Title: The aperiodic exponent of resting-state pupil fluctuations predicts excitation/inhibition balance in attentional processing

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Abstract

The aperiodic exponent features neural signals in various modalities and has been linked to the excitation/inhibition balance of the neural system. Leveraging the rich temporal dynamics in the resting-state pupil fluctuations, the present study took the first attempt to investigate the cognitive significance of the aperiodic exponent of pupil fluctuations and its association with the neural excitation/inhibition balance in attentional processing. In separate phases, we recorded participants' pupil fluctuations during the resting state and evaluated their visual attention using the Posner cueing task with varied cue validity (i.e., 100% and 50%). We found significant correlations between the aperiodic exponent of resting pupil fluctuations and both the microsaccadic and behavioral responses indicative of attentional processing characteristics. Critically, this association was particularly evident under the condition of high stimulus uncertainty (i.e., with 50% cue validity), highlighting the strong reliance on neural excitation/inhibition balance. Further analysis revealed that the aperiodic exponent of pupil fluctuations predicted the severity of ADHD symptoms of hyperactivity/impulsivity across individuals, suggesting its potential as a marker for attentional deficiency traits. These findings underscore the rich information contained in pupil fluctuations and provide a novel approach to evaluating the neural excitation/inhibition balance in cognitive processing, both in normal and abnormal attention.

Keywords

Aperiodic exponent, pupil size, excitation/inhibition balance, visual attention

Introduction

Brain activities at different spatiotemporal scales often exhibit a 1/f-like power spectrum – power decreases with increasing frequency, which represents aperiodicity of the neural activities. This aperiodicity has been demonstrated in the fluctuations of electrocorticography (ECoG, Manning et al., 2009), electroencephalogram (EEG, Waschke et al., 2021), magnetoencephalography (MEG, Dehghani et al., 2010), and functional magnetic resonance imaging (fMRI, He, 2011) signals. Additionally, 1/f-like power spectrums have also been observed in the behavioral recordings of animals (Jones et al., 2023) and humans (Palva et al., 2013). Intriguingly, while the slopes of the power spectrum are different for different neural-signal modalities, their aperiodic dynamics are strongly related across modalities (Jones et al., 2023), indicating a general neural mechanism underlying these dynamics. Evidence has suggested that the slope of the power spectrum (i.e., the aperiodic exponent) tracks the ratio of excitation and inhibition in the underlying neuronal circuits (Gao et al., 2017). For instance, causal evidence has been found where the anesthetic drug propofol, which enhances neural inhibition, increases the aperiodic exponent, while the anesthetic drug ketamine, which enhances neural excitation, decreases the slope of the aperiodic exponent (Waschke et al., 2021). As a result, the general mechanism underlying the aperiodic dynamics of the various neural signals is suggested to be a reflection of the excitation/inhibition (E/I) balance in the associated neural system.

Like neural activity and behavioral responses, the pupil fluctuates widely and dynamically over time (Reimer et al., 2016), even at rest. Extensive evidence has suggested that the raw pupil size tracks the degree of attention and the state of arousal (Contadini-Wright et al., 2023; Joshi & Gold, 2020), but direct examinations of characteristics of the pupil fluctuations, the aperiodic exponent of

pupil fluctuations in particular, are lacking. Indirect evidence supports the association between pupil fluctuations and the neural E/I balance. Pfeffer et al. (2022) recently revealed a negative correlation between the pupil size and the aperiodic exponent of the MEG signal, indicating a significant relationship between pupil size and neural E/I balance. Critically, pupil fluctuations, such as the hippus (oscillations below 1 Hz), reflect neural excitability better than raw pupil size (Binda & Lunghi, 2017; Pomè et al., 2020). These findings inspired the present study to investigate whether the aperiodic exponent of pupil fluctuations could also reflect neural E/I balance just like neural signals in other modalities. Furthermore, being controlled by the locus coeruleus-norepinephrine (LC-NE) and the basal forebrain-acetylcholine (BF-ACh) systems (Thiele & Bellgrove, 2018), the pupil fluctuations may specifically be associated with the E/I balance in attentional processing, which is controlled by the two neurotransmitter systems as well. As evidenced by Yu and Dayan (2005), both the LC-NE and BF-Ach neurotransmitter systems utilize information uncertainty for attentional processing. These findings raise a possibility that the aperiodic exponent of pupil fluctuations may reflect the characteristics of attentional processing by reflecting the E/I balance of the attentional system.

The abnormality of neural E/I balance could reflect the symptoms of attention-deficit/hyperactivity (ADHD). Experimental evidence has shown changes in the spectral slopes of neural signals in ADHD patients. For example, Robertson et al. (2019) reported that the spectral slopes were steeper in preschool-age children with ADHD. Consistently, children with ADHD have been found to show a greater theta-beta ratio (Arns et al., 2013), which also implies a steeper spectral slope (Finley et al., 2022). However, other researchers have found flatter spectral slopes in school-age and adolescent ADHD samples (Ostlund et al., 2021). Karalunas et al. (2022) reconciled this

conflict by showing that infants with a family history of ADHD had steeper spectral slopes, whereas adolescents diagnosed with ADHD had flatter slopes, suggesting that the trend reverses across age. Based on these findings, we further hypothesized that if the aperiodic exponent of resting-state pupil fluctuations could reflect the neural E/I balance, then this aperiodic exponent should be associated with ADHD symptoms.

