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Working together to orient faster: The combined effects of alerting and orienting networks on pupillary responses at 8 months of age

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ABSTRACT

Multiple visual attention mechanisms are active already in infancy, most notably one supporting orienting towards stimuli and another, maintaining appropriate levels of alertness, when exploring the environment. They are thought to depend on separate brain networks, but their effects are difficult to isolate in existing behavioural paradigms. Better understanding of the contribution of each network to individual differences in visual orienting may help to explain their role in attention development. Here, we tested whether alerting and spatial cues differentially modulate pupil dilation in 8-month-old infants in a visual orienting paradigm. We found differential effects in the time course of these responses depending on the cue type. Moreover, using Principal Component Analysis (PCA) we identified two main components of pupillary response, which may reflect the alerting and orienting network activity. In a regression analysis, these components together explained nearly 40 % of variance in saccadic latencies in the spatial cueing condition of the task. These results likely demonstrate that both networks work together in 8-month-old infants and that their activity can be indexed with pupil dilation combined with PCA, but not with raw changes in pupil diameter.

1. Introduction

How attentional control emerges has been perhaps one of the central themes of research in developmental cognitive neuroscience over the last few decades. Different strands of work sought to describe some of the basic attentional mechanisms (e.g. Colombo, 2002; Richards et al., 2010) and the role of changing neural organisation that supports their development (e.g. Amso and Scerif, 2015). While several models of attentional processes exist in the literature, the Petersen and Posner model (Petersen and Posner, 2012; Posner and Petersen, 1990) has been successfully applied in research with infants and young children (Rothbart et al., 1990). It postulates that three systems of attention exist, each of them dependent on the activity of separable brain networks and different neurotransmitters (Posner and Rothbart, 2007; Rueda et al., 2004; Xuan et al., 2016). The orienting system controls attention shifting towards stimuli and sustains attention on selected objects or events. It relies on the spatial orienting network encompassing superior colliculi, frontal eye fields, and parietal cortex. The alerting system maintains

appropriate levels of alertness, regulates the level of sensitivity to stimuli and depends on the activity of the locus coeruleus and the right fronto-parietal cortex. The executive attention system - the last one to emerge in development, supports endogenous attention control and stimulus selection. It relies on the activity of basal ganglia, anterior cingulate cortex, and lateral ventral prefrontal cortex. Since the development of executive attention control is protracted and extends well beyond infancy (Diamond, 2014), we focused on the alerting and orienting networks since they are already operational at 4 months of age (Amso and Johnson, 2006).

The orienting network undergoes significant changes throughout the first 6 months of life. Newborns readily orient to salient visual stimuli, especially faces and track them, largely thanks to subcortical mechanisms, mediated by the superior colliculus (see Johnson et al., 2015). Early on, they exhibit “obligatory attention”, a difficulty to disengage attention and to shift it voluntarily (Hood and Atkinson, 1993; Johnson et al., 1991). However, the cortical control of orienting is apparent by 3 months of age, when they show anticipatory looking (Haith et al., 1988)

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and begin to inhibit reflexive saccades in the antisaccade task from 4 months (Holmboe et al., 2008; Johnson, 1995). Crucially, 4-month-olds show evidence of covert orienting in the spatial cueing task, suggesting that parietal cortex and frontal eye fields are engaged in spatial orienting early in infancy (Butcher et al., 1999; Hood and Atkinson, 1993; Johnson and Tucker, 1996). ERP studies of the spatial cueing task complement these results, with spatial cue validity effects found for early responses (enhanced P1 component around ~135 ms from target onset when cue and target were in the same location) for infants aged 4.5 months, but not younger (Richards, 2000). Subsequent work localised cortical sources of this effect to clusters of activity in temporal and extrastriate occipital cortices (Richards, 2005), broadly replicating adult patterns of activation (Martínez et al., 1999). Altogether these results suggest that by the middle of the first year of life the orienting network is fully functional producing similar experimental effects in the spatial cueing task and engages broadly the same set of cortical areas as in adults.

The early development of the alerting network has been perhaps less explored. Alertness depends on the activity of several neural circuits, including the Autonomic Nervous Systems (ANS), which modulates slow changes in the level of arousal (tonic alertness) (Oken et al., 2006). The tonic mechanism maintains an appropriate level of responsiveness to incoming stimuli and research with 12-month-old infants indicates that fluctuations in arousal are associated with fluctuations in look durations (de Barbaro et al., 2017; for a review see Wass, 2018). Alertness also depends on rapid task- and stimulus-relevant (phasic) modulation of neural activity via noradrenergic projections by the locus coeruleus, which is sensitive to stimulus novelty or reward value (Gilzenrat et al., 2010; Keehn et al., 2013; Oken et al., 2006). The two mechanisms are interrelated and distinct levels of tonic or phasic activity may affect cognitive performance (see Wass, 2018 for review).

Both the alerting and the orienting networks are affected by the phasic activity of locus coeruleus-norepinephrine system (LC-NC) (Bast et al., 2018). The flexibility of the orienting response to silent stimuli depends on two orienting sub-networks: the dorsal and the ventral frontoparietal attention networks (Corbetta and Shulman, 2002; Corbetta et al., 2008). The dorsal frontoparietal attention network activates during focused attention to selected stimuli. When the organism detects a change in the environment the ventral frontoparietal attention network activates. The switch between these networks - "circuit break" - is modulated by the LC activity (Corbetta and Shulman, 2002). During phasic alertness the increased signal from the LC projects to the temporoparietal junction (TPJ). The TPJ activity initiates, in turn, switch from focused attention to orienting response on silent stimuli. To summarize, the LC plays a crucial role in the regulation of alerting and orienting networks.

