Persistence of the dark-background-contingent gaze upshift during visual fixations of rhesus monkeys

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Spivak O, Thier P, Barash S. Persistence of the dark-background-contingent gaze upshift during visual fixations of rhesus monkeys. J Neurophysiol 112: 1999–2005, 2014. First published July 23, 2014; doi:10.1152/jn.00666.2013.—During visual fixations, the eyes are directed so that the image of the target (object of interest) falls on the fovea. An exception to this rule was described in macaque monkeys (though not in humans): dark background induces a gaze shift upwards, sometimes large enough to shift the target’s image off the fovea. In this article we address an aspect not previously rigorously studied, the time course of the upshift. The time course is critical for determining whether the upshift is indeed an attribute of visual fixation or, alternatively, of saccades that precede the fixation. These alternatives lead to contrasting predictions regarding the time course of the upshift (durable if the upshift is an attribute of fixation, transient if caused by saccades). We studied visual fixations with dark and bright background in three monkeys. We confined ourselves to a single upshift-inducing session in each monkey so as not to study changes in the upshift caused by training. Already at their first sessions, all monkeys showed clear upshift. During the first 0.5 s after the eye reached the vicinity of the target, the upshift was on average larger, but more variable, than later in the trial; this initial high value 1) strongly depended on target location and was maximal at locations high on the screen, and 2) appears to reflect mostly the intervals between the primary and correction saccades. Subsequently, the upshift stabilized and remained constant, well above zero, throughout the 2-s fixation interval. Thus there is a persistent background-contingent upshift genuinely of visual fixation.

during a time interval of visual fixation, the eyes are maintained relatively immobile. The direction of the eye in the orbit during a visual fixation is commonly thought to be determined so that the image of interest (target) falls on the fovea, allowing high-acuity foveal vision to analyze the target’s image. In fact, the standard procedure for calibrating an eye position monitor is based on this assumption. However, this may not always be the case. Snodderly and colleagues (Snodderly 1987; Snodderly and Kurtz 1985) noticed that in monkeys the direction of gaze is not determined solely by the target’s location, but also by the background illumination. Dark background elicits a peculiar behavior: the eyes are directed above the target, so that the target’s image is not centered on the fovea, and may altogether fall off the fovea. Snodderly found that humans do not show this upshift (Snodderly 1987; Snodderly and Kurtz 1985); hence, it might be specific to monkeys. In a later study, we rediscove...
targets that appeared in 23 locations (Fig. 1) in alternating blocks of bright and dark background (for the reason for using 23, not 24 locations, see METHODS). We studied the fixations of each monkey in each of the 23 locations. We followed the eye position for 2 s after the eye reached the vicinity of the target. The rationale was that if the upshift lingers for the full 2 s of fixation, then it is an attribute of the fixation mechanism, not of the saccades that initiated the fixations.

METHODS

Three male rhesus monkeys were used in this experiment. The monkeys were all trained previously in other oculomotor tasks and were prepared for experiments combining eye position measurements and electrophysiological recordings. All experimental procedures are standard and have been described in detail in recent publications of Thier and colleagues (Caggiano et al. 2013; Dash et al. 2012). Scleral search coils were used for recording the eye positions. The heads were painlessly immobilized by a titanium head post. Surgeries were performed under intubation anesthesia with isoflurane and nitrous oxide, supplemented by continuous infusion of remifentanil (1–2.5 μg·kg⁻¹·h⁻¹) with full tight control of all relevant vital parameters (see Caggiano et al. 2013 and Dash et al. 2012 for full details). All procedures conformed to the National Institutes of Health Guide for Care and Use of Laboratory Animals and were approved by the local ethical committee (Regierungspräsidium Tübingen).