To investigate the association between the aperiodic exponent of pupil fluctuations and the neural E/I balance, we examined the relationship between the aperiodic exponent of the resting pupil and the characteristics of attentional processing across individuals. Pupil fluctuations were recorded in the resting state, while participants' behavioral and microssacadic responses to exogenous attention were measured during an independent Posner cueing task. Different cue validities were included to manipulate the E/I balance of attentional processing (Fig. 1A). Results showed a significant correlation between the aperiodic exponent of resting pupil and the behavioral and microsaccadic responses when stimulus uncertainty was high and thus an E/I balance during attentional processing was required (i.e., the condition of 50% cue-validity). The microsaccadic responses mediated the association between the aperiodic exponent of resting pupil and the behavioral response. To validate the association between the aperiodic exponent of the resting pupil and the E/I balance in attentional processing, we further measured participants' ADHD symptoms with the Adult Self-Rating Scale (ASRS, Kessler et al., 2005). Replicating the findings in the spatial attention tasks, the aperiodic exponent was also predictive of hyperactivity/impulsivity in ADHD symptoms. Together, the present study demonstrates for the first time that the aperiodic exponent of pupil fluctuations reflects neural E/I balance in attentional processing and further suggests that this exponent could be used as a measure of adult ADHD symptoms.

Methods

Participants

Seventy adult volunteers (33 males, 19-25 years old) were recruited. The sample size was chosen based on previous studies, which suggested that a sample of 70 participants was adequate for a reliable individual differences approach (Carter Leno et al., 2022; Karalunas et al., 2022). All participants had normal or corrected-to-normal vision and had no known neurological or visual disorders. The research protocols were approved by a local ethics committee at Hangzhou Normal University. Written informed consents were obtained from all participants prior to the experiment.

Apparatus and tools

The visual stimuli were presented on a CRT monitor (resolution: 1024×768; refresh rate: 85 Hz). The MATLAB (The MathWorks) and the Psychophysics Toolbox (Brainard, 1997) were used to present the stimuli and to record the behavioral responses. The experiment took place in a dimly lit and sound-proof room. The participants comfortably seated at a viewing distance of about 70 cm, with their heads stabilized on a chin rest.

Stimuli and procedure

The experiment consisted of a spatial cueing task phase and a resting-state eye-movement recording phase. The spatial cueing task was similar to that in our previous study on attentional shifting (Jia et al., 2017; Lv et al., 2022). Fig. 1A showed the events that occurred in a typical trial. At the beginning of each trial, a central fixation dot (0.16°×0.16°; 0 cd/m^2), flanked by two square place holders (4°×4°; 104 cd/m^2; distance to the fixation dot: 6°), was presented on a gray background (23 cd/m^2) for 1000-1500 ms. Then, the border of one of the placeholders suddenly dimmed to 0 cd/m^2 in luminance for 100 ms, serving as exogenous cueing on the corresponding

spatial location. Following an inter-stimulus interval of 1000-1500 ms, a bar (size: 1.30°×0.13°; luminance: 67 cd/m^2) tilted left or right by 2° appeared in one of the placeholders for 100 ms as a probe. The participants were instructed to report the tilt orientation of the probe by pressing one of two keys within 1000 ms. The participants were required to maintain fixation throughout the whole trial. The cue validity was manipulated within participants with a block design. Specifically, participants completed two blocks of the spatial cueing task, with the validity of the spatial cue being 100% and 50% in each block, respectively. The assignment of the validity to each block was balanced across participants, and participants were informed about the cue validity before starting each block. Each block contained 200 trials, taking about 20 minutes to complete. Participants completed 15 practical trials at the beginning of each block.

After the spatial cueing task, participants underwent a resting-state phase, in which their eye movement information was recorded. During this phase, participants were required to look at the fixation point (0.16°×0.16°; 0 cd/m^2) in the center of the gray screen (23 cd/m^2) for 300 s and were told to minimize blinking.

Eye movement recording

Eye movements were recorded binocularly at 1000 Hz with an EyeLink 1000 eye tracker (SR Research, Ottawa) during the whole experiment. A chin rest was used for each participant to maintain good tracking accuracy. The eye tracker was calibrated with a standard 9-point calibration procedure at the beginning of each experimental block.

Microsaccade detection in the spatial cueing task

Microsaccades were detected with an improved version (Engbert & Mergenthaler, 2006) of the algorithm originally proposed by Engbert and Kliegl (2003). Horizontal and vertical eye positions

were mapped onto a velocity space, to which a 5 standard deviations threshold was applied to detect saccades. For a detected saccade to be counted as a microsaccade, three criteria must meet: 1) a temporal overlap between the two eyes, 2) a minimum duration of 6 ms, and 3) an amplitude below 1°. Trials that contained saccades larger than 1° were excluded from the analysis. This data cleansing procedure excluded 8% and 10% of the trials from the 100% and 50% cue validity conditions, respectively.

Microsaccade rate analysis

A rectangular moving window of 100 ms (stepped in 1 ms) was used to examine the microsaccade rate in a time window of 100 ms before and of 1000 ms after the attention cue onset (Engbert & Kliegl, 2003). Considering that a microsaccade could land in the same or opposite hemifield as the cue, we will use "congruent" and "incongruent" to refer to these two types of microsaccades, respectively. The cueing effect was revealed in the rate difference between the congruent and incongruent microsaccades.

Resting-state pupillometry data processing

The PuPl toolbox (Kinley & Levy, 2021) was used for pre-processing of the raw pupil size data, which took the following three steps:

1. Raw pupil size data cleaning. The samples in which pupil size is changing at an implausibly high rate (i.e., where the "dilation speed" is too high) and samples that are small "islands" surrounded by missing data are recognized as outliers and deleted from the recordings (Kret & Sjak-Shie, 2019). The threshold of the dilation speed was set to be the median speed plus 3 times the median absolute deviation. The "islands" were defined as samples with a duration of less than 30 ms and are surrounded by missing data exceeding 25 ms.

