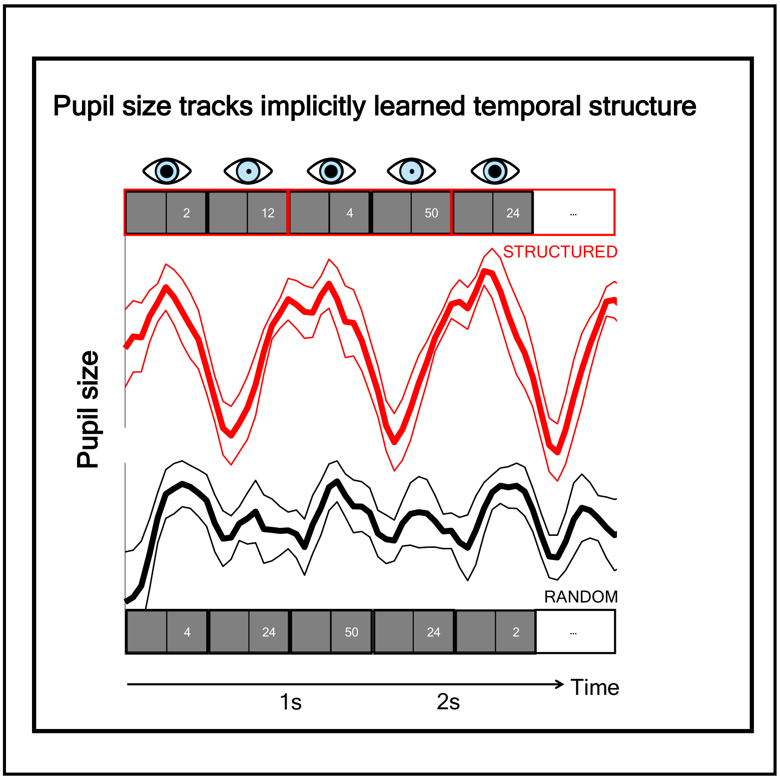
Current Biology

Report

Pupillometric signature of implicit learning of statistical regularities

Graphical abstract



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In brief

Binda et al. show that the diameter of our eye pupils tracks the statistical structure of visual stimuli, as implicitly learned through passive viewing. The learning occurs despite unawareness of the temporal structure and even when attention is not directed to the predictable feature.

Highlights

- When a temporal series is predictable, our eye pupils track its temporal structure
- The pupil-size changes generalize over local features and track abstract features
- This pupillometric signature of implicit learning does not require attention

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Report

Pupillometric signature of implicit learning of statistical regularities

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SUMMARY

Animals learn about the statistical regularities of their environment by a process of *implicit learning*, a powerful mechanism that may operate by mere exposure.[1] Implicit learning supports processes such as speech acquisition but also learning about the spatial and temporal structure of the world more generally, which is essential for effective interaction.[2] Here, we used a frequency-tagging technique to demonstrate a pupillometric signature of the learning of the temporal structure (pairing of numerosities) of sequential arrays. Although the numerosity pairings were unnoticed by all participants, the pupil responded clearly to their repetition frequency (1 Hz). Pupillometry allowed us to track the learning as it unfolded (the response became significant after less than 3 min of passive viewing), without ever directing attention to the temporal structure of the stimuli. Diverting attention away from the numerosity feature did not prevent learning, but it did affect the dynamics of the response acquisition. A clear pupillometric response was also elicited by pairing dyads of digits. In all our stimuli, the local features were randomized, implying that learning successfully generalized across stimuli that were locally different and only acquired a temporal structure once their global statistics (overall shape or numerosity) were extracted.

RESULTS

In eight separate sessions, we presented participants with 2-Hz sequences of random arrays of black and white lines (Figure 1). The number of lines varied from array to array, and so did their orientation (always homogeneous within the arrays), but as the lines comprised black and white stripes, all stimuli had identical mean luminance. In the *structured* condition, the stimuli were presented in pairs: arrays of 12 lines always followed arrays of 2, 50 followed 4, and 6 followed 24 (see STAR Methods and Figure S1 for full details). In the *control* condition, the same stimuli were presented randomly. Four sessions of the random control condition were always presented first, followed by four sessions of the structured condition. Each session lasted around 5 min.