The experimental setup was a standard electrophysiological setup. Monkeys faced a cathode ray tube (CRT) screen positioned 43 cm in front of them. The CRT was an Eizo Flexscan F730, 50-cm diagonal, displaying 1,280 × 1,024 pixels at a frame rate of 72 Hz. The room was lighttight to the level that after sitting in the closed room devoid of artificial light sources for 1 h, human viewers reported inability to see anything. During the experiments, the only active light source was the monitor in front of the monkeys. Each experimental session consisted of a series of blocks, with alternating blocks of bright and dark background. The same target was used in all blocks. Targets could appear in any of 23 positions, arranged in 3 concentric circles, 8 locations on each circle, as in the 1998 study but with the exceptions that, because of the size of the monitor, the extreme vertical positions on the outer circle were slightly shifted toward the center of the screen and one location was not used at all because its view was blocked (hence the 23 locations instead of 24). The target’s color was white, and its luminosity was 30.5–60 cd/m² (variation between sessions). Bright background consisted of luminosity of 0.1, 0.4, and 0.4 cd/m² for monkeys 1, 2, and 3, correspondingly. Target radius was 0.1°. Each trial lasted for 2.5 s. Monkeys had to bring their eyes into an invisible window (2–3° radius) centered on the target location. Because of a technical glitch, of 2,280 trials performed by the 3 monkeys, 204 (9%) of the trials contained fixations shorter than 2 s. These trials were discarded before analysis. Trials with fixation longer than 2 s were clipped so that all analyzed trials contained 2 s of fixation, or more precisely, 2 s of stay of the eyes within the invisible window around the target. Within each block, the order of target locations was randomized, and locations were used several times in each block.

In the present study, we focused on the vertical component of the eye position records. In the 1998 study, we analyzed both vertical and horizontal components of the eye position and showed that the dark-background-contingent upshift is directed upwards in head-centered coordinates, not upwards in earth coordinates. In the monkeys used in the present study, head-centered vertical and ground vertical appeared to be so close as to allow focusing on the vertical component of the eye movement. Henceforth, for simplicity, we will usually refer to the vertical component of the eye position as “eye position.”

Data from the first sessions in the 3 monkeys were divided into 3 × 23 × 2 groups: each group corresponded to a monkey × target location × background combination (background could get 2 values, “bright” and “dark”). For each of these groups, a running time-dependent median was computed as follows. Eye position, as reflected in the search coil signal, was sampled every 1 ms. Hence, a 2-s fixation interval contained 2,000 samples. For each ith sample, the vertical component of the eye position in each trial of the group was noted, and the median of these values was recorded and used in subsequent analysis. (The median was preferred to the mean because of the presence of outliers; see RESULTS and Fig. 2.) Thus, at the end of the first stage of analysis, we had 3 × 23 × 2 median eye position records, each corresponding to a monkey × target location × background combination. These records allowed us to inspect the time course of the upshift over a significant part of the oculomotor range in the first sessions of three monkeys.

We then computed the background-contingent upshift for each of the 3 × 23 monkey × target location combinations. A time-dependent upshift function was operationally defined for each monkey × target location combination as the vectorial, sample-by-sample difference of the dark-background median eye position minus the bright-background median eye position. The upshift functions were studied in the following ways. 1) A median upshift was calculated for each monkey as the time-dependent median of the upshift functions corresponding to the 23 locations of the relevant monkey. 2) The mean total upshift was calculated, pulling all the median upshift functions from all monkeys and locations together. A confidence interval was calculated in addition to the mean. The confidence interval allowed a millisecond-by-millisecond (ms-by-ms) testing of the null hypothesis, that the upshift is not significantly different from zero. 3) The mean upshift as a function of the vertical component of the location was calculated, pulling together data from all monkeys in groups of locations (see Fig. 6).

RESULTS

An example: fixations of one target location by one monkey. Figure 2 shows traces of the vertical component of the eye position for all trials of one monkey × target-location combination. Namely, Fig. 2 shows those trials in monkey 2’s first
session in which the target had appeared at 3.5° to the left, 3.5° above the center of the screen. Figure 2A shows trials recorded with bright background, and Fig. 2B shows trials with dark background. For the order in which these trials were recorded, see METHODS. Each trial is depicted in a different color.

Figure 2, A and B, does not show the target’s location. The reason has to do with the eye position calibration. We followed standard procedure and calculated a calibration transforming the search coil signal to eye position based on several dozens of trials. However, such calibration has limited precision. The hundreds of fixation trials that make up a session in the present study allow for greater precision. Thus a good approximation to the target location (3.5° for Fig. 2) would be the mean value of the median eye position after the initial 0.5 s of fixation. To be careful, we avoid marking absolute values of eye position. We do observe that, regardless of the precise target’s location, the y-axes of Fig. 2, A and B, are identical. This observation is critical for the rest of the analysis.

