = 9) acted as a prior for coefficients of the basis functions to improve the generalizability of the developmental curves. Gender was not entered as a variable in the GAM models given it was not evenly distributed across the adolescent age range (i.e., more females were recruited for participation in a separate eye-tracking study that was run in parallel), and previous studies with child participants have reported no differences in eye-tracking metrics (for review, see Salman et al., 2006).

To identify periods of statistically significant developmental change and age of maturation, the uncertainty of each estimated model was calculated using confidence intervals. Confidence intervals (95%) were computed via posterior simulation, a process described by Wood (2017) and previously implemented to identify periods of age-related changes across adolescence in white matter and brain functional connectivity (Simmonds et al., 2014; Calabro et al., 2020). During posterior simulation, 1000 random draws from a multivariate normal distribution whose vector of means and covariance corresponded to the fitted GAM parameters were taken, therefore each random draw represented a new trend that was compatible with the fitted trend but also reflected the uncertainty in the estimated trend (Simpson, 2018). The first derivative of the GAM fit was computed (at 0.1-year age intervals) for the 1000 random draws to identify time points x_i equal to zero, consistent with the null hypothesis of no change. Significant periods of age-related change were identified when the (simultaneous) confidence interval of the first simulated derivative did not include zero (p < 0.05). Points of maturation were identified as the maximum age when the confidence intervals were nonzero. GAM fit parameters (adjusted r^2, deviance explained, and p value) were reported for each statistically significant model, as well as the period of significant age-related change and maturation point. The Gaussian family of GAM models was used and modeling was performed with the mgcv package in R (Wood, 2009).

In general, statistical analyses were performed on population-level (i.e., averaged participant responses) data for saccade, blink, and pupil responses and on an individual trial level (i.e., trials with predictive saccades, for example). Before any group-level analysis was performed, normality of response parameters was assessed using the Kolmogorov–Smirnov test. Parametric tests were performed for normally distributed data and nonparametric tests were performed for skewed data. Means, SDs, and confidence intervals were reported for statistically significant main effects. All main effects were further assessed using t tests and reported significant p values were corrected for multiple comparisons using the Bonferroni method. t tests and regression analyses were two-tailed.
Participants therefore used the first approximately three target steps to identify the rate of the alternating target before launching predictive saccades to anticipate the timing of the next target’s appearance, in agreement with previously published predictive saccade results (Joiner and Shelhamer, 2006; Zorn et al., 2007). Nonparametric kernel density curves of SRTs in Figure 2A (predictive in red; express in light blue; regular in dark blue) show the probability of a given reaction time in the three saccade categories. Although the SRT range for each category was unique, the kernel nature of the density curves displays an artificial overlap among distributions. As the rate of the alternating target slowed from 2 to 0.66 Hz, the probability peak of predictive saccades correspondingly decreased and became broader (Fig. 2, red traces).

A main effect of metronome target rate was observed for percentage of regular (F(4,565) = 25.70, p = 1.39e-19, effect size $\eta^2 = 0.154$) and predictive saccades (F(4,565) = 22.16, p = 5.18e-17, effect size $\eta^2 = 0.136$) but not express saccades (F(4,565) = 1.26, p = 0.283). t tests revealed that the percentage of predictive saccades significantly varied among the following frequencies: 2 Hz [mean percentage: 55.6% (SD = 22.3)] and 0.8 Hz [42.2% (20.5); p = 3.10e-05], 2 and 0.66 Hz [34.9% (20.0); p = 1.09e-11], 1.33 Hz [57.5% (22.9)] and 0.8 Hz (p = 1.00e-06), 1.33 and 0.66 Hz (p = 9.89e-14), and 1 Hz [50.1% (21.1)] and 0.66 Hz (p = 1.00e-06). Target rates that differed by the percentage of regular saccades included: 2 Hz [33.9% (19.1)] and 0.8 Hz [47.7% (21.3); p = 6.00e-06], 2 and 0.66 Hz [56.7% (21.0); p = 8.13e-15], 1.33 Hz [33.6% (21.3)] and 0.8 Hz (p = 4.00e-06), 1.33 and 0.66 Hz (p = 3.86e-15), and 1 and 0.66 Hz (p = 2.25e-08). In summary, as target rates slowed, participants generated fewer predictive saccades and more regular saccades, while the frequency of express saccades remained unchanged.

