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Bisphenol A exposure inhibits contrast sensitivity in cats involving increased response noise and inhibitory synaptic transmission

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ABSTRACT

Contrast sensitivity (CS) is one of the primary fundamental factors determining how well we can see, and it directly influences object recognition. Whether bisphenol-A (BPA, an environmental xenoestrogen) can perturb contrast detection in the visual system has yet to be elucidated. In the present study, we analyzed CS of single neurons in the primary visual cortex (area 17, A17) of cats before and after BPA exposure using a multiple-channel recording technique. The results showed that CS of A17 neurons was markedly depressed with an increased contrast threshold after two hour of intravenous BPA administration, which had a positive correlation with decreased firing rates of A17 neurons. Additionally, responses of these neurons presented an overt increase in the trial-to-trail response variability (a kind of neuronal noise), which could disturb the information-filtering function of single neurons. We also found that neuronal CS in the visual relay station was not disturbed after BPA administration, which rules out the contribution of CS alteration in the optical pathway. Importantly, acute BPA treatment obviously increased the inhibitory innervation to the visual pyramidal neurons. This implies that alteration of intracortical inhibitory regulation contributes to the compromised contrast detection in the visual system after BPA treatment.

1. Introduction

Bisphenol A (BPA), a common xenoestrogen, has high exposure potential in our living environment due to its frequent application in the production of water bottles, canned-food and beverage packaging, dental fillings, hundreds of human daily necessities, etc. (Cao et al., 2009; Lorber et al., 2015; Wang et al., 2015). Without intentional exposure, BPA concentration in human blood serum has been reported to range from below 1 μg/L up to 19 μg/L (Volkel et al., 2005). It has been widely considered that BPA is implicated in many brain dysfunctions in humans and animals, such as deficits of motion, learning and memory (Braun et al., 2009; Hu et al., 2017; Roen et al., 2015). These functions, built through processing information from visual, auditory and other sensory cortices, are crucial for species survival under different environmental conditions (Zhang et al., 2015). Until now, studies of the underlying mechanisms of BPA-induced neural impairment have mainly focused on structural and molecular alterations of specific cortices, such as the hippocampus, prefrontal cortex, etc. (Eilam-Stock et al., 2012; Elsworth et al., 2015; MacLusky et al., 2005). However, it is unknown whether alterations in perception of sensory information contribute to the BPA-related cognitive impairment.

Humans can distinguish objects from background in natural views by their differences in luminance or color, which is defined as contrast (Avidan et al., 2002). This visual perception ability, contrast sensitivity, is mainly dependent on information encoded by the primary visual system, in which neurons can discern the different contrasts of grating stimuli presented in their receptive field (Mante et al., 2005). This is one of the important limiting elements in wide variety of visual tasks and can be modified by environmental visual experiences (Li et al., 2009). The decline of contrast sensitivity (CS) always is found in unique persons, such as those in professional jobs requiring excellent eyesight (Sekuler, 2000), people with amblyopia (Hess and Howell, 1977; Zhou et al., 2006) and aging populations (Tang and Zhou, 2009). CS deficits in humans provide enough evidences that visual perception could be impacted by aging and environmental factors. As an environmental xenoestrogen, BPA has been reported to disturb ocular dominance plasticity of the primary visual cortex (area 17, A17) after 4 days monocular deprivation in the critical period of brain development

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(Kelly et al., 2014). Our previous studies have also shown that BPA can decrease the activity and orientation discrimination of A17 neurons (Xu et al., 2018). Whether BPA disturbs the contrast detection for object recognition is still elusive. In the present study, we combined multiple-unit recording and patch clamping methods to directly and accurately dissect the effects of BPA on the contrast response function of A17 at the individual neuronal level.

Here, we used cats as an animal model for their visual system is as highly developed as that of humans. Neuronal responses and contrast sensitivities of visual neurons to grating stimuli with different contrasts were obtained and analyzed. We explored the neuronal contrast sensitivity of A17 neurons in cats before and after BPA exposure. Moreover, its underlying physiological mechanisms were uncovered by analyzing the response characteristics of A17 neurons and contrast sensitivity of neurons in the visual projection pathway (lateral geniculate nucleus, LGN). We also explored the underlying synaptic mechanisms by patch-clamp recording in pyramidal neurons of the primary visual cortex. It is known that contrast sensitivity is a pivotal property of the visual system. The alteration of information processing in visual neurons for contrast detection could be an important clue to how BPA affects cognitive functions dependent on other cortices in a physiological manner.

2. Materials and methods

2.1. Experimental animals and BPA treatment

Six healthy adult cats and 10 mice (21 ~ 24 days old) were used for the present studies. All related experiments were performed following the National Institutes of Health Guide for the Care and Use of Laboratory Animals and approved by the Institutional Animal Care and Use Committee of the University of Science and Technology of China, P. R. China. Cats were treated with BPA (0.2 mg/ml in saline, pH = 7.3) for 2 h by microinjection pumping (1 ml/kg/h) via a venous indwelling needle located in the leg. The responses of visual neurons in the primary visual cortex and LGN to stimuli with different contrasts were recorded before and after injection of BPA solution. Mice were used for patch-clamp recording of pyramidal neurons in the primary visual cortex to analyze the excitatory and inhibitory postsynaptic currents before and after BPA treatment.

2.2. In vivo electrophysiological recording in the primary visual cortex

The cat preparations were similar to those in our previous description (Xu et al., 2018). First, the cats were intramuscularly injected with ketamine HCl (20 mg/kg, Ben Venue Lab Inc., Bedford, OH, USA) for anesthesia. After the tracheal intubation and venous indwelling needles were placed, the cats were fixed in a stereotaxic apparatus and then a craniotomy was carried out. Lidocaine HCl (1 %; Abbott Labs, Chicago, IL, USA) was injected into all incisions points to reduce pain. During this experiment, atropine eye drops were administered to dilate pupils and corneal contact lenses were used for protecting their cornea. With continuous administration of gallamine triethiodide, sufentanil and propofol, the cats were kept anesthetized and immobilized. The EEG, EX.CO_2 and body temperature of cats were monitored throughout the experiments.