With both bright and dark background, the position of the eye varies, within trials, by fixation movements, as well as between trials. Despite the considerable variability of the eye position traces, even a superficial look shows that, except for the correction saccades evident during the first 0.5 s of fixation, the eyes do not converge on a single vertical value. Because the target location is common to the trials depicted in Fig. 2, A and B, had there been no dark-background-contingent fixation upshift, we would have expected the traces in Fig. 2, A and B, to converge on the vertical component of the common location of the target.

Figure 2, A and B, shows that fixation saccades directed both upwards and downwards, and intervals of drift, abound in both bright and dark background. Although of obvious interest in themselves, fixation movements are not directly pertinent to this study, because the fixation upshift is not an attribute of the rapid changes in eye position that vary from trial to trial, from one fraction of a second to the next, but rather to the central eye-positions around which fixation movements fluctuate. At issue in the present study is the question whether the central eye positions, recorded during fixation with dark and bright background, converge during fixations to one location or whether the background-contingent difference between the central eye positions persists throughout fixation.

To be able to follow the time course of the central eye positions of Fig. 2, A and B, we need to attain the vertical component of the central eye position for each millisecond during fixation. Because the variability is high but the number of trials is not that high (13 and 12 trials in Fig. 2, A and B, respectively), with some eye positions being outliers, we assessed the central eye position using median rather than mean. Thus, for each millisecond, we calculated the median value from the positions the eye took, at the millisecond at issue, in each of the 13 bright-background trials depicted in Fig. 2A. The
resulting time-dependent median is depicted in Fig. 2A as a thick black trace. A similar procedure yielded a time-dependent median for the dark-background (thick black trace in Fig. 2B). Henceforth, the median traces are used to represent the time course of the eye position of monkey 2 at the location tested in the trials depicted in Fig. 2.

Inspection of Fig. 2, A and B, shows that the dark-background median (thick trace in Fig. 2A), but both median traces fluctuate. The precise relationship of the two median eye positions is captured in Fig. 2C, which depicts the time-dependent upshift of monkey 2 at this location. The upshift is operationally defined as the ms-by-ms difference of the median eye positions depicted in Fig. 2A and B. Namely, the upshift is defined as the median eye position with dark background minus the median eye position with bright background. The upshift varies with time; although somewhat reduced about 1 s into the fixation, the upshift remains positive throughout the trial. Indeed, during the second half of the fixation interval, the upshift increases in size (Fig. 2C). But is this result, of the upshift being positive, statistically significant?

To assess whether eye position with dark background is significantly above eye position with bright background, we pulled together all the eye position samples during the final half-second from all trials illustrated in Fig. 2A and B. The results are shown in Fig. 2D, where the x-axis reflects eye position values. The entire x-axis of Fig. 2D represents 1.5°; the top histogram shows the distribution of the values of the 500 last samples (1 sample for each ms) of each of the 12 trials displayed in Fig. 2B (12 × 500 samples in total), and the bottom histogram shows the last 500 samples for each of the 13 trials illustrated in Fig. 2A (13 × 500 samples in total). The difference between the means of the two distributions is 0.32°, and this difference, tested with a t-test without assuming equal variances, yields a significance level of $P = 0$ (zero, as far as double-precision computer arithmetic is concerned).

Thus, in the trials of this example monkey × target position combination, the upshift persists throughout the 2-s fixation interval. But is this persistence typical? We now move to analysis of the $3 \times 23$ median vertical eye position traces calculated for each monkey, at each target location, for 2-s fixations of a target with dark and with bright background. **Mean eye position of the three monkeys with bright and dark background.** We first analyzed the $3 \times 23$ median vertical eye position traces to test for differences between monkeys. Toward this end, we computed a mean median trace for each monkey, for dark and bright background. We did this by calculating the mean across the 23 traces reflecting the response of the relevant monkey in all target locations. We ended with $3 \times 2$ traces showing, for each monkey, the mean vertical eye position. Each trace is the mean of the median traces calculated separately at each location, for each monkey, as illustrated in Fig. 2. Note that the thick traces are above the thin traces throughout the trial. The difference between the thick and the thin traces is the upshift, shown for each corresponding monkey in B, D, and F.

