Serial processing in scotopic vision: from upshift to the scotopic band

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Table of Contents

Summary

It has been known for decades that haplorrhine primates and other non-mammalian foveate vertebrates explore visual scenes by making fast eye movements that allow them to shift the image of an object of interest into the fovea. High spatial resolution accommodated by the fovea can be used to scrutinize the object of interest during a period of steady foveal fixation of the object's image, interrupted only briefly by different types of miniature eye movements, too small to jeopardize the image position within the confines of the fovea. In this dissertation I address a peculiar aspect of the object fixation of macaque monkeys observed under conditions of full darkness. Macaque monkeys exhibit an upward shift of gaze (for short, 'upshift') when asked to fixate a tiny fixation spot in an otherwise dark environment. Given the advantages of foveal vision, the upshift that moves the fovea away from the object of interest, the fixation point, must appear paradoxical. The upshift was first described by Snodderly (1987) in rhesus monkeys and rediscovered and investigated in detail by Barash et al. (1998) who studied it in cynomolgus macaques. Barash et al. (1998) demonstrated that the upshift depended solely on the background luminance and not on the contrast between target and background. Barash and coworkers could also show that the upshift increased with training on the fixation and typically started within seconds after turning the background from bright to dark. The view that the upshift was a hitherto undescribed illumination-dependent fixation offset not related to known features of the systems controlling saccades and fixation, was put into a question by Goffart et al. (2006). This group claimed that the upshift is nothing but a luminance dependent disbalance of the saccadic system for the vertical. However, based on this hypothesis, the upshift should be abolished soon after the onset of fixation.

To critically test this hypothesis, we carried out a first study in which we trained rhesus monkeys to fixate small targets on the screen. The monkeys fixated a target in two conditions: with bright or dark background. We investigated the time course of the difference between fixation in darkness and fixation in bright conditions that is the actual upshift. We showed that dark-background-dependent upshift persisted during at least during two seconds of fixation. Additionally, the size of the upshift depended on fixation location: fixation in the lower visual field resulted in larger upshifts whereas fixation in the upper visual field demonstrated smaller upshifts. These results clearly indicated that the upshift cannot merely be a consequence of a dysbalanced saccadic system causing hypermetric upward and hypometric downward saccades as both should be corrected within a few milliseconds, ultimately establishing foveal fixation.

Anecdotal observations seemed to suggest that the upshift might differ between monkeys. In an attempt to consolidate this impression and, moreover, to identify a cause explaining eventual differences, we embarked on study of a very large sample of 14 monkeys from two species. We tested the monkeys in the same task that we had used in the preceding study. The results were very clear: all monkeys in our sample had upshift and the monkeys lacked systematic horizontal deviation during fixation in darkness. However, the monkeys tested exhibited substantial differences as to the size of the upshift. The monkeys in our large sample differed by the level of 'habituation': dark habituated (monkeys that had been previously trained in tasks in full darkness with small bright stimuli) and bright-habituated (monkeys that had been trained in tasks with large bright stimuli without control for full darkness of experimental setup). We showed that the size of the upshift largely reflects the extent to which a monkey is habituated to work in the dark. Dark habituated monkeys with mostly belonged to

the group with higher upshift whereas bright habituated monkeys were very likely to demonstrate lower upshift. Species differences (cynomolgus vs. rhesus macaques) were not found.

In seeking to explain the upshift, we resorted to the geometry of the rod and cone densities, which constitute a hard bound for the resolution of the percept. Cones peak in the fovea; extrafoveally, cone density decreases as eccentricity increases. Rods are absent in the foveola. On going dorsally from the foveola, rod density increases, until reaching a peak in a location called dorsal rod peak, or rod hotspot (Packer et al., 1989; Wickler and Rakic, 1990; Wickler et al., 1990). We therefore started with the hypothesis that if any retinal location replaces the fovea in scotopic vision, it would be the rod hotspot. We therefore expected that the vertical component of the line of gaze would be distributed bimodally, one mode reflecting the fovea and the other the rod hotspot. However, the results did not corroborate this hypothesis. Upshift height varied a lot, from monkey to monkey, from condition to condition, even within session. Eventually we were led to an alternative hypothesis, that of a scotopic band. We now suggest that at any epoch during scotopic vision there might be a scotopic center, located dorsally to the fovea. The scotopic center replaces the fovea. During fixations, target images are projected on the scotopic center, not on the fovea. Saccadic trajectories show that saccades shift the target's image directly to the scotopic center. Therefore, not all saccades foveate. Scotopic saccades do not foveate. The relative weight of the scotopic center in the evolution of saccades remains open. In any case, scotopic saccades come with their own sensorimotor transformations, as do scotopic fixations.

Scotopic center is only the beginning of the scotopic analogy for high-acuity vision. Unlike the close, tight fovea of photopic vision, the scotopic center moves along a line extending dorsally from immediately dorsal to the fovea. We call this the scotopic band. The presence of the band makes scotopic sensorimotor transformations more complex than photopic because they rely on a parameter – the scotopic band setting, the current location of the scotopic center on the band. Scotopic band is set primarily according to the ambient light, reflected in the laboratory as background luminance. Increasing luminance results in lower upshift, or, equivalently, more ventral setting. However, other factors heavily influence the scotopic setting too. To mention but 2: habituation to darkness increases the upshift, that is, makes the setting more dorsal; and so do also threshold task conditions.

Thus, we suggest that ultimately, the scotopic band setting reflects the statistics of the scene and the monkey's task. The computational needs change gradually, and so do the anatomy (and physiology). Therefore, scotopic band setting is not limited to the two endpoints but occupy the points in between.

Introduction

For humans and other primates, vision is the most important sense. It provides a source of imperative sensory information that allows us to perceive the world and to orient in it. The first step in processing of visual information is accomplished by photoreceptors that collect the light emitted by objects and convert information on light into graded membrane potentials. After substantial processing in the retina, visual information is transferred through the optic nerve, comprising the axons of about 1 million (Perry and Cowey, 1985) of retinal ganglion cells to the brain. Photoreceptor cells form the first, the outermost layer body of the retina. They consist of two types: cones and rods. Cones are responsible for color vision and vision in day-light conditions. Rods in turn function primarily under conditions of dim light. The idea that the retina might consist of two complementary components for vision in night and in day-light conditions was first proposed by Max Schultze (1866) in his duplex theory of vision, based on histological studies of vertebrate retina. However, already earlier observations of Purkinje (1825) had noticed that the illumination condition matters for vision. His suggestion that the human vision may deploy two distinct mechanisms for high and for low illumination conditions, was prompted by his observation that blue objects that appeared brighter than red ones before sunrise reverse their color properties afterwards; phenomenon that nowadays is called “Purkinje shift” (Wade and Brožek, 2001). However, unlike Schultze, Purkinje did not yet know that the basis of this difference were architectural specializations of the retina. The consequences of these specializations for eye movements are the subject of this thesis, in particular the role of differences in the spatial layout of the two sets of photoreceptors. This is why, in this introduction I will first take a closer look at their distribution in the retina of two groups of primates, the human retina and the retina of macaque monkeys, a genus comprising several closely related species visual systems that are very similar to the human one. Because of this similarity macaque monkeys have played an enormous role as preferred “model systems” in invasive studies trying to advance further our understanding of human vision. The similarity of human and macaque monkey vision is a consequence of their phylogenetic relation which I will touch on before finally introducing the “upshift” phenomenon, a peculiar upward gaze shift found in macaque monkeys under certain visual conditions. Contributing to better understanding the properties of this phenomenon and its significance has been the main aim of this dissertation.

The mosaic of photoreceptors in the retina

As indicated in the beginning, the visual image is sensed by the retinal photoreceptors: rods and cones. Rods and cones have unique distributions in the retina. The first to study photoreceptor density and photoreceptor distribution in the human retina was Osterberg (1935). According to him, cones are densely distributed in the fovea and their number rapidly decreases with the eccentricity from the foveal center. Rods, in turn are absent from the fovea and unlike cones, their density increases with the eccentricity from the fovea. Similar results were obtained by the study of Packer et al. (1989) which examined the topography of photoreceptors in the pigtail macaque retina. According to this group, rods greatly outnumber cones: the retina contains on average 3.1 million cones and 60.1 million rods. The density of cones peaks in the center of the fovea (210,000 cones/mm2) and decreases exponentially with eccentricity down to 1,500 cones/mm2 in the periphery. Unlike cones, rods were not found in the fovea. Rather they were found to form a ring of elevated density at 3.4 mm eccentricity centered on the cone dominated fovea. The highest rod density (177,000 rods/mm2) was counted in the superior part of the ring, a density two times the density of the inferior part.

According to this study, the difference between superior and inferior retina disappears only gradually with eccentricity. Only at 17 mm eccentricity were the densities of rods in superior and inferior retina were to be identical. The exact location of the rod hot spot exhibited some variability between individuals: it was nasal to the vertical meridian in the retina of one individual and temporal to the vertical meridian in the other.

A study of Wickler et al. (1990) showed qualitatively similar results regarding the mosaic of rods and cones in the retina of rhesus monkeys. They reported the location of the rod hot spot in the dorsal retina further out than Packer et al., namely approximately 5 mm away from the center of the fovea. The density of rods in the rod hot spot reached a maximum of 218,000 rods/mm2, i.e. outnumbering density of cones in the center of fovea as seen in the same study by 18%. The density of rods in the superior pole of the ring was 30% higher than anywhere else on the rod ring, hence supporting the notion of a rod hot spot.

Analogous results were obtained for the human retina in a study by Curcio et al. (1990). This group found that the density of cones peaks in the foveal center (200,000-300.000/mm2), decreases rapidly away from the foveal center, so that half-maximum density is reached only 120 µm inferior to 150 µm temporal from the center of the fovea. As for rods, similar to primate retina, fovea appeared to be rod-free. Rods were mainly distributed in a rod ring at 3-5 mm eccentricity centered on the fovea. Also, in the human retina the highest density of rods was seen in the superior parts of the ring, hence also the human retina exhibits rod hot spot.

The vision of ancestor mammals

From an evolutionary point of view mammalian nocturnal vision seems to have evolved earlier than diurnal vision. According to Walls (1942) during the early evolution of mammals, the members of this group preferred a nocturnal lifestyle in order to avoid predation. Walls suggested that the demands of this lifestyle might explain the fact that modern diurnal mammals, no longer restricted to the ecological niche of their ancestors, still exhibit comparably large eyes endowed with large pupils. These features, not exhibited by non-mammalian vertebrates without the need to apt to a nocturnal lifestyle, maximize the amount of light that the eye can sample. Support for this notion comes from a study by Hall et al. (2012) who analyzed morphology of the eyes of 266 extant mammalian species representing 23 orders with diurnal, nocturnal and cathemeral activity pattern. The eyes of the majority of the species studied were similar in terms of the cornea size to those of nocturnal lizards and birds. Also, the analysis of fossils reports that the earliest true mammals that evolved from therapsid ancestors during the Early Jurassic were nocturnal (Kemp, 2005) as indicated by relatively large orbits. According to Heesy and Ross (2001) this also holds for primates: in their paleontological study they analyzed the orbit diameters of fossil primates, reconstructed their activity patterns by examining the correlation between the mediolateral orbit diameter and estimated body size, and concluded that the common ancestor of anthropoid and prosimian primates was nocturnal. The hypothesis that early mammals evolved under the pressure to adopt a nocturnal lifestyle is often referred to as ‘nocturnal bottleneck’ concept. During this period of nocturnal lifestyle, mammals developed various adaptations helping them to survive under these conditions such as superb olfactory sensitivity (Striedter, 2005) and high-frequency hearing (Coleman and Boyer, 2012). Originally, the forebrain of vertebrates was dominated by the olfactory region: relatively large olfactory bulbs with no exposure of midbrain structures dorsally (Kielan-Jaworowska et al. 1986; Krause and Kielan-Jaworowska 1993; Simpson, 1937), suggesting that the sense of smell was imperative. In parallel, the evolution of ear ossicles that allowed better sound reception and transmission indicates the importance

of the sense of hearing. In contrast, mammals lost many adaptations for photopic vision found in other vertebrate clades: the relatively small size of the optic lobes and the absence of color vision indicate that ancestor mammals relied less on sight (Kemp, 2005).