2. Blink identification. Blinks were identified with the pupillometry noise method (Hershman et al., 2018). The identified blink samples were deleted from the recordings along with their adjacent data within 50 ms prior to and 150 ms after the identified blinks.

3. Data interpolation, smoothing, and downsampling. The gaps in pupil size data resulting from data cleaning and blink removal were filled in using linear interpolation. High-frequency noises in the pupil size data were removed by data smoothing using a moving mean filter with a 150-ms length Hanning window (Zhao et al., 2019). Finally, the data were downsampled from 1000 Hz to 100 Hz and averaged across two eyes.

Spectral slope and power analysis

The 300-s pre-processed pupil data were first normalized for each participant to remove the mean difference between participants. Then, the power spectral density (PSD) of the normalized pupil data was calculated using the Welch's method with a window length of 5000 data points and 50% overlapping windows. The "Fitting Oscillations and One-Over-f" (FOOOF) toolbox was used to calculate the aperiodic exponent (Donoghue et al., 2020) of the PSDs, which uses a spectral parameterization algorithm to decompose the power spectrum into periodic and aperiodic components via an iterative model fitting process. Considering that the fluctuations of pupil size are mainly concentrated between 0-1 Hz (Pomè et al., 2020; i.e., pupillary hippus; Turnbull et al., 2017) and that the spectral plateau above 1 Hz could disrupt the estimation of aperiodic exponent (Gerster et al., 2022), we extracted aperiodic exponents from the 0-1 Hz frequency range of each power spectrum (aperiodic_mode = 'fixed', max_n_peaks = 'inf', default settings otherwise). The 'fixed' setting was used given that we did not anticipate a "knee" in the power spectrum. This assumption was supported by visual inspection of each PSD after spectral parameterization via FOOOF.

ADHD symptoms measurement

ADHD symptoms were measured using the World Health Organization Adult ADHD Self-Report Scale (ASRS; Kessler et al., 2005), an eighteen-item measure of ADHD symptoms that can be administered verbally or through survey. The "inattention" and "hyperactivity/impulsivity" symptoms were each measured with nine items of the ASRS. Participants select how frequently each statement is true in the past 6 months for them on a five-point scale from 0=never to 4=very often. Higher scores are indicative of more severe symptoms associated with ADHD.

Statistical analysis

Independent samples t-tests were used in tests for differences in behavioral accuracies and the behavioral cueing effects between groups of participants. The cluster-based permutation test (Maris & Oostenveld, 2007) was used to test the between-group differences in the cueing effects in the microsaccade rate. Pearson correlation was used for all correlation analyses.

As significant paths were found between the aperiodic exponent and the microsaccadic cueing effect, the aperiodic exponent and the behavioral cueing effect, and the microsaccadic cueing effect and the behavioral cueing effect, a formal participant-wise mediation model was implemented. The indirect path from the aperiodic exponent to the microsaccadic cueing effect (a) and the microsaccadic cueing effect to the behavioral cueing effect (b) was computed, as was the direct path from the aperiodic exponent to the behavioral cueing effect (c'). The indirect path and mediation were computed as the product of $a \times b$, with a resulting 95% confidence interval for each indirect path from 1,000 bootstrapped samples. The total, direct, and indirect effects were tested for significance at $P < 0.05$.

Results

The aperiodic exponent of resting pupil fluctuations associates with the attentional cueing effect

We first validated the pupil size recordings by examining the characteristics of pupil fluctuations. Fig. 1B shows that the pupil size in the resting state gradually decreased over time during the 300-s recording phase. The power spectral density (PSD) of the pupil size fluctuations (Fig. 1C) followed a 1/f power law distribution, with the power being higher at lower frequencies and decreasing rapidly with increasing frequency. The decrease in power was roughly linear in the double logarithmic coordinates (Fig. 1C). We used the FOOOF algorithm (Donoghue et al., 2020) to separate the aperiodic and oscillatory components of the pupil spectrum, which found that only 30 of the 70 participants (43% of the participants) had periodic oscillations in their pupil data. The remaining 40 participants (57% of the participants) did not have any oscillatory component in their pupil fluctuations (Fig. 1D) despite that the parameters were set to allow the algorithm to detect any possible oscillatory component. These results suggest that most resting pupil fluctuations are nonperiodic and do not contain periodic signals. We extracted the oscillatory components from the pupil data of the 30 participants who had oscillatory components. Results showed that the oscillatory frequency of the pupil was concentrated around 0.3 Hz (Fig. 1E), consistent with previous studies (Bouma & Baghuis, 1971; Turnbull et al., 2017; Ukai et al., 1997). Next, our analysis focused on the relationship between the aperiodic exponent of pupil fluctuations and the behavioral performance in the attention task.