Figures 2A and 2D show pupillary response time courses, averaged over all participants, at 4-s intervals. As expected, both conditions caused a modulation at 2 Hz, the stimulus presentation rate. However, the structured condition also showed a clear response at 1 Hz, the frequency of pairing, which was very clear in the Fourier spectrum of Figure 2B: no 1-Hz response is apparent in the Fourier spectrum of the control sequence (Figure 2E). Figure 2C shows the phase and amplitude of the 1-Hz responses of all 14 participants. In the structured condition, all vectors fall in the same hemiplane, within ±90° of the average response, and are highly statistically significant ($t(13) = 4.4$, $p < 0.001$). For the random control condition, there was no phase-coherent response ($t(13) = 1.0$, $p = 0.33$). The phase coherence of the 1-Hz response is particularly striking when compared with the scatter of the 2-Hz response elicited by the stimulus presentation (Figure 2F) and is much more variable across individuals and across conditions.

After experimentation, all participants were asked whether they noticed anything different about the sequences in the different sessions: none did. The sequences were too fast for participants to notice the pairings, which is easily verified by observing Videos S1 and S2.

To test whether the pupillometric signature of the pairing was influenced by attention, we collected data with identical sequences but two different tasks (see STAR Methods for details): reporting when two successive stimuli had the same numerosity or when they had the same line orientation. This procedure was designed to direct attention to either the numerosity or the orientation of the stimuli, without drawing attention to the structured pairings driving the 1-Hz response. Figure 3A plots the amplitudes of the 1-Hz response of all participants to paired stimuli (log normalized by the response to random stimuli) in the attend-to-orientation condition against the attend-to-numerosity condition. The average log-normalized amplitude of the attend-to-numerosity condition was slightly higher than the attend-to-orientation

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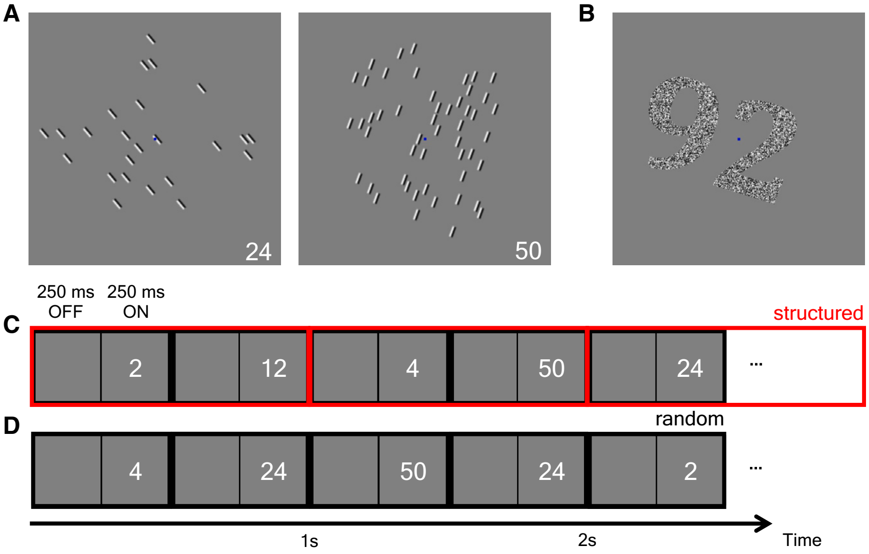

Figure 1. Stimuli used in the main study

(A) Example of the line array stimuli, each element comprising a black and white line, with average luminance equal to the background; the number in the bottom right corner shows the numerosity (but was not displayed). See also Figure S1 and Videos S1 and S2.

(B) Example of Arabic digit dyads, made from black and white texture of the same mean luminance as the background and presented with jittered orientation and position.

(C) Example sequence for the structured condition, with 12 following 2, 50 following 4, etc.

(D) Example of random sequence, comprising the same numerosities as the structured condition, but in a random order.

condition (0.33 vs. 0.24), but the difference did not approach significance ($t(13) = 0.9$, $p = 0.39$).