Photopic vision and the implications for eye movements

High-acuity trichromatic photopic vision is determined by three types of cones with different spectral sensitivities, 'blue', 'green' and 'red' cones which are most sensitive to wavelengths of 430, 531 and 561 nm respectively (Baylor et al., 1987). In the macaque and human retinae, the blue-sensitive cones are absent from the center of the fovea (Curcio et al., 1991, De Monasterio et al., 1985, Wald 1967; Williams et al., 1981). Simultaneous activation of the population of cones by photons emitted from visual scene enables parallel processing of the visual information. The spatial resolution of photopic vision is determined by the density of foveal cones because bipolar cells receive input from just one foveal cone and in turn contact just one ganglion cell (Wässle et al., 1995). Outside the fovea, spatial resolution decline not only becomes of an eccentricity dependent drop in cone density but also because bipolar cells receive converging information from several cones (Wässle et al., 1990). In order to make use of the high-resolution vision offered by the fovea under day light conditions, primates are able to utilize specialized eye movements. As the fovea can cover only a tiny part of the visual scene, amounting to 1° of visual angle, saccades are needed that shift the fovea from one location of interest to another. Saccades are high velocity eye movements taking just a few milliseconds, hence, interrupting visual scrutiny only briefly. The saccadic shift is usually followed by a period of fixation during which the eyes do not move, allowing the visual analysis of the features seen by the fovea. The larger the amplitude of the saccadic gaze shift the faster the saccade will be, a relationship called the main sequence (Bahill et al., 1975; Zuber et al., 1965). Saccadic eye movements are found in large variety of vertebrates, even non foveal ones and are arguably old from an evolutionary point of view. In extant fishes, lacking a fovea, saccades re-center the eye in the orbit once a vestibular ocular reflex should have driven the eye too far away from the straight-ahead orientation (Land, 2018). The fovealizing saccades of fovea endowed vertebrates including primates can build on the machinery for resetting saccades.

As said periods of fixations between saccades allow the fovea to scrutinize objects or object elements of interest. In case the object may be moving slowly, smooth pursuit eye movements can be deployed in order to stabilize the object image within the confines of the fovea (de Brouwer et al., 2002; Fuchs, 1967; Robinson, 1965; Westheimer, 1954). Moreover, on closer examination it becomes clear that also periods of fixation are interrupted by different types of small eye movements like microsaccades, drift and tremor, usually too small to move the object outside the confines of the fovea. Early work on these eye movements argued that they might be required to ensure the amount of image movement needed to prevent a fading of image percepts due to too strong adaptation (Ditchburn and Ginsborg, 1952). However, recent work on microsaccades has clearly changed this view by suggesting an active role: whereas macrosaccades are large gaze shifts moving the object image into the fovea, microsaccades position the relevant part of the object image onto the foveola, the fovea´s center, offering a spatial resolution that surpasses that of the more peripheral parts of the fovea. In other words, microsaccades are needed because also the fovea is not functionally uniform (Poletti et al., 2013).

Scotopic vision and its paradox

Scotopic vision is mediated by rods. Rod cells have a spectral sensitivity peak at 491 nm wavelength (Baylor et al., 1984). The circuitry processing rods derived signals in mammalian retina appears to be simpler than the one for cones: they are presynaptic to only one single type of rod depolarizing bipolar cells. These rod bipolar cells establish connection to AII amacrine cells that serve as interneurons, transmitting scotopic signals to both the ON and OFF bipolar cells of the cone circuit, that transmit the information further to the ON- or OFF-center ganglion cells (Bloomfield and Dacheux, 2001; Soucy et al., 1998). Under scotopic conditions there are very few photons available. Hence, under scotopic conditions the retina should be able to separate the activation of a limited number of rods by single photons noise generated in the remaining rods (Field et al., 2005). Photon-like noise events in the rods that limit photon detection are generated by spontaneous activations of rhodopsin – photosensitive receptor protein found in rods. The activation of rhodopsin starts a biochemical cascade that ultimately leads to membrane hyperpolarization (Barlow et al., 1957; Barlow et al., 1971; Mastronarde, 1983). Previous studies have shown that human retina is capable of detecting reliably <10 photons (Hecht et al., 1942; Sakitt, 1972; van der Velden, 1946).

While the luminosity may change instantaneously, the transition to scotopic vision is gradual, a relatively slow process referred to as dark adaptation. The term ‘adaptation’ was first introduced by Aubert (1864) who assessed the perceptual threshold of the eye in darkness. Aubert measured the electrical current needed to make the glimmering on a platinum wire visible. He found that the sensitivity of the eye increased largely with time in the dark. Hecht and Shlaer (1938) measured the time course of dark adaptation in more detail. For threshold measurements they used a red fixation point of adjustable intensity. The subject fixated this point while the test field of a short wavelength was briefly flashed around the point. This stimulus was presented at different times relative to turning off ambient illumination. During the first minutes in darkness the light appeared violet or blue to the subject. This period was characterized by a relatively fast drop of the intensity threshold. After about 7 minutes in darkness the light stimulus appeared almost colorless and blurry outlined to the observer. Since cones are responsible for color vision, they dominated the vision during the first minutes of dark adaptation. Later, vision relied mainly on the function of rods. The point at which the sensitivity of rods first exceeded the sensitivity of cones, and the color of the light stimulus reported by the observer changed from violet to colorless, was called ‘rod-cone break’. Beyond this point, the minimal intensity level required for perception declined further, yet, more slowly until after about 45 minutes from the start of dark adaptation, a plateau was reached. At this point the eye appears to be fully dark-adapted, arguably because all rods are fully activated, and cones are fully desensitized.

As we know, the pattern of fixation and saccades continues in scotopic vision. As said earlier, saccades have always been closely associated with foveating the target of interest. A survival of saccades under scotopic conditions seems paradoxical, given the fact that under these condition foveal cones are insensitive. Hence, why should the visual system still object images onto the fovea?

Dark background-dependent upshift of gaze

In the year 1987, Snodderly conducted an experiment in which he had both rhesus monkeys and humans fixate a central target on the screen. The fixation of the target was performed alternatively in a completely dark or in an illuminated room. The monkeys demonstrated an upward shift of their vertical eye position while fixating in the dark as compared to the light. Although a fixation trial lasted for several seconds, the monkeys did not correct the upshift in

order to return to an eye orientation prevailing while fixation in the illuminated room. Humans in turn did not show differences in eye position between the two lighting conditions.

Later in 1998, Barash and colleagues rediscovered the results of Snodderly (1987) by demonstrating that during the fixation of a small target on dark background monkeys tend to shift their gaze upwards with respect to their eye position during fixation of the same target on illuminated background. Barash and colleagues investigated different aspects of this dark-background-dependent upshift of gaze (for short – upshift) and determined that the upshift is elicited by the luminance of the background rather than by the contrast between background and the target. They also identified a gradual nature of the upshift: the upshift increased as a function of decreasing background luminance with the dimmest background inducing the largest upshift. The size of the upshift was shown to be determined by the level of illumination around the line of sight: the size increased nearly linearly as the radius of the dark circle surrounding the fixation target increased. Moreover, Barash et al. (1998) demonstrated that the upshift could be induced if during ongoing fixation background luminance was suddenly lowered. In this case, the upshift had the appearance of a saccade-like movement having a duration of just a few milliseconds that brought the eye to an upshifted location within half a second from the onset of the dark background. An inverse saccade-like eye movement abolished the upshift and brought the eye back to the location of the target if in turn the background luminance suddenly returned to bright. The size of the upshift was noted to be experience dependent. Whereas the upshift in the first days of training an animal on the fixation task was still relatively small, its size increased as the behavioral experiments progressed over the following months. The study of Barash et al. (1998) tested four monkeys for upshift. The size of the upshift varied from monkey to monkey from relatively small (1.09 deg) to relatively large (4.3 deg). In case of small upshift, the target's image will fall onto the borders of the fovea. If the upshift is relatively large, as demonstrated by one of the monkeys in the study, the image will completely fall off the fovea during fixation. Barash and colleagues suggested that the existence of the rod hot spot in the superior retina, described earlier, may at least partially explain the emergence an upshift. They hypothesized that monkeys' strategy during fixation in darkness may be to direct the line of gaze towards a point in superior retina located between the fovea and the rod hot spot. However, the notion that the upshift is a consequence of aligning the line of sight during fixation differently depending on the lighting conditions has been contentious: Goffart et al. (2006) argued that the upshift is the result of a luminance-depended disturbance of the metric the saccades that initiates fixation in darkness. However, if this were the case, a second corrective saccade should follow that eliminating the upshift. Barash et al. (1998) reported in one monkey that upshift stayed for at least 2.5 s of fixation and no saccadic corrections were observed. However, this monkey had been trained in oculomotor tasks in darkness and specifically in the upshift-inducing paradigm several months before the actual experiment took place, whereas the study of Goffart et al. (2006) was based on the first sessions in which monkeys were tested for upshift. This fact may be the reason for the contradicting conclusions by the study of Goffart et al. (2006) because, as previous study (Barash et al., 1998) stated, upshift's size can be increased with training. Therefore, in our first study we decided to focus on the very first sessions of three monkeys being tested in the upshift-inducing paradigm to minimize the effect of training. The persistence of upshift for the full 2 s of fixation would support the hypothesis that the upshift is an attribute of visual fixation and not only of the saccade that initiated fixation.

In study 2 we test the upshift phenomenon in more detail by considering an unusually large cohort of 14 monkeys: 10 rhesus monkeys recorded in Tübingen, to which we added the

published data from 4 cynomolgus monkeys from the study of Barash et al. (1998). This large sample size would allow us to delineate the limits of upshift and test the existence of upshift on a population level within 2 macaque species. In addition, the monkeys in our sample varied by the level of habituation to ambient light months before the experiment: one group of monkeys was used to train in the dark whereas the other group was used to train in bright room light. We hypothesized that the type of habituation may affect the size of the upshift, namely higher upshift in dark-habituated group, and lower upshift in bright-habituated group. This observation would suggest that the upshift is a reflexive behavior, modulated by cumulative experience that plays a role in improving vision in darkness.

The next two studies are based on a hypothesis on scotopic and mesopic vision, which suggests the presence of a scotopic band in the retina, and a scotopic center that is located on the band and relocates along the band according to visual and other conditions. The scotopic center parallels the fovea in scotopic vision. In photopic vision, the fovea is singled out by its superior visual resolution, based on its dense cones. Specialized fixation and saccadic eye movements shift the fovea to interesting locations of the scene ('targets') and fixate the fovea long enough for visual sensation to take place. A major element of study 3 provides evidence that in scotopic vision fixation direct the target's image to the scotopic center, not the fovea; and that saccades do not foveate, but rather shift the target's image directly to the scotopic center. Moreover, the location of the scotopic center on the scotopic band ('scotopic band setting') is strongly influenced by the ambient light, within the scotopic range. The higher the background luminance is, the more dorsal is the scotopic band set (that is, farthest from the fovea). Scotopic band setting does not only reflect background luminance. It is also influenced by the task situation. In particular, near threshold the scotopic band is set more dorsally (that is, the upshift is higher) than at salience, when the target is salient. Thus, a scotopic band is present in scotopic vision of monkeys.

Study 4 describes the experiments that led us to the notion of the scotopic band. One of the original aims was finding out whether the upshift is associated with scotopic vision. It could, alternately, be an attribute of photopic dark, the first few minutes after darkness' onset. Towards that aim we compared 4 experimental conditions. Bright targets over bright background was at one end, of photopic vision. Scotopic targets over scotopic dark background (that is, after 45 min dark adaptation) was at the other. In between, there were bright targets over photopic dark background, the conditions of the previous upshift studies; bright background over scotopic dark background; and mesopic background. The results were that scotopic vision had a large upshift, larger than the intermediate sets. Mesopic background induced some upshift. These results support the scotopic background hypothesis. Importantly, they also extend the scotopic band to comprise a ventral region which is used in mesopic vision.

Thus, scotopic vision appears to comprise a serial processing system, much like the fovea-saccades+fixation system of photopic vision.

Aims of this dissertation

The overall objective of the research described in this dissertation was to understand the phenomenon of dark-background-contingent upshift of gaze, for short, upshift. This phenomenon was viewed as an idiosyncrasy of the monkey oculomotor system; eventually, we have suggested that it reveals a serial processing scheme, hitherto unknown, in scotopic system. Snodderly (1987) first noticed the upshift. Barash et al. (1998) rediscovered the upshift and studied its basic properties, but it was called into question by Goffart et al. (2006), who maintained that this was not an effect of fixation but of the saccade initiating the fixation.

The aim of the first study was testing Goffart's suggestion, and testing whether the upshift remains stable during fixations or dissipates. Goffart's notion implies that the upshift should be dissipated in the course of the fixation. To examine Goffart's hypothesis we tested the stability of the upshift during two seconds of fixation (1st study, Appendix 1). After the first 0.5 s, the upshift remain stable, well above the target. Thus, Goffart's hypothesis was rejected (Spivak et al., 2014).

The aims of study 2 were (1) testing whether upshift is a general phenomenon among monkeys; (2) Understanding the sources of the considerable inter-individual variability in the size of the upshift from monkey to monkey. In order to find answers to these questions we investigated the upshift in the large sample of 14 monkeys (2nd study, Appendix 2). We found that the main component of variability appeared to be the monkey's habituation – how used the monkey was to working in the dark in the months preceding the testing.