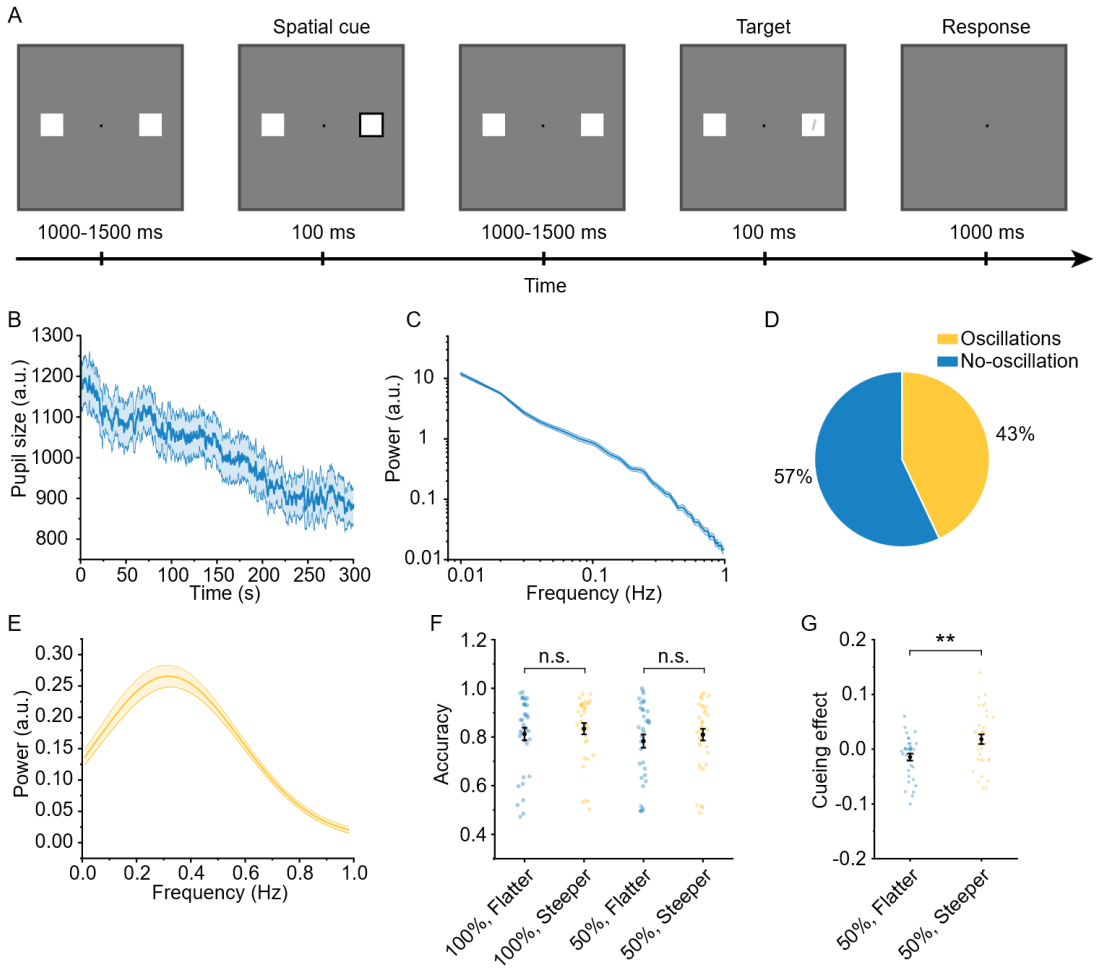

Fig. 1. Trial structure of the experimental task and pupil fluctuations in the 300-s resting state. (A) Trial structure for a valid-cue condition of the experimental task. A fixation dot flanked by two placeholders (white squares) was first presented on the screen. After 1000-1500 ms, the border of one placeholder turned into black for 100 ms (spatial cue). After another interval of 1000-1500 ms, the probe appeared briefly in one of the placeholders. The probe was a bar tilted left or right by 2°, and participants judged the tilt orientation by a keypress within 1000 ms. The cue predicted the probe location on 100% and 50% of the trials in the two blocks of the experiment, respectively. (B) Fluctuations of the raw pupil size in the 300-s resting state. (C) PSD of the resting-state pupil fluctuations shown in the double logarithmic coordinates. (D) The proportion of participants with and without periodic oscillations in the pupil fluctuations. (E) PSD of the pupil oscillations within

0 - 1 Hz. (F) Behavioral response accuracy in the 100% and 50% cue-validity experiments for participants with flatter and steeper spectral slopes. (G) Spatial cueing effects in the 50% cue-validity experiment for the two groups of participants, i.e., participants with flatter or steeper spectral slopes. The shaded areas in B, C, E and the error bars in F, G indicate the standard error of the mean (SEM).

To examine the relationship between the aperiodic exponent of pupil fluctuations (i.e., the slope of the spectrum) and the behavioral performance, we divided the 70 participants into two halves based on the median value of the aperiodic exponent. The slope of the spectrum was relatively steeper in participants with a high aperiodic exponent and flatter in participants with a low aperiodic exponent. In the experiment with 100% cue validity, both groups of participants had high response accuracies ($M \pm SD$: flatter group, 0.81 ± 0.15; steeper group, 0.83 ± 0.14), and there was no significant difference between the two groups ($t(68) = -0.63$, $P = 0.529$, Cohen's $d = -0.15$; Fig. 1F). For the 50% cue-validity experiment (i.e., where the cue failed to predict where the probe would appear), the two groups of participants also had high response accuracies ($M \pm SD$: flatter group, 0.78 ± 0.16; steeper group, 0.81 ± 0.14), which did not differ significantly between groups ($t(68) = -0.73$, $P = 0.468$, Cohen's $d = -0.17$; Fig. 1F), either. These results suggest that the aperiodic exponent of pupil fluctuations is not predictive of the overall accuracy in attentional tasks, either in the 100% or 50% cue validity conditions.

