What is the best fixation target? The effect of target shape on stability of fixational eye movements

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A B S T R A C T

People can direct their gaze at a visual target for extended periods of time. Yet, even during fixation the eyes make small, involuntary movements (e.g. tremor, drift, and microsaccades). This can be a problem during experiments that require stable fixation. The shape of a fixation target can be easily manipulated in the context of many experimental paradigms. Thus, from a purely methodological point of view, it would be good to know if there was a particular shape of a fixation target that minimizes involuntary eye movements during fixation, because this shape could then be used in experiments that require stable fixation. Based on this methodological motivation, the current experiments tested if the shape of a fixation target can be used to reduce eye movements during fixation. In two separate experiments subjects directed their gaze at a fixation target for 17 s on each trial. The shape of the fixation target varied from trial to trial and was drawn from a set of seven shapes, the use of which has been frequently reported in the literature. To determine stability of fixation we computed spatial dispersion and microsaccade rate. We found that only a target shape which looks like a combination of bulls eye and cross hair resulted in combined low dispersion and microsaccade rate. We recommend the combination of bulls eye and cross hair as fixation target shape for experiments that require stable fixation.

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1. Introduction

When people fixate a visual target, they intend to keep their gaze still. Nonetheless, the eyes make small, involuntary movements (e.g. tremor, drift, microsaccades) (for reviews see for example Martinez-Conde, Macknik, & Hubel, 2004; Martinez-Conde et al., 2009; Rolfs, 2009). This can be a problem during experiments that require participants to keep their gaze stable for extended periods of time. For example, eye movements during fixation shift the location of a stimulus on the retina, which introduces noise into retinal receptive field measurements acquired with neurophysiological recording techniques, multifocal Electroretinograms (Sutter & Tran, 1992; Zhang et al., 2008), or high-resolution fMRI (e.g. Schira et al., 2009). In addition, the planning and execution of eye movements during fixation results in neural and muscular activity, as well as physical motion of the eye ball, all of which affects measurements that are based on electric and/or magnetic field strength, such as EEG, MEG and fMRI (Dimigen et al., 2009; Tse, Baumgartner, & Greenlee, 2010; Zhang et al., 2008). Thus, from

a methodological point of view it would be good to minimize involuntary eye movements during fixation for experiments that require stable fixation.

Previous research has shown that fixational eye movements are affected by variables, such as attention to the process of ocular fixation itself (Steinman et al., 1967), selective attention to aspects of the visual display (e.g. Engbert & Kliegl, 2003a; Hafed & Clark, 2002), precision requirements of the response (Ko, Poletti, & Rucci, 2010), presence of visual 'distracters' (Otero-Millan et al., 2008), changes in the visual display (Engbert & Kliegl, 2003a; Sinn & Engbert, 2011), or the experimental viewing condition (i.e. free viewing vs. fixation) (Ko, Poletti, & Rucci, 2010; Otero-Millan et al., 2008). None of these variables are easily manipulated within the context of an experimental paradigm. Properties of the fixation target that would perhaps be easier to manipulate, such as blur, color, luminance and/or luminance contrast, have no effect on fixational eye movements unless they render the target barely visible, in which case fixation is bad (Boyce, 1967; Steinman, 1965; Ukwade & Bedell, 1993). Finally, it has been shown that changes in the size of a fixation target result in changes in both dispersion (drift) and microsaccade rate (Steinman, 1965). However, even though it is the case that target size has an effect on fixational eye movements, it is not the case that a specific target size would

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generally reduce both drift and microsaccades during fixation. The shape of a fixation target can be easily manipulated in the context of many experimental paradigms. Therefore, the shape of a fixation target might be a good variable to manipulate in order to reduce involuntary eye movements during fixation.

To survey which fixation target shapes researchers typically use in their research, i.e. if there is already some sort of 'gold-standard' in place, we surveyed the shape of fixation targets that were used in experiments published in Journal of Vision. We included articles from regular and special issues of Journal of Vision published between 2001 (volume 1, issue 1) and 2009 (volume 9, issue 1), we excluded issues consisting of conference abstracts. For the purpose of the survey, the article text (including footnotes, captions, figures and tables) was searched for the letter combination 'fixa'. If the search produced a hit, the article was manually searched to determine if the research had used a fixation target. If so, the article was manually searched for the most detailed verbal description of the fixation target's shape. Experiments that involved fixation, but did not mention or describe a visual target, were not included in the survey. If an article contained multiple experiments and used different fixation target shapes for each experiment, each description was counted separately. This resulted in a sample of 500 fixation target shapes. The results of the survey are shown in Table 1. Two things are evident. First, we found a large number of descriptions such as 'target', 'spot', 'point' or 'mark', without any reference to a specific shape and/or size. Second, those descriptions that are more specific indicate that a wide variety of fixation target shapes and sizes are in use, even though there appears to be a preference towards circular target shapes or crosses.

In summary, the shape of a fixation target can be easily manipulated in the context of many experimental paradigms, and there is currently no 'gold-standard' for a specific target shape in the literature. At the same time, stable fixation is required for many behavioral and neuroimaging experiments (e.g. Electroretinograms, EEG, MEG, fMRI). Thus, from a purely methodological point of view, it would be good to know if there was a particular shape of a fixation target that minimizes involuntary eye movements during fixation, because that target shape could then be used in experiments that require stable fixation. Based on this methodological motivation, the current experiments tested if the shape of a fixation target can be used to reduce involuntary eye movements during fixation.

2. Methods and materials

2.1. Experiment 1

2.1.1. Ethics statement

Two subjects performed the experiment at the University of Western Ontario, Canada, and 10 subjects performed the experiment at Giessen University, Germany. All testing procedures were approved by the ethics board at the University of Western Ontario, and by the ethics board at Giessen University, respectively. Participants gave written informed consent prior to testing. Subjects (except the first author) were paid 10 CAD, or 8 Euro, respectively, for participation.

2.1.2. Subjects

Twelve subjects (incl. the first author) participated in the experiment. Subjects had normal or corrected to normal vision.

2.1.3. Apparatus and eye-movement recording

At the University of Western Ontario visual stimuli were presented on a 19 in. LCD monitor (Dell Ultrasharp) with an ATI Radeon HD 2400XT graphics card at a temporal resolution of 75 Hz and a spatial resolution of 1280(H) × 1024(V) pixel. The active display area subtended 37.5(H) × 30(V) cm, and the display was positioned at a distance of 46 cm from the observer. At Giessen University, visual stimuli were presented on a 21in CRT monitor (ELO Touchscreen) with an Nvidia Quadro NVS 285 graphics card at a temporal resolution of 100 Hz and a spatial resolution of 1280(H) × 1024(V) pixel. The active display area subtended 37(H) × 29.6(V) cm, and the display was positioned at a distance of 47 cm from the observer. Eye position signals were recorded by a separate PC with a head-mounted, video-based eye tracker (EyeLink II; SR Research Ltd., Osgoode, Ontario, Canada) and were sampled at 250 Hz. At the University of Western Ontario we used 'pupil with corneal reflex' mode to record eye position signals for both subjects. At Giessen University, we used 'pupil with corneal reflex' mode for one subject, 'pupil only' mode for five subjects, and for the remaining four subjects we used 'pupil only' mode in one session, and 'pupil with corneal reflex' mode in the other. The system was calibrated at the beginning of each experimental session by instructing the observer to fixate single dots that appeared successively at nine different positions on the monitor. Based on the results of this calibration, the better eye was chosen automatically by the system, and eye position was recorded from this eye. Observers were seated with their heads stabilized with a chin rest. They viewed the display binocularly through natural pupils. Experimental software was written using the Eyelink SDK, Windows API, OpenGL and C/C++ programming language.

