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In peripheral vision, it becomes harder to identify a target if presented with nearby flankers. This deleterious effect of nearby flankers on target identification, is referred to as visual crowding (Levi, 2008; Whitney & Levi, 2011). The crowding effect occurs with various kinds of visual stimuli and tasks and is thought to be an essential bottleneck of object recognition and visual awareness. Because crowding represents a ubiquitous limit on visual processing in the periphery, studying visual crowding cannot only advance our understanding of the mechanisms of visual awareness and object recognition, but also have potential clinical implications for patients with visual deficits, such as amblyopia (Hussain, Webb, Astle, & McGraw, 2012). To date, perceptual learning (or training) has been demonstrated to be an effective way to improve spatial vision and alleviate visual crowding (Chung, 2007; Huckauf & Nazir, 2007; Le Dantec, Melton, & Seitz, 2012; Zhu, Fan, & Fang, 2016). However, perceptual learning is time-consuming because it necessitates intensive training and sustained attentional engagement to gain improvement. Therefore, there is growing demand to find other ways to alleviate the crowding effect rapidly (relative to perceptual learning).

Transcranial direct current stimulation (tDCS), is a noninvasive brain stimulation technique that is capable of modulating brain activity and cognitive processes (Paulus, 2011). By applying weak current through the scalp, tDCS alters cortical excitability orchestrated in a polarity-dependent manner: anodal stimulation increases excitability, whereas cathodal stimulation reduces it (Nitsche & Paulus, 2000). The tDCS technique, characterized by its high-safety and easy-operation, has been widely used in basic and clinical neuroscience researches. Over the last 2 decades, many studies have used tDCS to modulate visual sensory processing, such as orientation discrimination (Pirulli, Fertonani, & Miniussi, 2013), surround suppression (Spiegel, Hansen, Byblow, & Thompson, 2012), contrast sensitivity (Antal, Nitsche, & Paulus, 2001), motion discrimination (Battaglini, Noventa, & Casco, 2017), and collinear lateral inhibition (Raveendran, Tsang, Tiwana, Chow, & Thompson, 2020). These studies showed that tDCS was effective in altering various human visual functions. Notably, Reinhart et al. (2016) reported that vernier acuity at 5 degrees eccentricity could be improved immediately following 20 minutes of anodal tDCS, suggesting that offline anodal tDCS could improve peripheral vision. However, to date, there is no study investigating whether

tDCS can improve peripheral vision in crowded scenes and reduce visual crowding.

We conducted a single-blind sham-controlled study to investigate whether tDCS could alleviate visual crowding at different visual eccentricities and with different visual tasks. Specifically, subjects were instructed to perform an orientation discrimination task or a letter identification task with isolated and crowded targets in the periphery, before and immediately after applying 20 minutes of anodal tDCS to the visual cortex of the hemisphere contralateral or ipsilateral to visual stimuli. In experiment 1, we investigated whether tDCS was able to reduce orientation crowding. Experiment 2 was designed to examine whether the tDCS effect found in experiment 1 (if there was any) could be generalized to a smaller eccentricity. Furthermore, experiment 3 tested whether the tDCS effect could be found with a completely different task – letter identification. We hypothesized that contralateral anodal tDCS was able to improve peripheral vision in crowded scenes and effectively alleviate visual crowding across different visual eccentricities and with different visual tasks.

METHODS

Participants

There were totally 118 subjects in 3 experiments (experiment 1: $n = 45$, 23 men, 18 to 32 years old; experiment 2: $n = 45$, 21 men, 18 to 26 years old; and experiment 3: $n = 28$, 16 men, 18 to 32 years old). In each experiment, subjects were divided into three groups at random - the contralateral group (visual stimuli were presented in the hemifield contralateral to the stimulated hemisphere), the ipsilateral group (visual stimuli were presented in the hemifield ipsilateral to the stimulated hemisphere), and the sham group (subjects received sham electrical stimulation). There were 15 subjects in each group in experiments 1 and 2. In experiment 3, there were 9 subjects in the contralateral group and the ipsilateral group each, and 10 subjects in the sham group. All subjects were right-handed, reported normal or corrected-to-normal vision, and had no known neurological or visual disorders. Exclusion criteria included personal or family history of mental illness or neurological disorders (e.g. epilepsy), drug abuse, pregnancy, and metallic implants. They all gave written, informed consent in accordance with the procedures and protocols approved by the human subject review committee of Peking University. This study adhered to the Declaration of Helsinki.