**Age and SRT**

GAM models, corrected for multiple comparisons (required p value for significance = 0.05/5 target rate conditions; p = 0.01), revealed a significant relationship among age and percentage of predictive saccades for the following target rates in the metronome task (Fig. 3, red data points and traces; adjusted $r^2$ values and period of age-related changes are reported in the figure): 2 Hz (deviance explained = 15.5%, p = 0.0011), 1.33 Hz (22.5%, p = 2.29e-05), 1 Hz (10.1%, p = 0.00843), and 0.66 Hz (8.58%, p = 0.01). Age of maturation for predicting short and medium target rates (2, 1.33, 1 Hz) were highly similar (~14 years), whereas maturation for the longest target rate (0.66 Hz) was delayed until age 16. There was no significant age × percentage of predictive saccade effect observed for 0.8 Hz (p = 0.10).

**Results**

Data were excluded from three participants in the random task and four participants in the visual metronome task because of poor quality eye-tracking. Therefore, statistical analyses were performed on 115 participants in the random task and 114 participants in the metronome task (see Fig. 1D for the distribution of participants’ age and gender).

**Task metrics**

**SRT**

Figure 2 shows all participants’ SRTs to target steps 1–12 for each trial of the visual metronome task (~7000 saccades per target frequency; 34497 total saccades) and the random task (35811 saccades). In the metronome task, the first three target steps largely consisted of regular and express saccades (SRT > 90 ms), whereas saccades to target steps 4–12 showed an increased incidence of predictive behavior (SRT < 90 ms).
The paucity of predictive saccades to rhythmic targets in young children across all presentation rates coincided with a higher prevalence of regular saccades. GAM models revealed a significant inverse relationship between age and percentage of regular saccades in all target rates of the metronome task (Fig. 3, dark blue data points and traces). Significant periods of age-related change for percentage of regular saccades in the 2-Hz condition were observed from 7.5–14.1 years (22.8%, \( p = 1.87 \times 10^{-5} \)), 7.6–13.8 years for 1.33 Hz (30.5%, \( p = 3.91 \times 10^{-7} \)), and 7.4–16.7 years for 1 Hz (14.0%, \( p = 5.12 \times 10^{-4} \)), 6.5–23 years for 0.8 Hz (7.51%, \( p = 0.00319 \)), and 7.8–17.3 years for 0.66 Hz (13.2%, \( p = 6.51 \times 10^{-4} \)). The percentage of express saccades × age GAM models revealed a significant effect of age on express saccades for targets that alternated at 0.8 Hz (Fig. 3D, light blue data points and traces), with 8.98% deviation explained \( (p = 0.00121) \). In the random task, a significant interaction between age and the percentage of express saccades was observed (14.1%, \( p = 0.00914 \)).

**Metronome saccade metrics and age**

A multivariate ANOVA was run with individuals’ mean saccade amplitude and peak velocity toward all target steps entered as dependent variables and saccade type in the metronome task as the independent variable. A main effect of saccade type was observed on the saccade metrics \( [F_{4,674} = 28.89 (p = 3.49 \times 10^{-22}) \)\), partial \( \eta^2 = 0.146 \). As expected (Bronstein and Kennard, 1987; Evans et al., 1999; Shelhamer and Joiner, 2003; Wong and Shelhamer, 2011) predictive saccades were hypometric \( [\text{mean} = 17.7^\circ (SE = 0.16)] \) relative to the 20° targets, likely because the target appearance was not yet visually processed when the saccade was initiated, resulting in increased neural noise in the brainstem for the saccadic command. Regular saccades were also hypometric \( [16.4^\circ (0.16)] \), although we attribute this to the abundance of regular latency saccades to the first target step 10° away from FP (see Fig. 4D). Amplitudes differed among predictive and regular saccades \( (p = 8.70e-09) \), express \( [18.0^\circ (0.16)] \) and regular saccades \( (p = 2.90e-12) \), but not among predictive and express saccades. Peak velocity differed among all saccade types (mean peak velocity for predictive saccades = 433.9°/s; express = 496.4°/s; regular = 459.1°/s; \( p < 0.001 \)).