For decades, changes in pupil diameter (PD) have been used to index arousal and attention (Beatty and Lucero-Wagoner, 2000). More recent studies have also linked it with a host of other cognitive processes, e.g. memory, rationality, object representation, or surprise (for review see Hepach and Westermann, 2016; Laeng et al., 2012). Like many psychophysiological responses, PD can either develop gradually over time in a tonic manner or at short latency in a phasic task-relevant fashion. Neurophysiological and neuroimaging research to date suggests that baseline pupil diameter reflects both tonic and phasic changes in the activity of the locus coeruleus (LC) (Rajkowski et al., 1994; Murphy et al., 2014), with high baseline PD indicating the tonic mode and low baseline PD indicating the phasic mode. Moreover, phasic and tonic changes in pupillary diameters are dependent on each other (Aston-Jones and Cohen, 2005). Additionally, recent studies have shown that PD is modulated by the predictiveness of the cue location in the orienting task in adults (Dragone et al., 2018) and it reflects the likelihood and speed of orienting in visual search task in infants (Kleberg et al., 2018). Geva et al. (2013) investigated more closely PD as a marker of attention network activation in adults by measuring the time course of PD responses to different visual cues in the Attention Network Test

(ANT), a behavioural paradigm for studying the orienting, alerting and executive attention networks (Fan et al., 2002). In this task, the target stimulus is preceded by a cue, which is thought to activate one of the networks. Orienting is activated by a cue that provides information about the location of the target - Spatial Cue (SC), in comparison with a cue that provides information only about the onset time of a target - Central Cue (CC). Alerting network is activated by a warning cue that provides only information about the time of a target onset - Double Cue (DC), in comparison to the condition without any cue - No Cue (NC). Geva et al. (2013) found differential PD changes in the alerting, orienting and executive components of the ANT, which were limited to specific time windows relative to the onset of the visual cue. They interpreted these results as evidence for tonic and phasic PD modulation by specific attention networks, such that pupillary responses to DC relative to NC reflected selective activation of the alerting network, while responses to spatially valid Spatial Cues (SCValid) relative to CC reflected activation of the orienting network. However, this interpretation was challenged by Ambrosini et al. (2013), who pointed out that the alerting and orienting conditions of the ANT activate to some extent all attention networks and thus PD responses in a given condition cannot be attributed exclusively to the activity of one network. Earlier studies using the ANT already showed that increased alertness influenced spatial cueing orienting effect providing additional evidence that the attention networks might interact (e.g. Callejas et al., 2004). In order to isolate the effect of each attention network in the ANT Ambrosini et al. (2013) proposed to use Principal Component Analysis (PCA), which isolates independent components in the PD responses that correspond to the activity of each network (Nuthmann and Van der Meer, 2005).

Developmental literature on the pupillary indices of attention networks is very limited. To shed more light on this topic, we investigated the activity of the alerting and orienting attention networks in 8-month-old infants. We adapted component tasks from the ANT to measure pupillary responses and saccadic reaction time to different visual cues. Thus, first, we tested whether, similarly to adults, the activity of the alerting and the orienting networks is reflected in the dynamics of PD (Geva et al., 2013). Since both the alerting and the orienting network are already operational at this age, we hypothesised that the activity of each network is related to the differential dynamics of pupil dilation in different task conditions. Particularly, we predicted that the activity of the alerting network will be reflected in PD changes in response to the DC relative to a control NC condition. Also, the activity of the orienting network should be reflected in PD changes in response to spatially valid SC relative to the control CC condition. Moreover, we also tested the time course of the effects of each network, hypothesising that alerting-related PD changes will precede the orienting-related PD changes (Bast et al., 2018). Secondly, since recent data suggests that the dynamics of PD may reflect the interactions between these attention networks (Ambrosini et al., 2013), we sought to further separate out the effects of each network by means of covariance PCA (Lemercier et al., 2014). The advantage of PCA for the evaluation of pupillary responses lies in the fact that all information in the PD data is taken into consideration rather than that of single data points and this information is used to identify the components that explain the greatest proportion of variance (Nuthmann and Van der Meer, 2005). Finally, we used a hierarchical regression model to test whether individual differences in the activity of both attention networks measured with PD changes are associated with saccadic latencies in the spatial cueing task. If the PCA components reflect the activity of separate attention systems, then individual differences in those components should predict unique portions of variance in infant saccadic latencies in the orienting task.

2. Method

2.1. Participants

Forty participants were recruited to take part in the study (age in

days $M = 238.05$, $SD = 15.82$, range 213–270; 22 girls). Five participants did not complete the study because of fussiness, while additional four were excluded from the analysis because they contributed less than 50 % of valid trials in each condition. The final sample consisted of 31 infants (age in days $M = 238.39$, $SD = 14.51$, range 213–270; 19 girls and 12 boys). All infants were healthy, delivered at term and except one without any complications (birthweight $M = 3455$ g, $SD = 331$, range 2450–4020 g; gestational age $M = 39.33$ weeks, $SD = 1.47$, range 36–42). Participants were middle-class families from a city with >1.5 million inhabitants. The study was approved by the local ethics committee and conformed with the standards of the Declaration of Helsinki. Prior to the testing all parents gave written informed consent.