The visual stimuli (drifting sinusoidal grating with different contrasts) were presented via a CRT monitor (640 × 480, 75 Hz, G220, SONY, Japan) placed 57 cm in front of the cats' eyes. The average luminance of the display was 45.2 cd/m^2 and the environmental luminance was approximate 0.11 lx near the eye lens of cats. The visual stimuli were created by a program written by Psychtoolbox-3 in MATLAB™ (Mathworks, Natick, MA, USA) (Brainard, 1997). The visual stimuli were presented 10 times (10 sweeps) with 12 different contrasts and a blank stimulus in every sweep. Every stimulation trial in a sweep lasted 1 s. Each contrast and blank stimulus was randomly presented only once per sweep. The blank stimuli were shown as the average brightness on the CRT monitor. The optimal orientation, spatial frequency, and temporal frequency of each neuron were determined with tuning curve experiments. For the contrast response function test, the temporal frequency and spatial frequency were fixed at the optimal values of each neuron.

2.2.1. Data collection and analysis of in vivo electrophysiological recording

During neurophysiological recording, extracellular spiking activity of neurons in V1 and LGN was recorded by 1 × 32 linear arrays (NeuroNexus) and 1 × 16 linear arrays (Plexon), respectively. All cells for recording had receptive fields within 25 degrees of the area centralis and most of cells were within 10 degrees. Their response signals were amplified by a front-end amplifier (cut-off frequency: 10 kHz, BLACKROCK) and filtered by a neural signal processing system (sampling frequency: 30 kHz, 16 bits, BLACKROCK). Neuronal spikes were saved and detected by cluster analysis (Offline Sorter V3.3.5, NeuroNexus).

For spike sorting of neuronal responses in the recorded channels, we used a four-pole high-pass Butterworth digital filter (300 Hz cutoff) to filter the raw data, and set the threshold at 3 SD to extract multi-unit (MU) activity. Then, we used Offline-Sorter software for classification of spike waveforms to isolate single units (SUs). Sorting was initiated by the "semi-automatic K-means" algorithm, based on a 2D or 3D feature space. SUs were further isolated using a manual clustering procedure, which used additional 2D/3D feature spaces, autocorrelograms, cross-correlograms, and refractory periods (Li and Baker, 2012). Spike clusters which were clearly separated from the background noise could be accepted as SUs. Next, we compared the spike waveforms of SUs in the same channel recorded pre- and post-BPA exposure by calculating the Spearman correlation coefficient (SCC). When the SCC values exceeded the 99 % confidence interval, the spike clusters were considered to be from the same neuron (Xu et al., 2018).

The contrast response function of a single neuron is alway fitted by the Naka-Rushton equation (Albrecht, 1995), which is defined as follows:

$$R = \frac{R_{means} C^n}{\left(C^n + C_{50}^{\frac{n}{50}}\right)} + M$$

Here, R is the response of a single unit to visual stimuli with different contrasts; R_{max} is the fitted maximum firing rate; con is the contrast of the visual stimuli; n is the waveform parameter which determines the shape of the waveform; C_{50} is the contrast of visual stimuli which can evoke half of the maximum firing rate. Smaller C_{50} values correspond to higher contrast sensitivity; R_{min} represents the fitted baseline of firing rate.

The contrast threshold of visual neurons was calculated by the receiver operating characteristic (ROC, a signal detection theory) analysis (Thiele et al., 2000). In this approach, the overall probability of contrast detection is supposed that a neuron detects a stimulus whenever its activity elicited by that stimulus is greater than its baseline activity. The ROC values for each contrast stimuli are fitted with Weibull function, which is defined as follows:

$$\text{Accuracy} = a - (a - b) \times e^{-(con/c)^d}$$

Here, accuracy (ROC value) is the percentage of A17 neurons with responses more than 5 spikes/s to the grating stimuli within a specific period. a is the true hit rate (a neuron responses to stimuli); b is the false hit rate (a neuron responses with no stimuli); con is stimulus contrast; c corresponds to contrast threshold (75 % ideal observer performance); d corresponds to the slope of the function.

In the visual system, the responses of neurons (spike counts) to repeated stimuli are extremely variable. This variability is calculated by the ratio of variance to mean spike counts, as follows:

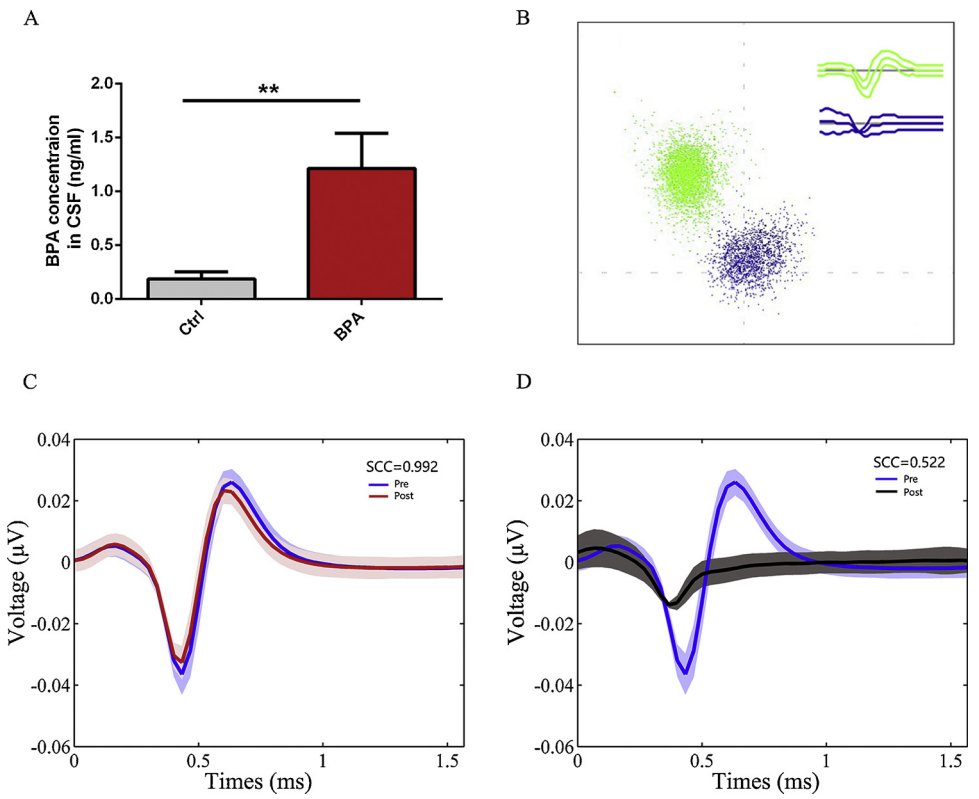


Fig. 1. A) BPA penetrated the blood-brain barrier (BBB) of cats. Before and after 2 h BPA exposure on cats, the cerebrospinal fluid (CSF) was collected from their medulla. The BPA levels were determined by UPLC. Histogram showing the BPA concentration in the CSF samples of cats before and after BPA exposure. (**$p < 0.01$, $n = 5$ cats). B) Spike sorting results from a recording channel. Scatter plot of spike-waveform principal component analysis components showed two well-isolated single units (blue and green). C) Two representative pairs of spikewaves with different similarities for different SCC values. The blue spikewaves from one neuron responses recorded in a channel pre-BPA treatment. The red and gray spikewaves of two neurons were from the same recording channel as the blue spikewaves post-BPA treatment. Only upper paired-spikewaves (blue and red lines), with the SCC value of 0.9912, were judged belonging to the same neuron. Voltage values in neuron spikewaves at each time point were shown as mean ± SEM.

$$\text{Fano Factor} = \frac{variance^2}{R_{means}}$$

Here, variance is the standard deviation of neuronal responses (the spike counts) to the repeated stimuli and R_{means} is the mean response of neurons to each trial of visual stimuli.

2.3. BPA detection

Cerebrospinal fluid (CSF) samples were used for analysis of BPA concentrations in the central nervous system of the cats. Sample preparation and BPA detection were conducted in reference to previous studies (Elsworth et al., 2013; Hu et al., 2017). In brief, a CSF sample (~100 μl) from the medulla of each cat was collected with 1 ml syringes after its vital signs (EEG, EX.CO_2 and body temperature) were stable under our maintenance system and before visual electrophysiological recording. After recording in the A17 of cats with BPA treatment, we recollected their CSF sample. Then, CSF was diluted with methyl tertiary butyl (3 ml) with an oscillator for twice. The supernatant was collected from two extraction and evaporated to dryness under N_2 flow. The residue was reconstituted in acetonitrile solution (100 μl). Finally, the concentration of BPA was measured using Ultra Performance Liquid Chromatography (UPLC), which had a fluorescence detector (Acquity UPLC BEH C18 1.7 μm, Waters, USA). The column oven temperature was 40 °C and the injection volume was 10 μl. The mobile phase, water and acetonitrile, was degassed using an ultrasonic bath before using. For detecting BPA, the excitation wavelength and the emission wavelength were set as 227 nm and 310 nm, respectively. The BPA sustained time in the UPLC column was ~3.65 min in our present study.

2.4. Patch-clamp recording in the primary visual cortex of mice

All electrophysiological experiments were conducted on neurons located in layer II/III of the primary visual cortex (V1) from C57/BL6JCnc mice. Animals were anesthetized with isoflurane and brains were isolated following decapitation. Brain tissue was cut into acute slices by a vibratome (VT1200S, Leica, Germany). Patch pipettes were pulled from borosilicate glass capillaries (O.D.:1.5 mm, I.D.:0.86 mm, SUTTER) on a horizontal puller (model P-1000, SUTTER Instrument, Novato, CA) to the tip resistance of 4~7 MΩ when filled with intracellular solutions. Before drugs were perfused into the slice, the baseline of spontaneous excitatory and inhibitory currents (sEPSC/sIPSC) was recorded during 10 min in the ACSF solution: 125 mM NaCl, 1.25 mM KCl, 25 mM $NaHCO_3$, 1.25 mM KH_2PO_4, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 25 mM D-Glucose, 2 mM Na-Pyruvate, and 0.4 mM L-Ascorbic acid (pH = 7.20 ± 0.10, Osmotic = 305 ± 5, bubbled with 95 %O_2/5 %CO_2). The glutamate receptor antagonists (AP5, 20 μM; CNQX, 20 μM) were added into the ACSF for recording sIPSC, and the GABAA receptor antagonist (PTX, 50 μM) was added for recording sEPSC. The intracellular solution in the patch pipettes contained: 135 mM K-gluconate for sEPSCs, 135 mM Cs-Methanesulfonete for sIPSCs, 10 mM KCl, 10 mM Hepes, 0.1 mM EGTA, 5 mM ATP-Mg^{2+}, and 0.5 mM GTP-Na^+. Then, 10 μM BPA (drug) was added into the extracellular solution for drug section. Finally, BPA was washed off using BPA-free extracellular solution for 20 min.

sEPSCs and sIPSCs were recorded in voltage clamp mode under the whole cell configuration. Data were acquired at a sampling rate of 20.0 kHz and low-pass filtered at 10.0 kHz using a Digital 1440A and MULTICLAMP Axon 700B patch-clamp (Axon Instrument, Burlingame, CA). Data analysis was performed using Clamp fit9.

2.5. Statistical analysis

All results in our present study were expressed as the means ± SEM. When comparing the differences in average value between 2 groups, we used the paired *t*-test or Mann-Whitney test based on the sample distributions. Spearman correlation analysis was applied for calculating the correlation relationship between two parameters.

