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Fixational saccadic eye movements are altered in anisometropic amblyopia

Xue-feng F. Shi[a,b,*], Li-min Xu[a], Yao Li[a], Ting Wang[a], Kan-xing Zhao[a,b] and Bernhard A. Sabel[c]
[a]*Tianjin Key Laboratory of Ophthalmology and Visual Science, Tianjin Eye Institute, College of Clinical Ophthalmology, Tianjin Medical University, Tianjin, China*
[b]*Department of Pediatric Ophthalmology and Strabismus, Tianjin Eye Hospital, Tianjin, China*
[c]*Institute of Medical Psychology, Otto.-v.Guericke University of Magdeburg, Magdeburg, Germany*

Abstract. *Purpose:* Amblyopia develops during a critical period in early visual development and is characterized by reduced visual sensory functions and structural reorganization of the brain. However, little is known about oculomotor functions in amblyopes despite the special role of eye movements in visual perception, task execution and fixation. Therefore, we studied the relationship of visual deficits in anisometropic amblyopia and fixational saccadic eye movements.
Methods: We recruited twenty-eight anisometropic amblyopes and twenty-eight age-matched control subjects. Using a high-speed eye-tracker, fixational eye-movements of both eyes were recorded. A computerized fixational saccadic component analysis of eye-movement waveforms was developed to quantify the parameters of fixational saccades (FSs) and a simulation model was developed to help explain the FS performances.
Results: Amblyopic eyes, but not control eyes, showed fewer FSs, but these had increased amplitudes, increased peak velocities, and longer inter-saccadic intervals. The reduced FSs occurred mainly in the 0- to 0.6-degree amplitude range, and the probability of FSs with larger amplitudes and longer inter-saccadic intervals was significantly higher than in controls. A new simulation model analysis suggests that an excitatory-inhibitory activity imbalance in superior colliculus may explain these FSs changes.
Conclusions: We propose that the abnormal visual processing and circuitry reorganization in anisometropic amblyopia has an impact on the fixational saccade generation. We see two possible interpretations: (i) altered FSs may be an attempt of the visual system to adapt to the deficit, trying to capture more information from a broader spatial domain of the visual world so as to enhance the contrast sensitivity to low spatial frequencies viewed by the amblyopic eye, or (ii) it may be the cause of amblyopia or a contributing factor to the original deficit that aggravates the early deprivation.

Keywords: Anisometropic amblyopia, fixational eye movements, microsaccade, amplitude, inter-saccadic interval, excitation-inhibition balance

1. Introduction

Amblyopia is a classic example of experience dependent synaptic plasticity of the central nervous system (Wiesel and Hubel, 1963; Johnston, 2004; Hensch, 2005) which affects visual perception and behavioral performance in multiple ways, such as reduced visual acuity, decreased contrast sensitivity (Hess, 1979; Hess et al., 1983; McKee et al., 2003) deficits in global form (Jeffrey et al., 2004; Levi et al., 2007) or motion detection (Simmers et al., 2003), the crowding phenomenon (Stuart and Burian, 1962; Bonneh et al., 2007; Levi et al., 2002) and abnormal spatial interactions (Polat et al., 1997; 2005; Levi et al., 2002; Ellemberg et al., 2002). The brain regions responsible for these functional deficits are believed to be mainly V1 and downstream (higher) visual areas (Levi, 2006). However, in addition to deficits in the primary

*Corresponding author: Xue-feng F. Shi, Tianjin Eye Hospital, 4 Gansu Road, Heping District, Tianjin 300020, China. Tel.: +86 22 27313336 83031; Fax: +86 22 27313133; E-mail: shixf2004@yahoo.com.cn.

visual pathway that directly controls visual sensations, subcortical regions which control eye movement and movement perception via alternative (extrastriate) visual pathways may be part of the problem. These extrastriate pathways may also be altered and contribute to abnormal visual experience in amblyopia (Ciuffreda et al., 1979; Srebro, 1983; Martinez-Conde, 2006a).

Among the different kinds of eye movements, fixational eye movements fixational saccades, FSs are the most important for maintaining visual sensation and perception, as they keep a target relatively stable with respect to the retinal photoreceptors (Rolfs, 2009). Fixational eye movements are classified into three types: tremors, drifts, and microsaccades (Krauskopf et al., 1960; Steinman et al., 1973; Moller et al., 2002). In all three, microsaccades are the most prominent and fastest eye movements that occur during fixation (Rolfs, 2009; Martinez-Conde et al., 2009). Recent neurophysiological and psychophysical results using advanced eye tracking techniques, analysis and modeling of eye movements have raised the interest in studying FSs under different conditions (Rolfs, 2009). However, most available studies on FSs were carried out with subjects that have normal vision. Disease-related research on FSs is very scarce except for one recent publication (Otero-Millan et al., 2011a). A quantitative analysis of FSs in visual diseases such as amblyopia has not been performed, despite its significance in diagnosis and therapy (Martinez-Conde, 2006a).

Because the role of FSs in amblyopia is also unknown, a detailed quantification of FSs in amblyopia is an important issue to be looked at. Therefore, we now quantified FSs in monocular anisometropic amblyopic eyes, fellow eyes and normal eyes to investigate if and how the visual deficits in anisometropic amblyopia, a mild form of visual input deprivation, affect fixational saccadic eye movements.

As we show for the first time amblyopic eyes make fewer FSs than fellow eyes or normal eyes and show increased amplitudes, increased peak velocities, and longer inter-saccadic intervals (ISIs).

2. Methods

2.1. Ethical statement

The study was approved by the Tianjin Eye Institute Ethics Committee. All of the experiments were performed in accordance with the Declaration of Helsinki, and all individuals gave their informed consent written by their parents or guardians on the behalf of them to participate in the study.

2.2. Participants

Twenty-eight anisometropic amblyopes and twenty-eight normal control subjects of the same ages were recruited from the outpatient clinic at the Department of Pediatric Ophthalmology and Strabismus of Tianjin Eye Hospital (see Table 1). The visual acuities of the amblyopic eyes were <20/30, with their fellow eyes at 20/20. All of the normal subjects had visual acuities of 20/20, in whom 17 subjects had weak eye dominance and 11 subjects had strong eye dominance assessed by hole-in-card test.

2.3. Experimental setup and eye-movement recordings

Participants were tested individually in a silent, dimly lit room; they were seated with their heads positioned on a chin rest. They viewed a visual target on a projection screen from a distance of 2.0 meters. Eye-movement data were recorded by an EyeLink-1000 system (SR Research, Ontario, Canada) with a temporal resolution of 1 ms, a noise-limited instrument spatial resolution of 0.01 degree and a FS resolution of 0.05 degrees. Visual stimuli were presented against a black background on a Hitachi projection screen (1024×768 resolution; frame rate 120 Hz). The data collection was implemented by a program made with EyeLink Experiment Builder (SR Research, Osgoode, Ontario, Canada).

2.4. Stimuli and procedure

The fixation spot was a bright dot with a diameter of 0.15 degree displayed at the center of the screen on a black background. The contralateral eye was covered in all of the experimental trials because amblyopic eyes need to be measured during monocular fixation (Ciuffreda et al., 1980; Srebro, 1983; Niechwiej-Szwedo et al., 2010). Each subject initiated a recording trial by pressing a key after being sure that he/she fixated on the spot. All subjects were required to maintain fixation throughout the entire experimental trial, which lasted for 6 seconds. The measurement of FS consisted of at least 6 trials, but the actual number of trials