2.2. Procedure

Following a familiarisation with the testing room and staff, the infants were seated in a high chair ($n = 12$) or on parent's lap ($n = 28$), approximately 60 cm away from the eye-tracker 24" screen, on which the stimuli were presented. The experiment took place in a purpose-built, infant-friendly testing room. The area around the eye-tracker monitor was covered with a black cloth to provide a uniform background and occlude potential visual distractors. The eye-tracking task reported here was part of a longer testing session consisting of two eye-tracking tasks and a parent-child interaction. Eye-tracking data (gaze position and pupil dilation) were collected using Tobii T60XL (Tobii AB, Sweden) with a 24" monitor, recording at a 60 Hz sampling rate. At the beginning of the testing session an infant-friendly five-point calibration procedure was conducted (four points in corners of the screen and one in the centre). All participants successfully calibrated at least 4 points (calibrated from 1 to 3 times; $M = 4.64$, $SD = 0.66$). The entire task did not exceed 10 min. Luminance levels were measured immediately after the orienting experiment using a light meter (Digital Lux Meter, LX-1010B) and ranged from 32 to 160 lux, on average 88.31 lux ($SD = 32.38$) at the same distance as infant's eyes from the screen. The stimuli were presented using Psychtoolbox (Kleiner et al., 2007) and MATLAB R2013a (The MathWorks, Inc., Natick, MA, US) running on a MacBook Pro laptop.

2.3. Task

We adapted component tasks from the Attention Network Test (ANT; Fan et al., 2002) to measure the activity of the alerting and the orienting networks. We applied a gaze-contingent paradigm to maximise infant performance and to adjust the timing of stimulus presentation to individual differences in saccadic responses (see example in Fig. 1). All the

stimuli in all conditions were presented over a uniform pink background (224 cd/m²). At the beginning of each trial, the infant's attention was focused in the centre of the screen using a rotating and slightly looming colourful animation (a sunflower, subtending 3–5 deg in diameter, 86.40 cd/m²). As soon as the infant fixated the central stimulus for at least 500 ms, the trial sequence was initiated with a presentation of a cue (a baby rattle, subtending 3 deg, 47.48 cd/m²) for 100 ms with the cue stimulus being located either peripherally (spatial cue, SC – along the midline, left or right side of the screen, 14 deg from the centre; double cue, DC – both sides of the screen), or centrally (central cue, CC – replacing the attention-getter in the middle of the screen). On trials without any cue the central attention-getter stimulus remained on the screen for an additional 100 ms - same duration as the cue stimulus (no cue condition, NC). After 100 ms from the cue offset, a peripheral target (a cloud, subtending 5 deg diameter and 221.32 cd/m²) was presented peripherally 12 deg from the centre along the midline either on the left or the right side and remained until fixated by the participant or for a maximum of 1300 ms. In all conditions the target was the same stimulus in one of two positions on the screen, it had the same eccentricity, and was equally often on the left and right side so the saccadic responses were comparable across all conditions. Similarly, the cue was always of the same size, always located along the vertical midline (with the exception of the no cue condition) and had the same colour and the same luminance to reduce the effect of on-screen luminance in the pupil response (Ajasse et al., 2018). Once fixated, the target was replaced by an animated, looming reward in the same location and remained on the screen for 1500 ms. The inter-trial interval was set to 1000 ms.

2.4. Saccadic responses

Data from trials classified as valid (infant successfully fixated the central attention getter and the peripheral target) were analysed to extract saccadic latencies and pupil dilation. The task was presented in blocks. Each block consisted of eight conditions - two NC, two CC, two DC and two SC. In addition, the spatial cue tests were divided into two tasks in which the target appeared in the same (SCValid) or opposite place (SCInvalid) with a 1:4 ratio. In the final sample, participants contributed on average 8.90 blocks ($SD = 2.79$). Saccadic response times were calculated as the latency from the target onset to the first sample where the eyes entered the target AOI (area-of-interest) and were analysed in a one-way ANOVA, followed up by Bonferroni-corrected pairwise comparisons between relevant conditions.

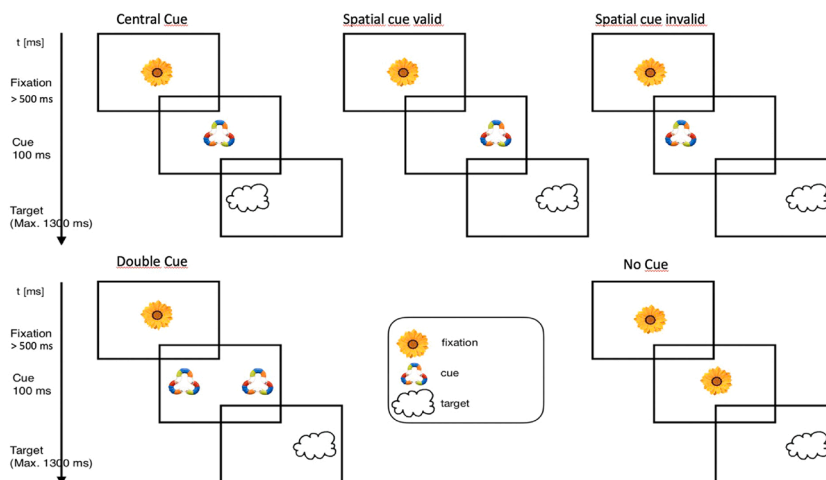


Fig. 1. Adaptation of the Attention Network Test to measure the activity of the alerting and the orienting networks. For clarity, the picture does not include the final reward that appears after the target has been fixated.