How long does statistical learning take? Contrary to most behavioral techniques, our approach did not require explicit testing of learning on separate trials, so we could monitor the learning as it unfolded by tracking the amplitude of the pupil response to pairings. The traces of Figures 3B and 3C show that the response develops quite quickly, especially when attention is directed to numerosity rather than orientation. Figure 3B shows a very orderly learning curve, reaching statistical significance (between participants) after less than 3 min of testing. The significant response is maintained and increases further in the second numerosity session. The curves for the attend-to-orientation condition (Figure 3C) are far less orderly and often fall below significance. Considering only the second half of the experiment, the learning was significantly stronger when attention was directed to numerosity than to orientation ($t(13) = 2.5$, $p = 0.025$), although the two were indistinguishable in the first half ($t(12) = 0.1$, $p = 0.946$). Taken together, these results suggest that attention to numerosity is not essential for implicit learning of structure in the numerosity sequences but that it does facilitate and accelerate the learning process.

Do arrays need to be visually discriminable to yield a pupillometric signature? We repeated the experiment with pairs of arrays that were closer in numerosity: 20 and 22, 23 and 24, and 28 and 26. These pairs differ in numerosity by less than 15%, near or below the just-discernible difference (JND) for numerosity in this range[3] and not obviously different on inspection (see Figure S2). These stimuli led to no significant 1-Hz pupillary modulation, showing that the stimuli had to be discernibly different to drive the pupil. Whether the threshold for pupil response is the same as the psychophysical discrimination threshold remains an open question.

Does the pupil response to temporal structure occur only for spatial arrays of varying numerosity or for any discernible repetition? We repeated the experiment with Arabic digit dyads, made from black and white texture of the same mean luminance as the background (Figure S1B, using the same pairing principle as before; see STAR Methods for details). The results are shown in Figure 4, showing the response to the pairing (as in Figure 3, normalized by the 1 Hz response in the random condition and log transformed). Like the array stimuli (first two bars), the digit dyads elicited a strong and reliable 1-Hz response ($t(7) = 2.6$, $p = 0.038$). To be certain that the response was to the information contained in the digit dyads (e.g., 98) rather than the local spatial information, we randomly varied the orientation of the digits between ±30° and the position between ±1° of screen center (Figure 1B) and still found a reliable response ($t(7) = 2.9$, $p = 0.024$). Lastly, we mixed digits with arrays of lines, with specific digits paired with specific arrays of lines (e.g., 98 paired with 24 lines; see Figure S1C). Again, this produced a reliable response ($t(7) = 5.1$, $p = 0.001$). One-way ANOVA comparing data across experiments revealed no significant variations ($F(4,47) = 0.6$, $p = 0.6$). The same statistics for the 2-Hz response were distributed around zero, indicating that the response elicited by the stimulus presentation was undistinguishable between the structured and the random conditions in all conditions (all $t < 2$, all $p > 0.05$; not shown).

DISCUSSION

This study shows that the visual system is exquisitely sensitive to the structure of the numerosity in rapid sequences of random arrays and that the structure drives a clear pupillary response.

The pupillary response was not driven by low-level aspects of the image, such as local luminance or contrast, but by a global feature, the number of elements. On each presentation, the line elements were positioned randomly and at random orientations: the only structure in the sequence was that twelve lines predicted that the next array would contain two lines (and so forth), not their position or orientation: detecting this structure requires encoding of numerosity. When the difference in numerosity was insufficient to be detected, no response was measurable. Although the line arrays do vary slightly in contrast energy, the digits—constructed by modulation of random noise—do not, further evidence that contrast was not driving the effects. Again, the meaning of the digit dyads had to be interpreted for the temporal structure to emerge as the local structure was not informative, and the response also occurred when their orientation and