2.1.4. Stimuli

Our survey of fixation target shapes showed that experiments that require stable fixation commonly use circle and cross shapes, as well as their combinations, as fixation target shapes (Table 1). Thus, we decided to use circles and crosses and their combinations as target shapes in our experiment. Fig. 1 illustrates the seven different targets shapes that were used. In addition, we included a small and a large circle shape (target shape A and B) as control conditions. Previous research has shown that shape A elicits less dispersion, but a higher number of microsaccades as compared to shape B (Steinman, 1965). Thus, if our experimental setup is sensitive enough to measure variations in eye movements during fixation, we would expect to see a negative relationship between these two dependent measures for target shape A and B. All stimuli were shown in front of a homogeneously gray background. Stimuli were shown both black-on-gray (illustrated in Fig. 1, top panel), as well as white-on-gray. Code for drawing the ABC target using Matlab (The Mathworks, Natick, MA, USA) and Psychtoolbox (Brainard, 1997) is given in Appendix A.

2.1.5. Task and procedure

Subjects were instructed to keep their gaze directed at the center of the fixation target and as stable as possible throughout a trial (trial duration 17 s). Before the onset of a trial the subjects saw the target shape colored in red. Once the subject was ready, they pressed a button with their right index finger to start a trial. Once they pressed the button, the target shape changed from red to black. After 10 s, the target changed from black to white. Then, after another 7 s the target disappeared. The screen remained gray for 3 s, before the next target would appear. The combination of luminance change and 3-s 'blank' minimized the presence of afterimages. Two subjects each performed four separate sessions on four separate days. In each session, each of the seven target shapes was shown nine times, so that each session contained 63 trials total. The other 10 subjects each performed two separate sessions on two separate days. In each session, each of the seven target shapes was shown twelve times, so that each session contained 84 trials total. For all subjects and sessions presentation of target shapes within each session was block-randomized in order to balance presentation order over the course of the experiment. Eye movement

Table 1
Results of survey of fixation target shapes (*n* = 500) published in Journal of Vision between 2001 (volume 1, issue 1) and 2009 (volume 9, issue 1). The left and right columns list descriptions of target shapes and sizes, respectively. Numbers in parentheses in the right column represent the number of times that a particular shape/size combination occurred. Unless otherwise stated, units are in degrees visual angle. It is evident that a wide variety of fixation target shapes and sizes are in use, even though there appears to be a preference towards circular target shapes or crosses.

Shape description	Size (number of reports)
'+'	0.3 (2), 0.5 (1), 0.6 (1), 1 (2), size 30 courier new font (1), unspecified (3)
'+' within 2.36 bounding square	Unspecified (1)
'='	Unspecified (1)
'=' and '+' superimposed	Unspecified (1)
6/12 Snellen 'E'	Unspecified (1)
6/12 Snellen 'E' inside elliptical field	0.7 × 1 (1)
Annulus	Unspecified (2)
Arrow	0.7 × 0.7 (1)
Asterisk	Unspecified (1)
Bar	Unspecified (1)
Box	0.46 (1), unspecified (1)
Bullseye	0.2 (1), 0.35 (1), 0.5 (1), 0.6 (1), 0.8 (1), unspecified (1)
Circle	0.05 (1), 0.1 (1), 0.12 (1), 0.57 (1), 1.0 (1), 1.5 (1), unspecified (3)
Circle of LEDs	1.2 (1)
Circular dot	11′ (1)
Circular point	11′ (1), 0.46 (1), 0.5 (1)
Open circle	0.26 (1)
Cross	0.1 (2), 6.3′ (4), 0.15 (1), 10′ (1), 0.17 (1), 0.2 (3), 0.25 (1), 15.5′ (1), 0.35 (1), 0.4 (2), 0.5 (5), 0.54 (1), 0.57 (1), 0.63 (1), 0.7 (1), 0.75 (1), 0.8 (2), 0.84 (1), 1.0 (5), 1.5 × 0.5 (1), 2.0 × 0.6 (1), 23 × 16.7 (1), unspecified (101)
Cross hair	0.14 (1), 0.3 (1), 0.47 (1), 0.7 (1), unspecified (1)
Cross surrounded by square	4.0 (1)
Cross with central gap	Cross 3.3 gap 1.1 (1)
Cross with dot at center	Unspecified (1)
Cross with nonius lines	Unspecified (2)
Crosses, dumbbells, etc.	About 2.0 × 6.0 (1)
Cue	Unspecified (1)
Disk	0.15 (1), 0.2(1), 0.3 (1), 0.4 (2), 61.6′ (1), Unspecified (1)
Disk with central dot	Disk 0.6 dot 0.15 (1)
Disk with cross	Disk 0.4 cross 0.3 (1)
Small disk superimposed on big disk	Small 0.2 big 1.0 (1)
Dot	1.6′ (1), 4.1′ (1), 0.1 (2), 0.2 (3), 0.28 (1), 0.3 (1), 0.4 (2), 0.5 (2), 0.76 (1), 0.8 (1), 1.0 (1), unspecified (43)
Gaussian Blob	SD = 0.04 (1)
'L' or 'T'	1.0 (1)
Laser target	0.2 (1)
Laser spot	0.2 (1), unspecified (1)
Laser dot	Unspecified (1)
LED	0.26 × 0.53 (1), unspecified (10)
L-shaped marks at four corners of image	Image size 2.133 (1)
Maltese cross	1.0 (1)
Maltese Star	Unspecified (2)
Mark	1.7 (1), unspecified (12)
Mark and two nonius lines	Unspecified (1)
Marker	0.08 (1), 0.2 (1), unspecified (10)
Marks	Unspecified (1)
Nonius target	Unspecified (1)
Numbers from 2 to 9	0.22 × 0.57 (1)
Patch	Unspecified (1)
Pattern	26′ (1), unspecified (1)
Point	0.1 (3), 0.114 (1), 8.3′ (1), 0.2 (3), 0.3 (1), 22.5′ (1), 0.5 (3), 0.8 (1), 2 pixel (1), 4 pixel (3), unspecified (75)
Pinhole point	Unspecified (1)
Point over circular mask	Mask 1.0 (1)
Point overlaid on circular region	Point 0.32 circular region 1.8 (1)
Point source	Unspecified (1)
Point with nonius lines	Unspecified (3)
Raster	1.0 × 1.0 (1)
Rectangle	1.0 × 0.5 (1)
Ring	0.4 (1), 3.1 (1), unspecified (2)
Ring with inset	Ring 0.8 inset 0.1 (1)
Spot	0.15 (1), 0.2 (1), 0.4 (1), 2 pixel (1), 4 pixel (1), unspecified (20)
Spot and letter	Unspecified (1)
Spot surrounded by larger disk	Spot 0.24 disk 0.97 (1)
Square	0.05 (3), 3.75′ (1), 4.2′ (1), 4.36′ (1), 6′ (3), 0.15 (2), 10′ (1), 0.2 (2), 0.25 (1), 0.35 (2), 2.0 (1), unspecified (9)
Square with center	Square 0.87 center 0.37 (1)
Stimulus	Unspecified (1)
Sunburst figure	Unspecified (1)
Target	2.0 (1), 3.0 (1), 4.0 (1), unspecified (2)
Target with small hole	Unspecified (1)
Two concentric circles	Unspecified (2)
Two concentric circles and nonius lines	Unspecified (3)
Two sets of four 12′ dots placed on perimeter of inner and outer circles	Inner circle: 4 outer circle 12.2 (1)