The next two studies sought to form a more general understanding of the upshift. Toward that aim, the first aim was testing whether a 'scotopic center' could replace the fovea in scotopic vision. The answer appeared to be positive, based both on fixation and saccade data. The second aim tried to get at the noticeable variability in the size of the upshift by suggesting the notion of a scotopic band, over which the scotopic center moves. For that matter the first aim was to map the effect of background luminance on the upshift; the second aim, to test scotopic band setting in the context of threshold performance. Both approaches supported the existence of a scotopic band, whose properties gradually change (3rd study, Appendix 3).

The final study was the one that led us to the notion of the scotopic band. We aimed to compare the upshift generated with very small targets in several conditions – photopic vision, photopic dark with bright targets, scotopic dark with bright targets, scotopic dark with dim targets, and mesopic background. The results all strongly supported the notion of the scotopic band: the closer a condition was to full scotopic, the higher was its upshift, that is, the more dorsal was the scotopic center located on the scotopic band. In addition to the general support, this study extends the notion of the scotopic band to mesopic vision (4th study, Appendix 4).

Summary of scientific findings

Study 1:

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

O. Spivak, P. Thier and S. Barash

Journal of neurophysiology, *112*(8), 1999-2005

At issue beyond this study was the question: is the dark-background-contingent upshift an attribute of visual fixation, or of the saccadic eye movement that precedes the fixation? The implications of this question are far-reaching, because finding that the upshift is an attribute of visual fixation would indicate that we do not entirely understand the function of fixation while the background is dark. The contention that the upshift is related to fixation was called into question by a study that argued the upshift was specific to a brief post-saccadic period, hence an attribute of the saccade rather than fixation. Thus, the crucial question here is whether the upshift indeed decreases and disappears during fixation.

To test whether the upshift decreases with fixation, we trained monkeys to sustain fixation of targets for long time intervals. In the data described in this study we analyzed full 2 s fixations. These are very long fixations; in natural conditions, saccades occur at a rate of several per second.

The analysis of the time-course of the upshift showed, as expected, a high variability immediately after the saccade. As is well known, saccades often fail to precisely end at the target; if the endpoint is not very close to the target, a second, correction saccade shifts the eye to the target's location. Thus, during an initial phase of about 0.5 s, gaze direction variability was high. Interestingly, the median upshift was also high during this 0.5 s interval. After 0.5 s, gaze direction stabilized. Individual trials continued (as expected) to show fixation movements, including fixation saccades up and down, but the median upshift was not annihilated. Indeed, it was stable, very significantly above zero. Thus, the specific testing of the null hypothesis, that upshift occurs only at the start of fixations, was rejected.

In addition to the main result, we characterized in this study a dependence of saccade and upshift size (in dark background) on the vertical component of the orbital position. The size of the upshift was strongly dependent on the location of fixation target: targets in the lower part of the screen resulted in higher upshift whereas targets in the higher part of the screen, in lower upshift. Nonetheless, in spite of this size dependence, it should be emphasized that upshift was present in all orbital positions.

Study 2:

Dark-habituation increases the dark-background-contingent upshift of gaze in macaque monkeys

O. Spivak, P. Thier and S. Barash

Revised version under review at Journal of Vision

This study had 2 objectives. The first objective was that of evaluating the generality of the upshift. Is the upshift specific to one monkey species? If the upshift were an idiosyncrasy, that could be the case. What are the inter-individual differences, is upshift present in some monkeys but not all? Could we identify a specific factor driving the inter-individual differences? The second objective, apparently unrelated, relates to the effect of perceptual-motor learning. If the upshift has anything to do with vision in the dark, is there any learning effect induced by the experience of working in the dark? More specifically, is upshift larger in monkeys used to working in the dark, than in monkeys used to work in photopic conditions?

In approaching these questions, we took advantage of the unusually large sample of monkeys that we studied. Overall, we tested the upshift behavior in 10 monkeys. These monkeys were used in other tasks in Prof. Thier's lab. We studied monkeys who participated in two groups of studies. In one study, primarily of cerebellar physiology, monkeys were trained to make fixation and saccadic eye movements in dark rooms. Recording single-unit in the dark pre-empts the possibility of contamination by visual artefacts. The other group was trained and studied in full light, similar to daylight conditions. In this group single-cell recordings were made from areas of the cerebral cortex. The tasks in which this group was trained were also based on fixations of fixation spots and saccadic eye movements. Thus, we had one group of 3 monkeys habituated to working in the dark, and another group of 7 monkeys habituated to working in bright-light conditions. We analyzed these 10 monkeys both alone, and also in conjunction with published data from 4 cynomolgus monkeys which were studied in another lab. All these 4 monkeys were dark-habituated.

The first result of this study was that all 14 monkeys showed upshift. Thus, upshift is a universal phenomenon in macaque monkeys. Notably, upshift was not particular to either rhesus or cynomolgus monkeys, but common to both. In contrast, there was no systematic horizontal shift. Thus, dark-background-induced upshift is omnipresent and specific.

We then proceeded to compare upshift in the dark-habituated monkeys to that in the bright-habituated. We made this comparison twice; once, within the 10-monkeys Tübingen sample; second time, within the 14-monkey sample comprising the 10 rhesus-monkeys Tübingen sample and the 4 cynomolgus-monkeys Rehovot sample. The results of the two comparisons were very similar to each other.

The main result was that the dark-habituated monkeys showed a large upshift, larger than the bright-habituated monkeys. The mean upshift was 2.20 deg in the dark-habituated monkeys, compared to 0.50 deg in the bright-habituated monkeys. The difference between the distributions of the upshift in the two habituation groups was so large that, in some cases, knowing the upshift even of a single trial was enough to classify the monkey as dark-habituated with high confidence. This result reflects the observation that an upshift greater than 2 to 3 deg was almost never observed in the bright-habituated monkeys but was not rare in the dark-habituated monkeys. Thus, large upshift, even in a single trial, is robust evidence that the monkey is dark-habituated.

In sum, the upshift depends on habituation. The upshift is a reflexive behavior whose size reflects months-long cumulative experience.

Study 3:

Scotopic band: serial processing through foveal substitution in scotopic vision of monkeys

S. Barash, O. Spivak, and P. Thier

Submitted

In this study we suggested a new perspective on the upshift in scotopic and mesopic conditions. The new perspective focuses on the notions on a 'scotopic center' and 'scotopic band'.

We first suggest that a 'scotopic center' replaces the daytime fovea in scotopic and mesopic vision. The hypothesized scotopic center covers only a small patch of space; we suggest that specialized eye-movements have evolved to get the target's image directly onto the scotopic center. These movements are fixation and saccadic eye movements, well known from photopic vision. The difference is that in scotopic (and mesopic) vision, these movements shift the 'target', the interesting next location in the scene, not to the dysfunctional in scotopic vision fovea but to the scotopic center. Importantly, together, the scotopic center and nighttime eye movements turn night-vision into a serial processing scheme, much like the fovea and saccades do for day-vision.

So, the scotopic center is in many ways like the fovea. However, we suggest that there is a fundamental difference between photopic vision, and scotopic-mesopic vision. Whereas the fovea is always fixed on the retina, the scotopic center moves. The range over which the scotopic center can move makes up the scotopic band. The scotopic band is an elongated region which ventrally starts just dorsal to the fovea and extends dorsally. We know from previous anatomical studies that light reception gradually changes along the scotopic band. Going dorsally, cones turn scarce, rods dense. Why is a band of retina needed in scotopic vision whereas only one locus suffices in photopic vision? The needs of mesopic and scotopic vision might in some ways be more intricate than those of photopic vision and might require the rather unique band structure. In any case, the question comes up, how is the current location of the scotopic center on the scotopic band set? We call this operation scotopic band setting and show that it can be passive or active.

To support these suggestions, we carried out three sub-studies. We first studied fixations and horizontal saccadic eye movements before and after proper dark adaptation, accomplished by the monkey spending 45 min in full darkness. This duration is known from many previous studies to be enough to bring the system to maximal sensitivity. We trained 3 monkeys in this task. The target stimuli were within the scotopic range. All three monkeys showed a clear upshift. We computed the foveal gaze direction ('eye position') for each fixation, before and after the saccade. Virtually all the scotopic gaze directions were above the photopic. This was true before and after the saccades, in central and peripheral location.

We then looked at the saccadic trajectories themselves. We followed the experimental prediction that scotopic saccades do not foveate the targets. Instead, they shift the target to the proximity of the previous scotopic center. Indeed, the foveation prediction failed completely. Almost none of the saccades directed the target's image to the fovea. Instead,

the trajectories were near-horizontal, supporting the alternative prediction, that targets are shifted to the scotopic center, at its new, post-saccadic location.

Other than their start and end points, scotopic saccades looked like any saccades. Their main sequences looked normal, though velocities were slightly lower than those of photopic saccades.

To support the notion of the scotopic band we made 2 sub-studies. The first recorded upshift size while background luminance increased in small steps from complete darkness, scanning the scotopic luminance range. As background luminance increased, upshift slowly decreased. Thus, scotopic band setting is set according to the visual conditions. A relevant condition is ambient luminance. In darkness (after dark adaptation) the setting is to the dorsal regions of the scotopic band. As ambient light increases (within the scotopic range), the scotopic center is set closer to the fovea.

However, scotopic band setting is not only a passive process. We showed this by studying the upshift in near-threshold and salient conditions. Upshift is higher in threshold conditions.

Study 4:

In mesopic and scotopic vision monkeys use the scotopic band, a retinal region dorsal to the fovea

O. Spivak, P. Thier and S. Barash

In final stage of preparation

This study establishes and extends the concept proposed in Study 3. It actually preceded and led to Study 3; in retrospect, it reinforces the conclusions of Study 3 and extends them, in particular to mesopic vision. This reinforcing is important, because the database showing the scotopic band in Study 3 is not large, even though the results are significant. Study 4 is based on a very large dataset (thousands of trials, in multiple conditions). The study begins with a systematic presentation and justification for the analysis used throughout the thesis, in which data from several retinal target locations are pooled together. Thus, the notion of shift is properly defined. Although the shifts are not identical in all locations, they make a coherent unimodal distribution and the mean is well defined. The core of this study is a comparison of the upshift in several conditions. Condition 1 is the standard upshift as measured in previous studies (except for study 3). The target is small and bright, in the photopic range. The time after dark onset is short, so that vision is dominated by cones (we call it photopic dark). Condition 2 uses the same small but intense target, however after dark adaptation. Condition 3 uses a less intense target, at the upper end of the scotopic range. Thus, we kept the target at the same small size; in condition 3, the luminance is minimal for the monkey to note the target. The results showed large inter-trial variability. At the sample level, differences were clear. As expected, even in condition 1 there was upshift. Conditions 2 and 3 showed upshift, too. Thus, upshift is not specific to photopic dark. Moreover, the upshift in condition 2 was larger than in condition 1. In spite of the brightness of the target, using dark adaptation to drive the system into near-scotopic conditions had a significant effect. (The conditions are not fully scotopic because of the exposure to the bright small targets). Condition 3 had the maximal upshift. Thus, scotopic vision involves setting the scotopic center to more dorsal

locations of the scotopic band. The likely mesopic condition 2 involved more ventral setting, though not as ventral as that of condition 1.

Condition 2 can be described as ‘mixed mesopic’. To test mesopic vision more systematically, we applied a mesopic background. The result was upshift, but, as expected, less dorsal (lower upshift) than scotopic.

Finally, we made the important control of light adaptation. Light adaptation led to no upshift. Hence, the challenging experimental procedure of 45 min in full darkness is not involved in the upshift.

Conclusions and outlook

Taken together, our results indicate that monkeys optimize the conditions of vision during scotopic conditions by deploying the extrafoveal part of the retina. The results presented in this dissertation first establish the ubiquity of upshift in non-photopic conditions. The upshift is a method of measurement for the location of the current scotopic center used for vision, on the scotopic band: retinal region rich in rods that extends dorsally from the fovea. The scotopic band is revealed by the patterns of eye movements that we recorded. The scotopic center and scotopic band resolve the paradox of why saccades and fixation linger on in scotopic vision.

Because the upshift reflects the activation of the scotopic center, its size does not dissipate during fixations, if the visual conditions remain unchanged. The location on the scotopic center appears to be influenced primarily by the current visual conditions, mainly the luminance level of the background, that is, the ambient light. However other factors influence the scotopic band setting as well. We defined salient and threshold conditions of fixation target. Threshold conditions, invoking low signal to noise ratios, result in higher upshifts by pushing the setting of the scotopic band dorsally. Interestingly, habitually working in the dark has a similar effect. Monkeys trained for months in the dark before being tested also showed higher upshift – that is, use of more dorsal position on the scotopic band.

The observations of non-foveating saccades, saccades that transfer the target's image directly to the scotopic center, are of particular interest. The vast majority of the research on vision deals with photopic vision. It therefore has become common wisdom that saccades have evolved to shift the target's image to the fovea. That may be so, but it might also be the case that the weight of scotopic vision in the evolution of saccades was greater than we now appreciate.