To evaluate the slope of the main sequence by saccade type, a square root model \( (\gamma = V\sqrt{x}) \) was fit to all saccades made in the metronome task with a peak velocity <1000°/s, resulting in 34416 saccades (Fig. 4A–D). As can be seen, compared with the express epoch (Fig. 4C), there are a significant number of saccades in the regular epoch (Fig. 4D) with amplitudes around 10°, contributing to the previously reported difference in amplitude among express and regular saccades. Given that peak velocity changes with saccadic amplitude, we evaluated whether the participants’ mean peak velocity varied among saccade types for saccades with amplitudes that ranged between 18° and 21°, inclusive. Indeed a main effect of saccade type was still observed \( F_{(2,325)} = 22.12 (p = 9.88e-10) \); however, post hoc \( t \) tests revealed that at these amplitudes, peak velocity for express and regular saccades did not differ \( (p = 0.67) \), whereas predictive and express \( (p < 0.001) \) and predictive and regular saccades \( (p < 0.001) \) did. Indeed, predictive saccades are known to have slower peak velocities compared with reactive saccades (Bronstein and Kennard, 1987; Smit and Van Gisbergen, 1989).

Square root model fits were derived to estimate the main sequence of individual subjects’ predictive, express, and regular saccades (including all amplitudes) in Figure 4B–D and a Kruskal–Wallis test was performed to test whether they shared the same underlying distribution. A main effect of saccade type was observed on the model fits \( (X^2_{(2.236)} = 47.76, p = 4.26e-11) \), where model coefficients varied between predictive and express saccades \( (p < 0.001) \) and predictive and regular saccades \( (p < 0.001) \), but not between express and regular saccades \( (p = 0.81; \text{Fig. 4A}) \). Spearman \( \rho \) correlations were run for age and the main sequence model fits for the three saccade types, and as expected (Fischer et al., 1997; Munoz et al., 1998; Calancie et al., 2022).
Luna et al., 2001), there was no observed association with age (Fig. 4E).

**Pupil size, temporal prediction, and age**

All participant pupil size values were combined (Fig. 5C) and categorized according to their saccade (e.g., predictive, express, regular) to target on given metronome trial (Fig. 5A). A Kruskal–Wallis test supported a main effect of saccade type on pupil size in the metronome task ($\chi^2(2) = 369.82, p = 4.94e-81$), with post hoc $t$ tests supporting differences among all saccade types ($p < 0.001$; see Fig. 5A,B). In Figure 5C, participants’ corresponding pupil size for all metronome trials that met the pupillary analysis criteria are plotted against the RT of the saccade to target, showing an increased density of bigger pupil sizes at around the time that the visual transient signal is converted to a saccade motor command (SRT = 90 ms). A significant positive Spearman’s rank-order correlation was observed between pupil size and SRT ($r_s = -0.07, p = 0.43$), express saccades ($r_s = -0.01, p = 0.90$), or regular saccades ($r_s = -0.02, p = 0.79$). Individual participant’s pupil size was measured on each trial and averaged based on their SRT category (predictive, express, and regular). GAM models were applied to estimate how pupil size changed with age across the three saccade categories. Indeed, pupil size significantly varied with age for predictive saccades (adjusted $r^2 = 0.118$, deviance explained = 12.6%, $p = 0.000115$), express saccades (adjusted $r^2 = 0.0985$, deviance explained = 10.7%, $p = 0.000421$) and regular saccades (adjusted $r^2 = 0.12$, deviance explained = 12.8%, $p = 9.29e-05$; see Fig. 5D). As can be seen with the GAM model fits, the difference among pupil size by saccade type is not observed at an individual-subject level. The SD for pupil size was compared across participants and no significant difference was observed across saccade types ($\chi^2(2) = 4.44, p = 0.1084$).

**Blinks**

Inspection of blink and saccade probability density plots in Figure 6A revealed an anti-correlated relationship between saccades and blinks. During visual fixation, participants show the highest rate of blinking, which is reduced during target steps 1