Table 1 (continued)

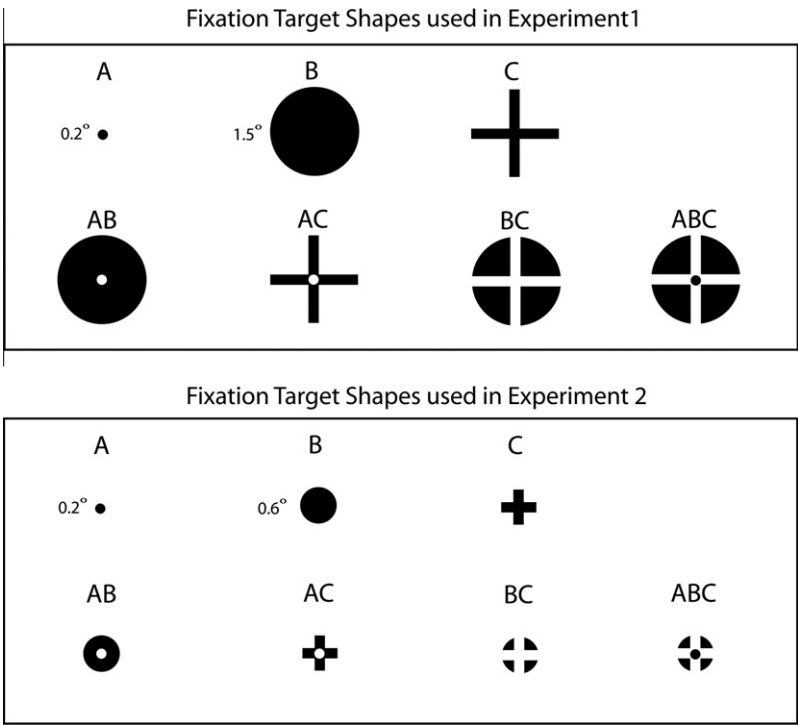| Shape description	Size (number of reports)
Two vertically aligned squares with gap in between; observer was instructed to fixate gap	Unspecified (1)
Up or down arrow	Unspecified (1)
Vertical bar	Unspecified (1)
Vertical line	0.13 × 1.0 (1)
'x'	0.6 (2), unspecified (2)

Fig. 1. Illustrations of the fixation target shapes used in Experiments 1 and 2.

data were saved to disk for off-line analysis. Before the experiment proper, subjects performed one practice trial for each target shape. The experiment was self paced and one session took approximately 45 min to complete.

2.1.6. Analysis of eye movement data

We characterized performance by computing microsaccade rate (saccades per second) and dispersion of gaze position in the plane (degrees). Data samples during which the subjects had blinked, as well as samples 200 ms before and 500 ms after a blinks were excluded from analysis. This way we were able to retain a high number of data samples, while avoiding contamination of samples through eye movements that occur before and after blinks (e.g. Collewijn, van der Steen, & Steinman, 1985; Riggs et al., 1987). Microsaccades were detected using the velocity-based algorithm by Engbert and Kliegl (2003a). The algorithm performs an initial smoothing by computing a moving average of velocities over five data samples. The velocity criterion parameter for the detection of microsaccades was $\lambda = 6$, and the duration criterion was set to three data samples (12 ms) to further reduce noise.

In addition, to further reduce noise we used a 'linearity' criterion, which exploits the fact that the trajectory of microsaccadic eye movements measured in the plane is typically straight (e.g. Engbert, 2006). Specifically, for each group of samples that was labeled a microsaccade according to the initial velocity based analysis (Engbert & Kliegl, 2003a), we computed both the sample path length (movement path summed across all samples), as well as the sample amplitude (length of the straight line connecting the first and last sample). We then computed the ratio of path length to amplitude, and considered only those groups of samples for which the ratio exceeded 0.5. To compute microsaccade rate in saccades per second for each trial, we then divided the number of microsaccades for each trial by the duration of that trial (i.e. duration of samples with blinks removed). To inspect quality of microsaccade data, we also calculated microsaccade amplitudes and directions. To compute dispersion of eye movements in the plane (2DSD) for each trial we generally followed the analysis of Steinman (1965). Specifically, we fitted a minimum variance ellipse to samples and computed the area of this ellipse in degrees visual angle. Free parameters for the ellipse were radius in x and y, center and orientation. Steinmann and collaborators additionally scaled the area of the minimum variance ellipse to compute the area of a 68% confidence ellipse. We decided to keep with the area of the minimum variance ellipse itself as we felt that this descriptive statistic was suitable to characterize performance of subjects for the current experiments. To confirm the suitability of our dispersion analysis for our data, e.g. see Castet and Crossland (2012), for a recent investigation of dispersion analysis methods, we also calculated the spatial distribution of dispersion data. Averages and standard deviations (SD) of microsaccade rate and dispersion were then computed across all trials for a particular target shape. There is evidence to suggest that there are both conjugate and

non-conjugate microsaccades (e.g. Engbert, 2006; Engbert & Kliegl, 2003b). In the context of our experiment we recorded eye movements only monocularly so that we did not dissociate between conjugate and non-conjugate microsaccades.

2.1.7. Statistical data analysis

To analyze if target shape had an effect on raw dispersion and raw microsaccade rate measured separately, we applied repeated measures ANOVA with factor 'target shape' to these data. In addition, we performed an analysis of normalized data to determine if target shape had an effect on fixation stability taking into account both microsaccade and dispersion data combined. For this analysis we first normalized data for each participant using a linear remapping of the data across its range. This way, for each participant, the minimum value across all seven targets was assigned a value of zero, and the maximum a value of one, and for example a value halfway between the minimum and the maximum was assigned a value of 0.5. Normalized values were computed separately for microsaccade and dispersion data. To determine if a particular target shape was successful at reducing fixational eye movements, we then compared normalized microsaccade and dispersion values to a pre-defined success criterion. For example, using a success criterion of 0.5 we would consider those target shapes as 'successful' that reduce both microsaccade as well as dispersion to at least 50% of the range present in the data. We computed the number of 'successes' for each target shape across participants, and used a binomial test to statistically evaluate the number of successes for each target shape. The binomial test compares the number of successes observed in a sample to an expected number of successes. For small samples it is preferable to a Chi-Square test of proportions (e.g. Bortz, 1999, pp. 154–155). For our analysis we defined the expected number of successes to be 50% or less. Thus, in the case of a significant test result ($p < .05$) we would reject the Null hypothesis that the number of successes is equal to or less than 50%, and conclude that the number of successes is higher than 50%. Tests were computed for each target shape separately.

The numerical value of the success criterion will affect the number of successes. For example, if the success criterion is zero, only data points that have the minimum dispersion and microsaccade values will be considered successful, which would make success very unlikely. Conversely, if the criterion is one, all data points will be considered successful, which would make success certain. Thus, the success criterion will affect the probability of retaining the Null hypothesis (p-value) in the context of our analysis. Specifically, we would expect that the probability of retaining the Null hypothesis decreases as the success criterion increases from zero to one, so that p-values might be biased towards falsely rejecting the Null hypothesis.

To investigate to what degree p-values in our analysis might be biased towards falsely rejecting the Null hypothesis, we performed numerical simulations. The simulations assumed that there is no effect of target shape on fixation stability. Under this assumption, we then estimated the probability of falsely rejecting the Null hypothesis (p-value) for success criteria ranging from zero to one in 0.1 steps for each of the seven target shapes. Subsequently, we computed p-values for our participants' data in the same way as for the simulated data. This way we were able to determine (A) if p-values for our participant's data are statistically significant (i.e. $p < .05$), and (B) if p-values for our participants data are expected from bias.