Table 1
Clinical data of the subjects with anisometropic amblyopia

Patient	Sex	Age (y)	Refractive error (D)		Best corrected VA	
			RE	LE	RE	LE
1	Female	7	Plano + 0.25 × 90	+4.00–0.75 × 70	20/20	20/60
2	Female	11	+1.00–3.25 × 10	+0.75	20/50	20/20
3	Female	6	+0.25	+0.50 + 2.25 × 80	20/20	20/40
4	Female	10	Plano	+3.50 + 0.75 × 100	20/20	20/60
5	Female	6	–1.00 + 1.25 × 80	–2.75 + 3.25 × 75	20/20	20/40
6	Male	10	+2.75 + 0.75 × 60	–0.75	20/50	20/20
7	Female	10	+3.00–1.25 × 10	Plano	20/40	20/20
8	Male	8	+0.50	+2.00	20/20	20/30
9	Female	9	–1.00 + 2.75 × 90	Plano	20/50	20/20
10	Male	8	+3.75	+0.25 + 0.50 × 10	20/50	20/20
11	Male	6	+1.00	+4.50	20/20	20/40
12	Male	7	–0.25 + 0.50×20	–0.75 + 2.50 × 10	20/20	20/60
13	Female	9	+2.50	Plano	20/30	20/20
14	Female	11	+0.75 + 0.25 × 80	+1.25 + 2.75 × 90	20/20	20/60
15	Male	9	+0.25 + 2.75 × 60	–0.75 + 0.25 × 70	20/40	20/20
16	Female	12	–1.25–2.25 × 70	–1.00–0.25 × 90	20/40	20/20
17	Male	12	+1.00–0.75 × 10	+5.00	20/20	20/50
18	Female	7	+1.25	+3.75	20/20	20/40
19	Male	10	–0.25 + 0.50 × 20	Plano + 2.25×10	20/20	20/30
20	Male	6	–0.75 + 3.25 × 70	–0.50–0.50 × 10	20/60	20/20
21	Male	7	–1.00–2.50 × 90	–0.25–0.75 × 80	20/40	20/20
22	Female	7	+2.75	+0.75	20/30	20/20
23	Female	8	–0.75 + 0.25 × 90	+3.75	20/20	20/30
24	Male	10	+4.25	+1.00	20/40	20/20
25	Female	11	+1.25–3.25 × 10	+1.00–1.25 × 20	20/50	20/20
26	Male	9	0.75 + 0.50 × 90	–0.50 + 2.75 × 80	20/20	20/40
27	Male	9	–1.00–0.25 × 50	–1.50–2.25 × 60	20/20	20/30
28	Female	8	Plano + 2.75	–0.75 + 1.00 × 110	20/40	20/20

VA, visual acuity; RE, right eye; LE, left eye.

depended on the individual's cooperative level. The trials were separated by a brief rest period for each eye. Calibration and validation was performed before each trial to ensure sampling accuracy. The calibration method was applied according to the manufacturer's handbook and carried out in an identical manner for each eye and each patient/subject. We used nine-point calibration and when this was completed, the validity of it was checked again to show the experimenter the gaze position accuracy achieved by the current calibration parameters and to exclude the possibility of a wrong calibration reading. This was done by the eye tracker program according to device specifications. The calibration, validation and recording were done in the same environment with the same luminance for each eye and each patient/subject. We also avoided abrupt luminance change in the room which is thought to cause pupil size change and influence the spatial accuracy. These ensured the validity and consistency of the spatial accuracy. Finally, we obtained a total number of 1299 trials in amblyopic eyes, 1249 trials in fellow eyes, and 1288 trials in non-dominant, normal eyes.

2.5. Saccade detection

An adapted FS detecting algorithm programmed with Matlab (Mathworks, Massachusetts, U.S.A) was used to identify FSs (Engbert and Kliegl, 2003; Engbert and Mergenthaler, 2006). A FS was defined by the following criteria: (1) the angular velocity exceeded six median-based standard deviations of the velocity distribution (Engbert and Kliegl, 2003; Rolfs et al., 2008), hence, the computed average velocity thresholds are as follows: horizontal components: $9.12 \pm 1.40^\circ$/sec (amblyopia), $9.14 \pm 1.66^\circ$/sec (fellow), $9.20 \pm 1.65^\circ$/sec (normal); vertical components: $9.21 \pm 1.41^\circ$/sec (amblyopia), $11.82 \pm 1.30^\circ$/sec (fellow), $11.36 \pm 1.98^\circ$/sec (normal); (2) the duration exceeded 12 ms (Engbert and Kliegl, 2003); and (3) the inter-saccadic interval (ISI) preceding the measured FS exceeded 20 ms so that potential overshoot corrections

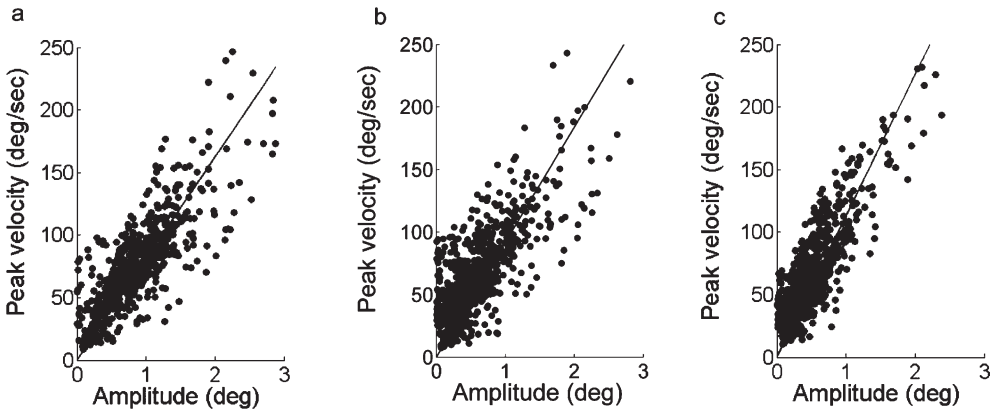


Fig. 1. Examples of velocity-amplitude relationship of FSs in three representative eyes: amblyopic, fellow eye and normal eye from typical single cases. In each panel, the peak velocity of each fixational saccadic event is plotted against its amplitude. The linear correlation between peak velocity and amplitude is called the "main sequence" and it shows for all three cases that peak velocity and amplitude change with each other in the same manner. a: Amblyopic eye, $r = 0.846$, $P < 0.001$. b: Fellow eye, $r = 0.738$, $P < 0.001$. c: Normal eye, $r = 0.851$, $P < 0.001$. These high linear correlations indicate that the events detected by the algorithm are valid FSs. The subjects of these examples were tested for 85, 87 and 92 trials, respectively. Other subjects were tested at least for 6 trials according to their ability to cooperate. Some patients (3/28) showed much greater variability in fellow eyes (as in Part b). However, there is no significant difference of amplitude or velocity between the groups of fellow eyes and normal eyes.

might not be regarded as new FSs (Otero-Millan et al., 2008). We did not use a binocular criterion for saccade detection (Martinez-Conde et al., 2006b; Levi and Li, 2009b). One reason is that we had to cover the contralateral eyes to study the characteristics of FSs in amblyopic eyes. Another reason is that the binocular criterion of saccade detection may be a double-edged sword, especially under pathological conditions (Van Horn and Cullen, 2012). Generally, according to the Hering's law, the movements of both eyes are thought tightly coupled. However, it has been recently shown that saccadic neurons encode the movement of each eye individually during FSs (Van Horn and Cullen, 2009; 2012; Cullen and Van Horn, 2011) which is contrary to the Hering's law. In fact, Engbert (2006) also showed that FSs can be binocular and monocular.

Trials that included saccades larger than 3 degrees of visual angle or for which data was lost (for example, due to eye blinks) during eye-movement recording were discarded (Troncoso et al., 2008a). In the end, a total of 952 trials (73.3%, max 85 trials in one eye) for amblyopic eyes, 971 trials (77.7%, max 87 trials in one eye) for fellow eyes, 1063 trials (82.5%, max 92 trials in one eye) for non-dominant normal eyes were included for further analysis. The main sequence analysis (Zuber and Stark, 1965) was performed to verify the validity of detected FSs (Fig. 1). The basic parameters for the measurement of FSs included amplitude, peak velocity, occurrence rate, and ISI. In addition, to analyse the distribution of the probability of occurrence, we defined FS as the proportion of those events that have a specified amplitude or eccentricity. For this analysis, we only selected the data of eyes from which we were able to collect >500 fixational saccadic events. In the end, 6 amblyopic eyes (average 69.9 ± 15.2 trials in each eye), 6 fellow eyes (average 70.8 ± 17.3 trials in each eye), and 8 normal eyes (average 74.1 ± 18.3 trials in each eye) were included for the analysis of the distribution of the occurrence probability.