2.5. Pupil dilation pre-processing

Pupil diameter data was extracted for each participant for all valid trials and processed further in Matlab 2016a (The MathWorks, Inc., Natick, MA, US). First, eye tracking video recordings were inspected to identify and reject trials, where the infant was moving excessively or not looking at the screen, followed by automatic rejection of trials with >50 % of missing samples from both eyes (rejected on average $M = 10.78$ trials, $SD = 11.86$). Additionally, trials were removed if the infant fixated initially on the cue and not on the attention getter. The remaining data were interpolated and processed following the procedure described by Jackson and Sirois (2009) using their *DataCleanLR.m* function. High correlations between the left and the right eye were present when data for both eyes were available (Pearson's $r > 0.85$ for all participants). Thus, when data was missing for one eye, data from the other was regressed to interpolate the missing samples. Otherwise, linear interpolation was done between the average of the last three values and the first three values before and after the gap. To prevent an increase in variability a low-pass digital filter with a sampling frequency of 15 Hz was initially applied. The filter was applied twice to cancel out phase-lags (Jackson and Sirois, 2009; Winter et al., 1974). Finally, pupil dilation data from both eyes were averaged at each sample, and cut into 3 s long segments encompassing 40 samples before and 140 samples after the cue onset. The period of ~200 ms (12 samples) prior to the cue onset was used for baseline correction of each segment, during which the infant should be focused on the fixation point, causing PD to be at its lowest values for this experiment. Thus we report relative changes in pupil dilation (PD) in mm. For each participant, median PD curves were calculated from all trials available for each condition (SCvalid, CC, DC, NC).

2.6. Pupil dilation peak analysis

First, we compared the average PD change in successive time windows (Lemercier et al., 2014). Therefore, we computed the moving average of the change in PD using non-overlapping time windows of 100 ms for each condition. Next, we used paired-samples *t*-tests to identify periods of significant differences ($p < .05$) for the alerting (DC vs. NC) and the orienting (SCvalid vs. CC) networks (Fig. 2). Next, we clustered together consecutive significant segments into larger periods and we identified three fixed time windows: 1) early „negative peak” (200–500 ms from cue onset); 2) middle „positivity” (1400–1800 ms), and 3) late „positivity” (1900–2400 ms). Average PD change values were calculated for each of these three time windows and each condition except for SCinvalid. Statistical analysis was carried out separately for each attention network (alerting, orienting network) with repeated-measures

ANOVA with condition (for alerting network: DC vs. NC; for orienting network: SCvalid vs. CC) and time window (200–500 ms, 1400–1800 ms, and 1900–2400 ms) as within-subject factors. Significant differences in each time window were further tested with Bonferroni-corrected pairwise comparisons.

2.7. Principal component analysis of PD and statistical analyses

We used Principal Component Analysis (PCA) to separate out the effects of the two attention networks on changes in PD. PCA is a non-parametric method that allows a complex dataset to be reduced to a lower dimension and reveal the sometimes hidden, simplified structure that often underlie it (Shlens, 2005). We adopted an approach proposed by Lemercier et al. (2014) and ran covariance PCA on average PD time series for each condition and each participant using their script (function *DataProcess.m*). In this approach, a set of possibly linearly correlated data (i.e., pupil responses) was divided into a set of linearly uncorrelated variables (i.e., principal components). This transformation is performed in such way that the principal components are ordered by decreasing the amount of contained information (i.e., the first component contains as much of the variability in the data as possible). Finally, we identified the number of the components that explained at least in total 95 % of the variance in the pupil data (see Table 1). We specifically tested whether PCA will identify two separate variance components: one potentially related to the alerting network in the first two time windows and another one related to the orienting network in the last time window. We limited the analysis to the three time windows, where we found significant differences between conditions (200–500 ms, 1400–1800 ms, and 1900–2400 ms).

The alerting network was evaluated using the NC and DC conditions, while the SCvalid and CC conditions were analysed for the orienting network. In both networks, we identified two components, which together explained nearly 100 % of variance in the PD data (Component 1 [88–93 %]; Component 2 [6–11 %]). Next, we tested these components by statistically comparing the component scores between conditions and time windows using a separate two-way ANOVA for each network (alerting network: NC vs. DC; orienting network CC vs. SCvalid). Finally, we ran correlations between component scores and saccadic latencies for the SCvalid condition and a hierarchical regression was used to test whether the component scores explain a significant a unique proportion of variance in infant saccadic latencies in the spatial cueing task. Since the onset of the visual target fell within the first time window, in which we analysed PD (i.e., 200–500 ms), we opted for a more cautious approach and analysed component scores only for the middle (1400–1800 ms) and late (1900–2400 ms) time windows, which occurred after the saccadic response was made. All variables