Next, we calculated the spatial cueing effect (i.e., the difference between the response accuracy in the cued direction and that in the uncued direction) in the 50% cue validity experiment. The results showed that there was a significant difference in the cueing effect between the two groups ($t(68) = -3.00$, $P = 0.004$, Cohen's $d = -0.72$; Fig. 1G). Furthermore, the cueing effect was

numerically lower than 0 ($M \pm SD$: -0.02 ± 0.04) for participants with a flatter slope (lower aperiodic exponent), but was numerically greater than 0 ($M \pm SD$: 0.02 ± 0.05) for participants with a steeper slope (higher aperiodic exponent). The clear association between the aperiodic exponent and the attentional cueing effect, rather than the overall accuracy, suggests that the aperiodic exponent of pupil fluctuations closely reflects the balance of attention in the cued and uncued directions.

The aperiodic exponent of the resting pupil fluctuations correlates with microsaccadic IOR in high stimulus-uncertainty

Attentional effects elicited by exogenous cues are reflected by dynamic changes in the microsaccade rate (Laubrock et al., 2005; Lv et al., 2022). The microsaccade after the presentation of an exogenous cue is oriented more in the opposite direction of the cue, reflecting a microsaccadic inhibition of return (IOR). To confirm the findings above, we further examined the relationship between the aperiodic exponent of the pupil fluctuations and the microsaccadic response. As in the analyses above, we divided the participants into two groups according to the aperiodic exponent value and examined the microsaccadic responses of each group of participants. We calculated the cueing effect for the microsaccade rate by subtracting the rate of microsaccade in the cued direction from that in the uncued direction. For the 100% cue-validity experiment, the cueing effect of the microsaccade rate was negative after cue presentation (Fig. 2A), replicating the classic microsaccadic IOR effect (Laubrock et al., 2005; Lv et al., 2022). Critically, the cueing effect of the microsaccade rate was not significantly different between the two groups of participants with high and low aperiodic exponents. To further validate the results of median splitting, we extracted the microsaccadic cueing effect in 200-500 ms for all participants and calculated its correlation with the aperiodic exponent of the pupil fluctuations (Fig. 2B). We found that the aperiodic exponent was

not significantly correlated with the microsaccadic cueing effect in the 100% cue-validity experiment ($r = 0.01$, $P = 0.946$).

For the 50% cue-validity experiment, the cueing effect of microsaccade was also negative after cue presentation (Fig. 2C). Interestingly, the cueing effect of microsaccade was significantly different between the two groups of participants (from 216 to 381 ms), with the participants having lower aperiodic exponent (i.e., flatter spectrum) showing greater microsaccadic IOR. To validate the results of median splitting, we also extracted the averaged microsaccadic cueing effect in 200-500 ms for all participants and calculated its correlation with the aperiodic exponent of the pupil fluctuations (Fig. 2D). We found that the aperiodic exponent was significantly correlated with the microsaccadic cueing effect in the 50% cue-validity experiment ($r = 0.36$, $P = 0.002$). These results replicated those of behavioral performance, showing that the aperiodic exponent was associated with microsaccadic and behavioral responses only at 50% cue validity, i.e., when stimulus uncertainty was high. More importantly, these results further demonstrate that participants with low aperiodic exponent exhibit hyperexcitability to nonpredictive cues and fail to suppress automatic microsaccadic responses to nonpredictive cues. The behavioral and microsaccade results provide direct evidence for the hypothesis of this study, suggesting that the aperiodic exponent of resting-state pupil fluctuations reflects E/I balance in attentional processing.

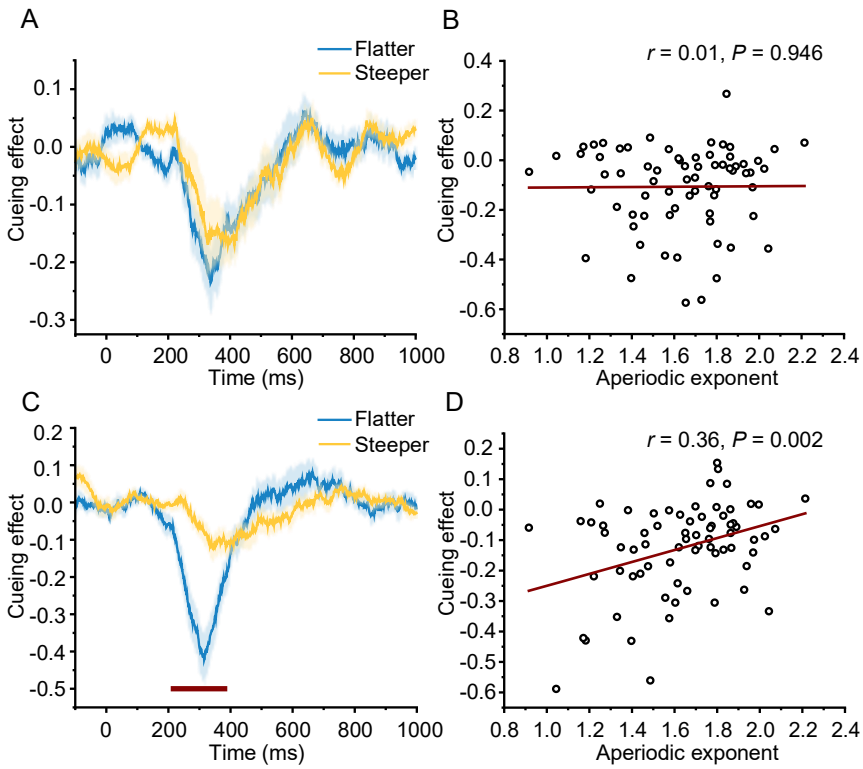

Fig. 2. Relationship between the pupillary aperiodic exponent and the cueing effect in microsaccade rate. (A) Microsaccadic cueing effects for two groups of participants with flatter and steeper spectral slopes in the 100% cue-validity experiment. (B) Correlation between the microsaccadic cueing effect and the aperiodic exponent in the 100% cue-validity experiment. (C) Microsaccadic cueing effects for two groups of participants with flatter and steeper spectral slopes in the 50% cue-validity experiment. (D) Correlation between the microsaccadic cueing effect and the aperiodic exponent in the 50% cue-validity experiment. The shaded areas indicate the SEM.