The validity and suitability of the recorded data were confirmed in our study. Firstly, to exclude the possibility of the influence of a selective change in noise, we measured the noise levels of eye movement of the three groups. We found that the RMS of the inter-sample distances in the three groups were comparable as follows: horizontal components: $0.0060 \pm 0.0015^\circ$ (amblyopia), $0.0059 \pm 0.0010^\circ$ (fellow), $0.0061 \pm 0.0007^\circ$ (normal), ANOVA test, $F = 0.287$, $P = 0.751$; vertical components: $0.0063 \pm 0.0010^\circ$ (amblyopia), $0.0064 \pm 0.0011^\circ$ (fellow), $0.0063 \pm 0.0009^\circ$ (normal), ANOVA test, $F = 0.146$, $P = 0.865$. To further check the influence of the noise level, we re-analysed the data with the detection threshold of 4 or 5 SD of velocity distribution which is a method used by others (Engbert and Mergenthaler, 2006; Cui et al., 2009; Mergenthaler and Engbert, 2010). We found that with

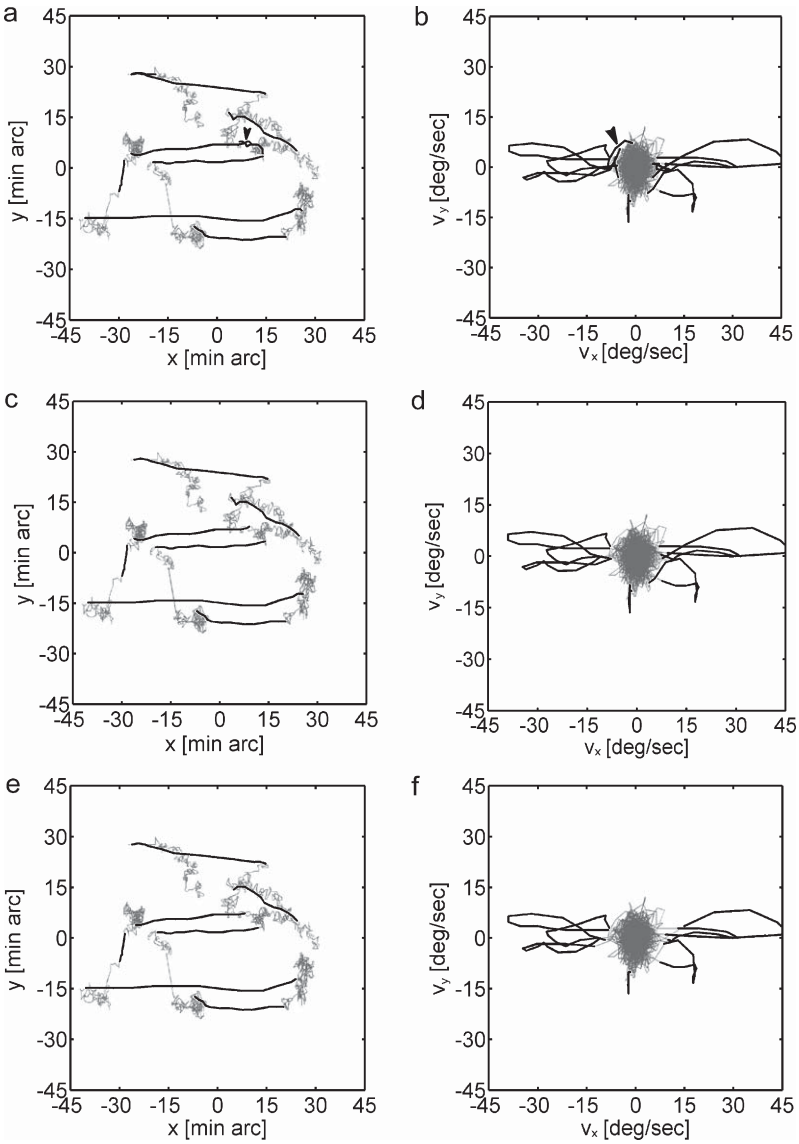


Fig. 2. An example of fixational eye movements in an amblyopic eye and the detected FSs under different velocity thresholds. Left column (a, c, e): The trajectory of fixational eye movements in an amblyopie eye. FSs are indicated by bold black lines. Right column (b, d, f): FS detection after transformation of the trajectory into 2D velocity space. The FSs indicated by bold black lines are identified by their higher velocity exceeded the specified thresholds. The thresholds are 4 SD (in Part a and b), 5 SD (in Part c and d), and 6 SD (in Part e and f) of the velocity distribution, respectively. The threshold of 4 SD may occasionally include some noise as "signal", for example the event indicated by the black arrow in Part a and b. The threshold of 6 SD ensured a sufficiently defined event detection which avoids any possible contamination by noise.

a lower threshold, the FS detection were still not significantly influenced, thus the detection threshold of 6 SD ensured that all the detected events were clean enough and avoid the interference of noise (Fig. 2). In addition, the noise level in real subjects is still rather low even if the heuristic filters (Stampe, 1993) of the eye tracker were set to "off"-status (data not shown), which further confirmed that the FS signals were not affected by the noise, regardless whether or no filtering was applied.

Secondly, besides system noise, other factors may produce artifacts interfering with the FS events. They include pupil size changes and head movements. However, these signals are generally very slow compared

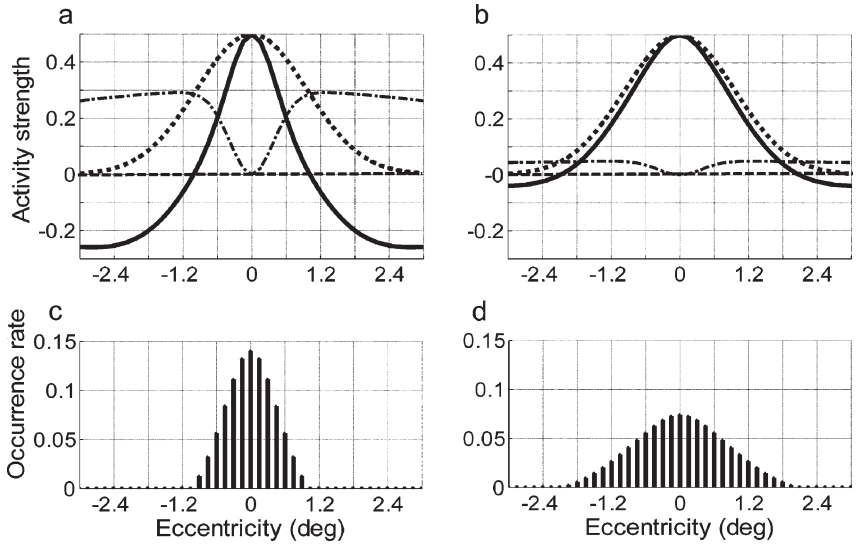


Fig. 3. Model analysis of FS generation determined by SC activities under normal versus amblyopic viewing conditions. All the data were obtained from modeling simulation. For this simulation, the values of parameters set in the model provided a spatial profile of activity similar to experimentally observations by previous studies (Hafed et al., 2008a; 2009; 2011). The values of activity strength in the simulation data were relative quantities. The results of the theoretical spatial distributions of the probability of FS occurrence are not very sensitive to the absolute activity level, but sensitive to the excitation-inhibition balance. a, b: The activity strength distribution along with eccentricity under normal viewing condition (Part a) and amblyopic viewing condition (Part b). The size of the simulated SC activity map is up to 12-degree eccentricity, but only the eccentricity range within 3-degrees is shown here. The dot, dash dot and solid lines describe the excitatory activity level, the inhibitory activity level and the difference of both, respectively. The dashed lines represent the threshold for eliciting FSs. Parts c-d: Simulated spatial distributions of the probability of FS occurrence as a function of endpoint eccentricity. Given that the occurrence rate is positively and linearly correlated with suprathreshold SC activity, the spatial distributions of FS occurrence probability in normal condition (c) and amblyopic condition (d) are shown here. Bin size = 0.15°.

to the very short life time of FS events or the time-scale involved in noise contributions. The measured amplitudes of pupil size changes during FS events were as follows: $0.107 \pm 0.032\%$ (amblyopia), $0.109 \pm 0.025\%$ (fellow), $0.110 \pm 0.026\%$ (normal). There was no significant difference among the three groups (ANOVA test, $F = 0.098$, $P = 0.906$). The estimated pupil-originated apparent eye movements were all $<0.002^\circ$ for a given mean pupil size of 3 mm in diameter, according to the relationship between the pupil diameter change and apparent eye movements (Wyatt, 2010). These changes are much lower than the noise levels of eye movement traces. Other abrupt pupil size changes caused by inevitably and occasionally occurring eyelid movements (but not completely closing the eye) always lead to very large apparent saccade-like traces, but this kind of artifacts can be easily excluded either by the 3 degree amplitude limit or by the velocity based FS detection algorithm.

In all, the combination of the low-noise data recording and an offline noise resistant saccade detector algorithm ensured the effectiveness and validity of detected FS events. This is in agreement with a recent paper (Kimmel et al., 2012) in which the authors showed broad agreement in detection of fixational saccades between a search coil system and a video eye tracker just as the one used in our study.

2.6. Modeling

We developed a computational model to explore the neural mechanism of changes in fixational saccadic performance of amblyopic eyes. In this model, the FS generation is controlled by the SC (superior colliculus) activity which is similar to Hafed's physiological observation in rhesus monkeys (Hafed et al., 2008a; 2008b). In addition, the SC activity level was defined as the difference of excitatory and inhibitory activities (Fig. 3). The modeling functions were as follows:

$$A_e = \beta_e \exp(-e^2/2(rb\sigma)^2) \tag{1}$$

$$A_i = \beta_i(\exp(-e^2/2(rb\sigma_1)^2) - \exp(-e^2/2(rb\sigma_2)^2)) \tag{2}$$

$$A_s = A_e - A_i \quad (3)$$

Where Ae is the excitatory activity strength, Ai is the inhibitory activity strength and As is the difference of them, βe and βi are the scaling parameters, e is the eccentricity of retinotopic site, r is the radius of the modeled retinotopic activity map, b is the bin size, σ describes the spatial spread of the excitatory SC activity with Gaussian distribution, $\sigma 1$ and $\sigma 2$ describe the spatial spread of the inhibitory SC activity calculated as the difference of double Gaussians distribution.