2.2. Experiment 2

Even though the sizes of target shape that we use in Exp. 1 are commonly used in research (see Table 1), one may argue that they are rather large. To test, if the findings generalize to smaller targets, we conducted Exp. 2.

2.2.1. Ethics statement

2.2.2. Subjects

Twelve undergraduate volunteers participated in the experiment. Subjects had normal or corrected to normal vision.

2.2.3. Stimuli

The stimuli used in Exp. 2 had the same basic shape as those used in Exp. 1. The only difference was that the largest target size was now 0.6° instead of 1.5° (Fig. 1, bottom panel).

2.2.4. Apparatus and eye-movement recording, task and procedure, analysis of eye movement data, statistical data analyses

Apparatus, eye-movement recording, task and procedure and data analysis were the same as those used for Exp. 1 at Giessen University. We used 'pupil only' mode to record eye position signals for all subjects. Each subject participated in two sessions of 84 trials each.

2.3. Simulation details for Exp. 1 and 2

Simulations were implemented in Matlab7 (R14, The Mathworks), separately for Exp. 1 and 2. Microsaccade and dispersion data for each experiment were simulated by resampling each subject's original data 5040 times (5040 = all possible permutations of data across seven target shapes). Because the simulation assumed that there was no effect of target shape on stability of fixation, microsaccade and dispersion data were resampled independently from one another. Each simulated data set consisted of 12 (subjects) × 7 (target shapes) = 84 numbers. Microsaccade and dispersion data for each sample were normalized for each simulated subject separately using the linear remapping procedure described above. Then, we computed the number of successes across simulated subjects for each of the seven target shapes, for success criteria ranging from zero to one in 0.1 steps. We then computed the p-values for each target shape in each sample using the binomial test described above. Averages and standard deviations of p-values were then computed across the 5040 samples for each of the seven target shapes.

3. Results

3.1. Simulation results

The top left and right panels of Fig. 2 show the simulation results for Exp. 1 and 2, respectively. Curves are color coded for the different target shapes. It can be seen that curves for the seven target shapes overlap, both for Exp. 1 and 2. This indicates that, under the assumption that there is no effect of target shape on fixation stability, p-values for the seven target shapes are indistinguishable from one another in both Exp. 1 and 2. In addition, it is evident that average p-values decrease as the success criterion increases, but that p-values only drop below 0.05 as the success criterion approaches one.
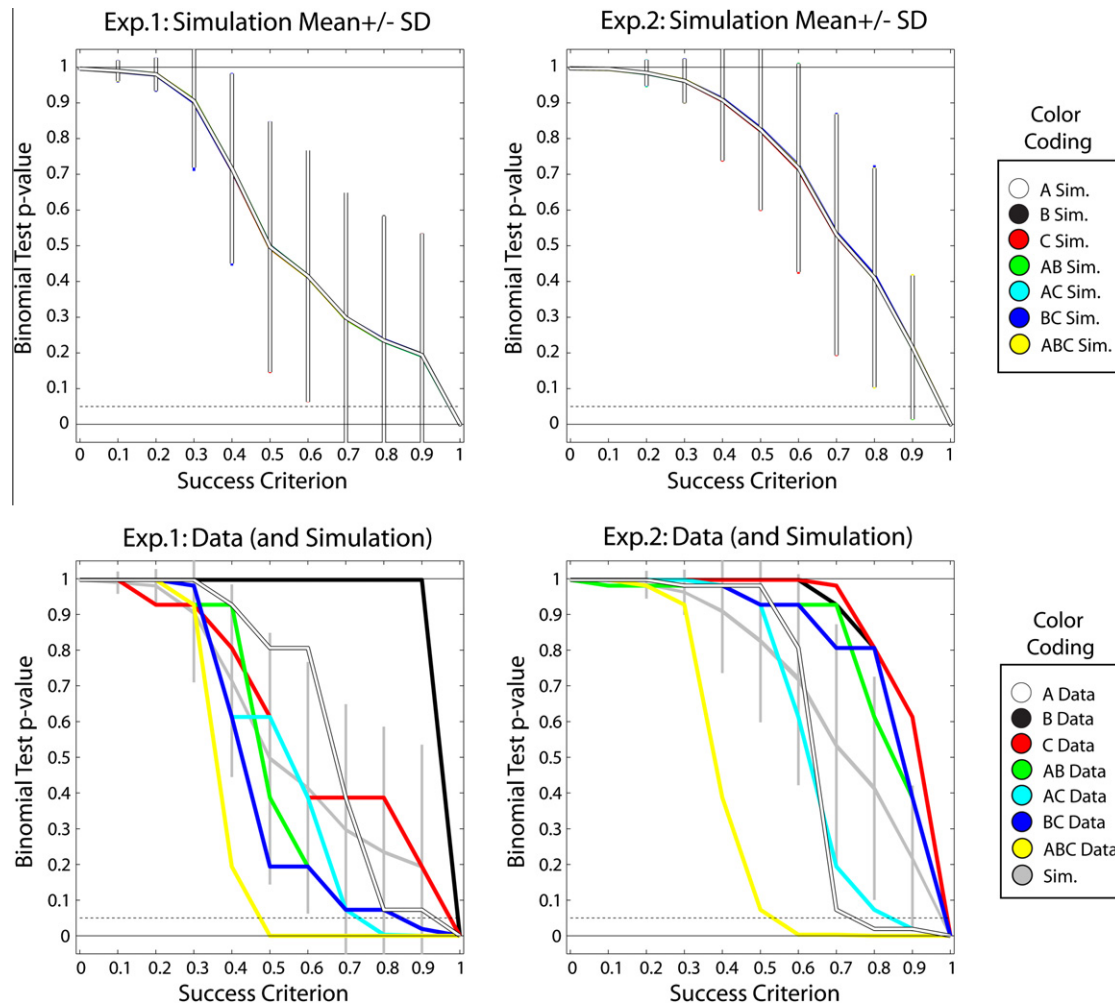
Fig. 2. Results from numerical simulations for Exp. 1 and 2. Top panels: Curves show averages of *p*-values ±standard deviations as a function of success criterion computed across 5040 samples separately for each of the seven target shapes. Curves are color coded for each of the seven target shapes as denoted in the legend, but curves overlap one another. Dashed horizontal lines indicate the threshold for significance (0.05). Bottom panels: Curves show *p*-values as a function of success criterion separately for each of the seven target shapes computed for participants data. Curves are color coded for each of the seven target shapes as denoted in the legend. Also shown (in gray) are the simulation results from the top two panels. Dashed horizontal lines indicate the threshold for significance (0.05). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

3.2. Experiment 1

The data for the two subjects who had performed 252 trials and the 10 subjects who had performed 168 trials were considered together in subsequent analyses. Due to blinks 7% of data were excluded from analysis. The algorithm used to detect microsaccades detects saccades of any amplitude. We found that over the course of the whole experiment (i.e. across all subjects and sessions) only 0.97% of all saccades exceeded 2°. We conclude that subjects had followed the fixation instructions well. We analyzed data both including and excluding saccades that exceeded 2°. The differences were negligible. Since we assume that all saccades during fixation are involuntary, we here report the results from the analyses that included all saccades, but we use the term microsaccades to describe our results.