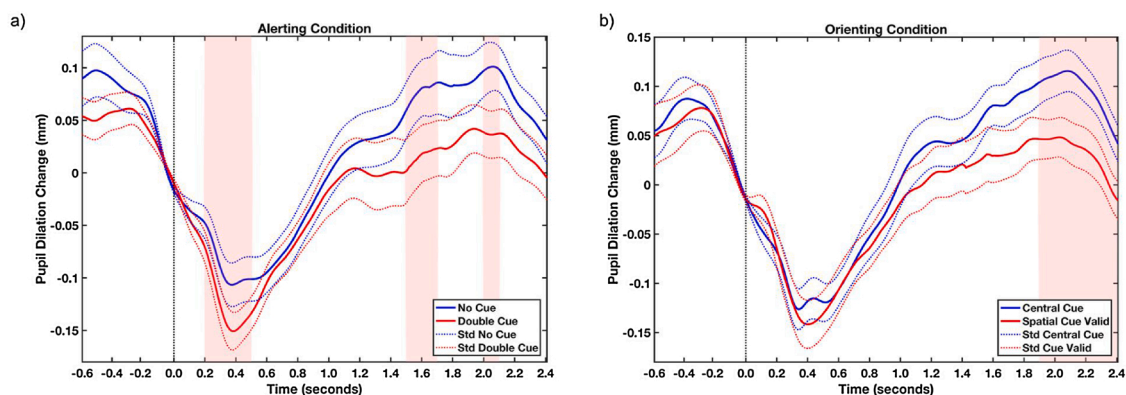


Fig. 2. Average pupil dilation change for the alerting (panel a) and orienting (panel b) conditions. The light dashed lines represent one standard deviation (SD) above and below the mean. The red shaded area represents the time window where significant differences were found between conditions ($p < .05$). The vertical black line at time 0 indicates the cue onset, which was preceded by a central attention getter presented for at least 500 ms (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

Table 1

Percentage of variance in PD data explained by PCA Component 1 and 2 for the alerting and orienting networks in each of the three time windows.

	Accounted Variance (%)					
	Alerting Network			Orienting Network		
	Early Window	Middle Window	Late Window	Early Window	Middle Window	Late Window
Component 1	87.99	92.45	90.62	88.10	87.24	87.40
Component 2	10.50	5.77	5.77	10.05	9.92	8.64

entered in the regression were mean-centred.

3. Results

Saccadic latencies differed as a function of visual cue type (main effect of condition, $F(4, 108) = 21.41, p < .001, \eta^2_p = .44$). For the orienting network conditions (SCvalid vs. CC), we found a robust spatial cueing effect with latencies to targets in the cued location being significantly shorter ($M_{SCvalid} (\pm SD) = 381.56 \pm 106.96$ ms) than in the opposite location ($M_{SCinvalid} (\pm SD) = 638.72 \pm 193.63$ ms; $t(27) = -5.55, p < .001, d = 1.62$). Latencies to targets in the central cue condition (CC: $M_{CC} (\pm SD) = 519.29 \pm 46.89$ ms) were significantly longer than in the valid spatial cue condition ($t(30) = -7.01, p < .001, d = 1.66$) and shorter than in the invalid condition ($p = .022$). For the alerting network conditions (DC vs. NC), pairwise comparisons showed an approaching significance trend for latencies in the double cue condition to be shorter than in the no cue condition ($M_{DC} (\pm SD) = 494.03 \pm 66.26$; and $M_{NC} (\pm SD) = 522.84 \pm 64.45$; $t(30) = -1.66, p = .107, d = .44$).

Fig. 2 shows the pupillary responses for the alerting (Fig. 2a) and the orienting (Fig. 2b) networks. A two-way ANOVA showed that in the alerting network, there are significant differences in pupil dilation (PD) between conditions (main effect of condition, $F(1,30) = 5.32, p = .028, \eta^2_p = .15$) as well as greater PD in both conditions as the trial progressed (main effect of time window, $F(1,30) = 37.33, p < .001, \eta^2_p = .55$). Pairwise comparisons for each time window showed significantly greater pupil dilation for the NC than the DC condition in the early (200–500 ms: NC $M(\pm SD) = -.094 \pm .10, DC M(\pm SD) = -.134 \pm .09, p = .010$) and the middle time window (1400–1800 ms: NC $M(\pm SD) = .069 \pm .15, DC M(\pm SD) = .008 \pm .15, p = .037$), but not in the late window (1900–2400 ms: NC $M(\pm SD) = .073 \pm .11, DC M(\pm SD) = .021 \pm .12, p = .09$).

In the orienting network, we found significantly greater PD for the CC than the SCvalid condition ($F(1,30) = 4.95, p = .034, \eta^2_p = .14$), along with an overall increase in PD as the trial went on ($F(1,30) = 39.98, p < .001, \eta^2_p = .57$). Subsequent pairwise comparisons confirmed a highly significant difference between the two conditions in the late time window (1900–2400 ms, SCvalid $M(\pm SD) = .030 \pm .10, CC M(\pm SD) = .090 \pm .10, p = .002$), but not in the early window (200–500 ms, SCvalid $M(\pm SD) = -.125 \pm .12, CC M(\pm SD) = -.117 \pm .10, p = .69$) or the middle window (1400–1800 ms, SCvalid $M(\pm SD) = .026 \pm .13, CC M(\pm SD) = .068 \pm .11, p = .11$).