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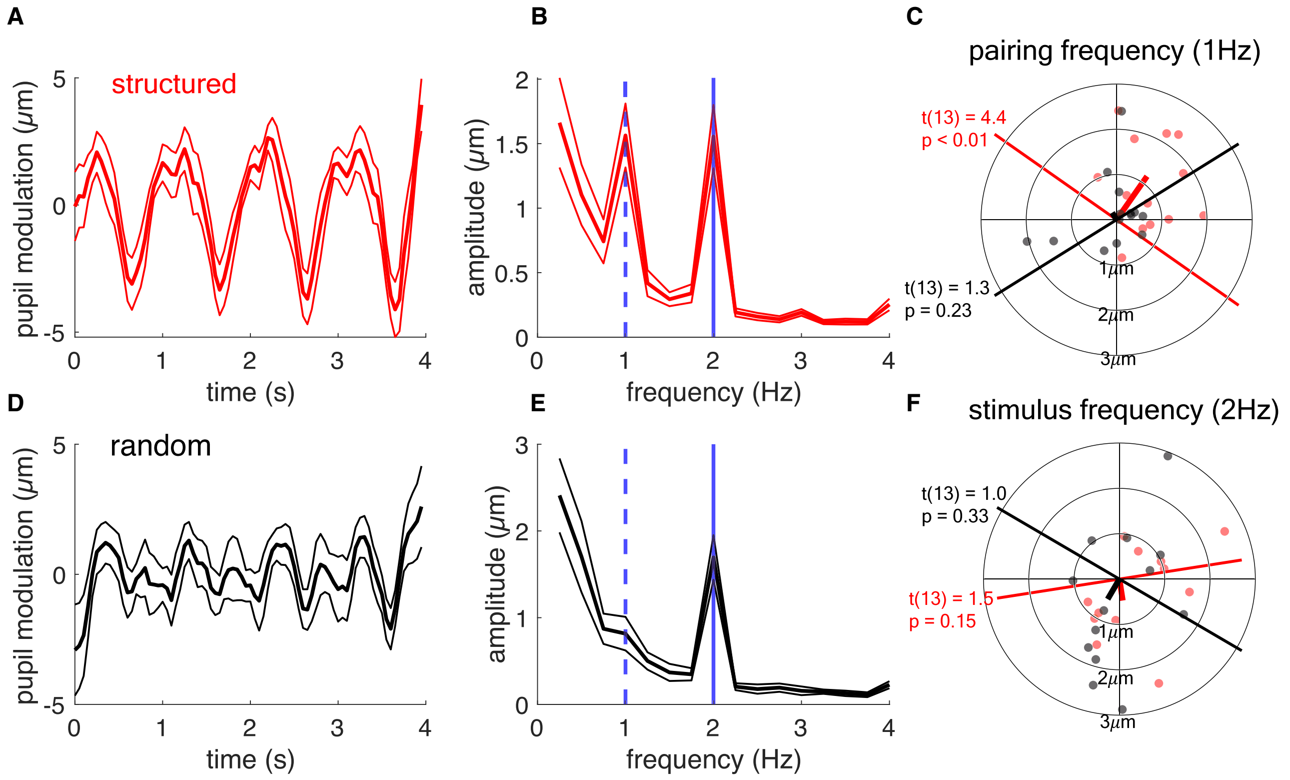

Figure 2. The pupil responds to the learned temporal structure

(A) Pupillary response to the structured (paired) stimulus presentations, averaged over all presentations and participants for a 4-s interval and high-pass filtered to eliminate components below 1 Hz. The four peaks of the 1-Hz modulation are clearly visible. Thin lines represent mean ± SEM.

(B) Fourier transform of the pupillary responses (as in A, but unfiltered), showing two clear peaks—at 1 Hz (the frequency of pairing) and at 2 Hz (the frequency of the stimulus). Thin lines represent mean ± SEM.

(C) Amplitude and phase of the 1-Hz response for the structured (red) and random (black) stimuli for all participants. The color-coded lines are drawn orthogonal to the average in that condition to highlight that most vectors of the structured condition lie in the same hemispace, leading to a significant t test (inset), whereas those of the random condition show no clear tendency.

(D) As for (A), for the random control condition, showing no obvious modulation at 1 Hz.

(E) Fourier transform of the pupillary response, with a unique peak at 2 Hz, the frequency of stimulus presentation.