The scotopic band explains the gaze direction in scotopic vision, but not only. Mesopic vision is considered to be more and more significant. That mesopic vision is integrated in the concept of the scotopic band and might be a main user of the ventral part of the band, is significant.

It makes a lot of sense for mesopic and scotopic vision not to have one fixed processing focus as photopic vision. Throughout the range of photopic vision, the photon density is high, so that the information processing needed remains quite fixed. In mesopic and scotopic vision there is more variability.

What is the function of this framework? Why does the retina use an elongated band for scotopic vision, rather than a fixed location, as photopic vision? A hint might be given by the retinal geometries of rod and cone densities. Cone and rod densities change along the dorsal dimension of the scotopic band. Going dorsally, cones become more scarce, whereas rods more dense. High cone density is a signature characteristic of the fovea. Perhaps the highly variable visual conditions of mesopic and scotopic vision are best served by selecting the appropriate combination of rod and cone densities, offered by the extended scotopic band.

References

Aubert, H. (1864). *Physiologie der Netzhaut*. Morgenstern.

Bahill, A. T., Clark, M. R., & Stark, L. (1975). The main sequence, a tool for studying human eye movements. *Mathematical biosciences*, *24*(3-4), 191-204.

Barash, S., Melikyan, A., Sivakov, A., & Tauber, M. (1998). Shift of visual fixation dependent on background illumination. *Journal of neurophysiology*, *79*(5), 2766-2781.

Barlow, H. B., Fitzhugh, R., & Kuffler, S. W. (1957). Change of organization in the receptive fields of the cat's retina during dark adaptation. *The Journal of physiology*, *137*(3), 338.

Barlow, H. B., Levick, W. R., & Yoon, M. (1971). Responses to single quanta of light in retinal ganglion cells of the cat. *Vision research*, *11*, 87-101.

Baylor, D. A., Nunn, B. J., & Schnapf, J. L. (1984). The photocurrent, noise and spectral sensitivity of rods of the monkey Macaca fascicularis. *The Journal of physiology*, *357*(1), 575-607.

Baylor, D. A., Nunn, B. J., & Schnapf, J. L. (1987). Spectral sensitivity of cones of the monkey Macaca fascicularis. *The Journal of Physiology*, *390*(1), 145-160.

Bloomfield, S. A., & Dacheux, R. F. (2001). Rod vision: pathways and processing in the mammalian retina. *Progress in retinal and eye research*, *20*(3), 351-384.

Coleman, M. N., & Boyer, D. M. (2012). Inner ear evolution in primates through the Cenozoic: implications for the evolution of hearing. *The Anatomical Record: Advances in Integrative Anatomy and Evolutionary Biology*, *295*(4), 615-631.

Curcio, C. A., Sloan, K. R., Kalina, R. E., & Hendrickson, A. E. (1990). Human photoreceptor topography. *Journal of comparative neurology*, *292*(4), 497-523.

Curcio, C. A., Allen, K. A., Sloan, K. R., Lerea, C. L., Hurley, J. B., Klock, I. B., & Milam, A. H. (1991). Distribution and morphology of human cone photoreceptors stained with anti-blue opsin. *Journal of comparative neurology*, 312(4), 610-624.

de Brouwer, S., Missal, M., Barnes, G., & Lefèvre, P. (2002). Quantitative analysis of catch-up saccades during sustained pursuit. *Journal of neurophysiology*, *87*(4), 1772-1780.

De Monasterio, F. M., McCrane, E. P., Newlander, J. K., & Schein, S. J. (1985). Density profile of blue-sensitive cones along the horizontal meridian of macaque retina. *Investigative ophthalmology & visual science*, *26*(3), 289-302.

Ditchburn, R. W., & Ginsborg, B. L. (1952). Vision with a stabilized retinal image. *Nature*, *170*(4314), 36-37.

Field, G. D., Sampath, A. P., & Rieke, F. (2005). Retinal processing near absolute threshold: from behavior to mechanism. *Annu. Rev. Physiol.*, *67*, 491-514.

Fuchs, A. F. (1967). Saccadic and smooth pursuit eye movements in the monkey. *The Journal of Physiology*, *191*(3), 609.

Goffart, L., Quinet, J., Chavane, F., & Masson, G. S. (2006). Influence of background illumination on fixation and visually guided saccades in the rhesus monkey. *Vision research*, *46*(1-2), 149-162.

Hall, M. I., Kamilar, J. M., & Kirk, E. C. (2012). Eye shape and the nocturnal bottleneck of mammals. *Proceedings of the Royal Society B: Biological Sciences*, 279(1749), 4962-4968.

Hecht, S., & Shlaer, S. (1938). An adaptometer for measuring human dark adaptation. *JOSA*, *28*(7), 269-275.

Hecht, S., Shlaer, S., & Pirenne, M. H. (1942). Energy, quanta, and vision. *Journal of General Physiology*, *25*(6), 819-840.

Heesy, C. P., & Ross, C. F. (2001). Evolution of activity patterns and chromatic vision in primates: morphometrics, genetics and cladistics. *Journal of human evolution*, *40*(2), 111-149.

Kemp, T. S. (2005). *The origin and evolution of mammals*. Oxford University Press on Demand.

Kielan-Jaworowska, Z., Presley, R., & Poplin, C. (1986). The cranial vascular system in taeniolabidoid multituberculate mammals. *Philosophical Transactions of the Royal Society of London. B, Biological Sciences*, *313*(1164), 525-602.

Krause, D. W., & Kielan-Jaworowska, Z. (1993). The endocranial cast and encephalization quotient of Ptilodus (Multituberculata, Mammalia). *Palaeovertebrata*, *22*(2/3), 99-112.

Land, M. F. (2018). The evolution of gaze shifting eye movements. In *Processes of Visuospatial Attention and Working Memory* (pp. 3-11). Springer, Cham.

Mastronarde, D. N. (1983). Correlated firing of cat retinal ganglion cells. II. Responses of X- and Y-cells to single quantal events. *Journal of Neurophysiology*, *49*(2), 325-349.

Osterberg, G. A. (1935). Topography of the layer of the rods and cones in the human retima. *Acta ophthalmol*, *13*(6), 1-102.

Packer, O., Hendrickson, A. E., & Curcio, C. A. (1989). Photoreceptor topography of the retina in the adult pigtail macaque (Macaca nemestrina). *Journal of Comparative Neurology*, *288*(1), 165-183.

Perry, V. H., & Cowey, A. (1985). The ganglion cell and cone distributions in the monkey's retina: implications for central magnification factors. *Vision research*, *25*(12), 1795-1810.

Poletti, M., Listorti, C., & Rucci, M. (2013). Microscopic eye movements compensate for nonhomogeneous vision within the fovea. *Current Biology*, *23*(17), 1691-1695.

Purkinje, J. (1825). Observations and experiments investigating the physiology of senses. *Prague, Czech Republic*.

Robinson, D. A. (1965). The mechanics of human smooth pursuit eye movement. *The Journal of Physiology*, *180*(3), 569.

Sakitt, B. (1972). Counting every quantum. *The Journal of Physiology*, *223*(1), 131-150.

Schultze, M. (1866). Zur anatomie und physiologie der retina. *Archiv für mikroskopische Anatomie*, *2*(1), 175-286.

Simpson, G. G. (1937). Skull structure of the Multituberculata. Bulletin of the AMNH; v. 73, article 8.

Snodderly, D. M. (1987). Effects of light and dark environments on macaque and human fixational eye movements. *Vision research*, *27*(3), 401-415.

Soucy, E., Wang, Y., Nirenberg, S., Nathans, J., & Meister, M. (1998). A novel signaling pathway from rod photoreceptors to ganglion cells in mammalian retina. *Neuron*, *21*(3), 481-493.

Spivak, O., Thier, P., & Barash, S. (2014). Persistence of the dark-background-contingent gaze upshift during visual fixations of rhesus monkeys. *Journal of neurophysiology*, *112*(8), 1999-2005.

Striedter, G. F. (2005). *Principles of brain evolution*. Sinauer associates.

van der Velden H. A. (1946). The number of quanta necessary for the perception of light of the human eye. *Ophthalmologica*, 111(6):321-331.

Wade, N. J., & Brozek, J. (2001). *Purkinje's vision: The dawning of neuroscience*. Psychology Press.

Wald, G. (1967). Blue-blindness in the normal fovea. *JOSA*, *57*(11), 1289-1301.

Walls GL. (1942) The vertebrate eye and its adaptive radiation. *Bloomfield Hills, MI: Cranbrook Institute of Science.*

Wassle, H., Grunert, U., Martin, P. R., & Boycott, B. B. (1995). Immunocytochemical characterization and spatial distribution of midget bipolar cells in the Macaque monkey retina. *Ophthalmic Literature*, *2*(48), 118.

Wässle, H., Grünert, U., Röhrenbeck, J., & Boycott, B. B. (1990). Retinal ganglion cell density and cortical magnification factor in the primate. *Vision research*, *30*(11), 1897-1911.

Westheimer, G. (1954). Mechanism of saccadic eye movements. *AMA Archives of Ophthalmology*, *52*(5), 710-724.

Wikler, K. C., Williams, R. W., & Rakic, P. (1990). Photoreceptor mosaic: number and distribution of rods and cones in the rhesus monkey retina. *Journal of Comparative Neurology*, *297*(4), 499-508.

Wikler, K. C., & Rakic, P. (1990). Distribution of photoreceptor subtypes in the retina of diurnal and nocturnal primates. *Journal of Neuroscience*, *10*(10), 3390-3401.

Williams, D. R., MacLeod, D. I., & Hayhoe, M. M. (1981). Foveal tritanopia. *Vision Research*, *21*(9), 1341-1356.

Zuber, B. L., Stark, L., & Cook, G. (1965). Microsaccades and the velocity-amplitude relationship for saccadic eye movements. *Science*, *150*(3702), 1459-1460.

Appended papers/manuscripts

Appendix 1: Spivak, O., Thier, P., & Barash, S. (2014). Persistence of the dark-background-contingent gaze upshift during visual fixations of rhesus monkeys. *Journal of neurophysiology*, *112*(8), 1999-2005.

Appendix 2: Spivak, O., Thier, P., & Barash, S. (2020). Dark-habituation increases the dark-background-contingent upshift of gaze in macaque monkeys. *Revised version under review at Journal of Vision*.

Appendix 3: Barash, S., Spivak, O., & P. Thier (2020). Scotopic band: serial processing through foveal substitution in scotopic vision of monkeys. *Submitted.*

Appendix 4: Spivak, O., Thier, P., & Barash, S. (2020). In mesopic and scotopic vision monkeys use the scotopic band, a retinal region dorsal to the fovea. *In final stage of preparation.*

Personal contribution statement

Study 1: Spivak, O., Thier, P., & Barash, S. (2014). Persistence of the dark-background-contingent gaze upshift during visual fixations of rhesus monkeys. *Journal of neurophysiology*, *112*(8), 1999-2005.

S.O. and B.S. designed research, **S.O.** performed research and acquired the data, **S.O.** and B.S analyzed the data; **S.O.** and B.S. wrote the manuscript and revised it based on T.P.'s comments and suggestions. All authors reviewed the manuscript.

Study 2: Spivak, O., Thier, P., & Barash, S. (2020). Dark-habituation increases the dark-background-contingent upshift of gaze in macaque monkeys. *Revised version under review at Journal of Vision.*

S.O. and B.S. designed the experiment, **S.O.** performed research and acquired the data, **S.O.** analyzed the data; **S.O.** and B.S. wrote the manuscript and revised it based on T.P.'s comments and suggestions. All authors reviewed the manuscript.

Study 3: Barash, S., Spivak, O., & P. Thier (2020). Scotopic band: serial processing through foveal substitution in scotopic vision of monkeys. *Submitted.*

S.O. and B.S. designed the paradigms, **S.O.** performed research and acquired the data, **S.O.** analyzed the data; **S.O.** and B.S. wrote the manuscript and revised it based on T.P.'s comments and suggestions. All authors reviewed the manuscript.

Study 4: Spivak, O., Thier, P., & Barash, S. (2020). In mesopic and scotopic vision monkeys use the scotopic band, a retinal region dorsal to the fovea. *In final stage of preparation.*

S.O. and B.S. designed the experiments, **S.O.** performed research and acquired the data, **S.O.** analyzed the data; **S.O.** and B.S. wrote the manuscript and revised it based on T.P.'s comments and suggestions. All authors reviewed the manuscript.

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Appendix 1:

Spivak, O., Thier, P., & Barash, S. (2014). Persistence of the dark-background-contingent gaze upshift during visual fixations of rhesus monkeys. *Journal of neurophysiology*, *112*(8), 1999-2005.