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**Figure 4.** A, Square root model ($y = \sqrt{y}$) was fit to estimate the main sequence of predictive (in red), express (in light blue), and regular saccades (in dark blue) made by all subjects across the five target rates in the metronome task. Model fits significantly varied among nonvisually triggered predictive and express saccades, and predictive and regular saccades, but not among express and regular saccades. Raw datapoints of saccadic peak velocity and amplitude used to calculate the main sequence model fits are shown for predictive (B), express (C), and regular (D) saccades. A kernel density estimate was applied to the scatterplot data in B–D to visualize the density of the datapoints, with warmer colors indicating increased density. E, Square root models were performed for predictive, express, and regular saccades made in the metronome task by each individual subject. Participants’ main sequence model coefficients are plotted on the $y$-axis, and their age is plotted on the $x$-axis with lines representing linear polynomial curves. Spearman rank correlations revealed no significant association between age and main sequence model fits for participant age and predictive saccades ($r_s = -0.07, p = 0.43$), express saccades ($r_s = -0.01, p = 0.90$), or regular saccades ($r_s = -0.02, p = 0.79$).
and 2, and increased again for target steps 3–12. Participants' mean blink rate did not vary according to the metronome task's target frequency \((p = 0.323; \text{Fig. } 6B)\), nor did it vary among metronome target rates and the random task. On the other hand, the timing of blink onsets relative to target appearance in the metronome task significantly varied \((\chi^2(4) = 203.73, p = 5.91e-43; \text{see Fig. } 6C)\). Multiple comparison tests showed differences in blink reaction times (relative to target appearance) between all target frequencies at the \(p < 0.001\) level (participants' median blink reaction times: 262.2 ms for 2 Hz, 417.5 ms for 1.33 Hz, 501.3 for 1 Hz, 584.7 for 0.8 Hz, and 678.8 for 0.66 Hz), with the exception of frequencies 1.33 and 1 Hz \((p = 0.0155), 1\) and 0.8 Hz \((p = 0.0108),\) and 0.8 and 0.66 Hz \((p = 0.0569)\).

The relationship among participants' age and their corresponding mean blink rate and average blink reaction time to target was queried using Spearman's rank correlation coefficient tests across the metronome target frequencies and corrected for multiple comparisons. No correlation was observed among participants' age and blink reaction time (range of \(\rho\) values: \(-0.005--0.20\)). Similarly, no relationship was observed among participants' age and mean blink rate with the exception of target frequency 0.66 Hz \((p = 0.34; p = 5.15e-04)\).

Mean probabilities of participants making a saccade or blink during the 1000 ms before and after target appearance are plotted in Figure 6D–G, categorized by saccade type. Participant mean blink probability differed by saccade type across the \(-1000-+1000-ms\) epoch relative to target appearance at 0 ms (2000-ms epoch total; \(\chi^2(2) = 18.99, p = 7.53e-05\)). Multiple comparisons showed that participants were more likely to make a blink during this epoch if they generated a regular saccade to target as opposed to a predictive \((p = 0.0059)\) or express saccade \((p = 0.0001)\). In the 1000-ms period before target appearance, blink probability significantly varied by saccade type \((\chi^2(2) = 32.35, p = 9.43e-08),\) with post hoc differences among all saccade types \((p < 0.001)\). In the 1000-ms period following target appearance, blink probability also significantly varied by saccade type \((\chi^2(2) = 10.54, p = 0.005),\) with all saccade types differing at the post hoc level \([\text{predictive and express } (p = 0.0135),\] predictive and regular \([p < 0.001),\] and express and regular \([p = 0.0135)]\). These data support a novel finding in that when participants generated a predictive saccade, blinks were more likely to be inhibited in the 1000 ms prior to target appearance. Indeed, compared to visually triggered saccades, blinks on predictive saccade trials were more likely to occur in the 1000 ms following target appearance. Spearman's rank correlation coefficient tests revealed that blink probability across these tested epochs did not significantly vary with participant age in any of the saccade categories.

### Discussion

The goal of this study was to better understand temporal prediction of visual metronome targets in typically developing children, adolescents, and young adults. Temporal prediction significantly correlated with age in four of the five metronome target rates with adult-level performance being reached by age 16. Smaller pupil sizes were observed following a predictive versus reactive saccade, supporting autonomic relaxation when anticipating rhythmic stimuli. Blink rate did not differ among metronome and random targets, however, blink timing varied with target frequency and saccade type; blinks occurred at the middle of the ISI and were suppressed before target appearance on trials when participants predicted the target. Together, these results support a strong age effect on the temporal prediction of periodic targets and a coordination among saccade, pupil, and blink metrics.