(F) Amplitude and phase of the 2-Hz response for the structured (red) and random (black) stimuli for all participants. These are far less orderly than the 1-Hz response and do not reach statistical significance. See Figure S2 for a control experiment where the stimuli were not easily discriminable, and no pupillometric evidence for implicit learning was observed.

position varied from trial to trial. Although this is clearly not an easy task at the rate of two images per second (certainly impossible to verbalize at that rate), it is possible to saccade rapidly to the more numerous of two dot arrays in less than 300 ms, showing that rapid resolution of numerosities is feasible.[4]

One important implication is that the implicit learning measured here generalized across stimuli, as no two stimuli were ever identical. Previous work did not always find evidence for generalization, suggesting that implicit learning may not be a unitary phenomenon.[5] It may be best understood as an umbrella term encompassing very different abilities: from relatively simple "chunk learning" that emerges in most species and at all ages but has limited ability to generalize to complex and flexible "artificial grammars," which generally require active task engagement and may be more selectively observed, such as in older participants. The level of complexity required by our experiments is between these extremes, with relationships emerging between adjacent stimuli with fixed order yet variable in many other respects. Consistent with this, we find that attending to the concept of numerosity (by searching for repetitions) facilitated learning. However, it was not essential, with excellent pupillary responses on trials when attention was directed toward the orientation rather than the numerosity of the line elements. This is consistent with the idea that statistical learning, at least in its simpler form, is a very powerful mechanism that operates by mere exposure.[1]

Why should pupil diameter track implicit learning? A variety of studies in the last decade show how complex perceptual and cognitive phenomena can modulate pupil size (e.g., Tortelli et al.[6] and Turi et al.[7]). For example, the pupillary response to light (or other visual features, like contrast) is enhanced when stimuli are attended or perceptually more conspicuous.[8,9] More relevant to this study, numerosity modulates the gain of the pupillary light response.[10,11] In addition, pupil dilation systematically signals violations of expectations, typically generated in the context of complex sequence prediction tasks

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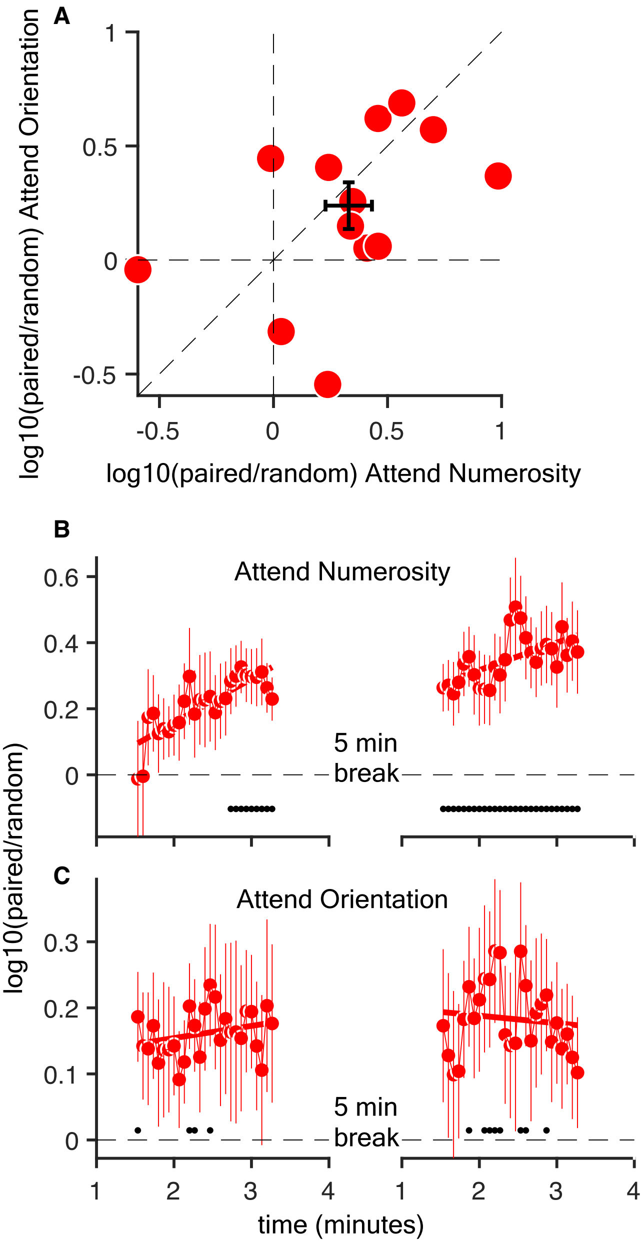

Figure 3. Attention is not essential for statistical learning but facilitates it

(A) Scatterplot comparing the average normalized amplitude (structured condition divided by random condition, log transformed) of the 1-Hz pupillary response for the attention-to-orientation condition with that of the attention-to-numerosity condition. The averages (black cross comprising error bars) are similar for the two conditions. The correlation between the normalized log-transformed amplitudes is significant ($r = 0.54$, $p < 0.05$), an indication of reliability.