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**Fig. 3.** Monkey-by-monkey analysis of the upshift. A, C, and E show the time-dependent mean fixation positions with bright background (thin traces) and dark background (thick traces). Each trace is the mean of the median traces calculated separately at each location, for each monkey, as illustrated in Fig. 2. Note that the thick traces are above the thin traces throughout the trial. The difference between the thick and the thin traces is the upshift, shown for each corresponding monkey in B, D, and F.
eye position trace for dark and for bright backgrounds. (We also repeated the computation using medians of the median traces, rather than means; the results were almost identical.)

For each monkey, we computed time-dependent mean eye positions for bright and for dark backgrounds. Figure 3 shows the resulting traces for the three monkeys (thick trace represents dark background, thin trace represents bright). As in Fig. 2, we avoid marking the target location but note that, critically, the vertical scale is identical for all thick and thin traces in Fig. 3, A, C, and E (see calibration bars at bottom). The thick traces are positioned above the thin traces for all monkeys, throughout the 2-s fixation intervals. The precise relationship between the thick and thin traces of each monkey is captured in the upshift, depicted in Fig. 3, B, D, and F (data for monkey 1 are illustrated in A and B, monkey 2 in C and D, and monkey 3 in E and F). During the first 0.5 s, the upshift of all monkeys is higher than during subsequent fixation. However, importantly, about 0.5 s after the fixation starts (that is, after the eye enters the invisible window around the target), the upshift stabilizes and persists almost without change throughout the interval. Importantly, this observation holds for each of the three monkeys.

The increased value of the upshift during the first 0.5 s of fixation is probably related to the saccades that initiate the fixation, and in particular to the correction saccades (visible in the example traces illustrated in Fig. 2, A and B). However, this decrease in the upshift during the first 0.5 s does not reflect behavior specific to dark background. With bright background, too, the eyes take time to stabilize. Monkey 2, in particular, shows a large drop after about 0.25–0.5 s of fixation with dark background, but a similar if smaller drop is present also with bright background (Fig. 3C). Thus the increased initial value of the upshift probably reflects a combined relationship of background luminosity on saccades and on fixation.

Confidence intervals for the upshift’s time course. The $3 \times 23$ monkey $\times$ target location combinations each yielded a time-dependent upshift trace, similar to the one illustrated in Fig. 2C. The mean of these $3 \times 23$ traces reflects the entire database, created from the first training sessions of all monkeys in the present task. Figure 4 shows the trace of this mean upshift, together with a running 95% confidence interval. That is, for each millisecond during the trial, $3 \times 23$ values could be read from each of the upshift traces values. These 69 values were used to calculate the mean and 95% confidence interval at this millisecond. The calculation used a $t$-test. The resulting time-dependent mean upshift and confidence intervals are plotted in Fig. 4. The mean upshift is depicted as the black trace, and the confidence interval as the gray region surrounding the mean.

Note that during the entire fixation interval, even the lower end of the confidence interval remains far above zero. For each of the 2,000 ms that made up the fixation interval, the $t$-test yielded $P = 0$ (that is, $P$ was smaller than the resolution of double-precision computer arithmetic).

During the first 0.5 s, the mean upshift is high and so is the variability, reflecting the presence of correction saccades at the start of fixation intervals. At about 0.5 s, the upshift stabilizes. This is illustrated in more detail in the subsequent analysis of time slices of the fixation interval.

Histograms of the upshift at 0.5-s time slices of the fixation interval. We also analyzed the time course of the upshift by splitting the data into four time slices and studying each separately. Figure 5 shows the results. Each panel corresponds to a 0.5-s time slice; time in trial goes from top to bottom. Each panel shows a histogram of the mean values of the $3 \times 23$ upshift functions at all monkey $\times$ target location combinations during the corresponding time slice. The first 0.5-s time slice (top) shows a large spread, reflecting the intervals between primary and correction saccades that take place at this time slice. After the end of the first time-slice, the upshift stabilizes. The means of the upshift during the subsequent three time slices do not differ statistically significantly ($2$-sample $t$-tests, $P = 0.7, 0.54$, and 1 for comparisons of the 2nd and 3rd, 2nd and 4th, and 3rd and 4th time slices, respectively).