We assumed that the inhibitory activity level is decreased in the retinotopic SC map due to deafferention from loss of corticotectal input driven by the amblyopic eye, which exerts a powerful suppression of retinotectal input. For the simulation data shown in Fig. 3, we defined βe = 0.5, βi = 0.3 (normal condition) or 0.05 (amblyopia condition), $r = 12$deg, $b = 0.15$deg, $\sigma = 0.1$, $\sigma 1 = 0.6$, $\sigma 1 = 0.04$. Note that in our model, the activity level not only could be supra-threshold, but also could be sub-threshold. The summation or build-up of excitatory and inhibitory inputs may reach a threshold required to trigger neuronal firing or firing burst, which, in turn, may drive fixational saccadic eye-movements (Hafed et al., 2009). Therefore, we defined a threshold T which was given a value of 0 in the simulation shown in Fig. 3. Given that the strength of suprathreshold SC activity is linear with the FS occurrence probability, the spatial distribution of FS generation can be obtained simultaneously (Fig. 3c, d). In the following function, A stands for the suprathrehold activity strength.

$$A = \begin{cases} A_s & (A_s > T) \\ 0 & (A_s \leq T) \end{cases} \quad (4)$$

2.7. Statistical analysis

Statistical analyses and graph plotting were done with Origin (OriginLab, Massachusetts, U.S.A), Matlab (Mathworks, Massachusetts, U.S.A.) and Illustrator (Adobe Systems, California, U.S.A).

3. Results

3.1. Amplitude, peak velocity and rate

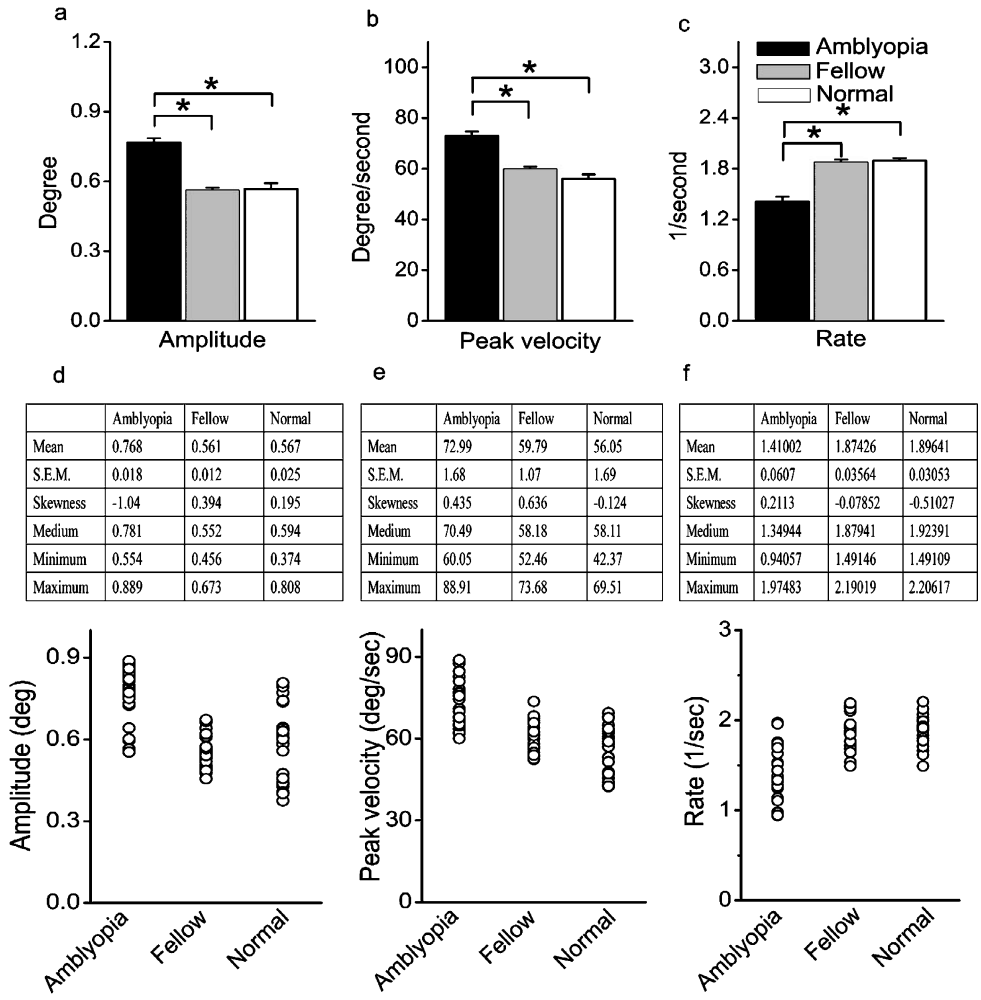
We first quantified the parameters of FS amplitude, peak velocity and rate for the amblyopic eyes, fellow eyes and normal control eyes. We calculated the average measures for each subject and between-subject means. Figure 4 depicts the mean values of these parameters for the various conditions. The differences among these means were evaluated using one-way ANOVA followed by *post-hoc* Turkey tests, and additional paired t tests were conducted for the comparison between the amblyopic eyes and fellow eyes. We found that the mean FS amplitude of the amblyopic eyes was larger than that of the fellow eyes ($q = 10.83$, $P < 0.01$; $t = 7.49$, $P < 0.01$) and that of the normal eyes ($q = 10.50$, $P < 0.01$). Also, the peak velocity of the amblyopic eyes was faster than that of the fellow eyes ($q = 8.77$, $P < 0.01$; $t = 9.28$, $P < 0.01$) and normal eyes ($q = 11.26$, $P < 0.01$). However, the FS rates of amblyopic eyes were reduced compared to both fellow ($q = 10.48$, $P < 0.01$; $t = 6.50$, $P < 0.01$) and normal eyes ($q = 10.98$, $P < 0.01$).

3.2. Inter-saccadic interval

To explore potential causes for the suppression of FS rates in amblyopic eyes, we first compared the ISIs among amblyopic eyes, fellow eyes and normal eyes. One can expect that a longer ISI corresponds to a lower FS rate, and vice versa. The results showed that amblyopic eyes demonstrated longer ISIs than did normal eyes and fellow eyes (Kruskal-Wallis ANOVA test, $\chi = 15.18$, $P < 0.001$). We next investigated the issue if the ISI depended on the FS amplitude. As Fig. 5 shows, the ISIs increased with the amplitude in all three groups. However, there was no difference of ISI at any specified amplitude among the three groups (ANOVA test, n.s.). In addition, an interesting phenomenon observed in all groups was that in the 0- to 0.6-degree amplitude range (which corresponds to the territory of the foveola, the area of best acuity), ISIs increased linearly with amplitude. Within the foveal range, but outside the foveolar border (0.6- to 2.6-degree), the ISIs nearly reached saturation.

3.3. Spatial distribution of the probability of occurrence

Next, we investigated the spatial distribution of the probability of the occurrence of FSs, as amplitude differences may influence ISIs and change the FS rate. The scatter of FS endpoint positions were plotted (Fig. 6a–c), and from these plots, we measured the occurrence probability of FS in each $0.1^\circ \times 0.1^\circ$ bin



d

	Amblyopia	Fellow	Normal
Mean	0.768	0.561	0.567
S.E.M.	0.018	0.012	0.025
Skewness	-1.04	0.394	0.195
Medium	0.781	0.552	0.594
Minimum	0.554	0.456	0.374
Maximum	0.889	0.673	0.808

e

	Amblyopia	Fellow	Normal
Mean	72.99	59.79	56.05
S.E.M.	1.68	1.07	1.69
Skewness	0.435	0.636	-0.124
Medium	70.49	58.18	58.11
Minimum	60.05	52.46	42.37
Maximum	88.91	73.68	69.51

f

	Amblyopia	Fellow	Normal
Mean	1.41002	1.87426	1.89641
S.E.M.	0.0607	0.03564	0.03053
Skewness	0.2113	-0.07852	-0.51027
Medium	1.34944	1.87941	1.92391
Minimum	0.94057	1.49146	1.49109
Maximum	1.97483	2.19019	2.20617

Fig. 4. FS amplitude, peak velocity, and occurrence rate in amblyopic eyes, fellow eyes and normal eyes. a: Comparison of mean amplitudes, ANOVA test, $F = 37.95$, $P < 0.01$. b: Comparison of mean peak velocities, ANOVA test, $F = 34.97$, $P < 0.01$. c: Comparison of mean rates of FS occurrence rate, ANOVA test, $F = 38.44$, $P < 0.01$. *Post-hoc* Turkey test was conducted across groups. Additionally, paired t test was conducted between amblyopic eyes and fellow eyes. Error bars represent SEM. $^*P < 0.01$ for ANOVA *post-hoc* Turkey test, also for paired t test between amblyopia group and fellow group. d–f: upper row: statistical values for FS amplitude, peak velocity and rate in the three groups. Bottom row: scatter plots of amplitude means, peak velocity means and rate means in the three groups showing the density distribution of these values.

on a $3^\circ \times 3^\circ$ retinotopic map, as indicated in Fig. 6d–f. The results indicated a decrease in the peak probability of smaller-amplitude FS and a less-focused spatial distribution of FSs in amblyopic eyes.