Raw pupillary measurements do not predict microsaccadic IOR

To explore whether the prediction of the microsaccadic cueing effect in the 50% cue-validity experiment was selective to the aperiodic exponent of the pupil fluctuations, we further median-split the participants using the mean, variance, and hippus power of the raw pupil size data. We found no significant differences between the microsaccadic cueing effects of the three median-split halves of participants (Fig. 3). This result suggests that the aperiodic exponent of the pupil fluctuations

predicts the attentional processing better than the raw pupillary measurements.

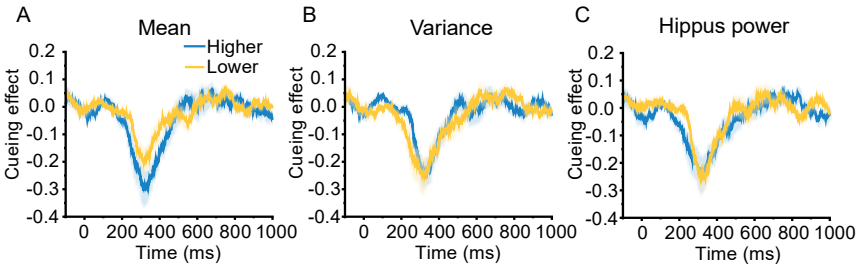

Fig. 3. Differences of microsaccadic cueing effects in the 50% cue-validity experiment using mean (A), variance (B), and hippus power (C) of the pupil size data to median-split the participants. The shaded areas indicate the SEM.

We found that the aperiodic exponent of pupil fluctuations was associated with cueing effects (both behavioral and microsaccade) selectively in the 50% cue-validity condition but not in the 100% cue-validity condition, tentatively suggesting that our analyses of the pupil data were valid. To further investigate whether the aperiodic exponent of the resting pupil fluctuations was affected by the interpolation of missing values (mainly caused by blinks) in the pupil data, we calculated the number of blinks of the subjects during the resting-state and examined the difference in the number of blinks between the two groups by median-splitting the subjects based on the aperiodic exponent, as above. The results showed that there was no significant difference in the number of blinks between the two groups of subjects ($M \pm SD$: flatter group, 80.66±69.29; steeper group, 67.29±57.92; $t(68) = 0.88$, $P = 0.384$, Cohen'$d = 0.21$). This further suggests that our analyses of the resting pupil data were valid and that the aperiodic exponent was not affected by missing-value interpolation.

Microsaccadic response mediates the effect of the aperiodic exponent of resting pupil fluctuations on the attentional effect

Since the aperiodic exponent of the pupil fluctuations was measured in the resting state

independent of the visual attention task, its correlation with microsaccadic IOR and behavioral performance in the 50% cue-validity experiment may indicate that the aperiodic exponent of the resting pupil fluctuations serves as an individualized trait of attentional processing. If so, then how would the pupil fluctuation influence the behavioral response to visual stimuli? We hypothesize that the pupil fluctuations may directly influence the microsaccadic IOR to the stimulus, which then influences the behavioral responses to the stimulus. Supporting this hypothesis, participant-wise mediation analyses revealed that the relationship between the aperiodic exponent of resting pupil fluctuations and the behavioral cueing effect was fully explained by the microsaccadic IOR (Fig. 4; indirect effect: $b = 0.02$, $P = 0.043$; direct effect: $b = 0.03$, $P = 0.166$; total effect: $b = 0.05$, $P = 0.018$). The total and indirect effects were significant in the mediation, but the direct effect was not significant. These outcomes indicate that the aperiodic exponent of resting pupil fluctuations influences individuals' microsaccadic responses to the cue which then influence the behavioral performance in spatial attention. These results support the hypothesis that the aperiodic exponent of resting pupil fluctuations serves as an individualized trait that is predictive of attentional processing and affects behavioral outcomes by influencing microsaccadic responses.

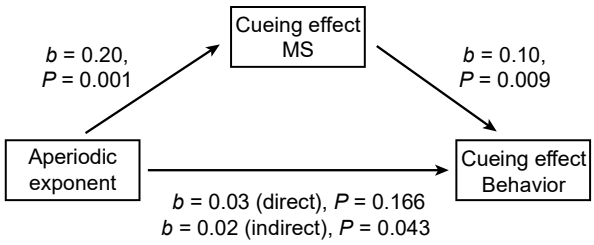

Fig. 4. Participant-wise mediation model for pupil and behavioral data from the 50% cue-validity experiment. The cue effect of microsaccade mediates the relationship between the aperiodic exponent and the cue effect of behavior.