The left panel of Fig. 3 shows normalized dispersion (2DSD) plotted against normalized microsaccade rate for each subject in Exp. 1. The different target shapes are color coded as indicated in the figure legend. The gray area in each plot denotes the range of values in which both dispersion and microsaccade rate are less than 50% of the range for a given subject. Where necessary, data points were offset from one another to avoid overlap. Note that

for 11 out of 12 subjects both dispersion and microsaccade rate are less than 50% for the ABC target.

The bottom left panel of Fig. 2 shows *p*-values of binomial tests for each of the seven target shapes in Exp. 1 plotted as a function of the success criterion. Curves for different targets are color coded, and for reference the simulation results for Exp. 1 are re-plotted in gray. It is evident that the *p*-value for the ABC target is below 0.05 at a success criterion of 0.5 or more. Furthermore, it is evident that the *p*-value of the ABC target at success criterium 0.5 is not expected from bias, i.e. there is no overlap between the yellow and gray curves and/or error bars. There is no other target for which these two criteria apply. To provide information about non-normalized performance values, the right panel of Fig. 3 shows average raw dispersion and average raw microsaccade rate (bars) and SE (error bars) for all subjects in Exp. 1. Repeated measures ANOVA with 'target shape' as factor reveals a significant effect on 2DSD ($F(6,66) = 8.118$; $p < .001$), but not microsaccade rate. In their entirety the data are consistent with the idea that the ABC target results in most stable fixation from our set of target shapes.

Beyond this main result, The bar graphs in Fig. 3 show that subjects show the expected negative relationship between microsaccade rate and 2DSD for target shape A and B, i.e. 2DSD is lower
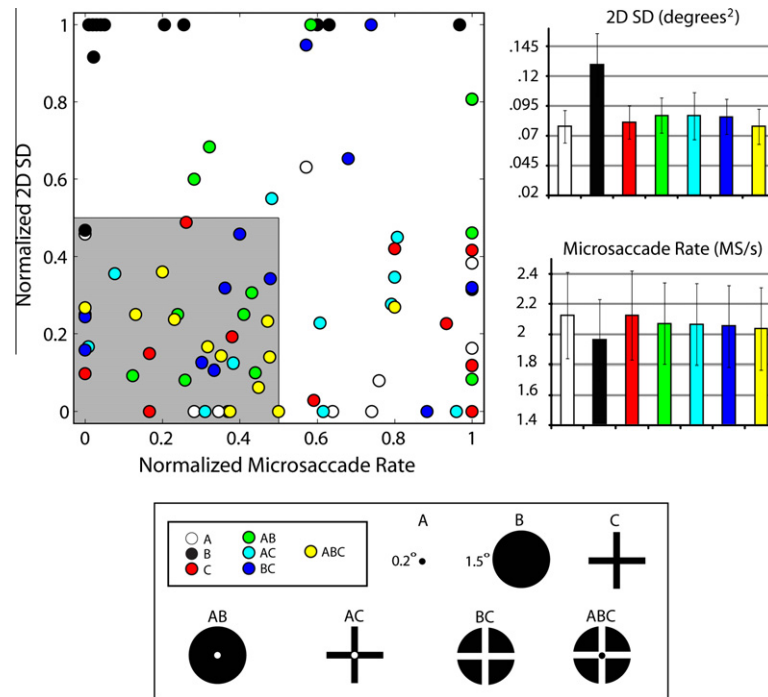
Fig. 3. Results from Exp. 1. Left panel: Normalized dispersion (2DSD) plotted against normalized microsaccade rate for each subject in Exp. 1. Microsaccade rate and 2DSD were normalized to remove individual differences, which makes it easier to plot data from all subjects together in one graph. The gray area denotes the range of values in which both dispersion and saccade rate are less than 50% of the maximum average value for a given subject. Note that for 11 out of 12 subjects dispersion and saccade rate are less than 50% for the ABC target. Right panel: Average dispersion and microsaccade rate (bars) and SE (error bars) for all subjects in Exp. 1.

for target shape A as compared to target shape B, and microsaccade rate is higher for target shape A than target shape B (Steinman, 1965). We used paired *t*-tests to confirm the reliability of this effect. The comparison between target shape A and B was significant for both 2DSD ($t(11) = 3.379$; $p = .006$) and microsaccade rate ($t(11) = 2.292$; $p = .043$). To confirm that the algorithm to detect saccades worked properly, we confirmed that microsaccade amplitude and velocity were linearly related, i.e. microsaccades follow the main sequence (data not shown). We also computed spatial distributions of microsaccades and histograms of microsaccade amplitudes (Fig. 4), as well as distributions of dispersion (Supplementary Fig. S1). As expected, the majority of microsaccades is oriented horizontally and the histogram of microsaccade amplitudes peaks at rather short amplitudes and is skewed to the right (e.g. Engbert, 2006), and dispersion distributions are elliptical in shape (Steinman, 1965). The overall impression gained from these analyses is that the data we measure appear to capture systematic movements of the eye rather than measurement noise.

In summary, our results in Exp. 1 are consistent with the idea that the ABC target is the 'best' target from our set of target shapes. Based on these results, we would therefore suggest that target shape ABC be used in experiments that require stable fixation.

One could argue however, that even though the sizes of fixation target shapes that we use in Exp. 1 are commonly used in research requiring subjects to fixate a visual target (compare Table 1), the size of the larger target is nevertheless relatively large (1.5°). To test if our findings generalize to smaller targets, we conducted Exp. 2, in which the size of the largest target was 0.6°.

3.3. Experiment 2

Due to blinks 3.4% of data were excluded from analysis. Due frequency of blinks did not differ significantly between Exp. 1 and 2 (two-sample *t*-test; $t(22) = 1.2$; $p = 0.24$). We found that over the course of the whole experiment (i.e. across all subjects and

sessions) only 0.35% of all saccades exceeded 2°. We conclude that subjects had followed the fixation instructions well. We analyzed data both including and excluding saccades that exceeded 2°. The differences were negligible. We here report the results from the analyses that included all saccades.

The left panel of Fig. 5 shows normalized dispersion (2D SD) plotted against normalized microsaccade rate for each subject in Exp. 2 in the same format as for Exp. 1. Note that for 8 out of 12 subjects both dispersion and saccade rate are less than 50% for the ABC target, and that for 10 subjects both dispersion and saccade rate are less than 60% (incl. those eight for whom dispersion and saccade rate were less then 50%). The bottom right panel of Fig. 2 shows *p*-values of binomial tests for each of the seven target shapes plotted as a function of the success criterion for Exp. 2. Curves for different targets are color coded, and for reference the simulation results for Exp. 2 are re-plotted in gray. It is evident that the *p*-value for the ABC target is below 0.05 at a success criterion of 0.6 or more. Furthermore, it is evident that the *p*-value of the ABC target is not expected from bias, i.e. there is no overlap between the yellow and gray curve curves and/or error bars. The only other target for which these two criteria apply in Exp. 2 is the A target, but this is only the case at success criterion 0.8.