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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 also 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.

gaze direction; eye position; visual background; luminosity; fovea; upshift; visual fixation; saccadic eye movements; correction saccade

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 in monkeys that 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 rediscovered this phenomenon and studied in detail many of its properties (Barash et al. 1998); we henceforth refer to this as "the 1998 study."

Of the properties of the upshift that were described in the 1998 study, the most relevant for the present study are that *1*) the upshift is a remarkably robust phenomenon. In the 1998 study, the upshift was demonstrated in four monkeys. In all monkeys, the upshift was highly statistically significant. However, *2*) the amplitude of the upshift depends on the level of training. One monkey was followed in the 1998 study from the start of training and on for several months. The upshift was conspicuous from the start but increased in amplitude during training. *3*) The size of the upshift depends on the target location; this dependence varies between monkeys. Some of this variability stems from the monkey's precise eye position; rotations with respect to the occipito-nasal axis showed that the coordinate frame of the upshift is head centered. Thus some of the location dependency may reflect the tilting of the head.

Therefore, even though the upshift might be an exceptional situation present only in monkeys, it still appeared to show that the heuristic that the fovea's line of gaze is always directed to the target is not universally valid. However, in 2006, Goffart and colleagues reported an upshift of saccades made in dark background (Goffart et al. 2006). Furthermore, Goffart et al. suggested that the upshift is actually an attribute of saccades; when the eyes fixate, they suggested, a series of fixation movements gradually eliminates that error so that eventually fixation is precise, that is, the target's image is foveated. Thus the question arises whether the dark-background-contingent upshift is an attribute of visual fixation or only of saccades.

In the 1998 study, we tested the time dependency of the upshift in one monkey (see Figs. 7 and 8 in Barash et al.). In that monkey, the upshift remained present for 2.5 s. However, this monkey was trained for a long time in oculomotor tasks in the dark, and specifically in the upshift-inducing paradigm (alternating blocks of bright and dark background). One difference between Goffart's study and the 1998 study is that Goffart studied the first sessions in which monkeys were tested for upshift, whereas the 1998 study was based on data from overtrained monkeys. The reason this might matter is that, as we already mentioned, we showed that, with training, the amplitude of the upshift significantly increases. Therefore, we decided to test three monkeys in the upshift-inducing paradigm, but concentrate only on the very first sessions of testing for the presence of an upshift in these monkeys, to minimize effects of training in the upshift-inducing paradigm. As in our previous study, we had the three monkeys fixate small bright

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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 2s 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 remifentanyl (1–2.5 $\mu g \cdot kg^{-1} \cdot h^{-1}$) 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,

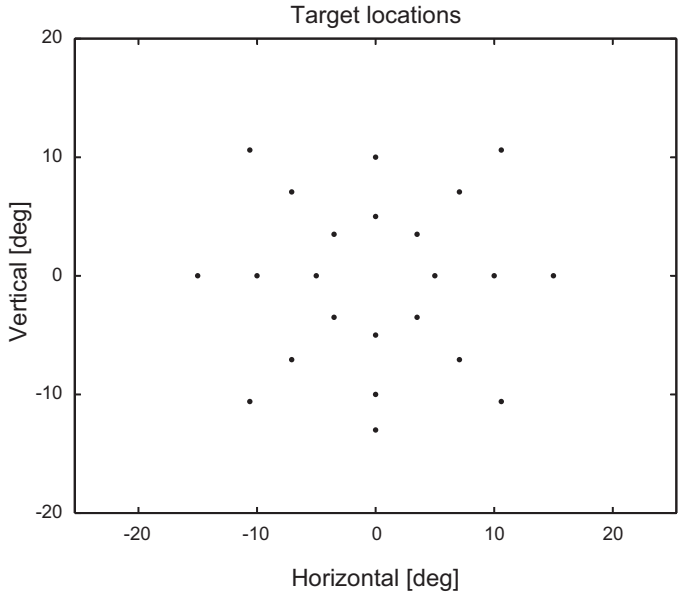

Fig. 1. The 23 locations in which a target could appear in the present study. The targets are arranged in 2 concentric circles and an outer modified circle adapted to the dimensions of the monitor. The top location of the outer circle was not used at all because the monkeys' view was blocked (hence the total of 23 locations).

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/m^2 (variation between sessions). Bright background consisted of luminosity of 0.1, 0.4, and 0.4 cd/m^2 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 *i*th 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

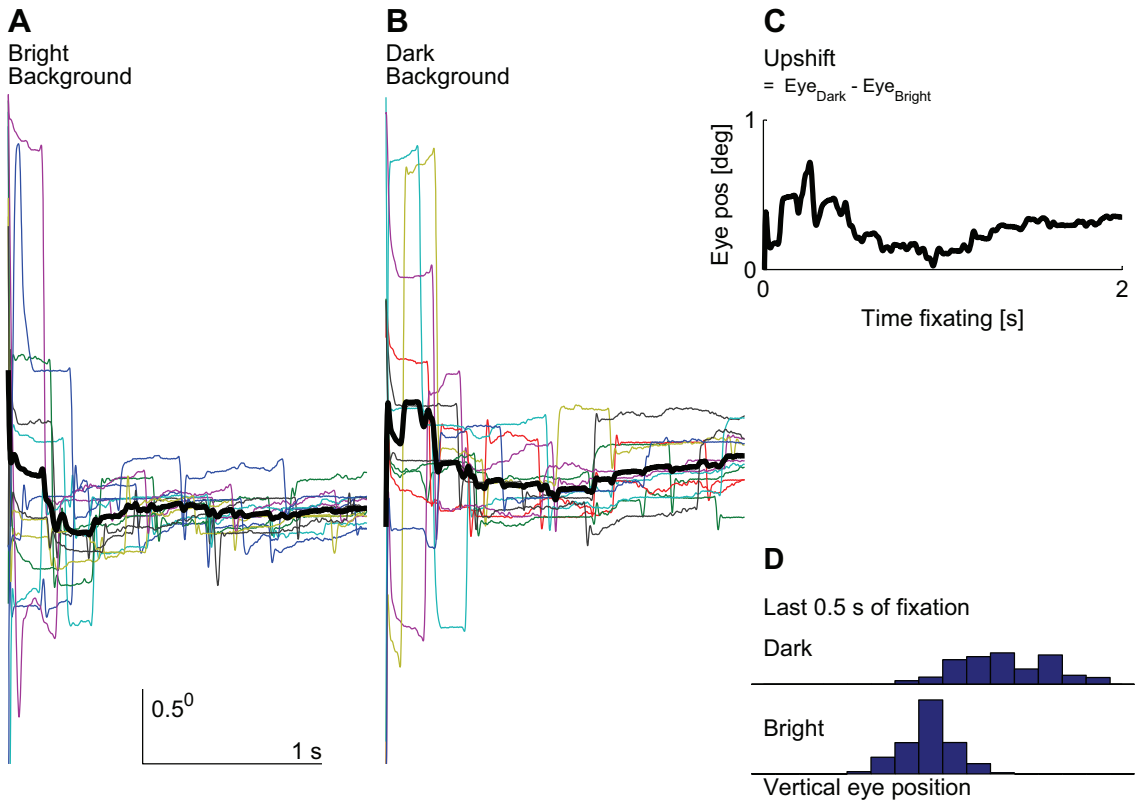

Fig. 2. Example vertical eye position traces of 1 monkey fixating 1 target with bright background (*A*) and dark background (*B*). The traces begin when the monkey's eyes get to within 3° of the target and continue from that epoch for another 2 s. Each thin colored trace shows the eye position of 1 trial; the thick black traces show the median eye positions, calculated for each millisecond. The traces in *A* and *B* are drawn on the same vertical scale. Note the correction saccades at the beginning of the traces in *A* and *B*, and the fixation eye movements that prevail during the residual fixations, with both bright and dark background. Note further that the bright background traces (*A*) are lower than the dark background traces (*B*). *C* shows the upshift, the time-dependent difference between the median traces for the dark and the bright backgrounds. Note that the trace of the upshift is not abolished during the fixation interval. *D* shows the vertical eye position values for the last 0.5 s of all trials, with dark background (*top* histogram) and bright background (*bottom* histogram). The means of the dark and bright histograms significantly differ.

session in which the target had appeared at 3.5° to the left, 3.5° above the center of the screen. Figure 2*A* shows trials recorded with bright background, and Fig. 2*B* 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. 2*A*. The

resulting time-dependent median is depicted in Fig. 2*A* as a thick black trace. A similar procedure yielded a time-dependent median for the dark-background (thick black trace in Fig. 2*B*). 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. 2*B*) is above the bright-background median (thick trace in Fig. 2*A*), but both median traces fluctuate. The precise relationship of the two median eye positions is captured in Fig. 2*C*, 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. 2, *A* 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. 2*C*). 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. 2, *A* and *B*. The results are shown in Fig. 2*D*, where the *x*-axis reflects eye position values. The entire *x*-axis of Fig. 2*D* 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. 2*B* (12 × 500 samples in total), and the *bottom* histogram shows the last 500 samples for each of the 13 trials illustrated in Fig. 2*A* (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 × 23 × 2 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 × 23 × 2 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 × 2 traces showing, for each monkey, the mean vertical

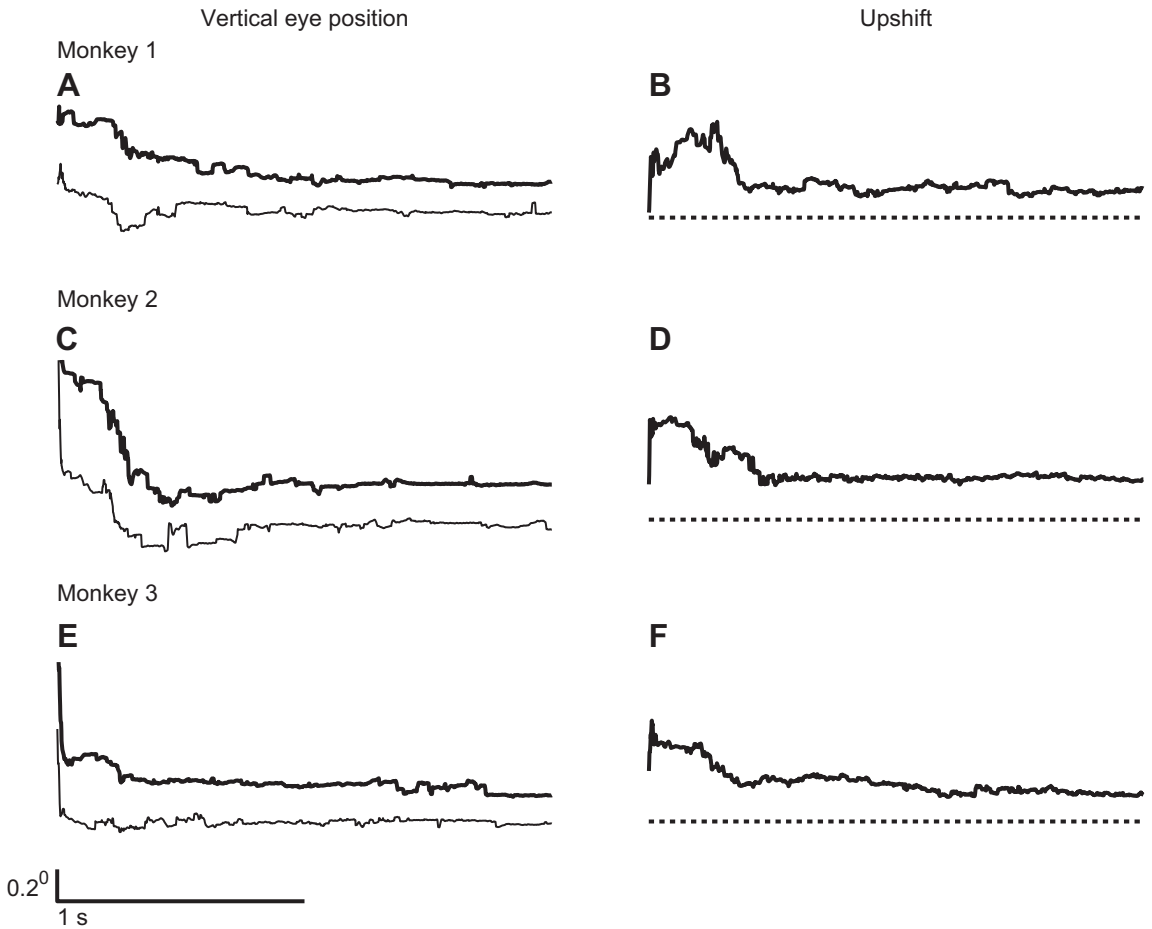

Fig. 3. Monkey-by-monkey analysis of the upshift. *A*, *C*, and *E* show the time-dependent mean fixation positions with bright background (thin traces) and dark background (thick traces). Each trace is the mean of the median traces calculated separately at each location, for each monkey, as illustrated 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 thus 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 m*onkey 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. 3*C*). 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 3×23 monkey × target location combinations have each yielded a time-dependent upshift trace, similar to the one illustrated in Fig. 2*C*. The mean of these 3 × 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 × 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 × 23 upshift functions at all monkey × 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).