Apparatus

In all three experiments, visual stimuli were displayed on a Sony Trinitron monitor (refresh rate: 100 Hz;

spatial resolution: 1024×768 ; size: 19 inch) with a gray background (luminance: 47.59 cd/m^2). Particularly, visual stimuli used in experiment 3 were rendered with a video card with an 8-bit input resolution and a 14-bit output resolution using Cambridge Research System Bits++. The output luminance of the monitor was linearized using a look-up table in conjunction with photometric readings from a colorCAL colorimeter (Cambridge Research System). The viewing distance was 70 cm, and we used a head and chin rest to stabilize subjects' head position. Subjects were asked to maintain fixation on a black dot at the center of the display and their eye positions were monitored with an Eyelink 1000 Plus eye-tracking system during the experiments. Subjects could maintain their fixation very well across experiments and stimulation conditions.

All experiments consisted of three phases: pre-stimulation test (Pre), tDCS phase, and post-stimulation test (Post; Figure 1A).

In experiment 1, subjects first received practice, which made sure they well understood the task. During the two test phases, subjects' orientation discrimination thresholds were measured with two test stimuli: the isolated target (only the target grating appeared) and the crowded target (the target grating and flanker gratings appeared simultaneously; the first column in Figure 1B). In the isolated condition, subjects were required to perform an orientation discrimination task with the target grating (radius: 1.75 degrees; spatial frequency: 2 cycles/degree; Michelson contrast: 1; mean luminance: 47.59 cd/m^2 ; eccentricity: 7.25 degrees) at an orientation of θ presented in either the upper-left or the upper-right visual quadrant, counterbalanced across subjects. For each subject, the orientation θ was chosen randomly from two perpendicular orientations (67.5 degrees and 157.5 degrees relative to the horizontal axis) prior to the experiment and was fixed throughout the whole experiment. We used these two orientations because they do not appear in daily life frequently, avoiding any potential ceiling effect. The crowded condition was similar to the isolated condition, except there were two flankers positioned abutting the target in the radial direction with respect to fixation. The flankers were identical to the target but with randomized orientations. The center-to-center distance between the target and flankers was 3.5 degrees. Ten QUEST staircases (Watson & Pelli, 1983) of 40 trials, 5 for each test stimulus, were completed alternately. In each trial, 2 targets with orientations of θ and $\theta \pm \Delta\theta$ were presented successively for 200 ms each and were separated by a 600-ms blank interval (Figure 1C). The temporal order of these two targets was randomized. Subjects were asked to make a two-alternative forced choice (2-AFC) judgment of the orientation of the

second target relative to the first one (clockwise or counterclockwise). The next trial began 800 to 1200 ms after subjects made a key press. The $\Delta\theta$ varied trial by trial and was controlled by the QUEST staircase to estimate subjects' discrimination thresholds at 75% correct. No feedback was provided during Pre or Post. Each test phase lasted approximately 30 minutes.

The experimental design and visual stimuli in experiment 2 were similar to those in experiment 1 except that both the eccentricity of the target and the radius of the gratings were reduced to 60 percent of those in experiment 1. The target grating was presented at 4.35 degrees eccentricity, and its radius was reduced to 1.05 degrees (the second column in Figure 1B). The target and flankers were still abutting and their center-to-center distance was 2.1 degrees. It was noteworthy that the target spanned 5.5 degrees to 9 degrees eccentricity in the visual field in experiment 1, and 3.3 degrees to 5.4 degrees eccentricity in experiment 2, meaning that there was no spatial overlap between them.