The aperiodic exponent of the resting pupil fluctuations predicts ADHD symptoms

Finally, if the aperiodic exponent of pupil fluctuations could reflect the trait of attentional processing, such a trait should correlate with the level of ADHD symptoms across individuals. To test this point, we calculated correlations between the aperiodic exponent of the resting pupil fluctuations and the ADHD symptoms measured by ASRS (Kessler et al., 2005). We calculated the correlation between the aperiodic exponent and the total ADHD score as well as its inattention and hyperactivity/impulsivity subcomponents. We found a significant negative correlation between the aperiodic exponent and the total ADHD score (Fig. 5A; r = -0.27, P = 0.023), suggesting that a lower aperiodic exponent (flatter slope) is accompanied by more severe ADHD symptoms. While negative correlations were observed in both subcomponents, the correlation was significant between the aperiodic exponent with hyperactivity/impulsivity (Fig. 5B; r = -0.35, P = 0.003) but not with inattention (Fig. 5C; r = -0.10, P = 0.472). Therefore, a lower aperiodic exponent is accompanied by more severe hyperactivity/impulsivity symptoms. These results suggest that the aperiodic component of pupil fluctuations, like the aperiodic component of the EEG signals (Karalunas et al., 2022), could reflect the level of ADHD symptoms.

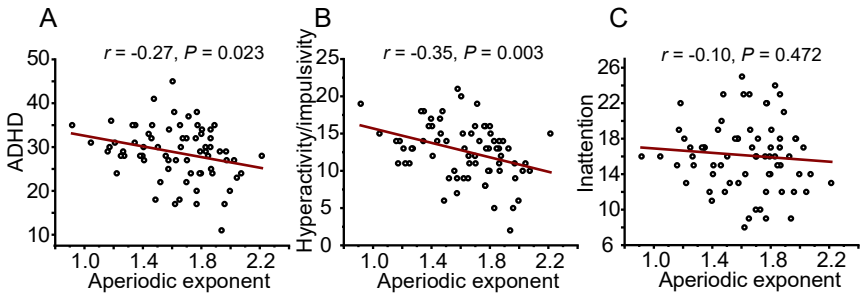

Fig. 5. Correlations between aperiodic exponent and ADHD (A), hyperactivity/impulsivity (B), and inattention (C) symptoms.

As a control analysis, we have also calculated the correlation between the total ADHD score and the mean, variance, and hippus power of the pupil fluctuations. Significant correlations were

observed in none of the analyses (Fig. 6). This result further confirmed that the aperiodic exponent is more predictive of the trait of attentional processing than other raw indices of pupil size.

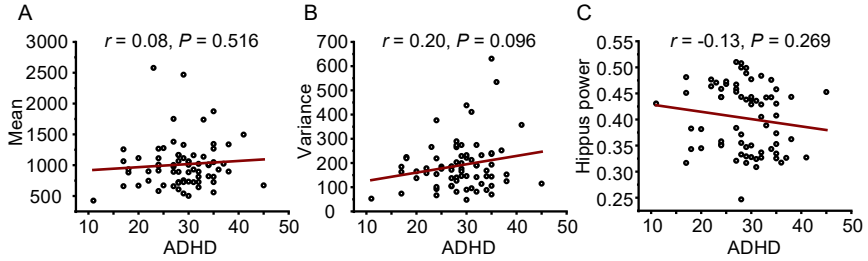

Fig. 6. Correlations between the total ADHD score and mean (A), variance (B), and hippus power (C) of pupil size.

Discussion

The present study explored the cognitive function of the aperiodic exponent of pupil fluctuations and the potential of using it as a reflection of neural E/I balance in visual attention. We recorded the pupil size of a group of participants in the resting state, examined the attentional E/I balance with an attentional task, and estimated the participants' attentional traits with an ADHD self-report scale. The aperiodic exponent of pupil size fluctuations correlated with the behavioral and microsaccadic cueing effects in the attentional task when the stimulus uncertainty is high. Specifically, a greater aperiodic exponent was accompanied by a larger cueing effect of behavior and a lower microsaccadic IOR in the 50% instead of 100% cue-validity condition. A mediation model further identified that the microsaccadic IOR mediated the effect of the aperiodic exponent on the attentional cueing effect, suggesting that the aperiodic exponent of the resting pupil fluctuations reflects individualized trait associated with attentional processing. Finally, we revealed that participants with a lower hyperactivity/impulsivity ADHD symptom exhibited a greater aperiodic exponent in their pupil fluctuations. Notably, because the pupil size information was recorded offline, these correlations were free from contaminations due to simultaneous eye movement or behavioral response recordings. Control analyses further showed that the prediction of the attentional effects and ADHD symptoms did not exist in other indices of the raw pupil size data. Taking these results together, the fluctuation information of pupil sizes is likely to reflect important cognitive functions and its aperiodic exponent in particular may reflect neural E/I balance in attentional processing.

Instead of focusing on the raw pupil size data, the present study analyzed the aperiodic exponent of pupil fluctuations during the resting state and explored its potential functions in

reflecting neural E/I balance. Our focus on the aperiodic exponent is inspired by the widely demonstrated aperiodic exponents in neural signals and behavioral responses. For example, 1/f-like power spectrum has been revealed in EEG (Waschke et al., 2021), MEG (Dehghani et al., 2010), ECoG (Manning et al., 2009), and fMRI (He, 2011) data. Functionally significant aperiodic exponents have also been found in the analysis of human behavioral responses (Palva et al., 2013). The aperiodic exponent in neural data has been related to E/I balance in the neural system (Gao et al., 2017), with a greater aperiodic exponent, i.e., a steeper spectrum, indicating more neural inhibition while a lower aperiodic exponent, i.e., a flatter spectrum, indicating more neural excitation. Here, the aperiodic exponent extracted from pupil fluctuations in the resting state also demonstrated an association with the neural E/I balance. Specifically, the aperiodic exponent of pupil fluctuations was correlated with two different indices of spatial attention, the microsaccadic responses and behavioral performance, in an attentional cueing task. Further, the aperiodic exponent of pupil size also predicted individuals' ADHD symptoms of hyperactivity/impulsivity. Because the attentional cueing effect was observed under high stimulus uncertainty (i.e., in the 50% cue validity condition) when E/I balance is required and individuals suffering from ADHD showed abnormalities in E/I balance, our results suggested the reflection of E/I balance in attention in the aperiodic exponent of pupil size.