### Maturation timeline of saccadic temporal prediction

Predictive saccades increased with age for nearly all target rates in the metronome task \((\text{Fig. } 3A–E, \text{red curves})\). This was not observed in the random task \((\text{Fig. } 3F)\), indicating that changes in performance were specific to conditions that had rhythmic stimuli, rather than any changes in anticipatory saccades toward visual targets in general. Developmental curves for predictive and regular saccades across target rates were inversely related, illustrating a shared maturation timeline; while the percentage of express saccades changed only slightly with age \((\text{Fig. } 3D,F)\). These data are in agreement with previous reports that young children \((<12 \text{ years})\) made fewer predictive saccades than adults.
Figure 6. A, Diagram of blinks (in purple) and saccades (in gray) made toward the alternating target of the metronome task at target frequency 0.66 Hz. Blinks and saccades are displayed such that each horizontal line marks a unique trial from a participant, making up 570 occurrences (114 participants × 5 trials of the alternating target at a rate of 0.66 Hz), and gray borders mark the timing of the target appearance. Histogram of blink probability (in purple) is scaled up by a factor of 5 so blink and saccade probabilities can be viewed on the same plot. B, Blink rate across metronome trial length. C, Blink reaction time for different target frequencies. D, Blink and saccade probability for predictive saccade trials. E, Blink and saccade probability for express saccade trials. F, Blink and saccade probability for regular saccade trials. G, Blink probability (uncalibrated) for predictive, express & regular saccade trials.
to target frequencies 0.5–1.33 Hz (Kowler and Martins, 1982; Ross and Ross, 1987; Lukasova et al., 2018). Similar to adult studies (Shelhamer and Joiner, 2003; Isotalo et al., 2005; Lee et al., 2016), we found that the highest rates of saccadic prediction occurred toward target frequencies of 1.33 and 2 Hz. Likewise, in subjects aged 4–38 years, the preferred motor tempi for spontaneous finger tapping was found to range from 1–2 Hz (McAuley et al., 2006). (McAuley et al., 2006). This frequency range closely aligns with the human heart rate (80 BPM = 1.33 Hz), raising interesting questions about whether individuals can more adeptly predict tempos that synch to their heart rate, or if tempo preference is shaped by exposure to a heartbeat in utero.

The prolonged period of age-related change for low-frequency (i.e., 0.66 Hz) versus high-frequency (i.e., 1–2 Hz) targets may reflect additional signaling of the striatum, thalamus and/or cerebral cortex during middle adolescence. The cerebellum and basal ganglia have unique temporal sensitivity for high and low frequencies (Ivry and Spencer, 2004; Buchusi and Meck, 2005; Allman et al., 2014). For example, when listening to auditory stimuli, patients with cerebellar lesions had reduced surface level EEG amplitude for beats with high frequencies, whereas patients with basal ganglia lesions demonstrated reduced EEG activity for low frequencies (Nozaran et al., 2017). Additionally, pharmacological silencing of the cerebellar dentate in monkeys impaired self-timed SRTs to fast versus slow-paced periodic targets (Kunimatsu et al., 2018). In a combined eye-tracking and fMRI study, Debrabant et al. (2013) observed that compared with age-matched controls, children diagnosed with developmental coordination disorder made fewer predictive saccades to a periodic target (ISI: 1200 ms) and exhibited less BOLD activity in cerebellar Crus I. The maturational timelines of predictive saccades in our participants toward target frequencies ≥1 Hz were remarkably similar, varying by only 1.3 years from the onset of performance-related change to its conclusion (Fig. 3A–C). These data, combined with previous reports of the specificity of cerebellar signaling for coordinating motor movements toward target frequencies ≥1 Hz (see Ivry and Spencer, 2004), together imply that in healthy children, the period of age-related cerebellar maturation to support saccades toward fast-paced rhythmic temps occurs from ages 8 to 14.

Previous nonhuman primate research has causally demonstrated that the caudate closely maintains neuronal activity to match the delay interval of an ISI, while, neuronal activity in the dentate is relatively constant for various target frequencies, showing characteristic ramping activity 500 ms before the self-timed saccade and peaked activity at saccade execution (Ohmoe et al., 2017). These results may explain why we observed fewer predictive saccades at the lowest frequency of 0.66 Hz, given low frequencies demand prolonged neuronal firing in the striatum to accurately signal the timing of saccade execution. Longitudinal cohort studies support age-related change of caudate gray matter volume from early childhood into young adulthood (Wierenga et al., 2014; Larsen and Luna, 2015). Furthermore, gray matter atrophy in the putamen in Huntington’s disease patients was negatively correlated with predictive saccade SRT (Vaca-Palomares et al., 2019). Other potential neural correlates for the prolonged maturation of predicting targets at low frequencies include the thalamus, which acts as a relay region for a disynaptic connection between the dentate and striatum (Hoshi et al., 2005; Bostan et al., 2013). The thalamus integrates timing-related subcortical signals with cortical signals (Matsuyama and Tanaka, 2021) and has strong buildup activity for self-timed saccades (Tanaka, 2006, 2007). Additional cortical regions that modulate their firing during timing-related tasks and may further contribute to young adolescents’ delayed temporal performance at 0.66 Hz include the Frontal Eye Fields (FEF) (O’Driscoll et al., 2000; Gagnon et al., 2002) and parietal cortex (Maimon and Assad, 2006; Jazayeri and Shadlen, 2015).