In experiment 3, there were 10 Sloan letters (C, D, H, K, N, O, R, S, V, and Z; 3 degrees \times 3 degrees) used as visual stimuli to measure subjects' letter identification thresholds. The target letter, randomly selected from the above letter set, was presented at 10 degrees eccentricity on the horizontal meridian of either the left or the right visual field, counterbalanced across subjects (the third column in Figure 1B). Similar to experiment 1, during each test phase, 10 QUEST staircases of 40 trials, 5 for each test stimulus (the isolated letter and the crowded letter), were completed alternately. In each trial of the isolated condition, the target letter was presented alone for 250 ms followed by a 500-ms blank interval, and then subjects were instructed to identify the target by making a key press (Figure 1D). The Weber contrast between the target letter and its background, (letter luminance - background luminance) / background luminance, varied trial by trial and was controlled by the QUEST staircase to estimate subjects' contrast thresholds for letter identification at 55% correct (the 55% correct rate for the 10-alternative forced choice in experiment 3 rendered the task difficulty in experiment 3 equivalent to those in experiments 1 and 2). In the crowded condition, two flankers were positioned abutting the target in the radial direction with respect to fixation and were randomly selected from the above letter set. The Weber contrast between flankers and the background was fixed at 0.33 throughout the experiment. The center-to-center distance between the target and flankers was 3 degrees.

The tDCS was delivered by a battery-powered, constant current stimulator (DC-Stimulator PLUS; neuroConn) and a pair of conductive rubber electrodes