3. Results

3.1. BPA inhibits the contrast sensitivity of A17 neurons

In the present study, BPA solution (0.02 mg/ml) was intravenously injected into the leg veins of cats for two hours systemic exposure. To confirm BPA can penetrate the blood-brain barrier (BBB) to affect brain function, we examined the BPA concentration in CSF samples from adult cats, which was determined using UPLC. As shown in Fig. 1, the BPA concentration in the CSF sample of cats for 2 h systemic exposure was increased to 1.33 ± 0.38 ng/ml compared to that of those cats before BPA treatment (0.17 ± 0.075 ng/ml, $p < 0.01$, Fig. 1). This implies that BPA has the potential to affect brain functions of cats after penetrating their BBB.

The contrast sensitivity of A17 neurons in cats was assessed using drifting grating stimuli with different contrasts. The contrast response function of each neuron was fitted with a Naka-Rushton equation function. The neurons recorded pre- and post-BPA exposure were first determined to be the same cells for further response property analysis as those in our previous reports (Xu et al., 2018). First, as Fig. 1B shown, the units in a recording channel were separated from each other by an offline sorter. Then, when the similarity between two spike waveforms from one neuron recorded pre-BPA exposure and from one neuron recorded post-BPA exposure in the same recording channel was more than 0.7441 (Spearman-correlation-coefficient, SCC), these two waveforms were judged as stemming from a same neuron (Fig. 1C). As a representative neuron pre- and post-BPA treatment in Fig. 2A, well-tuned neurons ($r^2 > 0.90$) before BPA treatment were selected for further contrast sensitivity analysis. After SCC and contrast tuning analysis, a total of 58 neurons from 3 adult cats were used to explore BPA-induced changes in the neuronal contrast threshold, which was calculated by the Weibull function. As shown in Fig. 2B, the contrast threshold of the representative neuron was 11.7 and 33 before and after BPA treatment, respectively. We also found that the contrast threshold of 3 cats was obviously increased after 2 h BPA treatment ($p < 0.05$, Wilcoxon matched pairs test, Fig. 2C and D). It implies that BPA exposure can induce a decrease in the contrast sensitivity of A17 neurons. According to previous studies, the decreased responsiveness of neurons is implicated in the decline of contrast sensitivity (Hua et al., 2010). We found that the peak firing rates (with no spontaneous responses) of those A17 neurons was obviously decreased under BPA treatment (Fig. 1E). In addition, there was a significant negative correlation between the changes in firing rates and contrast threshold (Pearson's ratio: −0.5381, $p < 0.0001$, Fig. 2F). Since the activity fluctuation of neurons during long-time recording may disturb their response properties, the neuronal firing spikes and contrast threshold of adult cats without BPA treatment were assessed at 0 h and 2 h after electrophysiological recording. These results showed that there were no differences between the first two time points (data not shown), demonstrating that the decrease in contrast sensitivity of A17 neurons was not due to the extended recording time but was only a result of BPA exposure.

3.2. BPA increases the response variability of A17 neurons

For obtaining visual information, A17 neurons should discriminate the signal from background noise for further information processing. In the primary visual cortex, the responses of a neuron (firing rates) to repeated presentations of the same stimulus always show high variability, which is considered to be a noise for information processing and can reflect perceptual stability to visual stimuli at the single neuron level (Carandini, 2004). To further explore noise contribution to BPA-induced visual dysfunction, the variance (standard deviance) of A17 neuronal firing rates to repeated grating stimuli was assessed. As shown in Fig. 3A and B, there was a considerable increase in response variance of A17 neurons ($n = 131$, $p < 0.0001$, paired Wilcoxon matched test). We found that the response variability had a positive correlation with neuronal activity and it was increased when the firing rates were between 20 and 40 spikes/s after BPA treatment ($p < 0.001$ and $p < 0.05$, Fig. 3C). To exclude the sampling effect on variability in neurons with different activity levels, the Fano Factor (ratio of response variance to mean spike rates) was applied to analyze variability. As shown in Fig. 3D and E, the Fano Factor was considerably increased after BPA exposure ($n = 131$, $p < 0.0001$, paired Wilcoxon matched test). These results suggest that BPA exposure can cause increased noise to perturb contrast information processing in the primary visual cortex.

3.3. BPA did not change the response properties of LGN neurons

The limit of contrast sensitivity to the visual environment is set by the beginning nodes in the visual pathway (Ress and Heeger, 2003). To detect the sources of contrast perception changes in the A17, we also assessed the effects of BPA on the responses of LGN neurons to grating stimuli with different contrasts. The chosen standard for LGN neurons was same as that of A17 neurons. A total of 35 neurons from 3 adult cats were used. As shown in Fig. 4A, compared to the contrast values pre-BPA treatment, BPA did not cause a contrast threshold change in the LGN neurons (median were 35 and 39, respectively, $p > 0.05$). The threshold cumulative curves had no shift after BPA treatment. In addition, there is no change in the peak firing rates of those LGN neurons to grating stimuli ($p > 0.05$, Fig. 4C). These findings suggest that the contrast sensitivity decrease in the A17 does not stem from BPA-induced contrast perception changes in the LGN and its neuronal activity change induced an information input decrease.