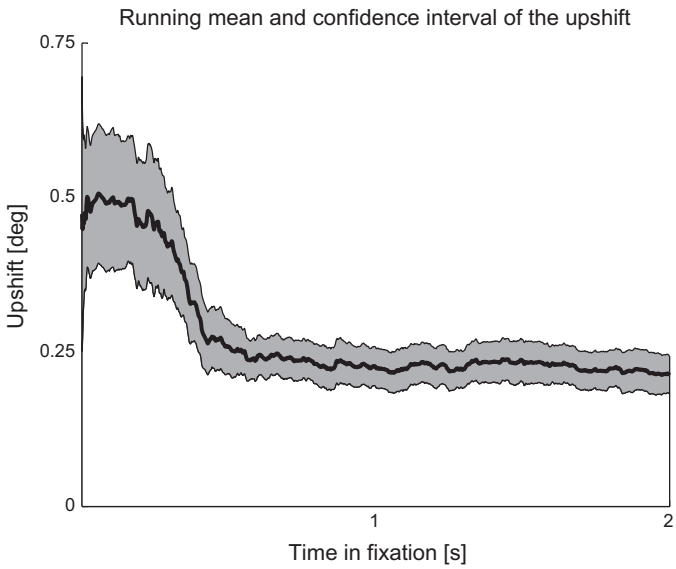

Fig. 4. Time-dependent running mean and 95% confidence interval for all the data pulled together. Note that the entire confidence interval remains stable, far from zero.

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

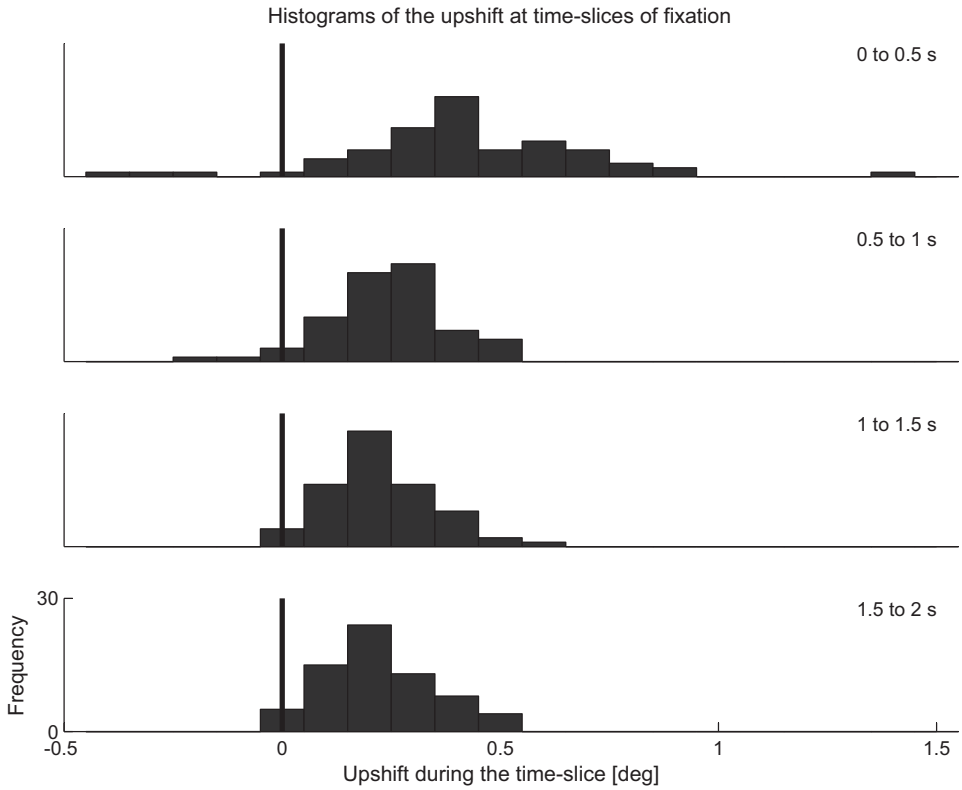

Fig. 5. Analysis of the time course of the distributions of the upshift performed by splitting the fixation interval into four 0.5-s time slices. The histograms reflect the values of the 3 × 23 upshift functions at all monkey × target location combinations during each time slice. Time in trial goes from *top* to *bottom*. After the correction saccades, reflected in the broader histogram at *top*, the histograms remain stable.

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.

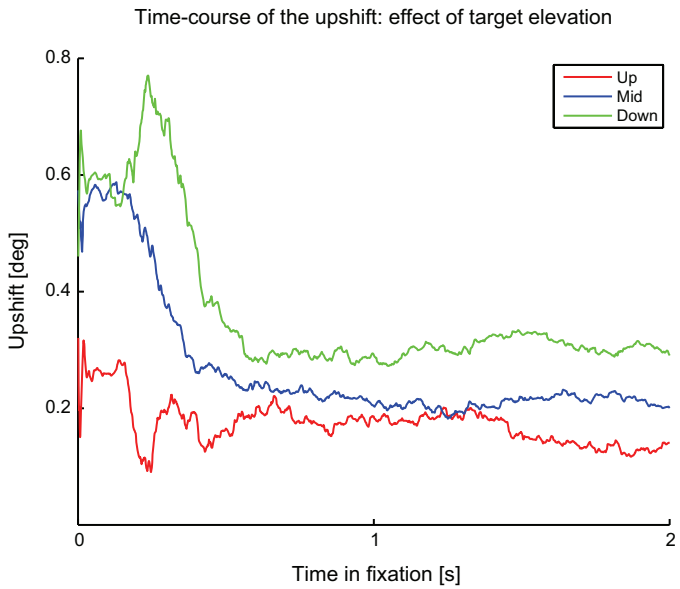

Fig. 6. Effect of target elevation on the time course of the upshift. The initial high value of the upshift, during the first 0.5 s of fixation, depends on the location of the fixated target. For upward locations, in particular, the upshift shows little if any initial high value. The persistent level of the upshift is also affected by the target elevation.

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. 2*C*). 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.

GRANTS

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

O.S. and P.T. performed experiments; O.S. and S.B. analyzed data; O.S., P.T., and S.B. interpreted results of experiments; O.S. and S.B. prepared figures; O.S., P.T., and S.B. edited and revised manuscript; O.S., P.T., and S.B. approved final version of manuscript; S.B. conception and design of research; S.B. drafted manuscript.

REFERENCES

Barash S, Melikyan A, Sivakov A, Tauber M. Shift of visual fixation dependent on background illumination. *J Neurophysiol* 79: 2766–2781, 1998.

Caggiano V, Pomper JK, Fleischer F, Fogassi L, Giese M, Thier P. Mirror neurons in monkey area F5 do not adapt to the observation of repeated actions. *Nat Commun* 4: 1433, 2013.

Cherici C, Kuang X, Poletti M, Rucci M. Precision of sustained fixation in trained and untrained observers. *J Vis* 1231: 1–16, 2012.

Cui J, Wilke M, Logothetis NK, Leopold DA, Liang H. Visibility states modulate microsaccade rate and direction. *Vision Res* 49: 228–236, 2009.

Curcio CA, Allen KA. Topography of ganglion cells in human retina. *J Comp Neurol* 300: 5–25, 1990.

Curcio CA, Sloan KR, Kalina RE, Hendrickson AE. Human photoreceptor topography. *J Comp Neurol* 292: 497–523, 1990.

Dash S, Catz N, Dicke PW, Thier P. Encoding of smooth-pursuit eye movement initiation by a population of vermal Purkinje cells. *Cereb Cortex* 22: 877–891, 2012.

Goffart L, Quinet J, Chavane F, Masson GS. Influence of background illumination on fixation and visually guided saccades in the rhesus monkey. *Vision Res* 46: 149–162, 2006.

Packer O, Hendrickson AE, Curcio CA. Development redistribution of photoreceptors across the *Macaca nemestrina* (pigtail macaque) retina. *J Comp Neurol* 298: 472–493, 1990.

Packer O, Hendrickson AE, Curcio CA. Photoreceptor topography of the retina in the adult pigtail macaque (*Macaca nemestrina*). *J Comp Neurol* 288: 165–183, 1989.

Snodderly DM. Effects of light and dark environments on macaque and human fixational eye movements. *Vision Res* 27: 401–415, 1987.

Snodderly DM, Kurtz D. Eye position during fixation tasks: comparison of macaque and human. *Vision Res* 25: 83–98, 1985.

Wikler KC, Williams RW, Rakic P. Photoreceptor mosaic: number and distribution of rods and cones in the rhesus monkey retina. *J Comp Neurol* 297: 499–508, 1990.

Appendix 2:

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Dark-habituation increases the dark-background-contingent upshift of gaze in macaque monkeys

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Running title: Monkey dark-induced upshift of gaze

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Abstract

What is the effect of prior experience on sensorimotor behavior? We studied the following intriguing behavior: monkeys fixating a small target direct their gaze above the target if the background is dark. We refer to the foveal line of sight. Fixating a target once on a bright background, then on a dark background, yields 2 gaze directions, typically one above the other; hence the name, ‘dark-background-contingent upshift of gaze', which is abbreviated as ‘upshift’. Is the upshift only an attempt to avoid using the fovea in the dark? If it is, we might expect to also observe a downshift and a sideshift. We studied gaze direction in a large group of 10 rhesus monkeys from Tübingen, to which we added published data from 4 cynomolgus monkeys from Rehovot. The upshift was ubiquitous, and there was no systematic sideshift. What is the function of the upshift? Is it related to vision in the dark? Here, we concentrate on the effect of the monkeys’ previous training. Seven of the 14 monkeys were accustomed to working in the dark (‘dark-habituated’), while the other 7 had worked in bright ambient light (‘bright-habituated’). The main result of this study is that the dark-habituated monkeys had a much larger upshift: the mean upshift was 2.2° in the dark-habituated monkeys, versus 0.5° in the bright-habituated. Thus, the upshift depends on habituation; the size of the upshift reflects months-long cumulative experience. These findings suggest that the function of the upshift is indeed related to seeing in the dark.

1 Introduction

1.1 The foveation rule and scotopic vision

The role of saccadic eye movements and fixations is usually thought to be 'foveation'. Foveation consists of directing the target image to the fovea and keeping the target stationary enough on the fovea while vision occurs. That foveation is the outcome of fixation and saccades can be called the 'foveation rule'. However, is foveation the sole function of fixation and saccades? More specifically, does the importance of foveation for vision of bright scenes generalize to situations with less intense ambient light? In scotopic vision, there are few rods in the fovea, and none in the foveola. Foveating the target in scotopic vision does not immediately make sense. Is there any evidence that foveation does not fully explain saccades and fixation?

1.2 The dark-background-contingent upshift appears to break the foveation rule

One apparent exception to the foveation rule appears to involve monkeys who fixate on small targets superimposed on a dark background. Monkeys turn their eyes so that the foveal line of gaze is directed *above* the target rather than at it (Snodderly, 1987; Barash et al., 1998; Spivak et al., 2014). This effect is called the dark-background-contingent upshift; for short, we will call it 'upshift'. Fig 1 schematically illustrates this effect. Note that the target spot is identical in both conditions; only the background differs, but the different background is enough to shift the gaze directions upwards. The fact that the gaze direction changes while the target remains unchanged is inconsistent with the foveation rule.

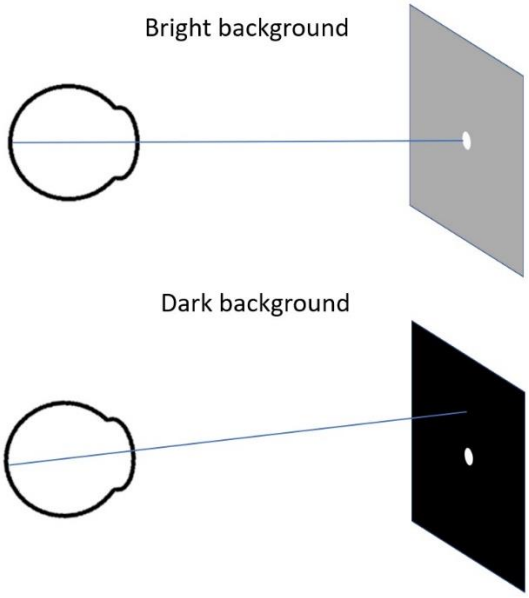

Figure 1: Schematic illustration of two fixation conditions: with a bright or a dark background. Fixation with a bright background results in precise foveation of the target on the screen: the blue line of gaze is directed at the target. A dark background elicits an upward gaze shift, and the line of gaze is directed above the target.