Effect of target location on the initial increased upshift. Although this study was not directed at the saccades that initiated the fixation intervals (there are factors that should be controlled in such a study), an indication regarding a possible source for the increased initial value of the upshift is shown in Fig. 6. We separated the fixation positions according to their height on the screen into positions close to the middle of the screen, positions high on the screen, and positions low on the screen. We plotted the mean upshift separately for each group. Remarkably, the initial high value of the upshift is limited to target locations close to the center and below it. Upward target locations show little if any increased initial upshift. Interestingly, the persistent value of the downward fixations also appears somewhat higher than at the medium and high positions.

DISCUSSION

The aim of this study was finding the time course of the dark-background-contingent upshift of the direction of gaze. In particular, we sought to clarify whether the upshift is gradually abolished during fixations (Goffart et al. 2006). The results show that the upshift is not abolished. On the contrary, after an initial phase of about 0.5 s that probably reflects imprecise fixations followed by correction saccades, the upshift remains almost without change until the end of the tested 2-s fixation.
interval. Thus the upshift is not a transient phenomenon reflecting only the saccades that precede fixations; it is an attribute of the fixations themselves.

This is the first systematic study of the time course of the fixation upshift. In the results of Goffart et al. 2006, there is no full study of the fixation position as a function of time during fixation. Rather, the positions at the start and end of the fixation interval are compared (see their Fig. 2). However, because the duration of the fixation interval varies in their experiment between 0.5 and 2 s, the values they present as end of fixation are some average of the upshift of this range. The full reconstruction of the upshift as a function of the time in the trial shows that after the initial 0.5 s, which probably reflects the eye position preceding the correction saccade, the upshift stabilizes. It is interesting to note that the examples of eye position traces in Goffart et al.’s study are also stable and do not show gradual abolition of the upshift during fixation.

The standard procedure for calibrating eye position assumes that the eyes are always directed at the target during fixation. Of course, for many practical purposes the standard calibration procedure may suffice. The presence of the upshift suggests, however, that the application of the standard calibration procedure should be practiced with caution. In fact, even with bright background, the eye position may take as much as 1 s to stabilize (see Fig. 2C). Most calibration procedures do not require the subject to fixate for more than 1 s.

Many questions remain open regarding the upshift. One group of questions refers to fixation eye movements, particularly to microsaccades. Cui et al. (2009) reported that monkeys make microsaccades primarily in the vertical directions, mostly downwards, and showed that the instantaneous rate of microsaccades changes with target visibility. These results are intriguing; in the present study we observed vertical saccades, although in both upward and downward directions. However, in our experiment the target is visible throughout the trial. A complementary viewpoint is offered by Cherici et al. (2012), who have tested visual fixations of humans in the presence and absence of a fixation spot, comparing highly trained and naive subjects. Interestingly, naive human subjects had significantly greater eye position variance, perhaps corresponding to the smaller upshift we have observed, particularly in the 1998...
study. The absence of a fixation spot led to more variable eye positions (greater fixation span). In the absence of a target, fixation saccades were larger but less frequent. Cherici et al. (2012) emphasized the significance of drift for maintaining direction of gaze. It is interesting to speculate whether the target-absent condition has analogy to the conditions in which upshift is observed in monkeys. Thus characterizing the fixation eye movements that go with the upshift is a pertinent open issue. This and other open issues go beyond the scope of the present study. To be meaningful, a study of fixation eye movements during the upshift has to include additional conditions, except for the basic ones that we have tested.

In the 1998 study we suggested two possible explanations for the upshift, a motor explanation and a sensory explanation. The sensory explanation was based on the discoveries of Curcio and colleagues and Rakic and colleagues of a region in superior retina very dense with rods (Curcio and Allen 1990; Curcio et al. 1990; Packer et al. 1989, 1990; Wikler et al. 1990). Whether this “rod hotspot” has a function remains unclear. Goffart et al. (2006) saw the same two possible explanations. At present it is unclear if the upshift has a function. It is clear, however, that in macaque monkeys this is a very robust phenomenon. For some reason, monkeys direct their eyes above the target when the background is dark, and they do so very systematically, right from the first session in which they are tested in the upshift-eliciting paradigm. The upshift is retained throughout the 2-s fixation. Hence, there are implications to the commonly held heuristic, that the objective of visual fixation is bringing the image of the target to the fovea. Namely, this heuristic appears to be not universally valid.

REFERENCES