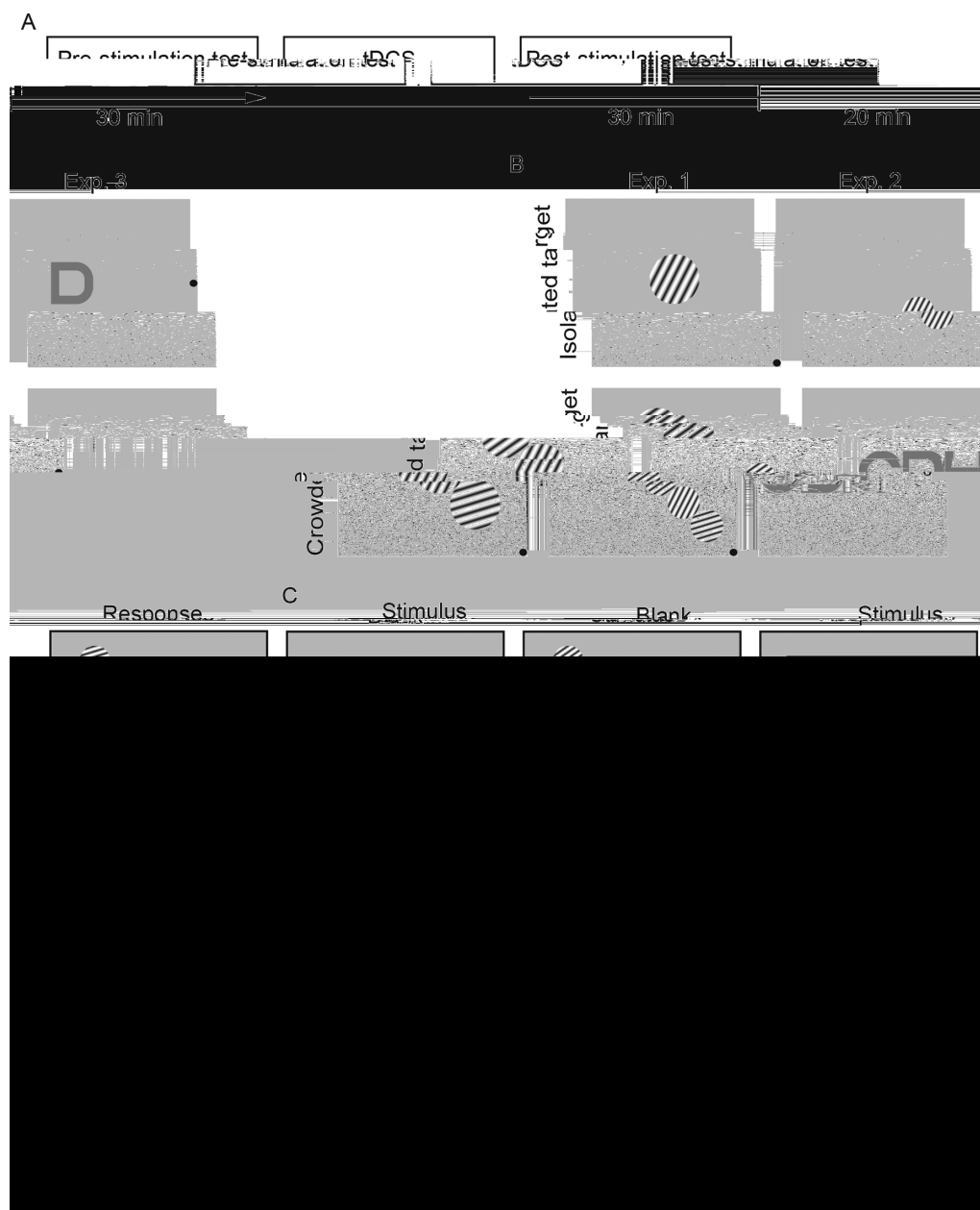


Figure 1. Experimental designs and stimuli. **(A)** All experiments consisted of three phases: pre-stimulation test (Pre), tDCS phase, and post-stimulation test (Post). **(B)** Visual stimuli in experiments 1 to 3. Black dots indicate the fixation point. In experiments 1 and 2, oriented gratings were presented in the upper-left or the upper-right (not shown in this figure) quadrant. In experiment 3, letters were presented on the horizontal meridian of either the left or the right (not shown in this figure) visual field. **(C)** Schematic description of a two-alternative forced choice trial in a QUEST staircase for measuring the orientation discrimination threshold with a crowded grating in experiments 1 and 2. **(D)** Schematic description of a 10-alternative forced choice trial in a QUEST staircase for measuring the contrast threshold for identifying crowded letters in experiment 3.

(size: 35 cm²). The electrodes were enclosed in saline-soaked sponges and held in place by two elastic bands. The anodal electrode was placed over the visual cortex in either the left or the right hemisphere (P1 or P2 in the international 10–20 electroencephalogram [EEG] system), whereas the cathodal electrode was placed over the cheek ipsilateral to the anodal electrode. The electrical current flowed from the anode to the

cathode (i.e. from visual cortex to the ipsilateral cheek). The impedance was kept below 10 k Ω . In the contralateral and the ipsilateral groups, the electrical current was ramped up to 2 mA over 10 seconds, held at 2 mA for 20 minutes, and then ramped down to zero over 10 seconds. In the sham group, the electrical current was ramped up to 2 mA over 10 seconds at the beginning of the 20-minute phase, but held at 2 mA

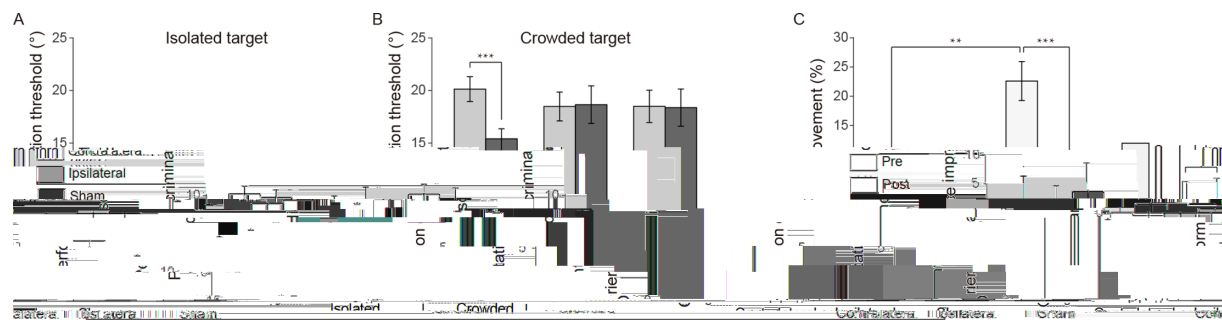


Figure 2. Results of experiment 1. Orientation discrimination thresholds with the isolated (A) and crowded (B) gratings at Pre and Post. (C) Percent of improvements in discrimination performance with the isolated and crowded gratings from Pre to Post.