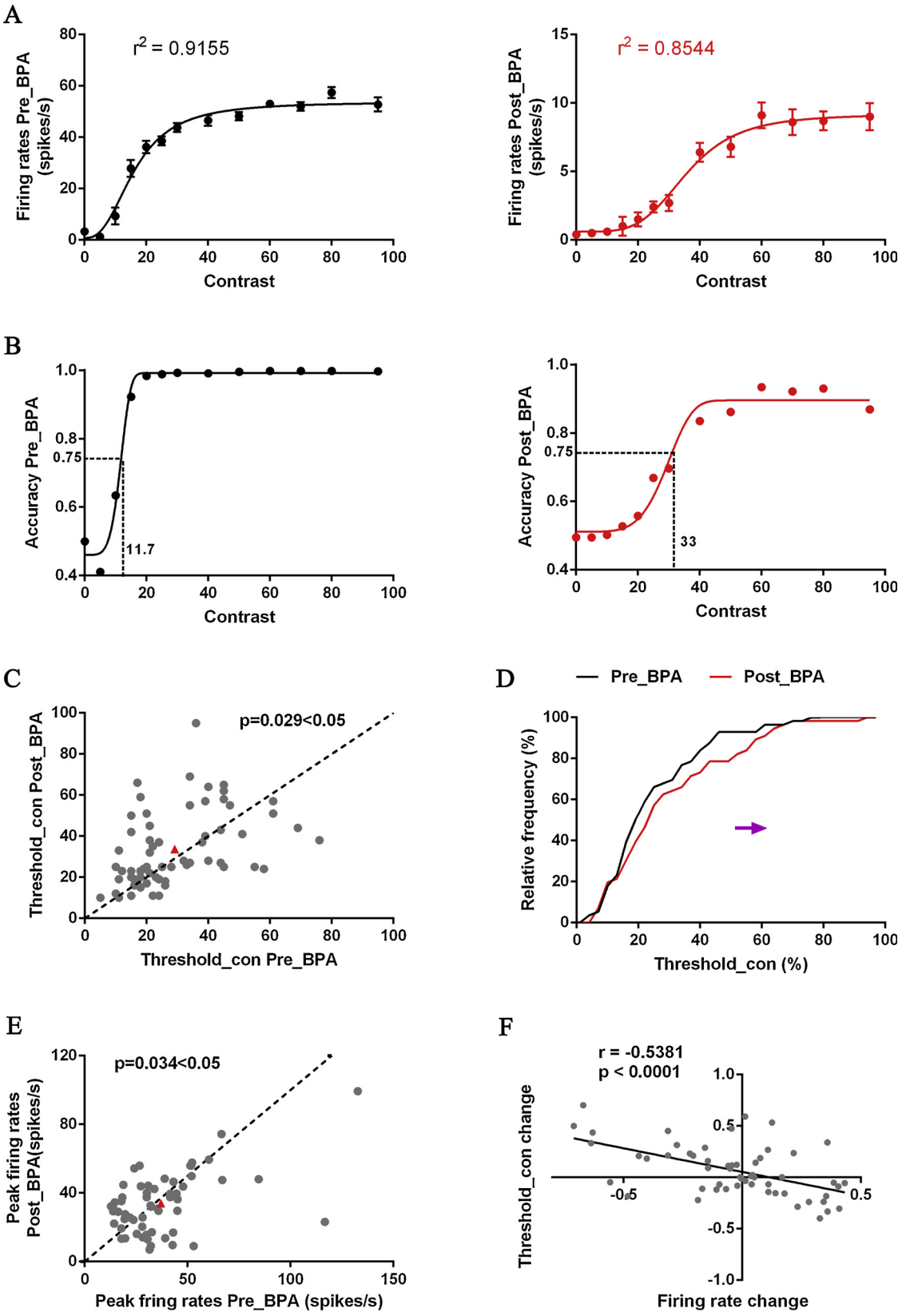
3.4. BPA increase inhibitory synaptic transmission in the primary visual cortex

The excitatory and inhibitory balance plays a pivotal role in the visual function depending on the activation of NMDA, AMPA and GABAA receptors on the pyramidal neurons (Beston et al., 2010). To understand how BPA impacts excitatory or inhibitory innervation of pyramidal neurons in the V1 of mice, we analyzed the frequency and amplitude of sEPSC and sIPSC in layer 2/3 pyramidal neurons by patch-clamp recording (Fig. 5B). After 20 min BPA solution (10 μM) perfusion, we found that both frequency and amplitude of sEPSC had no changes compared to their values pre-BPA perfusion ($n = 6$, $p > 0.05$, Fig. 5C). However, the frequency, not amplitude, of sIPSC was significantly increased by approximately 20 % after BPA perfusion ($n = 6$, $p < 0.05$, $p > 0.05$, Fig. 5D). This increase in frequency of sIPSC was washed out after 20 min of ACSF perfusion without BPA ($p < 0.05$, Fig. 5D). These results imply that BPA could perturb the activity of inhibitory interneurons within the V1, not the projection neurons (excitatory neurons) from the subcortical visual pathway or other cortices, to decrease the contrast sensitivity of the primary visual cortex.

4. Discussion

To study the effects of BPA on visual functions, we investigated contrast responses to grating stimuli and synaptic transmission in the primary visual system by multiple electrode recording and patch-clamping methods. Our results, for the first time, showed that contrast sensitivity of A17 neurons were markedly decreased in cats after 2 h BPA administration, accompanied by increased response variability of single neuron to the same stimuli (a kind of neuronal noise). Importantly, such neuronal compromise of contrast perception in BPA-treated cats was not attributable to the projection perturbation of contrast information in the LGN but to the increases in inhibitory transmission within the primary visual cortex.

The present exposure condition in adult cats (BPA 0.2 mg/kg/h, 2 h) could be considered to be an exposure dosage of 400 μg/kg/day. Although this dosage is higher than those used in many reports by other



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groups (50 μg/kg/day), it is 10 times less than the currently accepted no-observed-adverse-effect-level (NOAEL, 75 ppm, 5 mg/kg/day) (Tyl et al., 2002). Based on the ratio of surface area-to mass within adult human and cats, our exposure dosage of BPA (400 μg/kg) can be converted to 148.6 μg/kg in humans. In addition, our results showed that the BPA concentration in CSF samples after electrophysiological recording was 1.33 ± 0.38 ng/ml, which was similar with that in human fluid (1～19 ng/ml) without intentional exposures to BPA