3.4. Rightward shift of cumulative probability

Figure 7a indicated that the mean FS occurrence probabilities of all of the subjects changed with amplitude. It also showed that, in normal eyes, most FSs occurred in the foveolar range, whereas in amblyopic eyes an increased number of larger FSs occurred outside the foveolar border. It is interesting that in amblyopic eyes FSs within the foveolar region were greatly reduced in number compared with normal eyes. The cumulative probability (CP) values in Fig. 7b, derived from Fig. 7a, indicated that the CP curve of amblyopia shifted to the right. The amplitude at half CP increased in amblyopic eyes ($t = 13.269$, $P < 0.001$).

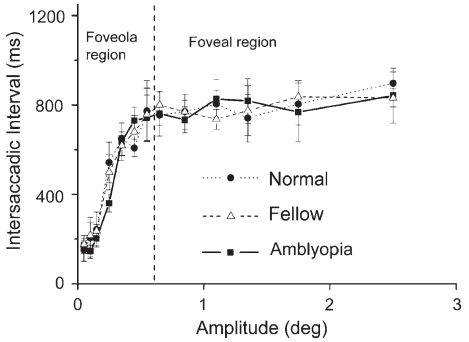


Fig. 5. Dependence of ISI on FS amplitude. The vertical dashed line represents the border between the foveola (0- to 0.6- degrees of visual angle) and the foveal region (0.6- to 2.6- degrees of visual angle). In the foveolar range, the ISIs of the three groups increased linearly with increasing amplitudes. Within the foveal range, but outside the foveolar range, the ISIs reached saturation in all groups. As the graph shows, there is no difference of ISI at each specified amplitude among the three groups (ANOVA test, n.s). Data shown as mean ± SEM. The amplitude ranges for the data calculation are as follows, respectively: 0–0.075°, 0.075–0.125°, 0.125–0.2°, 0.2–0.3°, 0.3–0.4°, 0.4–0.5°, 0.5–0.6°, 0.6–0.8°, 0.8–1.0°, 1.0–1.2°, 1.2–1.5°, 1.5–2.0°, 2.0–3.0°.

3.5. A model disclosing that excitation-inhibition balance in SC results in the changes of FS performances in amblyopia

To explain the changes of FS performances of amblyopic eyes, we developed a simulation model based on previous studies (Rolfs et al., 2008; Hafed et al., 2008a; 2009). In this model, we changed the inhibitory activity level since the corticotectal input influencing the inhibition level in SC may be functionally impaired (Hoffmann and Sherman, 1974; Berman and Sterling, 1976; Shibata et al., 1990; Hirai and Okada, 1993). Fig. 3a, b showed that the change of excitation-inhibition balance due to decreased global inhibition level significantly changes the activity distribution in SC, which causes a wider spread of spatial distribution of FS occurrence under amblyopia compared to normal conditions (Fig. 3c, d). Our model also produced results which correlated well with observed data both for normal condition (Fig. 8a) and amblyopia condition (Fig. 8b). The predicted occurrence probabilities along with amplitude (Fig. 8c) and CP curves (Fig. 8d) were also similar to those of observations displayed in Fig. 7a, b. In summary, the results of our modeling work indicate that a changed balance between excitation and inhibition may be the principal cause of abnormal fixational saccadic eye-movements in amblyopia.

4. Discussion

Although FSs are of great functional significance in neural coding (Leopold and Logothetis, 1998; Martinez-Conde et al., 2000; Martinez-Conde et al., 2002; Reppas et al., 2002; Herrington et al., 2009), visual perception (Martinez-Conde, 2006a; Laubrock et al., 2008; Martinez-Conde et al., 2006b; 2008; Troncoso et al., 2008a; 2008b), and visual task execution (Ko et al., 2010), their behavioral characteristics in visual and neurological diseases have rarely been studied. Only one disease-related quantitative study on FSs was conducted in patients with progressive supranuclear palsy (Otero-Millan et al., 2011a). With regard to amblyopia, only a few papers provided some qualitative description of FSs (Ciuffreda et al., 1979; Srebro, 1983; Martinez-Conde, 2006a). Ciuffreda et al., (1979) studied FSs in amblyopia without strabismus and reported that they are only rarely found. After Kowler and Steinman (1980) published their letter to R.W. Ditchburn and declared that small saccades "serve no useful purpose", Srebro (1983) reported that there was no difference of occurrence probability of large FSs between amblyopic eyes and normal eyes. However, these two studies used recording systems with very low spatial and temporal resolutions so that some very small eye movements must have escaped the experimenters' observation. Another shortcoming of research on FS behavior in amblyopia was that no effective computational algorithm for precise automatic detection of FSs was available (Martinez-Conde et al., 2000; Engbert and Kliegl, 2003; Engbert and Mergenthaler, 2006). We have therefore carried out for the first time quantitative and detailed analysis of FSs in amblyopia, using a high-speed, high-precision, non-invasive eye-tracking technique and an automatic FS-detection algorithm (Engbert and Kliegl, 2003; Engbert and Mergenthaler, 2006) known to be effective and reliable (Rolfs et al., 2008; Troncoso et al., 2008a; Otero-Millan et al., 2008; Poletti and Rucci, 2010). These tools allowed us to obtain objective and accurate results from our subjects.

We observed that in amblyopic eyes the mean FS amplitude and peak velocity were larger than those observed in fellow eyes or normal eyes. This coincidence of FS amplitude and peak velocity change reflects the strong relationship between the two. This coincidence also exists in voluntary saccades (Zuber and Stark, 1965; Schulz, 1984). In addition, we observed that the FS rate was suppressed in amblyopic