To provide information about non-normalized performance values, the right panel of Fig. 5 shows average raw dispersion and average raw microsaccade rate (bars) and SE (error bars) for all subjects in Exp. 2. Repeated measures ANOVA with 'target shape' as factor reveals no significant effects of target shape on either 2DSD or microsaccade rate. Subjects performed overall better in Exp. 2 as compared to Exp. 1 in that their overall dispersion and microsaccade rates were lower, even for target shape A which was identical across Exp. 1 and 2 (compare right panels in Figs. 3 and 5). Thus, the reduced effect of target shape on fixation stability (i.e. 50% reduction for 8 out of 12 (Exp. 2) instead of 11 out of 12 (Exp. 1) may be due to a ceiling effect.
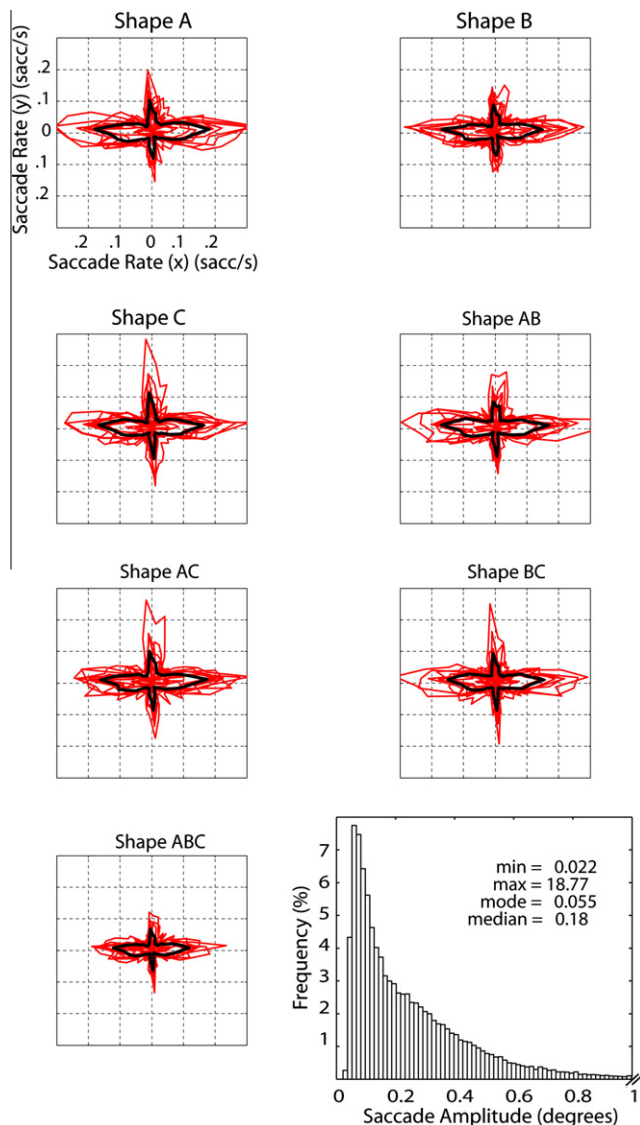
Fig. 4. Spatial characteristics of microsaccades for Exp. 1. The bottom right panel is a histogram of microsaccade amplitudes. The majority of saccades were less than 1°. Thus, for better visibility, the histogram is only shown up to 1°. Plots containing red/black curves are spatial distributions of microsaccade rate plotted as a function of direction separately for each of the seven different target shapes used in Exp. 1. Red curves denote the average microsaccade rate for each subject averaged across trials, and black curves denote the average microsaccade rate for each target averaged across subjects (*n* = 12). The majority of saccades are horizontally oriented. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

To confirm that the algorithm to detect saccades worked properly, we confirmed that microsaccade amplitude and velocity were linearly related, i.e. microsaccades follow the main sequence (data not shown). We also computed spatial distributions of microsaccades and histograms of microsaccade amplitudes (Fig. 6), as well as distributions of dispersion (Supplementary Fig. S2). As expected, the majority of microsaccades is oriented horizontally and the histogram of microsaccade amplitudes peaks at rather short amplitudes and is skewed to the right (e.g. Engbert, 2006), and dispersion distributions are elliptical in shape (Steinman, 1965). The overall impression gained from these analyses is that the data we measure appear to capture systematic movements of the eye rather than measurement noise.

In summary, our results in Exp. 2 are consistent with those obtained in Exp. 1 and suggest that the ABC target is the 'best' target

from our set of target shapes. Based on these results, we would therefore suggest that target shape ABC be used in experiments that require stable fixation.

4. Discussion

Many behavioral experiments require subjects to maintain fixation, but even during fixation people make involuntary eye movements. This can be a considerable problem for experiments that require stable fixation. As laid out in the introduction various variables such as attention, response requirements, visual 'distracters', display changes, or the experimental viewing condition (i.e. free viewing vs. fixation) affect fixational eye movements (e.g. Engbert & Kliegl, 2003a; Hafed & Clark, 2002; Ko, Poletti, & Rucci, 2010; Otero-Millan et al., 2008; Sinn & Engbert, 2011; Steinman et al., 1967), but these variables are not easily manipulated within the context of most experimental paradigms. Furthermore, properties of the fixation target that would perhaps be easier to manipulate, such as blur, color, luminance and/or luminance contrast, have no effect on fixational eye movements unless they render the target barely visible, in which case fixation is bad (e.g. Boyce, 1967; Steinman, 1965; Ukwade & Bedell, 1993). The shape of a fixation target can be manipulated easily in the context of many experimental paradigms. In addition, as laid out in the introduction, there is currently no 'gold standard' for a certain fixation target shape. Thus, from a methodological point of view it would be good if one could determine which shape of a fixation target minimizes eye movements during fixation. Consequently, here we investigated if the shape of a fixation target affects stability of fixation.

In an initial survey we found that even though a wide array of target shape is used in the literature, there seems to be a preference for circular shapes and crosses and combinations of these two basic shapes. Thus, for our experiments we chose a set of seven target shapes that were circular shapes and crosses and combinations of these two basic shapes. In two experiments in which subjects' primary task was to maintain fixation, we found that from our set of seven target shapes only target shape ABC, which looks like a combination of bulls eye and cross hair, resulted in combined low 2D-SD and microsaccade rate.