Fig. 2. BPA decreased the contrast sensitivity of A17 neurons in cats. A) The contrast tuning curve of representative neurons pre- (black) and post- (red) BPA treatment, fitted with the Naka-Rushton equation function. The fit index was 0.9155 and 0.8544, respectively. B) The accuracy curve for neuronal response to grating stimuli under different contrast pre- (black) and post- (red) BPA treatment. After being fitted with Weibull function, the contrast under the accuracy is 0.75 was defined as the contrast threshold. The contrast threshold of the representative neurons pre- and post-BPA treatment were 17.7 and 33, respectively. C) Scatter plot of the contrast thresholds of A17 neurons pre- and post-BPA treatment ($n = 58$, $p = 0.029 < 0.05$, paired Wilcoxon signed rank test). The red triangle (▲) indicates the median contrast threshold of A17 neurons pre- and post-BPA treatment. D) Cumulative distribution curves of the contrast threshold for A17 neurons pre- and post-BPA treatment. The curves shift to the right after BPA treatment. E) Scatter plot of the peak responses of A17 neurons pre- and post-BPA treatment ($n = 58$, $p = 0.034 < 0.05$, paired Wilcoxon signed rank test). The red triangle (▲) indicates the median peak firing rates of A17 neurons pre- and post-BPA treatment. F) The relationship between firing-rate change and contrast-threshold change. The Pearson correlation ratio is -0.5381 ($p < 0.0001$).

(Volkel et al., 2005). It also has been reported that BPA can accumulate in human tissues for long-lasting release even after a single treatment (Stahlhut et al., 2009; Volkel et al., 2005). These findings imply that the exposure model in our present study is suitable for exploring brain dysfunction in humans with unintentionally BPA exposure.

Contrast perception, determined by the contrast sensitivity of neurons, makes it possible for the visual system to distinguish objects from background in the natural images (Mante et al., 2005; Ress and Heeger, 2003). In the present study, our results showed that the contrast detection ability of A17 neurons in cats was severely decayed following 2 h BPA administration. Such decreased contrast sensitivity can cause no responses of neurons to low contrast information within the visual stimuli, which can perturb neuronal encoding of natural scenes in the A17 (Mante et al., 2005; Carandini and Sengpiel, 2004; Mante et al., 2008). Compared to people with good vision, people with decreased contrast sensitivity will have more difficulty dealing with a high-risk driving situation and have a worse reading and face-discrimination performance (McGwin et al., 2000; Mones and Rubin, 2005; Barnes et al., 2011). In addition, reduced contrast sensitivity was associated with increased risk of cognitive impairment (Ward et al., 2018). These findings imply that the decline of contrast sensitivity after BPA exposure can cause the visual information to be missed from natural scenes and also induce cognition deficits. In addition to the decline of contrast sensitivity, low-dose BPA exposure has been shown to impair the ocular dominance plasticity of rodents (Kelly et al., 2014). Our previous study also shows that acute BPA exposure causes orientation selectivity impairment in cats (Xu et al., 2018). These findings suggest that BPA exerts serious adverse effects on the visual plasticity and information processing. It is well known that many cortices for cognitive formation have broad innervations from the visual cortex (Davidson, 2002; Ji and Wilson, 2007), and BPA-induced cognitive dysfunctions also have been well documented (Braun et al., 2009; Hu et al., 2017; Roen et al., 2015). In future studies, we will focus on exploring whether BPA-induced visual dysfunction contributes to the high cognitive dysfunctions and the underlying mechanisms at the circuit regulation level. Since humans have a certain BPA dosage in their body fluids without intentional exposure (Volkel et al., 2005), we will also explore whether BPA causes contrast perception changes of human based on our previous psychophysical studies.

At the physiological level, there are two potential mechanisms underlying the alteration of contrast sensitivity after BPA treatment: 1) perturbed responsiveness of A17 neurons, and 2) decreased contrast perception of LGN neurons.

Within the A17, contrast response tuning is depended on the neuronal firing rates to grating stimuli with different contrasts and decreased responsiveness of A17 neurons can cause the contrast sensitivity decline (Hua et al., 2010). The responsiveness of A17 neurons was assessed by their peak-firing rates (signal) and response variability