** $p < 0.01$, *** $p < 0.001$, error bars denote 1 SEM across subjects.

for only 15 seconds. The stimulation parameters in our study were adopted from a previous study (Reinhart, Xiao, McClenahan, & Woodman, 2016). Reinhart and colleagues found that 20 minutes of 2.0 mA (but not 1.0 and 1.5 mA) anodal tDCS could significantly improve spatial vision.

D

For each test stimulus, subjects' performance at Pre and Post was quantified as the mean threshold from five QUEST staircases. Subjects' performance improvement with a test stimulus from Pre to Post was calculated as (pre-stimulation threshold – post-stimulation threshold) / pre-stimulation threshold \times 100%. Repeated-measures ANOVAs, with test (Pre versus Post) as a within-subject factor and group (contralateral versus ipsilateral versus sham) as a between-subject factor, and two-tailed t -tests were used to test whether there was any significant difference in threshold and improvement. It should be pointed out that, in all three experiments, for each test stimulus, no significant threshold difference at Pre was found among the three groups of subjects (1-way ANOVA, all p values $>$ 0.18).

E 1: DC

In the isolated condition, a repeated-measures ANOVA revealed that neither the main effect of test or group (test: $F(1, 42) = 0.07$, $p = 0.79$; group: $F(2, 42) = 0.16$, $p = 0.86$), nor the interaction between test and group ($F(2, 42) = 0.30$, $p = 0.74$) was significant, suggesting that offline tDCS had little effect on isolated orientation discrimination (Figure 2A).

In the crowded condition, however, we observed a significant main effect of test ($F(1, 42) = 13.42$, $p = 0.001$) and a significant interaction between test and group ($F(2, 42) = 13.89$, $p < 0.001$), yet the main effect of group ($F(2, 42) = 0.09$, $p = 0.91$) was not significant. A planned t -test showed that in the contralateral group, the orientation discrimination threshold at Post (15.41 ± 0.95 degrees, $X \pm Y$ indicates the mean \pm SEM) was significantly reduced compared with that at Pre (20.13 ± 1.18 degrees, $t(14) = 6.68$, $p < 0.001$; Figure 2B). No such effect was observed in the ipsilateral group or the sham group.

Then, subjects' performance improvements in orientation discrimination from Pre to Post were analyzed (Figure 2C). In the contralateral group, the improvement with the crowded gratings ($22.60 \pm 3.34\%$) was significant ($t(14) = 6.76$, $p < 0.001$), but not with the isolated gratings ($-0.46 \pm 6.79\%$, $t(14) = -0.07$, $p = 0.95$). And the improvement with the crowded gratings was greater than that with the isolated gratings ($t(14) = 3.415$, $p = 0.004$). There was no significant improvement with the isolated or crowded gratings in the ipsilateral group or the sham group (all t -values $<$ 0.77, p values $>$ 0.46). Notably, in the crowded condition, the improvement in the contralateral group was significantly larger than those in the ipsilateral and the sham groups (contralateral $>$ ipsilateral, $t(28) = 3.38$, $p < 0.001$; contralateral $>$ sham, $t(28) = 3.81$, $p < 0.001$).

The findings in experiment 1 revealed that offline tDCS contralateral to the visual stimuli could improve subjects' orientation discrimination performance with the crowded gratings, but not with the isolated gratings. No significant improvement was observed in the ipsilateral group or the sham group.