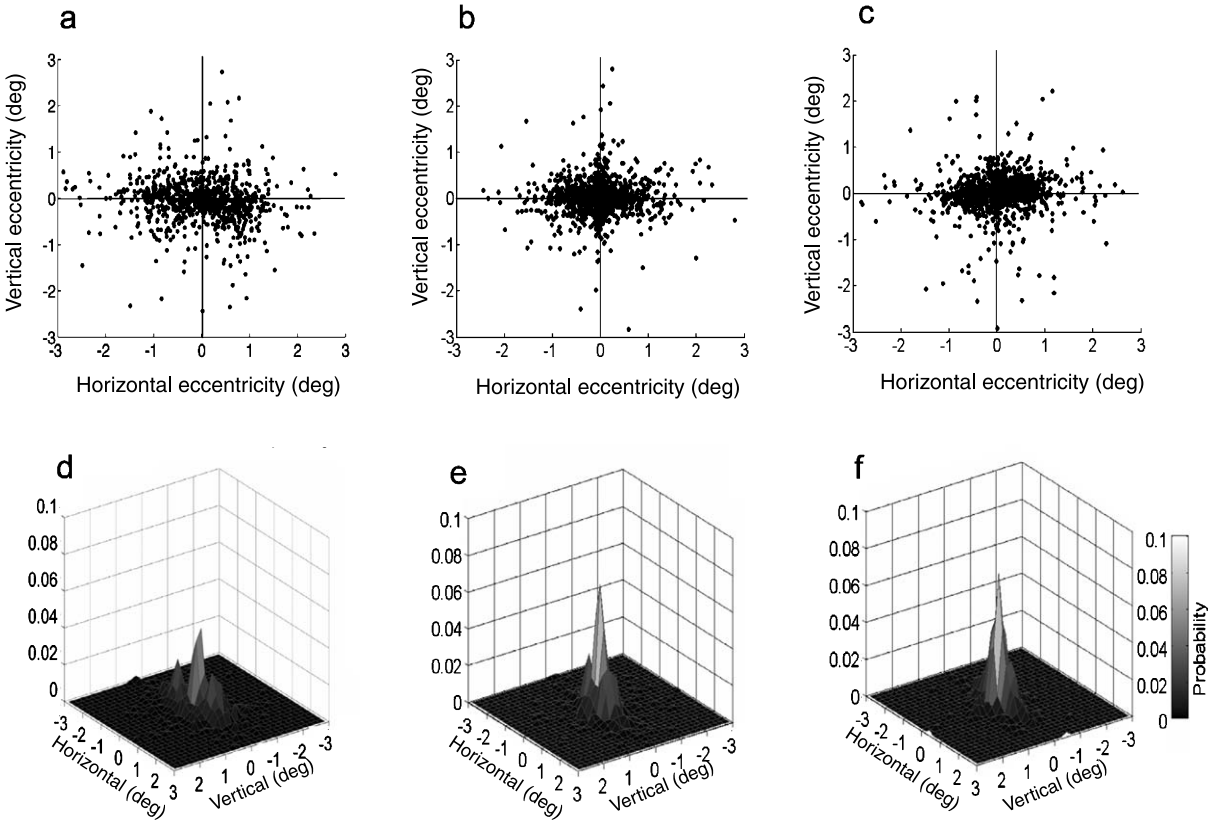


Fig. 6. Spatial distribution of FS occurrence. FS endpoint scatter plot of amblyopic eye (a), fellow eye (b) and normal eye (c). Each dot represents the endpoint spatial locus of a FS event, i.e. the position of each data point stands for the amplitude and direction of the vector of a FS event, but not for calculated absolute gaze position on the screen. The point of origin stands for the start of all FS vectors, but not for the center of the fixation point on the screen, hence, we can group all data from different trials based on the same point of origin which always corresponds to the retinotopic center. The scatter points spreads more widely in panel (a) than those in panels (b) and (c). This indicates that the amblyopic eye has less focused fixational eye movements, reflecting less fixational saccadic control. d–f: 3D spatial distribution of the probability of the FS occurrence in amblyopic eye (a), fellow eye (b) and normal eye (c), calculated from the above example plots. The peak in amblyopic eye (d) is obviously smaller than that in the fellow eye (e) or control eye (f). The amblyopic eye, on average, did not exceed a probability of 0.05; Bin size = $0.1^\circ \times 0.1^\circ$.

eyes, which confirms Ciuffreda et al.'s (1979) early qualitative observation but we studied FS rate suppression in greater quantitative detail. Considering that the ISI directly defines the instant FS rate, we chose ISI as a parameter for investigating the cause of FS rate impairments. We found that the overall ISIs in amblyopic eyes were significantly longer than those in fellow eyes and normal eyes. However, it is still not clear why the overall ISIs were prolonged because our further analysis intriguingly revealed that there was no significant difference of ISI at any specified amplitude among the three groups. This excludes the possibility that prolonging ISIs is a direct mechanism of FS rate suppression. Rather, we assume that there are differences of fixational saccadic magnitude distributions because we noted that ISIs increased with amplitude in all three groups.

To further clarify this point, we studied the spatial distribution of the occurrence probability and its correlation with the absolute amplitudes. We found that the endpoints of FSs in amblyopic eyes distributed more peripherally and the FS production was suppressed mainly for those with amplitudes <0.6-degree which resulted in a prominent rightward-shift of the cumulative probability in amblyopic eyes. Hence, we conclude that the change of the amplitude distribution is a dominant alteration in FSs of anisometropic amblyopia, while the changes of other parameters are alterations secondary to it. These characteristics of fixational saccadic behavior in amblyopia provide the

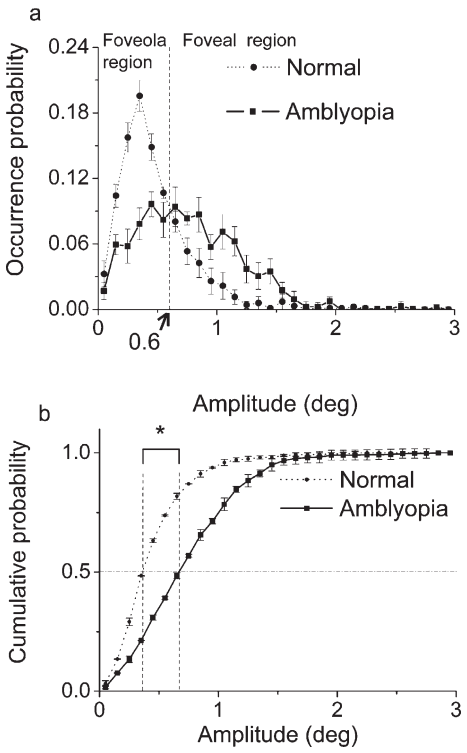


Fig. 7. Increased larger-amplitude FSs caused rightward shift of FS cumulative probability. a: Probability of FS occurrence as a function of amplitude. As in Fig. 3, the vertical dashed line represents the border between the foveola region and the foveal region. In normal eyes ($n = 6$) most FSs occurred in the foveolar range; in amblyopic eyes ($n = 8$), in contrast, a greater number of large-amplitude FSs were found outside the foveolar border. Of all FSs we measures, $74.5 \pm 1.9\%$ were within the foveolar regions in normal eyes but only $39.3 \pm 1.2\%$ in amblyopic eyes. Data represent mean $\pm$ SEM. b: Cumulative probability (CP) curves in normal and amblyopic eyes. The vertical dashed lines across the horizontal dash dot line represent the amplitudes at half CP in normal eyes and amblyopic eyes, respectively. Data represent mean $\pm$ SEM. $^*P < 0.001$.

necessary baseline information for future studies and should advance our understanding of the correlation between visual system impairment and eye-movement behavior.

Firstly, our results suggest that the amplitude threshold for simple identification of FSs should be set at higher levels in subjects with visual neural deficits. There has not been a general consensus of the magnitude of FSs in the literatures. Early studies set an arbitrary maximum amplitude of $\sim$12 arc minutes (Martinez-Conde et al., 2004), whereas recent studies set a more practical upper threshold of 1 degree of visual angle (Martinez-Conde et al., 2009), because this threshold captures the vast majority (>90%) of FSs (Otero-Millan et al., 2008; Martinez-Conde et al., 2009) according to the distribution of FS amplitudes in normal subjects. In agreement with recent studies, the distribution of FS amplitudes in our normal group showed that 93.8% of FSs had magnitudes below 1degree. However, the present study also suggests that the 1 degree threshold may not offer an appropriate or simple method of identifying FSs in studies of abnormal vision, because many FSs far exceeded this threshold in anisometropic amblyopia. Due to the scarceness of FS studies in visual or neurological diseases, the knowledge about the amplitude distribution of FSs in pathological conditions is not well explored. Similar to the finding of larger FSs in our study, a recent published disease-related study showed a greater number of larger horizontal saccades and rare saccades of "standard" size during sustained fixation in progressive supranuclear palsy patients (Otero-Millan et al., 2011a). Specifically, we also observed a greater number of larger horizontal saccades and fewer vertical components with increases of fixational saccadic amplitudes in all three groups (Fig. 9). Our study is different from the characteristics of FSs in progressive supranuclear palsy in that we did not observe a large number of FSs with amplitudes >2 degrees that may be attributable to more occurrences of square wave jerks. Therefore, we suggested that the change of FSs in amblyopic eyes is mild in comparison to other neurological diseases. Yet, both our study and that by Otero-Millan et al., (2011a) showed that the 1 degree threshold is not appropriate for identifying FSs in subjects with visual or neurological diseases.

Secondly, the combination of the observed results with computational modeling supports the idea that the excitation-inhibition balance shaped by visual experience can influence not only visual perception but also eye movement performance. FSs are believed to be controlled by the SC and downstream pre-motor and motor nuclei (Van Gisbergen et al., 1981; Munoz and Wurtz, 1992; Stampe, 1993; Rolfs et al., 2008; Hafed et al., 2008a; 2009; 2011; Poletti and Rucci, 2010; Otero-Millan et al., 2011b). The SC receives direct retinocollicular projections that are organized retinotopically, and (indirectly) cortico collicular projections that are aligned with the retino collicular map (Triplett et al., 2009). Theoretical models have shown that activity changes in the rostral part of SC coincide with changes in the generation of FSs (Otero-Millan et al., 2008; 2011b; Hafed et al., 2009; 2011) which was confirmed by experimental studies in primates (Hafed