1.3 The issue of habituation appeared from studying individual variation

The early studies, which showed that the upshift exists, did not take on the question of how general the upshift is. Do all monkeys show upshift of the same size? Although all the monkeys in our early study showed a large upshift after training (Barash, Melikyan, Sivakov, & Tauber, 1998), one subsequent study of eye movements in the dark reported little if any upshift (Goffart, Quinet, Chavane, & Masson, 2006). When we started to study the upshift in Tübingen, we were struck by the individual variability of the size of the upshift that we observed in the Tübingen monkeys. What caused this variability? We initially planned to study the individual variability of the upshift. However, it gradually became clear that the predominant factor that influenced the size of the

upshift was the monkey's prior training. More specifically, the monkeys that we tested were habituated, for months or years, to working either in bright background or in dark background. It appeared to us that the monkeys habituated to work in the dark had a large upshift that was similar in size to the Rehovot monkeys (Barash et al., 1998), whereas the monkeys habituated to work in bright light had smaller upshift.

The effect of the habituation on the upshift surprised us. The upshift appears to be an automatic response to the ambient light level. Why would this automatic response be modulated by the monkey's long-term experience? The effect of habituation appeared to dominate the response variability, so much so that it transpired that the effect of habituation must be described prior to any study of individual variability. We thus set out to describe the effect of habituation and the result is documented in the present manuscript.

Although the existence of the upshift was already confirmed prior to this study (Barash et al., 1998; Snodderly & Kurtz, 1985; Snodderly, 1987; Spivak, Thier, & Barash, 2014), we decided to view confirming the existence of the upshift as a main objective, or a main hypothesis to be tested. The most interesting and pertinent case is that of testing for the presence of the upshift in the bright-habituated monkeys (see ahead); because the upshift in these monkeys is small, showing that the upshift's existence is statistically significant is called for. In the other cases, the hypothesis that the upshift exists is useful too, because it unifies the separate cases, and because showing the existence integrates well with evaluating the size of the upshift in the various cases considered, habituation cohorts, Tübingen monkeys only versus the entire sample, etc.

1.4 A relationship to rod-density?

What could be the function of the upshift? A first possible explanation for the upshift could be that of avoiding the fovea because the fovea has low rod content. This observation alone is not sufficient to explain the upshift because fixations could occur all around the fovea in all directions to avoid the fovea. However, the data show that fixations in a dark background predominantly shift in the same direction; namely, upwards. Why do they shift upwards? The geometry of rod density appeared to provide a clue. Rods are denser in the dorsal retina. A well-defined location of maximal rod density is located dorsal to the fovea at an eccentricity approximately that of the blind spot. This location is called dorsal rod peak (DRP) or rod hotspot. (Packer et al., 1989; Wickler and Rakic, 1990; Wickler et al., 1990). Thus, a 'rod-peak hypothesis' was suggested; namely, the upshift reflects vision via the DRP/rod hotspot rather than via the fovea (Barash et al., 1998). However, this rod-peak hypothesis leads to an experimental prediction that fails.

More specifically, the rod-peak hypothesis implies that, in the presence of upshift, vision is mediated by rods. However, rods are activated only after lengthy dark adaptation (Hecht, Haig, & Chase, 1937; Reuter, 2011). Right after switching from a bright ambient environment to darkness, rods remain saturated for many minutes. Nonetheless, at least for some monkeys, the upshift starts via a small upward saccade that occurs within 1 s from the onset of a dark background (Barash et al., 1998). One second after the offset of the bright background, the rods are all saturated. More generally, lengthy dark adaptation was not implemented in most of the upshift studies to date. For this straightforward temporal rationale, the rod-peak hypothesis must be rejected. Upshift might be present in scotopic vision (Spivak, Thier, & Barash, 2018); regardless of whether it does, vision via rods is certainly not a necessary condition for the presence of upshift. Upshift is present long before the time-course of dark adaptation reaches rod-mediated vision.

With the rod-peak hypothesis rejected, we will briefly relate in the Discussion to the question whether the findings of the current study could relate to the function of the upshift.

1.5 Hypotheses

For the sake of clarity, we formalize the hypotheses of the current study.

Hypothesis 1. (a) A salient upshift exists. **(b)** There is no systematic analogous horizontal shift ('sideshift').

For Hypothesis 2, we need an additional definition. In accordance with the description above, a monkey is considered dark habituated if the monkey was trained during the months preceding the testing in a dimly lit or dark room with visuomotor tasks involving mostly small spots of light. A monkey is considered bright habituated if the monkey trained in a room with standard bright lighting with tasks requiring full photopic vision during the months preceding the testing.

Hypothesis 2. Extensive training in the dark in recent months significantly increases the size and prevalence of a monkey's upshift. In dark-habituated monkeys, the upshift is larger and more prevalent than in bright-habituated monkeys.

1.6 Terminology

To be consistent with past terminology, we discuss the upshift rather than the vertical shift. On the rare occasions in which the fixated foveal gaze in a dark background is directed below that in a

bright background, we discuss the ‘downshift’ or ‘negative upshift’. To emphasize that the upshift is indeed directed upwards, we occasionally refer to a ‘positive upshift’. In the horizontal direction, we discuss ‘sideshift’, ‘left-shift’, or ‘right-shift’, as appropriate. Unqualified ‘shift’ or ‘vectorial shift’ refers to the vector of both horizontal and vertical components, see ahead.

1.7 Two labs, 2 species

As part of a larger project, we studied in Tübingen the upshift of 10 *Macaca mulatta* monkeys. We have enclosed data from 4 *Macaca fascicularis* monkeys previously studied in Rehovot. Since the Rehovot study differed from the Tübingen study, we first analyzed the Tübingen data alone and only then the entire sample. We present the complete data because it is the more significant data. However, we also provide the data from 10-monkey Tübingen sample to show that the conclusions still hold. In this way, we extend the analysis to a total of 14 monkeys of 2 different species that were studied in 2 laboratories with slightly different apparatus and procedures. The consistency of the results of the 2 studies suggests that the properties of the upshift are robust.

1.8 Preliminary reports

The experimental procedure and analysis were previously described in detail (Barash et al., 1998; Spivak et al., 2014). Preliminary reports of the results have been published (Spivak, Thier, Barash bioRxiv (2018):290759 and poster presentations, at the Society for Neuroscience (Spivak, Thier, & Barash, 2016) and at the Primate Neurobiology Meeting 2019 in Goettingen.

2 Materials and methods

2.1 Database

In Tübingen, we used 10 rhesus monkeys in this study (specified as T1 to T10). The monkeys had been previously trained in various oculomotor tasks. Some monkeys were used in electrophysiological recordings unrelated to this project. The other 4 *Macaca fascicularis* monkeys (R1 to R4) were previously studied in Rehovot. The results were published; here we used data included in the paper (Barash et al., 1998). All monkeys were male.

The procedures for the Rehovot monkeys were published (Barash et al., 1998). Therefore, here we will describe mostly the procedures related to the Tübingen monkeys.

2.2 Animal preparation

All experimental procedures are standard and have been described in detail (Caggiano et al., 2013; Dash, Catz, Dicke, & Thier, 2012; Spivak et al., 2014). In brief, gaze direction was recorded using the scleral search coil method (Fuchs & Robinson, 1966; Judge, Richmond, & Chu, 1980). The monkeys were prepared for neurophysiological recordings (not part of the present study); their heads were painlessly immobilized by titanium head posts. Surgeries were performed under intubation anesthesia with isoflurane and nitrous oxide, supplemented by continuous infusion of remifentanil (1–2.5 (micro)g/kg^{-1}·h^{-1}) with full tight control of all relevant vital parameters. 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).

2.3 Experimental setup

The experiments were conducted in a lightproof electrophysiological setup. The monkeys were seated in front of a cathode ray tube (CRT) screen 30-40 cm from the screen center. The CRT was an Eizo Flexscan F730 with a 50-cm diagonal screen that displayed 1024x768 pixels at a frame rate of 60 Hz. The only source of light during the experiment was the monitor that the monkeys were facing. Gaze direction (eye position) was sampled at 1000 samples per second.

2.4 Paradigm and training

A trial began with the appearance of a small circular target spot at an unpredicted position on the screen. The size of the target was 0.02-0.1°. The monkeys had 1 s to make a saccade to the invisible fixation window centered on the target and to maintain their eyes within the fixation window for 1.5-2 s. The size of the window was small (2-3° radius) in bright-background trials and larger (5-15° radius) in dark-background trials so that shifted fixation would not be interpreted by the computer as a fixation failure (Spivak, Thier, & Barash, 2018); also, see the Results section. The invisible fixation window was symmetric around the scotopic target so that any shift, upwards or otherwise, would have the same opportunity to be detected. Upon error, the trial was aborted, and a new trial started; upon correct performance, the monkey was rewarded with a drop of water. The error rates, specifically, rates of premature fixation-breaks out of the invisible windows, occurred with similar frequencies in the bright-background and dark-background trials.

2.5 Visual stimuli in the blocks of a daily session

Target locations were arranged on 3 concentric circles of 5°, 10°, and 15° radii. In the Rehovot data, the outer circle had a 20° radius. Thus, the targets spanned the central region of the oculomotor range around the base position of the eye in the orbit. The location of every target was selected in a randomly interleaved order, with 1 target per trial. The experimental session consisted of a series of alternating blocks of dark-background and bright-background trials, usually with 120 trials per block. Every experimental session began with a standard gaze direction calibration using a bright background. The luminance of the target was set to 70 cd/m^2; however, in most cases, it was very small (0.02°) and only spanned a few pixels. The bright background was set at 7 cd/m^2, while the dark background was set at such a level that for humans sitting in the experimental chamber for up to 3 hours, the dark-background monitor remained completely indiscernible.

2.6 Database

A block of trials consisted of trials with each of the 24 target locations repeated 3-5 times. The experimenter determined the number of repetitions according to the monkey's performance each day. Only hits were counted; the proportion of errors (premature fixation breaks) was similar in bright- and dark-background conditions. A daily session consisted of an average of 7.3 ± 2.6 blocks. Bright and dark backgrounds were switched between blocks. The number of daily sessions was: T1, 15; T2, 20; T3, 11; T4, 4; T5, 11; T6, 7; T7, 34; T8, 2; T9, 3; and T10, 4. For a description of the database for the Rehovot experiment, see Barash et al., 1998.

2.7 Analysis

To calculate the mean gaze direction during visual fixation that represents each trial (Fig 2), we took the average across the interval of the last 1 s of the trial. Although the trial duration was variable, by considering the last second of fixation, we ensured that at least 0.5 s had elapsed from the time the eye entered the fixation window until the start of the time interval over which the gaze direction samples were taken for the calculation. Hence, during the 1-s interval used to calculate the trial's representative fixation position, all correction saccades were already completed, and the eyes were stably fixating. Trials with an atypical gaze direction (less than 0.5%) were excluded from the analysis. For every monkey and every target location, we subtracted the mean bright-background gaze direction, namely, the mean gaze direction of all bright-background trials, from each trial. Thus, the mean bright-background gaze direction is equivalent to an improved calibration of gaze direction while the monkey fixates on the pertinent target. This procedure allows calculation of the vectorial shift for each trial, regardless of its visual background, which reflects the trial's mean gaze direction compared to the improved calibration. See also (Spivak et al., 2018).

Thus, each trial had a background that was bright or dark and yielded upshift and sideshift values. We used these values to compute 4 histograms with 2 background states (dark and bright) for the upshift and sideshift dimensions for each monkey. Then, we summarized the histograms of the individual monkeys to yield the corresponding 4 histograms of the entire sample and the habituation subsamples. Each monkey contributed equally to the all-monkey histograms. We also developed 4 histograms each for the bright-habituated monkeys alone and the dark-habituated monkeys alone by restricting the summation to the members of each group. Within these groups, all members contributed equally. All analyses were performed using Matlab.

To define the reverse probabilities, it is useful to denote the histograms as XB_Y (upshift), where XB indicates either DB, which stands for the histogram of the dark background as illustrated in Fig 3, or BB, which stands for the histogram of the bright background. If the formula applies to a habituation group, it includes a Y, representing DH and BH, which are abbreviations for dark habituated and bright habituated, respectively. Assuming equal probabilities of DH and BH, given a trial with an upshift u, the probability this trial had a dark background is $(DB|u) = \frac{DB(u)}{DB(u)+BB(u)}$. The probability that the trial had a bright background (red trace in Fig 4) is $P(BB|u) = 1 - P(DB|u)$. The probabilities in Fig 7 are defined correspondingly. The black trace is $P(DB\ \&\ DH\ |u)$, which equals the product, $P(DH|u) \cdot P(DB|u)$. Because $P(DB|u)$ is nearly 1 for high values of *u*, the black trace is nearly equal to the cyan trace.