E 2: DC

In the isolated condition, a repeated-measures ANOVA revealed a significant interaction between test

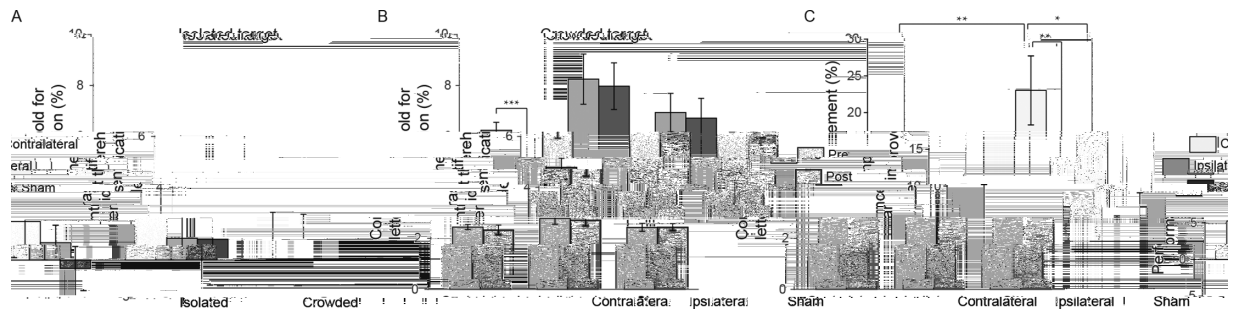


Figure 4. Results of experiment 3. Contrast thresholds for identifying the isolated (A) and crowded (B) letters at Pre and Post. (C) Percent of improvements in identification performance with the isolated and crowded letters from Pre to Post. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, error bars denote 1 SEM across subjects.

the sham groups (contralateral > ipsilateral, $t(16) = 3.61$, $p = 0.002$; contralateral > sham, $t(17) = 2.54$, $p = 0.02$).

These findings demonstrated that the contralateral tDCS could also improve performance in the crowded letter identification task and suggested that the ability of tDCS to reduce visual crowding might apply similarly to various visual tasks.

D

In the current study, we conducted three experiments to investigate whether tDCS was capable of alleviating visual crowding. We found that 20 minutes of anodal tDCS to the visual cortex of the hemisphere contralateral to the visual stimuli improved peripheral vision in crowded scenes and particularly alleviated the crowding effect, regardless of different visual eccentricities and tasks. To our knowledge, this is the first study substantiating that tDCS can effectively alleviate visual crowding.

Our study reveals that, compared with perceptual learning, tDCS has pronounced advantages in alleviating visual crowding (i.e. higher efficiency and lower attentional engagement), which can meet the demands for clinical application (Herpich et al., 2019). In a typical perceptual learning protocol, both intensive training and sustained attentional engagement are required to obtain considerable improvement in discriminating crowded targets (Zhu, Fan, & Fang, 2016; He, Wang, & Fang, 2019), whereas subjects just passively received electrical stimulation during the tDCS phase in our study. Therefore, our study demonstrated that tDCS is an effective and rapid way to alleviate visual crowding.

To optimize the modulatory effect of tDCS on visual crowding, we had carefully considered the stimulation protocol in our study. First, although

the effects of tDCS on visual function are mixed in previous studies (Costa et al., 2015; Reinhart, Xiao, McClenahan, & Woodman, 2016; He, Lin, Zhao et al., 2019), anodal tDCS was found to be more effective when applied before task execution than applied during task execution (Pirulli, Fertonani, & Miniussi, 2013; Barbieri, Negrini, Nitsche, & Rivolta, 2016). Therefore, offline tDCS was adopted. Second, regarding the stimulation site, we chose to stimulate visual cortex because it plays a key role in visual crowding (Bi, Cai, Zhou, & Fang, 2009; Chen et al., 2014; Millin, Arman, Chung, & Tjan, 2014; He, Wang, & Fang, 2019). For example, the C1 component, the earliest event-related potential (ERP) component, was found to be associated with the magnitude of the crowding effect, and the largest C1 amplitudes were observed at posterior electrodes, including P1 and P2 (Chen et al., 2014). In addition, it was also shown that tDCS at P1 and P2 could significantly modulate spatial vision in the parafoveal visual field (Reinhart et al., 2016). Therefore, we chose to deliver tDCS at P1 or P2 in the current study.