(B and C) Log-normalized 1-Hz response as a function of time in each of the two experimental runs (separated by a 5-min break) for the attention-to-numerosity condition (B) and attention-to-orientation condition (C). The black dots near the abscissae indicate statistical significance of the log-transformed amplitudes ($p < 0.5$, between participants). Error bars represent SEM.

(e.g., Nassar et al.[12] and Urai et al.[13]) but also observed in passive listening.[14] This work interprets pupil dilations as the consequence of "belief updating," where an internal model of the stimuli is distrusted in the face of inconsistent sensory evidence. Thus, pupil dilation may be a by-product of activation in the locus coeruleus, which broadcasts a norepinephrine signal to vast regions of the brain (including the pupil control centers), promoting the processing of bottom-up information, usually inhibited by top-down predictions.[15]

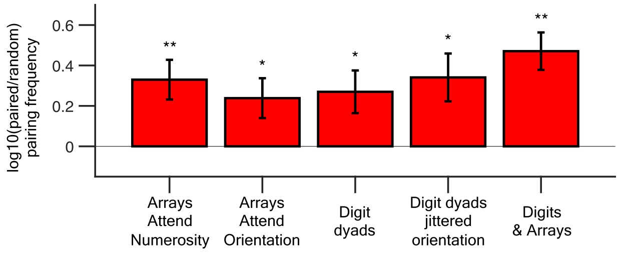

Figure 4. Generality of the pupillary signature of statistical learning

Average normalized amplitude (structured condition divided by random condition, log transformed, and compared against 0 by t test) for the various conditions tested in this study: dot arrays (attending to numerosity, $t(13) = 3.4$, $p = 0.005$), dot arrays (attending to orientation, $t(13) = 2.4$, $p = 0.031$), digital dyads (same orientation, $t(7) = 2.6$, $p = 0.038$), digital dyads (jittered orientation, $t(7) = 2.9$, $p = 0.024$), and digital dyads and dot arrays ($t(7) = 5.1$, $p = 0.001$). Error bars represent SEM.

Asterisks report the significance of the t tests (* for $p < 0.05$, ** for $p < 0.01$). For details of the stimuli, see Figure S1 and Videos S1, S2, S3, and S4.

This view fits naturally into the "predictive coding" framework,[16,17] which assigns crucial importance to *a priori* information that represents internal models of the world. Implicit learning of the statistical regularities in the sensory input could be the main mechanism to acquire these models.[2] In line with this, previous work has shown that consistent exposure to the stimulus statistics could be implicitly exploited to improve behavior, such as through learning the contingencies of task-relevant stimuli (e.g., location probability[18]) or the distributions of distracters.[19] We cannot be certain that the pupillary response to statistical regularities is driven by the same mechanisms tapped by psychophysical procedures,[18,19] but it does seem highly probable, particularly in the light of work showing that low-level sensory responses can be modulated by contextual information learned through passive exposure of stimulus pairs,[20] which could be tracked by pupillometry.[21]

The idea of pupil dilation signaling a belief update would be completely consistent with pupillary dilation with every unpredictable image: in our paradigm, with every first sample in a pair. However, with the current frequency-tagging steady-state technique, it is not possible to determine whether the pupillary response is driven directly by the *predicted* stimulus (the second element of the pair) or by violations of prediction (beginning of the next pair): both occur at 1 Hz and could drive the 1-Hz response. This could be readily examined by varying the temporal frequency of presentation (to obtain an estimate of response delay) or by sequences comprising both pairs and random coupling. The technique could also be readily adapted to test what the object of learning is: predictions (specific for the order of the stimuli, e.g., in a pair) or associations (order independent). These questions exemplify the many open issues related to implicit learning, which the pupillometry technique presented here could help advance.

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RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources should be directed to and will be fulfilled by the lead contact, Paola Binda: paola.binda@unipi.it.

Materials availability

This study did not generate new, unique reagents.