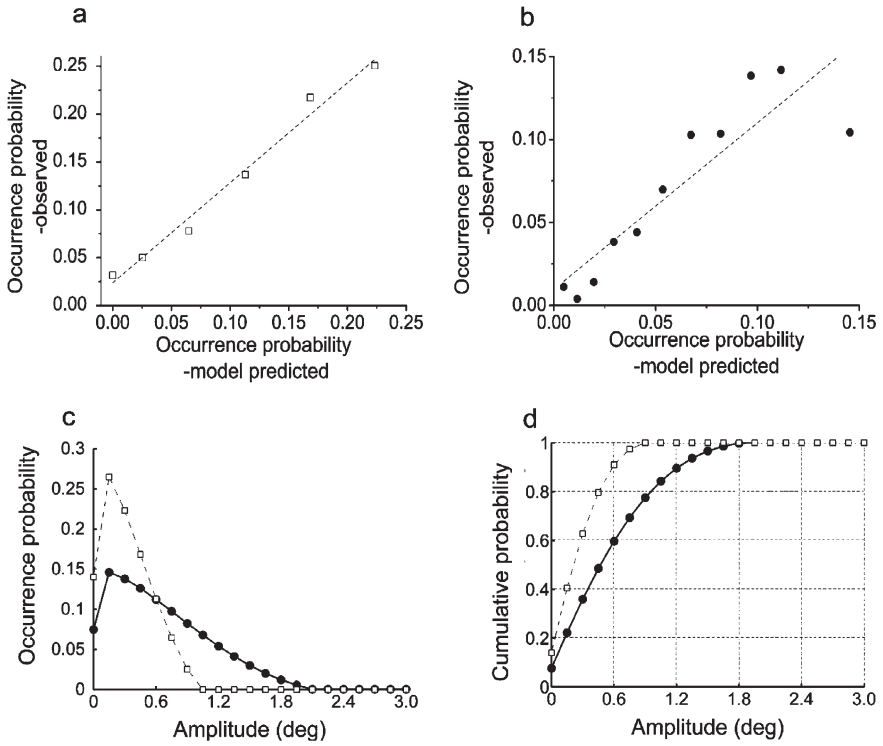


Fig. 8. Prediction of FS generation by a simulation model for normal and amblyopic conditions. a, b: Each data point is the occurrence probability as a function of FS amplitude. The observed data were obtained from 6 subjects in panel (a) and 8 subjects in panel (b) in whom the number of detected events was >500 so as to calculate the FS occurrence probability for each of the different amplitude ranges (bin size = 0.15°). The results obtained from the model-prediction correlate very well with the actual (observed) data both for the normal (panel a, $r = 0.992$, $P < 0.001$) and amblyopic condition (panel b, $r = 0.888$, $P < 0.001$). c, d: This shows the predicted occurrence probabilities (c) and cumulative probabilities (d) as a function of FS amplitude. White rectangles and dashed lines are the results of normal condition. Black circles and solid lines are the results of amblyopic condition.

et al., 2009). Populin (2005) suggested that the sensory processing in SC is governed by excitation-inhibition balance. Munoz and Wurtz (1992) found that injecting muscimol, an agonist of an inhibitory neurotransmitter, gamma-aminobutyric acid, into the rostral SC (corresponding to the center of retinotopic visual field) decreased the suppression of the initiation of saccades. Therefore, any impairment of the SC activity map by endogenous or exogenous inputs may affect the behavioral characteristics of FSs (Otero-Millan et al., 2008). Thus, if early visual experience influences the development and functioning of the circuitry and inputs to the SC (Hoffman and Sherman, 1974; Royal et al., 2010; Wang et al., 2010; Carrasco et al., 2011) so as to affect the excitation-inhibition balance in SC, then one would expect that the behavioral property of FSs in amblyopic subjects should be altered as well. It has been observed that monocular form deprivation in kittens during the critical period caused an impairment or even loss of the corticotectal pathway (Hoffman and Sherman, 1974) through which the cortex exerts a powerful suppression of the retinocollicular input via GABAergic interneurons within SC (Berman and Sterling, 1976; Shibata et al., 1990; Hirai and Okada, 1993). In addition, early visually driven activity is also necessary to maintain the inhibitory circuitry intrinsic to the SC (Carrasco et al., 2011). These studies provided the rational for our development of a computational model to simulate the inhibitory activity changes in anisometropic amblyopia. With this we wished to be able to predict how FS production is affected by an excitation-inhibition imbalance. Because the results of our modeling correlated well with our clinical observations, we propose that the global decrease of inhibition in SC caused by visual deficits may lead to an impairment of competitive priority of central SC activity versus peripheral

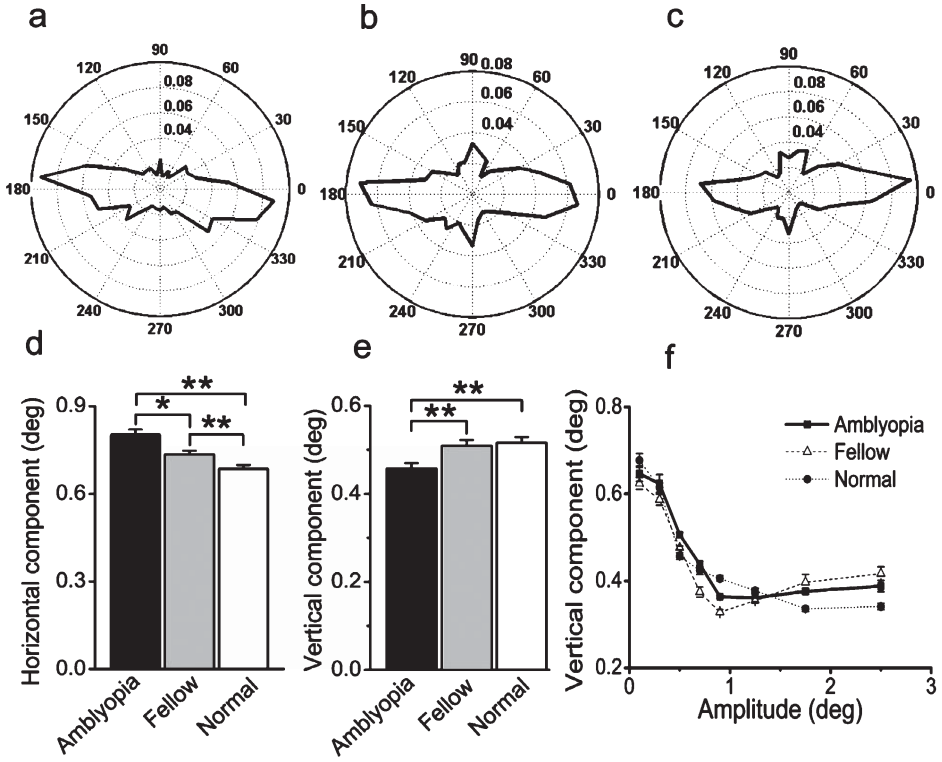


Fig. 9. Analysis of FS directions for the three groups. a–c: Polar histograms of occurrence rate of different FS directions in representative eyes of the three groups. a: an amblyopic eye; b: a fellow eye; c: a normal eye. There was a significant amount of vertical FSs in the fellow eye and normal eye. d–f: Analysis of horizontal (d) or vertical (e) components of FSs among the three groups. The magnitudes of all the saccades were normalized to 1deg to calculate the contributions of horizontal and vertical components of FSs of different sizes. Error bars represent SEM. d: ANOVA test, $F = 33.63$, $P < 0.001$; e: ANOVA test, $F = 86.06$, $P < 0.001$. $^{*}P < 0.05$, $^{**}P < 0.001$. f: Relationship between FS amplitude and vertical component.

SC activity, thus influencing the spatial distribution of FSs. This is compatible with the observations by other groups as follows:

Firstly, FSs in progressive supranuclear palsy patients showed more frequent, larger, and more pronounced horizontal saccades during sustained fixation (Otero-Millan et al., 2011a). One of the suggested reasons for larger saccades during fixation was an impaired inhibition of the SC by the substantia nigra pars reticulata (Otero-Millan et al., 2011a).

Secondly, the secondary saccades observed during the initial fixation period after primary saccades triggered by random stimulus onsets are distant-dependently larger than those observed in normal fixation conditions (Ohl et al., 2011). These authors claimed that a novel visual stimulus enhanced the activity in that SC hemisphere and the enhancement was stronger for distant than for close targets (Ohl et al., 2011). Thirdly, a transient irrelevant visual stimulus results in a fluctuation of FS amplitude or occurrence rate during the initial period after the stimulus onset (Otero-Millan et al., 2008), which may very well be mediated by the excitation/inhibition interaction in SC.

It should be pointed out that less global inhibition may change the distribution of local activity on the retinotopic SC map, but it may not change the activity center position on the SC map. Niechwiej-Szwedo et al., (2010) found that the mean amplitude and peak velocity of primary saccades were comparable between patients with anisometropic amblyopia and control subjects. In our study, in contrast, the mean FS amplitude and peak velocity in amblyopic eyes were larger than in fellow eyes and normal eyes. A possible explanation of this discrepancy is that the reduced global inhibition in SC does not interrupt the relocating process of the activity center dominated by an attentional shift, but it may change the

activity distribution at the new attention site. Hence the patients in the Niechwiej-Szwedo et al., study exhibited greater variability of saccade amplitude. As for FSs, the attention-induced activity in SC during fixation should always be located at the center of the retinotopic map. In this condition, the increased probability of larger FSs can be regarded as indicating a greater variability of zero-amplitude saccades. Therefore, we propose that the changes in involuntary FS and voluntary saccades in anisometropic amblyopia share a common pathological mechanism.