We further investigated the interactions of the attention networks by means of covariance PCA to determine the number of components that retained enough information to describe our PD data. For both attention networks, we found two components that explained nearly 100 % of the PD variance (see Table 1 and Supplementary Files for statistical comparisons between component scores).

Next, we tested whether the two components reflect differing attention mechanisms so that individual scores for each PCA component predict variability in saccadic responses to visual targets in the spatial cueing condition. Thus, we first ran correlations between component scores and saccadic latencies for the SCvalid conditions (see Supplementary Table 1 for correlation coefficients). Saccadic latencies were significantly positively correlated with Component 1 scores for the DC condition in both the Middle ($r = .433, p = .017$; Fig. 3a) and the Late

windows ($r = .416, p = .022$), but not with scores for the NC condition (both $ps > .18$). Moreover, Component 2 scores for the SCvalid condition only in the Late time window ($r = .528, p = .003$) were positively correlated with saccadic latencies (see Fig. 3b), while the scores for the CC in either time window were not (both $ps > .55$).

Finally, we used a hierarchical regression model to test whether both PCA components predict a unique proportion of variance in saccadic latencies in the SCvalid condition and thus reflect separable activity of different attention networks when the infants perform the spatial cueing task (Table 2). In the first step, the Component 1 for DC in the Middle Window ($\beta = .433, t = 2.55, p = .017$) predicted nearly 19 % of variance in saccadic latencies ($R^2 = .188, F(1,28) = 6.48, p = .017$). In the second step, the Component 2 for SCvalid in the Late Window ($\beta = .464, t = 3.05, p = .005$) predicted an additional nearly 21 % of variance ($\Delta R^2 = .208, F_{change}(1,27) = 9.32, p = .005$). The overall model was also highly significant and altogether explained nearly 40 % of variance in infant saccadic latencies for valid spatial cues ($R^2 = .396, F(2,27) = 8.86, p = .001$).

4. Discussion

In this study, we investigated the effects of alerting and spatial cues on changes in pupil dilation (PD) during a visual orienting task in 8-month-old infants. PD is directly controlled by the sympathetic and parasympathetic branches of the autonomic system (Loewenfeld, 1993) and indirectly reflects the activity of several neural mechanisms and neuromodulators, especially cholinergic and noradrenergic pathways (Larsen and Waters, 2018). Neural structures mediating both orienting and alerting attention mechanisms, such as the locus coeruleus (LC) and the superior colliculus modulate PD (for review see Larsen and Waters, 2018; Wang and Munoz, 2015). Thus, we expected both alerting and spatial cues to modulate PD in infancy. Since no previous study analysed the time course of pupillary responses in infants during the orienting task, we decided to apply a data-driven approach. We found differences in the time course of pupillary responses with earlier differences in average PD for the alerting (200–500 ms and 1400–1800 ms) than for the spatial cues (1900–2400 ms). Because the interaction between attention networks in ANT is complex (Callejas et al., 2004), we decided to use Principal Component Analysis (PCA) to disentangle their effects on PD. PCA identified two unique components that explained nearly all variance in the PD data: an earlier, larger component that we interpret as related to the alerting network, and a smaller, later component that we tentatively associate with the orienting network. Finally, we verified this interpretation of both PD components by showing that in a hierarchical regression each explained approximately 20 % of variance in saccadic latencies in the spatial cueing condition of the task. Our results demonstrate that the effects of alerting and spatial cues can be measured with pupil dilation in 8-month-olds. This may suggest that pupillary and saccadic responses reveal the additive effects of the alerting and orienting attention networks already in infancy. These results are broadly consistent with previous adult work (Geva et al., 2013), suggesting that the time course of pupillary responses to visual cues is similar across the lifespan.

PCA (e.g., Nuthmann and van der Meer, 2005; Lemerrier et al., 2014) identified two distinct components that explained nearly all variance in the PD data in all task conditions (Component 1 [87.24–92.45 %];

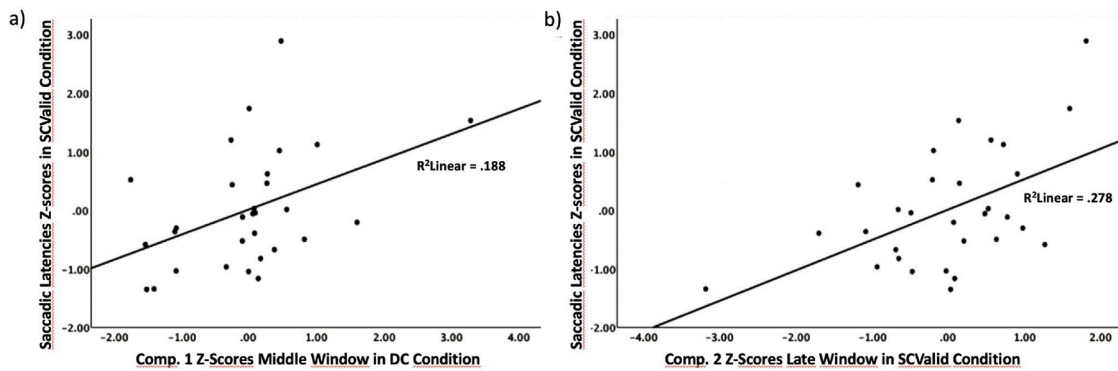


Fig. 3. Scatterplots showing correlations between saccadic latencies in the valid Spatial Cueing condition and PCA Component 1 scores in Double Cue condition the middle window (a) and PCA Component 2 scores in Spatial Valid Cue condition the late window (b).