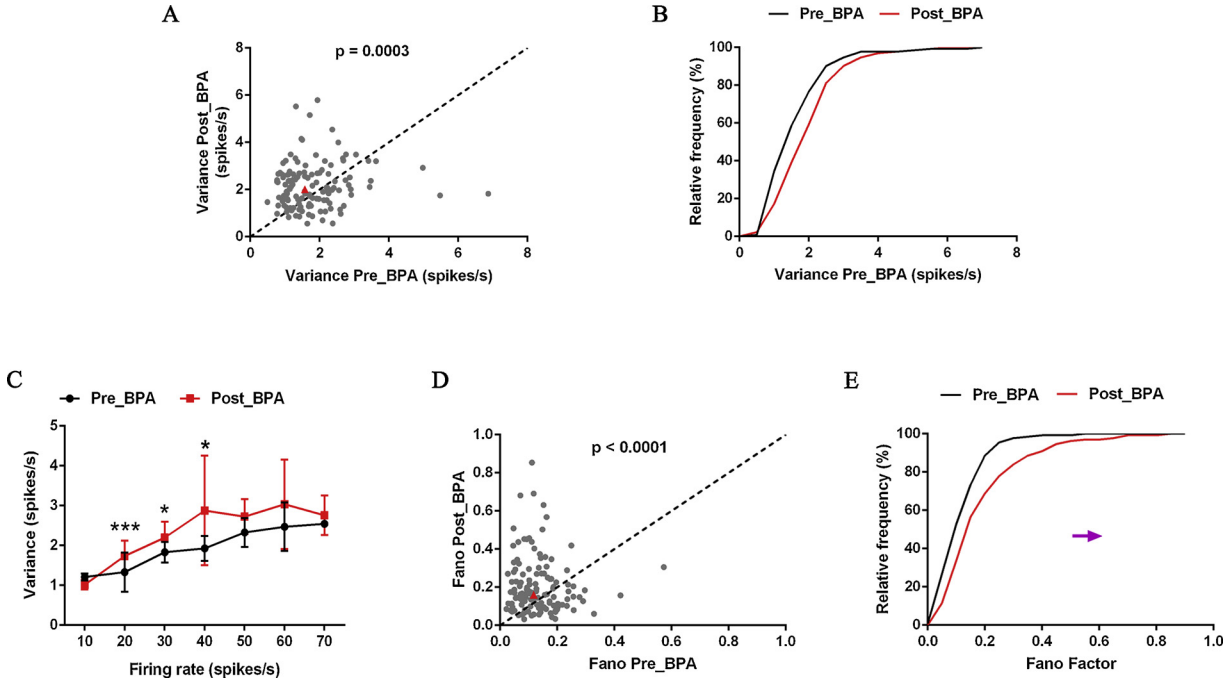

Fig. 3. BPA increased the noise when A17 neurons responded to the grating stimuli. A) Scatter plot of standard deviation (variance) of neuronal responses in the A17 pre- and post-BPA treatment ($n = 131$, $p = 0.0003 < 0.001$, paired Wilcoxon signed rank test). The red triangle (▲) indicates the median variance of A17 neurons pre- and post-BPA treatment. B) Cumulative curve of variance. C) The variance of neuronal responses at different firing rates (***$p < 0.001$, *$p < 0.05$, paired Wilcoxon signed rank test). D) Scatter plot of the Fano Factor of A17 neurons pre- and post-BPA treatment ($n = 131$, $p < 0.0001$, paired Wilcoxon signed rank test). The red triangle (▲) indicates the median Fano Factor of A17 neurons pre- and post-BPA treatment. E) Cumulative distribution curves of the Fano Factor for A17 neurons pre- and post-BPA treatment. The curves shift to the right after BPA treatment.

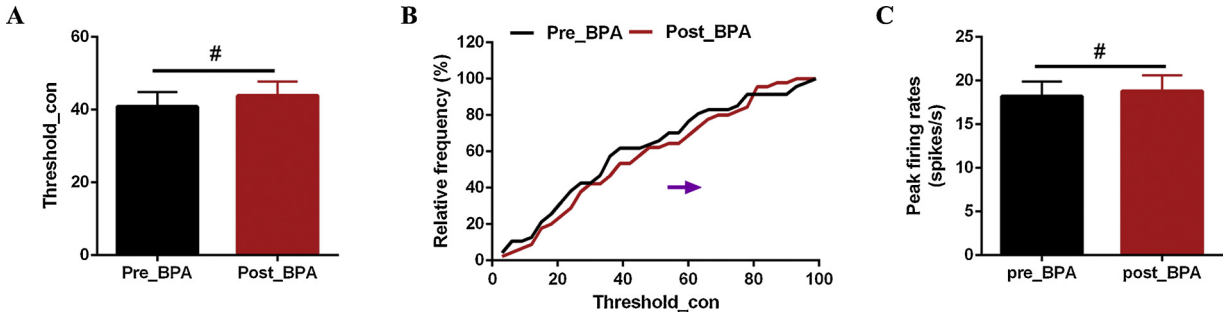

Fig. 4. BPA did not decrease the contrast sensitivity of LGN neurons in cats. A) Histogram of the contrast threshold of LGN neurons pre- and post-BPA treatment (n = 35, p > 0.05). B) Cumulative distribution curves of the contrast threshold for LGN neurons pre- and post-BPA treatment. C) Scatter plot of the peak responses of LGN neurons pre- and post-BPA treatment (n = 35, p > 0.05).

(noise) to the grating stimuli in the present study. After 2 h BPA treatment, we found that the peak firing rates were obviously decreased, which had a negative correlation with the increases in contrast threshold of A17 neurons. To some extent, such a decrease in neuronal evoked activity is consistent with our previous study, in which BPA exposure was found to inhibit the excitatory postsynaptic potential in the hippocampus when given a range of electric stimuli (0.1 ~ 0.4 mA) (Hu et al., 2017). In addition, we found that the decrease in evoked firing rates was accompanied by increased response variance (standard deviance), a noise reflecting the synaptic input's trail-to-trail variability (Carandini, 2004). This noise arises from fluctuations of neuronal membrane potential and can regulate the contrast detection of the cat visual cortex (Carandini and Ferster, 1997, 2004), which may be involved in the contrast sensitivity decline of A17 neurons after BPA