We propose two possible explanations for the tDCS effect on visual crowding. One is that anodal tDCS may alleviate visual crowding by reducing the concentration of GABA (γ -aminobutyric acid), a primary inhibitory neurotransmitter in the brain (Stagg et al., 2009). The GABA concentration reduction has been shown to be associated with reduced latent inhibitory connections in human cortex (Barron et al., 2016) and an improved ability to detect targets from a clutter (Frangou, Correia, & Kourtzi, 2018). Here, anodal tDCS may reduce inhibitory interactions between neuronal populations responding to the target and flankers, therefore alleviating visual crowding. A second explanation is that tDCS may alleviate visual crowding by activating μ 30500(b)o

to our target brain region were also stimulated (e.g. posterior parietal cortex [PPC], a core brain area of the attentional network; Okamoto et al., 2004; Roe et al., 2016; Falcone, Wada, Parasuraman, & Callan, 2018). Therefore, the tDCS effect found in this study might also result from the activation of the attentional network, which could improve the attention resolution and therefore alleviate visual crowding (He, Cavanagh, & Intriligator, 1996; Chen et al., 2014; Herzog, Sayim, Chicherov, & Manassi, 2015; He, Wang, & Fang, 2019). It might be argued that the alleviation of visual crowding can be simply explained by some test-retest effects (i.e. practice at Pre and Post). However, such alleviation was not observed in the ipsilateral group or the sham group, which rules out this explanation.

In the current study, tDCS not only alleviated visual crowding across three experiments, but also improved the performance in the isolated orientation discrimination task at a small eccentricity in experiment 2. As mentioned before, in experiment 2, the target spanned 3.3 degrees to 5.4 degrees in the visual field. In those studies showing that transcranial electrical stimulation was able to boost task performance in (isolated) orientation discrimination tasks (Fertonani, Pirulli, & Miniussi, 2011; Pirulli, Fertonani, & Miniussi, 2013; Sczesny-Kaiser et al., 2016), visual stimuli were presented within 4 degrees to 5 degrees eccentricity in the visual field, which fell into the eccentricity range of the target in experiment 2. Therefore, our finding in experiment 2 is in line with previous studies (Pirulli, Fertonani, & Miniussi, 2013; Sczesny-Kaiser et al., 2016).

Recent studies showed that transcranial alternating current stimulation (tACS) and transcranial random noise stimulation (tRNS) could also alleviate visual crowding (Contemori, Trotter, Cottureau, & Maniglia, 2019; Battaglini, Ghiani, Casco, & Ronconi, 2020). However, the neural mechanism of the tDCS effect on visual crowding might be different from other transcranial electrical stimulation techniques, which should be explored in the future. In our study, only anodal stimulation was applied to visual cortex. It would be useful to include a cathodal stimulation condition, although meta-analysis studies have shown that the effects of cathodal tDCS on cognitive functions are nonsignificant compared with sham stimulation (Dedoncker, Brunoni, Baeken & Vanderhasselt, 2016a; Dedoncker, Brunoni, Baeken & Vanderhasselt, 2016b; Salehinejad, Wischnewski, Nejati, Vicario, & Nitsche, 2019). Making comparison between anodal and cathodal stimulations will provide a more comprehensive understanding of both tDCS and visual crowding.

In addition to exploring the neural mechanisms of the effect of tDCS on visual crowding, it is also important to apply our current findings to clinical

practice. Previous studies found that tDCS could improve visual functions of patients with amblyopia, such as visual acuity (Bocci et al., 2018) and contrast sensitivity (Ding et al., 2016). We speculate that tDCS with proper parameters and design might be able to alleviate visual crowding in patients with amblyopia as well. Therefore, our findings might provide a promising neurorehabilitation way for patients with visual impairments or deficits.

C

We found that tDCS was effective in alleviating visual crowding across different visual eccentricities and tasks. These findings provide not only a promising way to alleviate visual crowding rapidly, but also a guide to clinical neurorehabilitation for patients with visual impairments or deficits in the future.

Keywords: visual crowding, transcranial direct current stimulation (tDCS), visual cortex, brain stimulation, cortical plasticity

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