Data and code availability

- The anonymized data reported in this study have been deposited at Zenodo (https://doi.org/10.5281/zenodo.14809868) and are publicly available as of the date of publication.
- All original code has been deposited at Zenodo at (https://doi.org/10.5281/zenodo.14809868) and is publicly available as of the date of publication.
- Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

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AUTHOR CONTRIBUTIONS

P.B. and D.C.B. conceived the study. P.B., D.C.B., and M.T. designed the research. P.B. and M.T. implemented the experiment. C.T. collected the data. P.B. and D.C.B. analyzed and interpreted the data, prepared the figures, and wrote the paper.

DECLARATION OF INTERESTS

The authors declare no competing interests.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.cub.2025.02.011.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Deposited data		
Psychophysical data	This paper	https://doi.org/10.5281/zenodo.14809868
Software and algorithms		
Online code	This paper	https://doi.org/10.5281/zenodo.14809868
MATLAB R2021b MathWorks	This paper	RRID: SCR_001622
Psychtoolbox-3	This paper	RRID: SCR_002881

EXPERIMENT MODEL AND STUDY PARTICIPANT DETAILS

Nineteen observers participated in the experiment (8 males, age range 23-31 years, mean 25.4 years, SD 3.1). All participants had normal or corrected-to-normal vision and provided written informed consent before taking part in the study. Different participants were engaged across various studies, with some taking part in multiple studies. Altogether, 14 individuals participated in the primary study (Line arrays), 6 in the numerosity array control study (small differences in numerosity), and 8 each in the two experiments with digit dyads and the cross-format experiment. Except for author CT, all participants were naive to the purpose of the experiment. Experimental procedures were approved by the regional ethics committee (*Comitato Etico Pediatrico Regionale, Azienda Ospedaliero-Universitaria Meyer*, Florence, Italy) and were in line with the principles of the declaration of Helsinki.

METHOD DETAILS

Participants sat 57 cm from an Acer LCD monitor (resolution 1920 x 1080 pixels), in a quiet, dimly lit room. They fixated a central blue square and observed a sequence of stimuli displayed at 2 Hz, each stimulus displayed for 250 ms, with 250 ms interstimulus interval. Head motion was minimized by chinrest, and pupil size and eye-position monitored by an eye tracker (EyeLink 1000) positioned below the monitor (more details below).

Stimuli

Line arrays

For the main experiment (N participants = 14), we generated arrays of lines randomly positioned within a 10 x 10° window (Figure 1A; Video S1). Each element in the array comprised two adjacent lines, one white (95 cd/m^2) and one black (5 cd/m^2), superimposed on a grey background of 50 cd/m^2 luminance. The number of elements in the array could be 99, 50, 24, 12, 6, 4, 3, 2, or 1. In the structured condition, numerosities were paired so that 2 was always followed by 12, 4 by 50, and 24 by 6. In addition, to create the numerosity attention task (see below), numerosities were caused to repeat on 20% of the trials; repetitions did not interrupt the pairing structure but were part of additional (infrequent) pairs: 12 followed by 99, 50 followed by 3 and 6 followed by 1. The sequences of pairs were totally random. In the control condition, the sequences were completely random except for the repetitions for the attention task. The same sequences were presented to all participants.

Within each array, all lines had the same orientation, but this orientation varied on each presentation. The variation was random, except for the constraint that 20% of the trials the orientation was repeated, which served as stimuli for the orientation-repetition task.

Line arrays with similar numerosities

For a control experiment (N participants = 6), the numerosity of the arrays were very hard to discriminate (20, 21, 22, 23, 24, 25, 26, 28, 30). All other parameters were the same as in the main experiment.

Digit dyads

In two additional experiments (N participants = 8), stimuli were double numbered Arabic digits made from random black-white texture of mean luminance matched to the background. The dyads (10, 11, 13, 15, 17, 20, 23, 26, 30, 35, 40, 46, 53, 61, 70, 80, 92 and 98) subtended 10 x 10°, positioned at screen centre (Figure S1B). Again, in the structured condition one dyad predicted another: six frequent pairs (15 followed by 30, 20 and 92, 40 and 46, 53 and 11, 61 and 13, 98 and 26) were intermixed with another six pairs (30 and 70, 92 and 17, 46 and 10, 11 and 80, 13 and 35, 26 and 23), which occurred infrequently (about 20%) and created the repetitions that participants detected for the vigilance task. The sequence was shuffled for the random condition.