Table 2

Hierarchical regression model with saccadic latencies in the valid Spatial Cueing condition as the dependent variable and PCA component scores as predictors. Time Windows of analyses: Middle: 1400–1800 ms; Late: 1900–2400 ms. Conditions: DC - Double Cue, SCValid - valid Spatial Cue. Significant results are marked with an asterisk: ** $p < .01$; * $p < .05$.

Predictor	B	SE	Beta	B CI 95 % Low. Upp.	
Step 1					
Middle WindowComponent 1 DC	0.429	0.168	.433*	0.084	0.774
R ²	.188*				
Step 2					
Middle WindowComponent 1 DC	0.345	0.15	.464*	0.037	0.654
Late WindowComponent 2 SCValid	0.457	0.15	.464**	0.15	0.764
ΔR ²	.208**				
R ²	.396**				

Component 2 [5.77–10.5 %]). PCA component scores indicate the relative size of each component in PD of each individual. When comparing component scores, we found significant differences between the double and no cue conditions for Component 1 in the early and middle time windows. These results together with the significant differences for relative PD found in the first two windows of the alerting condition suggest that Component 1 may reflect phasic changes in alerting. Therefore, we expected Component 2 to indicate the activity of the orienting network. However, we did not find any significant difference in the Component 2 scores between the spatial and central cue condition. This lack of significant differences is likely related to the fact that Component 2 explained only a very small proportion of variance in PD data - between 5.77 % and 10.5 %. As Component 1 explains the majority of PD variability, it is likely that alerting-related component may have masked the orienting-related changes. To further test the validity of our interpretation we ran correlations between Component 2 scores for a given condition and saccadic latencies in the same condition (see Supplementary Table 3). The default expectation was that Component 2 should predict saccadic latencies only when valid spatial cues were presented, while for other conditions there should be no correlations. We confirmed this pattern of results and found only a significant positive correlation between Component 2 scores in the late window and saccadic latencies in the SCValid condition, but in other conditions. Altogether, our data show that both alerting and orienting activity can be observed in PD, but the majority of phasic pupillary responses in 8-month-olds is explained by changes in the alerting activity.

If the two PCA components reflect the activity of separate attention systems, then the effects of each network on the average latency of infant saccadic responses should be additive. We tested this hypothesis on saccadic latencies in the spatial cueing condition because it ought to engage both, as attention is cued to a peripheral location, while the cue

itself signals an incoming target and mobilises resources necessary to respond (Galvao-Carmona et al., 2014). We found that higher scores of each component independently predicted longer saccadic latencies, with each explaining approximately 20 % of unique proportion of variance. These results suggest that PCA may provide a measure of specific attention processes in eye-tracking data that can be used to explain individual differences in orienting already at 8 months of age. Thus, our results demonstrate how the variability in the activity of attention networks revealed in psychophysiological, pupillary responses may contribute to early-emerging differences in visual attention at a behavioural level.

Previous studies (see Wass, 2018 for review) have found that larger phasic changes in alertness are associated with better stimulus encoding (Frick and Richards, 2001), faster habituation (Bornstein and Suess, 2000) and less distractibility (Lansink and Richards, 1997). Our findings complement this work and provide additional evidence for the influence of phasic alertness on orienting, but they also expand this work by showing the likely additive influences of the two attention networks on the speed of orienting in infancy. Altogether, our study highlights the utility of measures derived from pupillary responses for tracking the development of attention systems in infancy, whichobject knowledge (e.g. Jackson and Sirois, 2009) and attention (Kleberg, del Bianco & Falck-Ytter, 2018). Using PCA we were able to disentangle attentional processes that modulate pupillary responses and analysed their additive effects on saccadic reaction times. In this way, we likely provided evidence on the role of the LC-NC system in the regulation of attention in infancy. Thus, we believe that, given the diversity of applications of pupil measurement (see Hepach and Westermann, 2016 for review), the current approach might be used to map the activity of the LC and its relation to different cognitive domains (Wetzel et al., 2016).

When considering the use of PD measures of attention, it is worth taking into account the time course of these responses in relation to saccadic response latencies. While for Component 1 the changes are evident prior or concurrently with the eye movement to the peripheral target, our analyses showed that the component scores that best explain saccadic latencies are derived from PD changes that occur subsequent to the eye movement itself (in the middle and late time window). For this reason, we note that these slower PD changes might help to explain individual differences in rapid behavioural responses, but they may have limited use in predicting individual saccadic responses prior to their initiation. Nonetheless, our results illustrate, how task-related, phasic changes in PD may provide a quick and independent measure of attention systems in infancy.