One could argue that the measurement noise of the eye tracking system we used (Eyelink) poses problems for the conclusions we draw from the data. To investigate the quality of our data, we made use of descriptive data analyses such as spatial distributions of microsaccades, dispersion plots, histograms and numerical data summaries. The overall impression gained from these analyses is that the data we measure appear to capture systematic movements of the eye. For example, spatial distributions of microsaccades show that the majority of saccades are oriented horizontally, an orientation pattern typical for microsaccades (e.g. Engbert, 2006). If the data were dominated by measurement noise, spatial distributions would be isotropic. Furthermore, histograms and numerical data summaries clearly indicate not only that distributions of saccade amplitudes are skewed to the right as is typical for microsaccades, but also that mode and median saccade amplitudes well exceed the measurement noise of the eye tracking system as specified by the manufacturer (Eyelink2 RMS error 0.01° Pupil mode, 0.025° Pupil-CR mode). Furthermore, dispersion values we found in our participants are in reasonably good agreement with those reported by other researchers using eye-trackers with higher resolution. For example, in 'marker conditions' Cherici et al. (2012) measured dispersion in conditions where observers directed their gaze at a 4′ fixation marker. Using a method that directly estimated the 68th percentile of the 2D probability density of gaze position Cherici et al. (2012) reported dispersion to be 275 arcmin². Using 68% confidence ellipses, they report dispersion to be 483 arcmin².
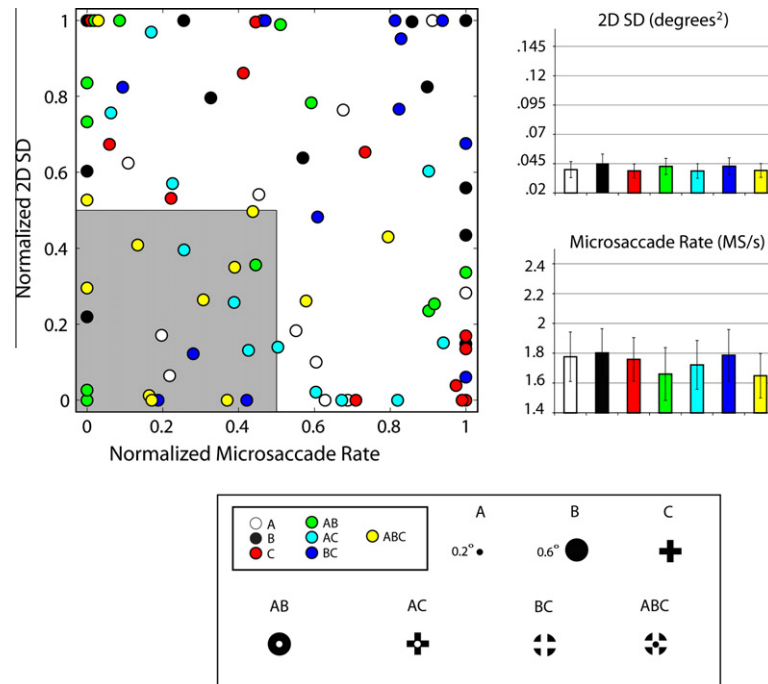
Fig. 5. Results from Exp. 2. Left panel: Normalized dispersion (2DSD) plotted against normalized microsaccade rate for each subject in Exp. 2. Microsaccade rate and 2DSD were normalized to remove individual differences, which makes it easier to plot data from all subjects together in one graph. The gray area denotes the range of values in which both dispersion and saccade rate are less than 50% of the maximum average value for a given subject. Note that for 8 out of 12 subjects dispersion and saccade rate are less than 50% for the ABC target. For two other subjects the reduction is close to 50%. Right panel: Average dispersion and microsaccade rate (bars) and SE (error bars) for all subjects in Exp. 2.

If we rescale our current data (which quantified dispersion using the area of minimum variance ellipses in degrees visual angle squared) to the area of an 68% confidence ellipse in arcmin^2, we measure dispersion in Exp. 1 and 2 to be 708 and 354 arcmin^2, respectively. Experimental conditions in our Exp. 2 are quite similar to those in Cherici et al.'s (2012) marker conditions. Thus, it is reassuring that the average dispersion value from our Exp. 2 is within the range reported by those authors, who used a higher resolution eye tracking system.

Finally, it is important to keep in mind that even though measurement noise will affect absolute values of dispersion and microsaccade rates we found, measurement noise cannot affect the relative differences we found between conditions, unless measurement noise systematically varied across conditions. This, however, is not possible, because target presentation order was randomized. Thus, since our conclusions are based on the relative differences we found across the conditions, not absolute values, measurement noise does not invalidate our interpretation of the data.

Microsaccades occur when stable fixation is required. Interestingly, when subjects are asked to keep their gaze stable in the absence of a visual fixation target, microsaccade rates decrease as compared to when a visual fixation target is provided (Poletti & Rucci, 2010). In the absence of a visual fixation target, however, accuracy of fixation is reduced and dispersion increases, and this is the case for both naïve and trained observers (Cherici et al., 2012). Thus, the method to fixate in the absence of a visual fixation target would be useful for experiments in which it is more important to suppress microsaccades than limiting the dispersion of eye position. However, this method would be problematic for experiments that require accurate and reliable fixation.

A previous investigation about the effect of target shapes on fixation eye movements did not find any systematic effect of target shape (Murphy, Haddad, & Steinman, 1974). Where comparable, i.e. a smaller vs. larger circular targets (target shape A and B from

our experiments; circles size 39 and 78 arcmin in Murphy, Haddad, & Steinman, 1974), our results replicate the findings from Murphy, Haddad, and Steinman (1974), i.e. we find a decrease of dispersion for the smaller as compared to the bigger target (microsaccades were not explicitly considered for that comparison in that study). However, the other conditions are not directly comparable, because Murphy, Haddad, and Steinman (1974) used different fixation target shapes as compared to us. Thus, the seemingly inconsistent finding that a previous study did not find a systematic effect of target shape on fixation eye movements, but our current study did, is most likely due to the fact that our array of target shapes was more effective at eliciting differences in eye movements than the array of shapes used by Murphy, Haddad, and Steinman (1974).

Target shape ABC gives good result under the current testing conditions. Most experiments that require stable fixation, however, will also present other stimuli in addition to the fixation target, and fixational eye movements are affected even in response to irrelevant auditory stimuli (Rolfs, Kliegl, & Engbert, 2008). It is unclear whether target shape ABC will provide the best results for any stimulus arrangement, but at the same time it is impossible for us to run even a fraction of possible stimulus arrangements. Therefore we want to emphasize that the recommended target shape gives good results under the current testing conditions and we encourage other authors to record eye movements in their experiments.

Even though our investigation was methodologically motivated, the data also relate to some theoretical questions.

In our experiments we measured both microsaccades and dispersion of fixational eye movements. With regard to microsaccades, it is an open question to what degree they affect visual perception (for reviews see for example Martinez-Conde, Macknik, & Hubel, 2004; Martinez-Conde et al., 2009; Rolfs, 2009). In the context of visual perception, it has for example been argued that microsaccades counteract visual fading (Martinez-Conde et al.,
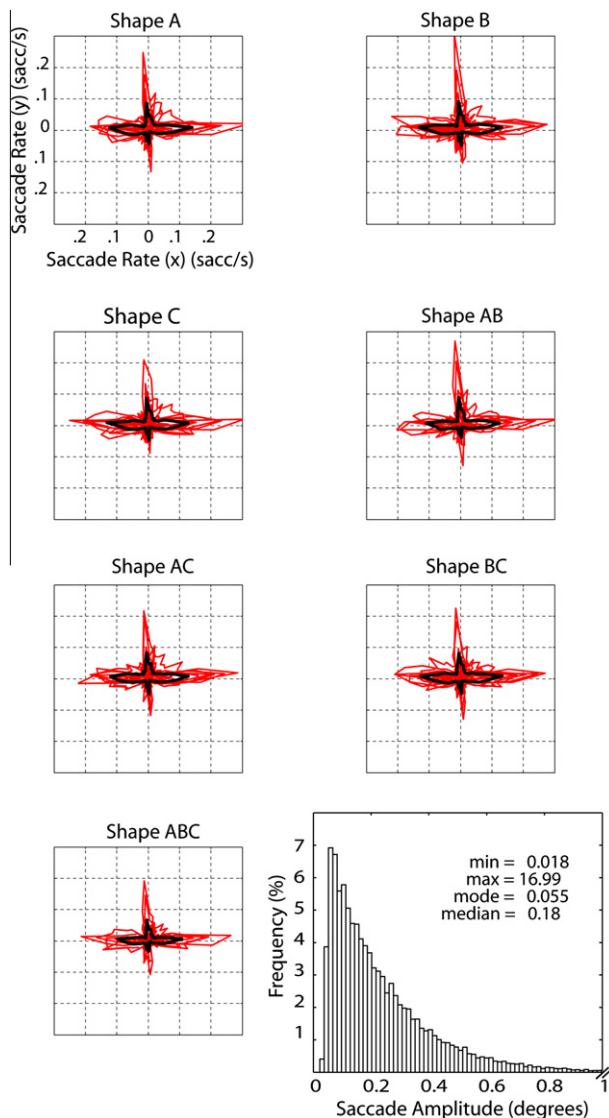
Fig. 6. Spatial characteristics of microsaccades for Exp. 2. The bottom right panel is a histogram of microsaccade amplitudes. The majority of saccades were less than 1°. Thus, for better visibility, the histogram is only shown up to 1°. Plots containing red/black curves are spatial distributions of micro saccade rate plotted as a function of direction separately for each of the seven different target shapes used in Exp. 2. Red curves denote the average microsaccade rate for each subject averaged across trials, and black curves denote the average microsaccade rate for each target averaged across subjects (*n* = 12). The majority of saccades are horizontally oriented. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