Pupil size and temporal prediction across age
Previous studies have reported a physiological relaxation effect when subjects were presented with rhythmic stimuli (Johnsson and Trawick, 1938; Brauchli et al., 1995; Berger, 2012). Indeed, Figure 5A,B shows that this relaxation effect was replicated using pupil size, which was smaller for predictive versus reactive saccade trials. NE has been shown to have a neuromodulatory effect on the parasympathetic oculomotor complex via LC inputs to SC (Edwards et al., 1979) and SC’s corresponding projection through Edinger–Westphal nucleus (Hartung et al., 1980) to the ciliary ganglion, which can be both excitatory and inhibitory (Barnersoi et al., 2017), providing a potential pathway for a decreased pupil size and thereby arousal for predictive saccades. This pupil effect is complementary to a previous fMRI study by Lee et al. (2016) that demonstrated strong activation of the default mode network (DMN), a neural network that increases its metabolic activity during relaxation, when adults made predictive saccades in the metronome task. Pupil size has been shown to negatively correlate with BOLD activity of the posterior cingulate cortex and parahippocampal gyrus of the DMN and positively correlate with LC (Almaes et al., 2014). Pupil size positively correlated with SRT (Fig. 5C), in accordance with a previous study that reported this coordination via shared signaling pathways in the SC (Wang and Munoz, 2021). Yet while differences in mean pupil size by saccade type were evident when all metronome trials were collapsed, this effect was not maintained when analyzed at the subject-level (Fig. 5D). This may be because of variability in the number of individual participants’ pupil trials that successfully met criteria for analysis for each saccade type. To address this, the field may benefit from incorporating biometric sensors that are additional to pupil size recording (e.g., respiration rate; skin conductance; heart rate) to measure sympathetic tone while individuals predict or react to rhythmic stimuli.

Pupil size reliably decreased with age for all saccade types (Fig. 5D). Previous studies have described age-related pupil size declines starting in later adolescence (for a detailed description, see Loewenfeld, 1999, p 501); however, our results suggest that this phenomenon may be observed earlier, beginning in childhood (onset range 7–12 years) and declining in size until age 24, the oldest we tested. Presumably this reduction in pupil size with age is driven by a diminished constriction capacity of the dilator pupillae muscle (Loewenfeld, 1999).

Coordination of blinks and predictive saccades
Despite not being provided with instructions regarding when to blink, participants exhibited a clear pattern in the timing of their
blinks relative to metronome target appearance, with median blink reaction times occurring at the halfway point of the ISI (Fig. 6C). Blink rate did not vary according to the rhythmicity of targets (Gagnon et al., 2002), but rather, blink timing varied according to frequency, and according to whether subjects predicted or reacted to targets (Fig. 6). It has been shown previously that blink reaction time variability decreases when participants view videos that have clear temporal event structures (Nakano et al., 2009). Plotting the blink probability leading up to target appearance allowed us to reasonably estimate the SRT category of the subsequent saccade (Fig. 6G), with increased blink probability for saccadic categories with slower RTs. Given that dopaminergic signaling within the basal ganglia has been shown to modulate blink excitability (Groman et al., 2014; Jongkees and Colzato, 2016) and adjust the precision of interval-based timing in mammals and humans (Kunimatsu et al., 2016; Soares et al., 2016; De Corte et al., 2019), the coordination we observed between blinks and temporal prediction may involve dopaminergic phasic activity. Kaminer et al. (2011) describe a neural mechanism whereby blinks are modified by striatal dopamine levels via substantia nigra pars reticulata inhibition of the SC, and the SC's excitation of the nucleus raphe magnus and the subsequent inhibition of the spinal trigeminal complex. However, more research is needed to elucidate the exact mechanisms underlying the coordination between saccade and blink reaction times toward periodic targets.

References