Our task, coupled with PCA of pupillary responses may prove useful in investigating the infant precursors of individual differences in attention and the trajectories of attention networks in children who later show attention difficulties, such as those with ASD. A recent meta-analysis on visual orienting in ASD using the Posner-type task showed that orienting was impaired in children with ASD (Landry and Parker,

2013). Also, differences in phasic attention were found in toddlers with ASD who outperformed the typically-developing controls in visual search tasks (Blaser et al., 2014). These early atypicalities in attention may result in later atypical development of social communication in ASD (for review see Keehn et al., 2013; Jones et al., 2014). We propose that pupillary indices of phasic modulation of attention may offer a non-invasive and relatively easy to use method for studying attention mechanisms in atypical development.

While the overall pattern of results for both attention networks is broadly consistent with the previous adult study (Geva et al., 2013), we note that infant responses to DC and SC relative to control cues were inverted in comparison to the adult data. These differences could potentially be attributed, for example, to luminance changes during the stimuli, variations of on-screen luminance or variations in gaze position, which can both affect the estimates of PD. However, the detected luminance was very similar across conditions (see Supplementary Table 2) and such small differences are insufficient to influence PD to such an extent. In fact, previous studies reported that small changes in the luminance level do not affect PD (e.g. Geva et al., 2013) and that the luminance level does not influence the phasic responses (Peysakhovich et al., 2017). On the other hand, variations of the on-screen luminance can also affect pupil size (Ajasse et al., 2018). However, in our stimuli, the luminance of the cues was constant across all conditions. For instance, in the CC and SCValid conditions the luminance is exactly the same and therefore the differences are most likely arising from attentional mechanisms affecting the pupil size given the correlations between Component 2 scores in the late window and saccadic latencies in the SCValid condition. There were slight differences in luminance between the DC (cue on both sides) and the NC condition (no cue presented, but attention getter remained for 100 ms, the same as the duration of the cue). The attention getter stimulus was brighter than the cue stimulus, which would mean greater pupil constriction for the NC than the DC condition early in the trial. However, we obtained a reverse pattern: early on there was greater pupil dilation for the NC than the DC condition, thus the difference in brightness between those stimuli is the unlikely explanation of our results. Altogether this suggests that on-screen luminance cannot explain our systematic condition differences in pupil responses. Finally, we controlled for gaze position in order to minimise its influence in the estimates of PD. Initially, for a trial to qualify as valid the infant needed to fixate the central attention-getter. After that, the cue appeared for 100 ms, which is not enough time to execute a saccade (e.g. Csibra et al., 1998). Additionally, we excluded those trials where participants made a saccade in responses to the cue. Finally, all the objects were in the same position on the screen and had the same eccentricity, and was equally often on the left and right side so the saccadic responses were comparable across all conditions. Altogether, it is highly unlikely that the observed PD patterns were elicited by eye-movements or differences in the luminance level between conditions.

We were unable to find a similar case in the developmental PD literature; however, we note that similar reversed patterns of responses are not uncommon in psychophysiological research, e.g. mismatch responses to acoustic change are positive in infancy and negative in the adult brain (see Kushnerenko et al., 2013). Also, a recent study has suggested that the parasympathetic inhibition and sympathetic activation may interact differently in infants in comparison to adults which can affect the activity of the LC and therefore the speed of the pupil response (Wetzel et al., 2016). Our spatial and alerting cues produced faster responses than in control conditions, consistent with the adult literature, thus we have no reason to doubt that each cue type activated selected attention network. Furthermore, several aspects of our infant task differed from the adult ANT in the Geva et al. (2013) study: we recorded only oculomotor rather than manual and eye-movement responses, the stimulus asynchrony between the cue and the target was much shorter than in the ANT, to maintain infant looking at the screen, while adults performed additionally a flanker task on each trial, which

may have further modulated PD on a trial-by-trial basis. It is possible that the spatial and alerting cues produce different pupillary responses due to the absence of stronger modulation from the executive attention network in our infant task. Thus, the reverse pattern of pupillary responses in our infant data compared to the adult warrants further research, but it is likely explained by the reduced engaged of the executive attention network.

Finally, we note several limitations of our study. First, with our paradigm, we could not test each attention network independently (Ambrosini et al., 2013), thus we had to use PCA to separate the effect of each network. Second, it is possible that increasing infant tiredness throughout the task may reduce the network-specific effects on PD and introduce an error into our measurement of individual differences with PCA component scores. Third, one could argue that in the alerting condition the double cue may lead to a greater arousal than the control condition, no cue. This in itself may modulate the pupil dilation. However, increased arousal typically affects response times and we did not see any significant differences in infant saccadic latencies between the two conditions, which is consistent with adult data (Fernandez-Duque, Posner, 1997). Finally, both control conditions involved cues presented in the centre, while alerting and spatial cues were presented in the periphery. It is, however, unlikely that this difference between conditions affected PD itself, as previous animal work did not find an effect of stimulus eccentricity on pupil size (Wang et al., 2014).

5. Conclusions

We analysed the time course of pupillary responses to alerting and spatial cues in 8-month-old infants. Using Principal Component Analysis, we found two separate components that peaked in different time windows, which we interpreted as reflecting separable contributions of the alerting and orienting attention network. Each of these components independently explained a substantial proportion (approximately 20 % each) of variance in infant saccadic latencies, likely suggesting that oculomotor responses are modulated by both these networks already in infancy. Our study provides new evidence for the early emergence of individual differences in pupillary responses, which reflect the activity of attention networks in 8-month-olds during visual orienting.

Declaration of Competing Interest

The authors declare they have no conflict of interest.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.dcn.2020.100763>.

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