Excitation and inhibition are two fundamental processes that govern the flow of information in the brain. The balance between excitation and inhibition is critical for many brain functions, including attention (Deiber et al., 2020). Attention, defined as the ability to focus on a specific stimulus or task while ignoring others, is a selective process that allows us to filter out task-irrelevant information and concentrate on what is important. Thus, E/I balance plays a key role in attention by

aiding in the selection of task-relevant stimuli for processing. Additionally, E/I balance is also important for maintaining attention over time. Initially, when we attend to a stimulus, the E/I balance is tipped in favor of excitation, enabling quick and efficient processing of the stimulus. However, over time, the balance gradually shifts back towards inhibition. This prevents our attention from being depleted on one stimulus, providing the opportunity to shift focus to other potential targets in the environment (Jia et al., 2019; Lv et al., 2022). In the present attention tasks, participants must suppress the automatic microsaccadic responses toward exogenous cues to maintain fixation after cue onset (Rolfs et al., 2004). Consequently, the automatic microsaccades towards the cue are suppressed, while the microsaccades opposite to the cue are enhanced. The response properties of microsaccades directly reflect the degree of neural excitation and inhibition in participants during the attentional tasks. As shown in our results, participants with a low aperiodic exponent of pupil fluctuations exhibit hyperexcitability to the exogenous cues and fail to suppress automatic microsaccadic responses to the cues. These results reveal an association between the aperiodic exponent of pupil fluctuations and neural E/I balance. We further demonstrated the mechanism underlying this association through a mediation model. We found that the association between the aperiodic exponent and the behavioral performance in the attention task was fully mediated by the microsaccadic responses in the attention task. This may suggest that variations in neural E/I balance traits lead to different patterns of automatic microsaccadic responses to the cue, which in turn result in different attentional effects in behavior.

In the present results, the aperiodic exponent of resting pupil fluctuations correlated with microsaccadic IOR in the 50% cue-validity experiment but not in the 100% cue-validity experiment. We speculate that this is because participants were certain about where the probe would appear in

the 100% cue validity experiment, such that they did not need to suppress the automatic microsaccadic response in the direction of the predictive cue. In contrast, in the 50% cue validity experiment, participants were completely uncertain about where the probe would appear. So, they tended to suppress the automatic microsaccadic response in the direction of a nonpredictive cue. Consequently, the neural E/I balance is critical for response control in the 50% cue-validity experiment. These results replicate the previous findings that the E/I balance in attention is sensitive to stimulus uncertainty (Feldman & Friston, 2010; Yu & Dayan, 2005) and demonstrate that the aperiodic exponent of resting pupil fluctuations reflects the neural E/I balance in attention better when under uncertain condition.

Previous studies have identified a strong relationship between the aperiodic exponent of neural signals and ADHD symptoms, albeit showing opposite trends across different age groups (Karalunas et al., 2022; Ostlund et al., 2021; Robertson et al., 2019). Despite numerous investigations linking various pupil indices during resting states, such as size and hippus, with ADHD symptoms in adults (Nobukawa et al., 2021), none have explored the aperiodic exponent of pupil fluctuations or its relationship with ADHD. For example, a recent study has examined the association between pupil size and ADHD symptoms but focused solely on the raw pupil size data (Bellato et al., 2023). In line with our results, this study failed to predict ADHD symptoms on raw pupil size. Here, by extracting the aperiodic exponent of the pupil fluctuations, we revealed a significant association between the aperiodic exponent and the hyperactivity/impulsivity symptom of ADHD, underscoring the functional significance of temporal dynamics in pupil size compared to raw pupil size (Binda & Lunghi, 2017). Notably, the pupil size data in the present study were merely recorded offline during a 300-s resting phase. It, thus, provides a possibility that offline eye recording is able to provide

important information about cognitive functions and associated traits. In fact, considering that the E/I balance indexed by the aperiodic exponent of neural signals has been linked to a variety of neurological disorders, including ASD (Bast et al., 2023), ADHD (Karalunas et al., 2022), and Parkinsonism (Wiest et al., 2023), our method holds promise as a valuable measure for assessing these neurological traits.

In conclusion, this study investigated the cognitive function of the aperiodic exponent of pupil size fluctuations. By utilizing independent assessments of behavioral sensitivity to spatial attentional cueing, self-reported ADHD symptoms, and resting pupil size fluctuations, the findings provide strong and consistent evidence for a link between the aperiodic exponent of pupil fluctuations and attentional processing and demonstrate the superiority of the aperiodic exponent over raw pupil size data in reflecting attentional processes. Critically, both normal and abnormal attention measures suggest a dependence on neural E/I balance in the process, further indicating that the aperiodic exponent reflects E/I balance in the attention system. Overall, this study offers a potentially valuable index for investigating neural E/I balance and neurological disorders associated with E/I balance. It also underscores the importance of analyzing the temporal dynamics of pupil size in future research.

Author contributions

Rongwei Wang: Investigation, Formal analysis, Visualization, Writing.

Jianrong Jia: Conceptualization, Formal analysis, Visualization, Writing, Supervision.

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