2006) and visual filling in (Troncoso, Macknik, & Martinez-Conde, 2008) and it would seem therefore, that microsaccades are useful for accurate visual perception. However, the interpretation of the functional significance of microsaccades is still a matter of scientific debate (Collewijn & Kowler, 2008). It has also been observed that visually evoked neural responses and detection of visual stimuli are enhanced after saccades (Cloherty et al., 2010). The latter has only been investigated in the context of saccades of amplitude 10°, but there is evidence to suggest that 'micro' and 'macro' saccades may have similar neural underpinnings (Hafed & Krauzlis, 2010). There are also results that support the proposal that 'micro' and 'macro' saccades serve the same exploratory function (Cunitz & Steinman, 1969; Ko, Poletti, & Rucci, 2010). Thus, considering these previous reports that link (micro) saccadic eye movements

to visual perception, the question arises if the different fixation target shapes used in our study not only lead to differences in microsaccade rates, but also to differences in visual perception. Future research is needed to address this question.

With regard to dispersion, or 2DSD, we want to emphasize that it reflects mainly slow drifts in eye position. Slow drift of eye position during fixation has also been termed 'slow control' as opposed to microsaccades, which are considered 'fast control' (e.g. Steinman et al., 1973). The idea is that both of these mechanisms control fixation location on the retina (for reviews see for example Collewijn & Kowler, 2008; Martinez-Conde, Macknik, & Hubel, 2004; Rolfs, 2009). Our measurements of spatial dispersion distributions of eye position in Exp. 1 and 2 (Figs. S1 and S2) indicate that average standard deviation of eye position in any direction does not exceed .13°, or 7.8 arcmin, respectively, in any of our experiments. Keeping in mind that our data also reflect measurement noise, the absolute values of .13°, or 7.8 arcmin will reflect the upper limit of the true values. It has been shown that the locus of fixation is on average 10 arcmin displaced from the area of highest cone density, but that there are individual differences as well (Putnam et al., 2005). In that sense, we may speculate that slow drifts in our experiments shift the locus of fixation, but only within parts of the retina with high cone density.

Finally, and most noteworthy in the context of the discussion about the potential role that drift and microsaccades play for the control of eye position (Collewijn & Kowler, 2008; Martinez-Conde, Macknik, & Hubel, 2004; Rolfs, 2009), it is interesting to note that the magnitude of dispersion (2DSD) and microsaccade rate do not appear to be systematically related in our experiment when we consider all target shapes that were used together. Previous experiments found a negative relationship between the magnitude of dispersion and microsaccade rate. However, this was only reported when the size of the fixation target was manipulated while shape remained constant (e.g. Steinman, 1965), and in fact, we replicated this finding in Exp. 1 (target shape A vs. B). A recently published model also suggests that slow movements (drift) and microsaccades might be controlled by the same laws of motion, which implies a dynamical coupling between slow movements and microsaccades (Engbert et al., 2011). Corresponding experimental results are based on a measure that counts the number of retinal cone receptive fields that the eye's trajectory covers during a certain time window (Engbert & Mergenthaler, 2006). Thus, there is the possibility that our current finding that dispersion and microsaccade rate were not systematically related, which is seemingly inconsistent with Engbert et al. (2011) might be a result of the dispersion measure we used. Cooperation between drift and microsaccades for maintenance of fixation has also been proposed by Cherici et al. (2012) who showed a compensatory interplay between direction of drift and the direction of microsaccades. They also showed that microsaccades are more frequent in subjects with faster drift, and larger in subjects who have less self-compensatory drift. Again, however, these results were obtained in conditions were the shape of the fixation target was constant. In sum, the relationship between dispersion and microsaccades when considering all target shapes together should be investigated in more detail in future experiments.

One question that arises is why target shape ABC produced the best fixation. Although we have no definite answer, it is possible to speculate. In particular, it has been shown that fixational eye movements improve the detection of high-spatial frequency gratings (Rucci et al., 2007) and based on these and similar results it has been suggested that fixational eye movements are an efficient way to acquire fine spatial detail (e.g. Ko, Poletti, & Rucci, 2010). Because the ABC target had the most high spatial frequency content, one might speculate that it provided the best control over fixation.

We observed reliable improvements in stability of fixational eye movements for the ABC target shape. Computed as the average difference between the ABC target and the average 'worst' target shape, the average non-normalized reduction in microsaccade rate and 2DSD was 0.12 saccades per second and .03 deg^2, respectively. This measure of effect size is tied to the set of targets shapes we used, and it does not take into account that the 'worst' target actually differs across subjects. As such it is a conservative estimate of effect size. As laid out in the introduction, for experimental paradigms that require precise and/or prolonged fixation, and/or that are sensitive to changes in neural or neuro-muscular activity even small changes in fixation stability affect data quality. Thus, we consider the magnitude of the effects we measured practically relevant.

5. Conclusion

Based on our results we would recommend the combination of bulls eye and cross hair (target shape ABC) as fixation target shape for experiments that require stable fixation. We want to emphasize, however, that our recommendation should not be understood as a 'wild card' to not record eye movements as long as the recommended target shape is used. Instead we want to encourage other authors to record eye movements in their experiments.

Acknowledgments

Appendix A

This code opens a window using Psychtoolbox, draws a black ABC target, and then closes the window again. The target has outer and inner circle diameter of 0.6° and 0.2°, respectively. The target should look like the ABC target for Exp. 2 (compare Fig. 1 in main text).

This code was written for Psychtoolbox 3 on the PC using Matlab (R2009a, The Mathworks, Natick, MA, USA).

```
width = 39;  % horizontal dimension of display (cm)
dist = 60;  % viewing distance (cm)

colorOval = [0 0 0];  % color of the two circles [R G B]
colorCross = [255 255 255];  % color of the Cross [R G B]

dl = 0.6;  % diameter of outer circle (degrees)
d2 = 0.2;  % diameter of inner circle (degrees)

screen = 0;

[w, rect] = Screen('OpenWindow', screen, [], []);
[cx, cy] = RectCenter(rect);
ppd = pi * (rect(3)-rect(1)) / atan(width/dist/2) /
  360; % pixel per degree

HideCursor;
```

```
WaitSecs(2);

Screen('FillOval', w, colorOval, [cx-dl/2 * ppd, cy-
  dl/2 * ppd, cx+dl/2 * ppd, cy+dl/2 * ppd], dl * ppd);
Screen('DrawLine', w, colorCross, cx-dl/2 * ppd, cy,
  cx+dl/2 * ppd, cy, d2 * ppd);
Screen('DrawLine', w, colorCross, cx, cy-dl/2 * ppd,
  cx, cy+dl/2 * ppd, d2 * ppd);
Screen('FillOval', w, colorOval, [cx-d2/2 * ppd, cy-
  d2/2 * ppd, cx+d2/2 * ppd, cy+d2/2 * ppd], d2 * ppd);
Screen(w, 'Flip');

WaitSecs(2);

Screen('Close', w);
```

Appendix B. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.visres.2012.10.012.

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