Thirdly, an increased number of larger FSs must have a significant impact on the visual perception and reorganization of neural circuitry in anisometropic amblyopia. It has been proposed that the visual system uses FSs as a preferred sampling strategy (Martinez-Conde et al., 2004; 2009; Troncoso et al., 2008b; Otero-Millan et al., 2008; Rolfs, 2009) which was supported by physiological studies (Martinez-Conde et al., 2000; 2002; Herrington et al., 2009). They improve the efficient sampling of fine spatial detail (Donner and Hemila, 2007) and elicit stronger responses in V1 neurons (Martinez-Conde et al., 2000). As for anisometropic amblyopia, Kiorpes et al., (1998) showed that the contrast sensitivity measured by the response behavior of V1 neurons decreased in all bands. Thus, larger FSs may be a compensatory/adaptive mechanism for the reduced sampling capability. One interpretation of this is that this may function to capture more information from a broader spatial domain so as to compensate the information loss due to impairment of neural connectivity. This, in turn, might help to enhance the impaired sensitivity of V1 neurons to low spatial frequency stimuli, but it may also aggravate the reduction of contrast sensitivity to high spatial frequency (Rucci et al., 2007). Hence, the deficits of contrast sensitivity in anisometropic amblyopia are not uniform in all bands of spatial frequency, but more severe in high spatial frequency (Bradley and Freeman, 1981; Smith III et al., 1984; Kiorpes et al., 1998). On the other hand, it has been observed that with increasing spatial frequencies (Polat et al., 2005) or increasing contrast (Polat et al., 1998) suppressive lateral interaction increases. Therefore, lateral interactions may mediate the reduction of contrast sensitivity to high spatial frequency in anisometropic amblyopia, since larger FSs should facilitate visual inputs from flanking stimuli.

Another effect of larger FS may be promoting remodeling of neural circuitry so as to adapt to the altered visual experience. Some studies have reported that the periodic eye movements of FSs modulate gamma-band synchronization from retina to visual cortex (Greschner et al., 2002; Yuval-Greenberg et al., 2008; Bosman et al., 2009; Melloni et al., 2009). The synchronization of neural activity plays an important role in signal transmission, visual awareness, synaptic plasticity and even in the vision restoration after visual system damage (Singer, 1993; Sabel et al., 2011). Therefore, a larger scale synchronization by larger FSs could promote the neural connectivity in a wider span and consolidate the ability of information capture to low spatial frequencies.

However, such a "compensation hypothesis" requires experimental testing in future studies. In particular, it would be of interest to determine if a compensation attempt by the oculo-motor system actually helps the patient's low-level visual perception capability in a long-lasting manner or, alternatively, if it prevents restorative plasticity of residual functions in the amblyopic eye through aggravating the state of visual deprivation to high spatial frequency. In this case, activation of residual functions (Greschner et al., 2002) might be impaired which, through behavioral training (Polat et al., 2004; 2008; 2009; Sabel et al., 2011), has a potential to be activated. On the other hand, the reorganization of neural circuitry in visual cortex by this adaptive mechanism through larger scale synchronization may successively reestablish the balance of excitation and inhibition, and furthermore gradually focus the spatial distribution of FSs. However, this natural rehabilitation process may come at a cost of a longer period for recovery.

The second possibility is that larger FSs are, maladaptive and that they may, in fact, be the cause rather than the consequence of amblyopia. According to Ewald Hering, both of our eyes move in a coordinated fashion. But another, opposite view proposed by Hermann von Helmholtz is that the movements of the eyes are individually controlled. However, over the past century, Hering's view was broadly accepted. Therefore, Krauskopf et al., (1960) emphasized that FSs occur synchronously in both eyes. Nevertheless, the view of the binocularity of FS faced a vigorous challenge over the past decade. Firstly, King and Zhou found that premotor neurons predicted by Hering's law to encode binocular neural commands to drive conjugate eye movements actually encode monocular commands (King and Zhou, 2000). They proposed that the network for binocular coordination must be trained

and calibrated during infancy and probably throughout life in order to maintain the precise binocular coordination characteristic of eye movements. Later, Engbert (2006) found that FSs can be binocular and monocular. Kloke et al., (2009) suggested that for the cases where a FS is detected in only one eye, the saccade in the other eye may happen to be too small to be detected due to binocular disconjugacy. Recently, Van Horn and Cullen (2012) verified that saccadic neurons encode the movement of an individual eye during FSs, which is contrary to Hering's law. Their findings implied abnormal frequency and size of FSs are characteristic of certain neurological diseases (Otero-Millan et al., 2011a; Van Horn and Cullen, 2012). Our study showed that in the amblyopic condition, the failure of binocular coordination may occur with characteristic manifestations of lower frequency and larger amplitudes of FSs in the amblyopic eye. We consider that the neural circuitry that controls binocular coordination may be maldeveloped, possibly due to abnormal visual experience in the early period of visual development. Thus, the misalignment of both eyes, although miniature, may cause one eye to be unfavorably neglected by the brain and through "non-used" which would induce or aggravate the development of amblyopia. One can easily imagine that if the eye coordination in a child is not aligned or in synchrony during fixation very early in life, the binocular disconjugacy (Kapoula et al., 1995; Maxwell et al., 1995; Bucci et al., 1999) of saccades may lead to unmatched images and then actually be the reason why the brain chooses a simple way out of this problem: it gates information of only one eye to reach conscious processing and interpret the visual world in such a way that continuous visual rivalry of the double images is avoided. This, in turn, would then lead to "non-use" of the "unfavored" eye with subsequent dominance of the fellow eye. The latter might cause further even more severe binocular disconjugacy of the eye movement control, and even strabismus (King and Zhou, 2000; Barrett et al., 2004), a condition in which the eyes are not properly aligned with each other. In any case, the possible binocular disconjugacy of eye movements in amblyopia will shed light on the cause of amblyopia and how the visual sensory system and the eye movement system interact with each other in early development.

Lastly, the present study implies that neural impairments in amblyopia not only include those in primary visual cortex (Barrett et al., 2004) and its downstream higher visual cortex (Kiorpes and McKeet, 1999; Levi, 2006), but that it also involves subcortical regions. As our simulation modeling analysis shows, the source of excitation-inhibition imbalance comes mainly from impaired cortico-tectal inputs but not from retino-tectal projections, which were found to be normal in monocularly deprived cats (Hoffmann and Sherman, 1974). The functional impairment in SC downstream from visual cortex has also been demonstrated by a recent physiological study in mice that showed alterations in spatial frequency tuning of SC neurons when cortical input was absent (Populin, 2005). Therefore, the FS impairment is similar to the deficits of other high-order visual functions in amblyopia that are located downstream from V1.

Understanding the functional impairment beyond V1 and the mutual connectivity of these areas recently promoted new approaches for visual restoration and therapy strategies in amblyopia and other visual deficits (Kasten and Sabel, 1995; Polat et al., 2008; Levi and Li, 2009a; 2009b; Sabel et al., 2011). Because the SC receives afferents from frontal eye fields (FEF) which play an important role in the control of visual attention, we hypothesize that the focused visual attention during visual training (Polat et al., 2004; 2008; 2009) may improve the FS performance through temporary modulation of excitation-inhibition balance in SC by the inputs from FEF to further strengthen the synchronization of a spatial domain representing higher spatial frequency. As a consequence, the improvement of sensory processing in visual cortex by visual training may help restore the intrinsic excitation-inhibition balance in SC and render the improvement of eye movements to be long-lasting. Indeed, vision restoration training in hemianopia has shown not only to improve visual fields but it also led to improved fixational performance in a task that required continuous fixation (Sabel et al., 2011; Kasten et al., 2006).

In summary, anisometropic amblyopes present in their amblyopic eyes increased overall mean FS amplitude, mean peak velocity and ISI, and decreased FS rate. FS rate suppression occurred mainly in the 0- to 0.6-degree amplitude range, whereas the occurrence probability of larger FSs increased. These changes in anisometropic amblyopia could be successfully predicted by a computational model based on the excitation-inhibition balance in SC. While it remains to be determined if altered saccadic behavior is adaptive or maladaptive, or even the cause of amblyopia, our current data provide the baseline information for future studies. Here, two issues are of particular interest:

firstly, are fixational saccade alterations the cause rather than the effects of amblyopia and secondly, can the effect of amblyopia treatment and visual training positively influence FS? To solve these questions will be a major step forward in our understanding of visual system and eye-movement impairments which then provides a new starting point for innovative therapeutic approaches.

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