In the first experiment, stimuli were always upright. In the second experiment, the orientation and position were randomized between trials within a range of ±30° of vertical and ±1 deg of screen centre.

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Arrays and digit dyad stimuli

A final experiment (N participants = 8) used both arrays of lines and digit stimuli, generated by the procedures explained before. The pairings (shown in Figure S1) are: 4 lines followed by 2 in digits; 50 lines followed by 12 in digits; 24 lines followed by 6 in digits. These frequent pairs were intermixed with another three infrequent pairs (about 20%): 2 in digits followed by 99 lines; 12 in digits followed by 3 lines; 6 in digits followed by one line. The sequence was shuffled for the random condition.

Session and Tasks

To ensure that participants attended to the images, they were required to perform simple vigilance tasks, identify repetitions of stimuli. The array experiment had two separate tasks: detect the occurrences of repetitions in numerosity of line arrays, or in the orientation of the lines. The repetitions of both features occurred in all sessions, only the task changed. For the digit and cross-model experiments, participants detected repetitions in numerosity. In all cases, the repetitions occurred on 20% of the trials. The motivation for using repetition-detection as the task was that it was independent of the sequence pairings, drawing attention to the general idea of numerosity, but not the specific structure being measured.

For the array experiments, all participants completed 8 sessions, each with 594 stimulus presentations, lasting just under 5 minutes: the first four sessions were random followed by four structured. In the first two sessions (both random and structured), half the participants (random assignment) attended to numerosity, the other half to orientation; in the second two to the other task. The two experiments with digit dyads, and the cross-modal condition comprised four sessions each, again with the two random sessions first. Participants were allowed a short break between sessions, usually lasting 1-5 minutes.

QUANTIFICATION AND STATISTICAL ANALYSIS

Eye-tracker data acquisition

Eye movement and pupil data were collected with an EyeLink 1000 system (SR Research, Kanata, Canada), positioned 55 cm from the eyes, below the monitor. It recorded eye-position and pupil size of the left eye at 1000 Hz. A 9-point calibration and validation procedure were carried out for each participant at the beginning of each session.

Eye-tracker data pre-processing

Data analyses were performed in MATLAB (R2021b MathWorks). Pupil data was recorded and pre-processed separately for each block. We linearly interpolated blinks and other missing values due to non-detected pupil during the recording or to pupil values varying at a physiological unrealistic speed higher than 10 mm/s. Data were down-sampled to 100 Hz, divided into epochs of 4s and each epoch was linearly detrended. For visualization purposes, epochs were further high-pass filtered to remove components < 1 Hz; all analyses, however, were performed on the unfiltered traces.

Data analysis

We calculated the mean pupil timecourse across epochs and we obtained one 4s pupil trace for each participant and condition (except for studying the learning curve, see below). We computed the Fast Fourier Transform (FFT) of the signal and extracted amplitude and phase of the two frequencies of interest: 1 Hz of the pairing frequency and 2 Hz of the image frequency. We evaluated the statistical significance of each response (at 1 Hz and 2 Hz) in two ways. We first looked at its phase-coherence across participants (Figures 2C and 2F). For this, we computed the vectorial sum of the participants' responses, then projected the individual responses to the orthogonal plane, assessing their distribution through a one-sample t-test of the distance from the plane. We also implemented a second approach that looked at the amplitude of responses in the structured condition, normalized by the same response in the random condition. The ratio was log-transformed to ensure normality, then tested by standard t-tests: paired t-tests to compare responses across attentional tasks (Figure 3A) or one-sample t-tests to assess the significance of the response (Figure 4). This second approach was applied to studying the learning curve of the response to pairs. Instead of computing a single 4s long pupil trace per participant and condition, we extracted a series of pupil traces by selecting segments of 45 epochs (each 4s long, corresponding to 180 ms) and shifting the selection in steps of 1 epoch. This running average was computed for the structured and random conditions; the structured values were divided by the random, and log-transformed, giving the timecourses in Figures 3B and 3C. In these figures, values are reported as a function of the average time from the beginning of each run.