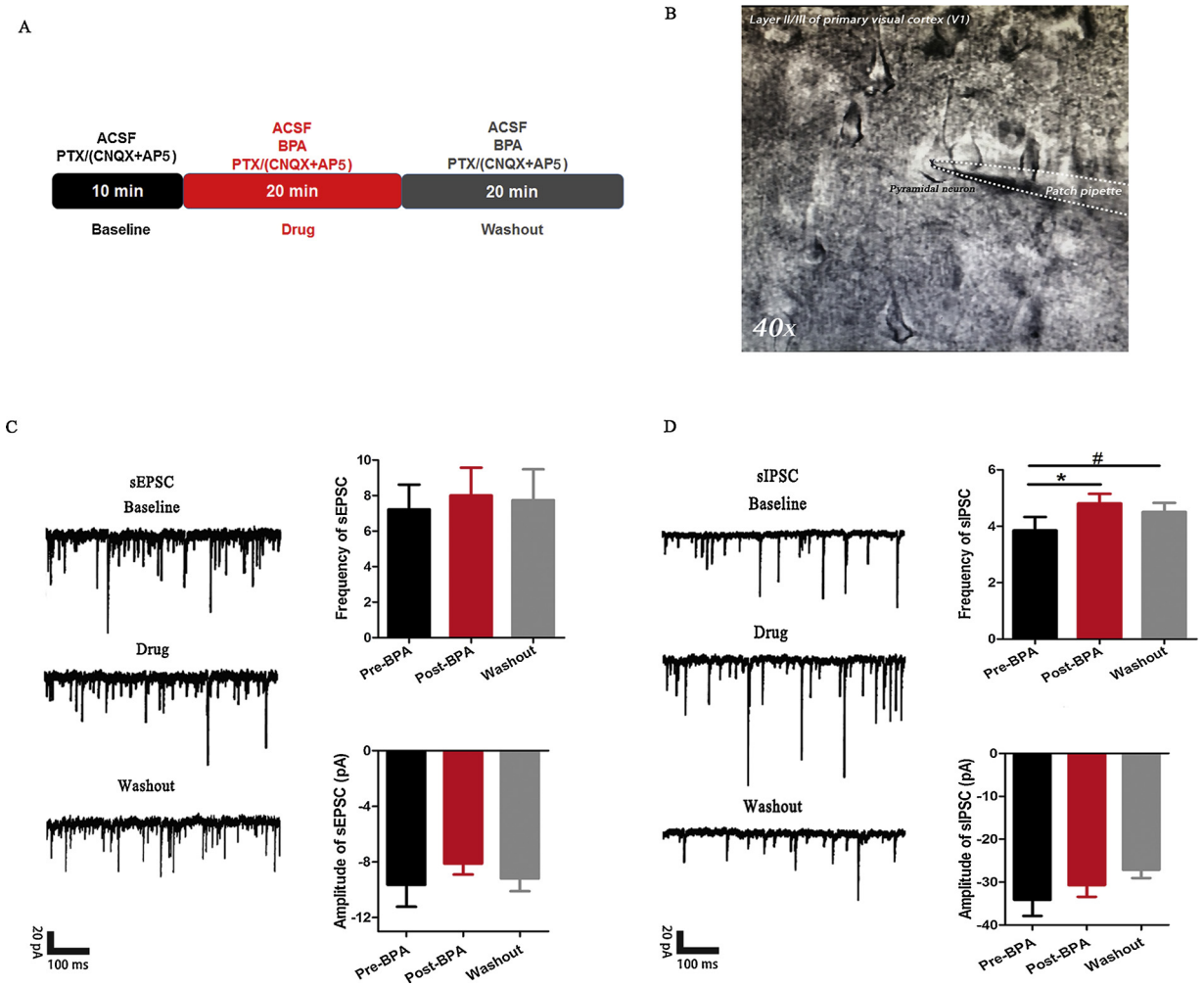

Fig. 5. BPA increased the inhibitory innervation of layer II/III pyramidal neurons in the primary visual cortex (V1) of mice. A) The schedule of patch-clamp recording in the V1 of a mouse brain slice. B) A path-clamp recording on layer II/III pyramidal neurons in the V1 of mice. C, D) Histogram of frequency and amplitude in sEPSC pre- and post-BPA perfusion and after BPA washout. (n = 6, p > 0.05). E) Representative spontaneous excitatory synaptic current (sEPSC) of pyramidal neurons in the V1 pre- (baseline) and post- (Drug) BPA perfusion and after BPA washout. F, G) Histogram of frequency and amplitude in sIPSC pre- and post-BPA perfusion and after BPA washout. (n = 6, *p < 0.05, #p > 0.05). H) Representative spontaneous inhibitory synaptic current (sIPSC) of pyramidal neurons in the V1 pre- (baseline) and post- (Drug) BPA perfusion and after BPA washout.

treatment.

To explore the underlying synaptic mechanisms of neuronal responsiveness changes in A17, we applied the patch-clamp recording method to examine the spontaneous excitatory and inhibitory innervations in layer II/III of V1 in mice. According to our results, BPA induced increases in the frequency of sIPSC but not sEPSC of pyramidal neurons. These growing inhibitory currents in pyramidal neurons of mice imply that there are also increased GABAergic inputs from inhibitory interneuron within the A17 of cats. This could induce the downregulated activity of the primary visual cortex (decreased firing rates to grating stimuli) after BPA exposure. To some extent, this finding is consistent with our previous report that BPA can increase the activity of interneurons in the A17 of cats (Xu et al., 2018). The excitatory and inhibitory balance is considered as the cornerstone regulation of neuronal functions in the visual cortex (Dehghani et al., 2016; Xue et al., 2014). In addition, inhibition has been proposed to regulate the gain of cortical responses, and activating interneuron (inhibitory) populations within the primary visual cortex (V1) can decay the contrast sensitivity of V1 neurons to grating stimuli (Wilson et al., 2012). It implies that increases in inhibitory innervations after BPA treatment could also contribute the decline in contrast sensitivity of A17 neurons.

The contrast sensitivity of the visual system is set by the beginning of the primary visual pathway (Heimel et al., 2010). According to our results, 2 h BPA treatment did not inhibit the contrast sensitivity of LGN neurons. This result rules out that the decreased contrast sensitivity in the A17 is attributable to alteration of contrast information projection from LGN neurons. In addition, there was no change in the neuronal responses (peak firing rates) to grating stimuli, which implies that the excitatory innervations from the LGN to the A17 are not changed by BPA exposure. These results were different with the neuronal response changes in the A17, which may be due to the difference of neural circuitry at the cortical and subcortical level. Compared to the LGN, there are more distribution of inhibitory interneuron and types of pyramidal neurons in the primary visual cortex (Kim et al., 2015; Seabrook et al., 2013). Combined with the inhibitory innervations increasing in the primary visual cortex after BPA treatment, BPA-induced declines in neuronal contrast detection may begin at the cortical level through disturbing the local intracortical connection, such as increasing the activity of inhibitory neurons. In future studies, we will further explore which kind of interneurons is BPA's "target" for modulating visual function or other cortical functions.

In conclusion, the present study showed that acute BPA treatment considerably inhibited the contrast perception of A17 neurons in cats. We provide valuable physiological evidence of neuron activity and synaptic innervations. The current investigations imply that perturbed contrast perception of environment information is involved in the vision-dependent cognitive impairments after BPA exposure. Additionally, our study opens a new "window" through exploring single neuronal function for discovering the occurrence and physiological mechanisms of BPA-induced cognitive deficits.

Declaration of Competing Interest

The authors declare that there are no conflicts of interest.

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