3 Results

3.1 Procedure in brief

The experimental procedure and analysis were previously described in detail (Barash et al., 1998; Spivak et al., 2014) (see also the Methods section). In brief, we tested 10 rhesus monkeys in Tübingen in the upshift task that will shortly be described. The monkeys were trained in other tasks for a long time before they participated in the present study. A daily session started in a bright-background condition. Gaze direction was first calibrated (see section 3.2). Then, blocks of fixation trials were run. There were 2 regimens, namely, bright and dark. The targets – very small bright spots – were identical in the bright-background and dark-background blocks. In a block with a bright background, the background was gray in the low photopic range (see the Methods sections for details). In a block with a dark background, the background was dark enough that human

observers could not see it after 1 h of dark adaptation. The room was light tight; the computer screen in front of the subject was the only light source present. In the dark-background condition, only the target spot was visible.

3.2 The scleral search coil is almost optimal, but requires calibration

Gaze direction, namely, the position of the eye in the orbit, was measured using scleral search coils with wires attached to a connector fixed to the skull (Fuchs & Robinson, 1966; Judge et al., 1980). This is the standard method for recording gaze direction in monkeys. Gaze direction is often called eye position. The scleral search coil has been used for decades in many laboratories. Compared with current methods such as video eye tracking, the measurements obtained with scleral search coils are stable and robust because the coil is implanted onto the eye eyeball and it adheres to the sclera via granulation tissue. As a result, the scleral search coil signal reflects the movements of the eyeball with high fidelity. The measurements are stable in space because the monkeys were fixed with a skull-based head holder. Another advantage of scleral search coils, which is crucial in the context of recording gaze direction in the dark, is that they give rise to gaze-direction signals that are independent of pupil size. In contrast, video-based eye trackers and other methods used in humans depend on pupil size and are prone to artifacts.

Nonetheless, scleral search coil require calibration. The standard method of calibration relies on the natural accuracy of gazing at small targets. Thus, targets in several screen locations are presented, and the search coil signal is recorded while the monkey appears to fixate each of the targets. Based on these measurements, an interpolating/extrapolating procedure is introduced for general screen locations. This standard procedure typically gives quite good results. In the case of

our study, which included many fixations, we could use these fixations to improve the calibration post hoc.

Bearing the calibration procedure in mind is critical for understanding the upshift. Performing the calibration procedure in bright background and in dark background would yield seemingly incoherent results. The upshift explains this seeming incoherence, but if one is unaware of the upshift the results would appear paradoxical.

3.3 Vision was not scotopic

We mentioned above that we started the daily sessions with a short sequence of one, or occasionally a few, bright-background blocks. The dark-background blocks were generally run almost immediately after the bright-background blocks. Thus, even during the dark-background blocks, vision was almost certainly photopic; nonetheless, we cannot rule out that some components of the rod response may have been present. In any case, the data analyzed in the current study were obtained predominantly during the initial leg of the dark-adaptation time course while the rods were mostly still saturated.

3.4 Relating gaze direction to the target

For each trial, we calculated a mean gaze direction by averaging each of the horizontal and vertical gaze direction components over the 1000 samples taken during the last 1 s of fixation in a particular trial. For each daily session, for each of the 24 target locations used in this study, we calculated the mean bright-background and mean dark-background gaze directions. The mean bright-background location is an improved calibration for gaze direction at the relevant location. We therefore

calculated vectorial shifts, namely, the differences in each trial's gaze direction from the mean bright-background gaze direction of the trial's target.

Fig 2 illustrates the shifts. There are 200 red dots; 20 dots were selected at random from the trials of each Tübingen monkey. A red dot reflects a trial with a bright background. The dots aggregate close to (0,0), which is unsurprising because the definition of a shift is the difference in the gaze direction of an individual trial from the mean gaze of all bright-background trials with the same target. In other words, (0,0) reflects the mean gaze direction with which the relevant target is fixated with a bright background. The deviation of the red dots from (0,0) reflects the well-known trial-by-trial variability of fixation positions even in bright light (Cherici et al., 2012).

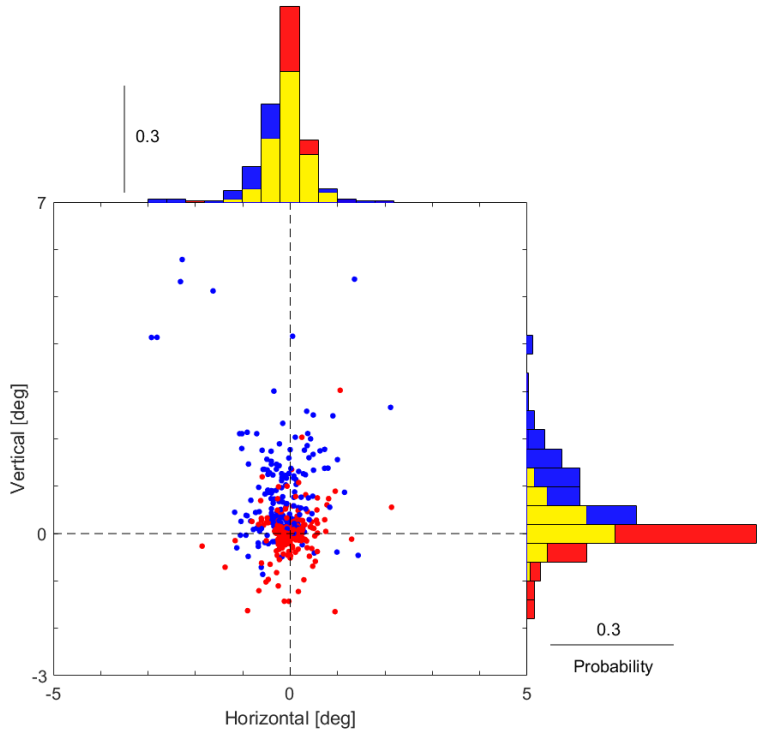

Figure 2: Scatter plot illustrating the shift in the mean eye position while fixating on bright (red dots) and dark (blue dots) backgrounds for all 10 Tübingen monkeys pooled together (20 dark-

background and bright-background trials for each monkey; 200 dots for each background in total). Each dot indicates the difference between the mean eye position during the fixation in one trial and the location of the fixation target. Histograms of the vertical and horizontal components are displayed on the right and the top of the scatter plot, respectively. Yellow bins depict the overlap between red and blue histograms.

Similarly, Fig 2 also contains 200 blue dots; a blue dot reflects the mean gaze direction in a single trial with a dark background. For the blue dots of Fig 2, 20 trials were selected at random from the trials of each Tübingen monkey. It is evident that (1) the blue dots concentrate above (0,0), and (2) the blue dots are more scattered than the red dots. The upshift is illustrated by the tendency of the blue dots in the scatterplot to be above the red dots.

The sideshift and upshift histograms for the blue and red dots in the scatterplots are displayed on top and on the right of Fig 2's scatterplot, respectively. Yellow bins denote overlapping bin regions. Thus, the histogram of the bright-background upshift is the union of the red and yellow bins on the right margin. Similarly, the histogram of the dark-background sideshift is the union of the blue and yellow bins on the top margin.

In the following subsections of the Results section, histograms similar to those shown in Fig 2 will be studied. However, they are not based on 200 random trials. Rather, they are based on all the trials of all monkeys pertinent to the current issue. All the monkeys contributing to a histogram always contribute equally; they are based on normalized histograms, with an area of 1, for each monkey. Thus, all trials contribute to the population measures; yet, the contribution of each trial is scaled so that all monkeys contribute equally.

3.5 Hypothesis 1A: Upshift is ubiquitous

Fig 3 shows the population histograms of the upshift computed from the combined 14-monkey sample. The red and blue histograms shown in Fig 3 reflect the foveal gaze direction while fixating on targets with a bright background (red histogram) or a dark background (blue histogram). The blue histogram differs from the red histogram in measures of central tendency and shape. The mean of the red histogram is zero, reflecting the monkeys' tendency to orient the fovea directly on the target as well as the definition of the upshift as a deviation from the mean bright-background gaze direction (see the Methods section). The mean of the blue histogram is 1.4°, with a zero probability of the mean being 0° (t-test). Thus, the probability of the histograms having the same mean is 0 (t-tests). The blue histogram is shifted to the right and has widened: the standard deviation of the red and blue histograms is 0.47 ° and 1.5°, respectively, and the interquartile range is 0.4° and 2°, respectively.

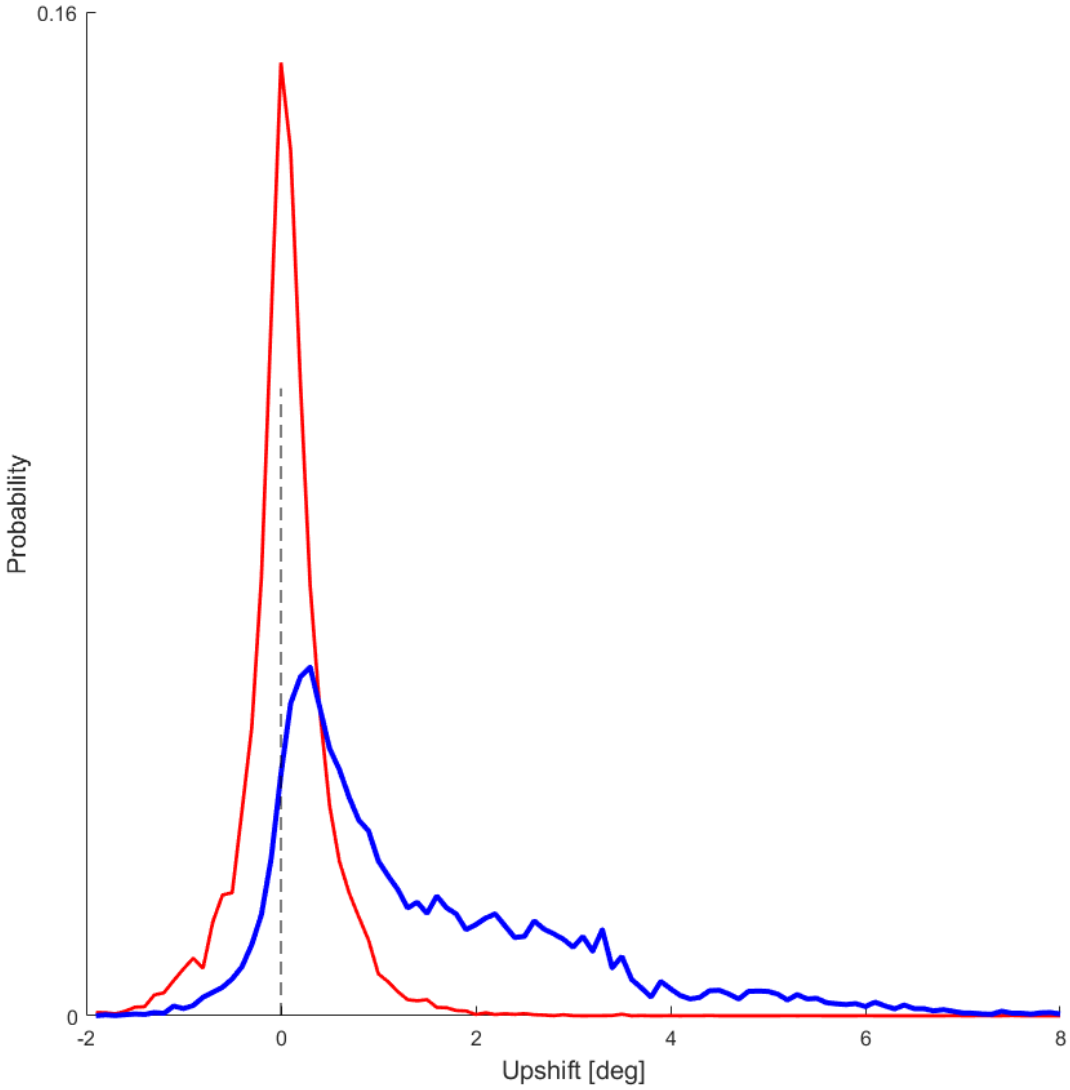

Figure 3: Trial-by-trial analysis of the entire sample. Cumulative histograms of the upshift of the entire 14-monkey sample. Red reflects a bright background, and blue reflects a dark background. All monkeys contributed equally to the histograms. The histograms show a salient upshift.

How likely is a trial to show a (positive) upshift? The histograms in Fig 3 show that this depends on whether the background was dark or bright. Indeed, a positive upshift was present in 85% of the dark-background trials but only 42% of the bright-background trials. (These frequencies reflect the

area under the red and blue curves in Fig 3, which is located to the right of the vertical dashed line at shift = 0).

Thus, in the dark-background condition, most trials showed a (positive) upshift. In the bright-background condition this was not observed. The trial-by-trial variability of the size of the upshift is greater in the dark-background condition than that in bright-background condition.

3.5.1 Hypothesis 1A, reverse direction: from a trial's upshift to the trial's background

Given the upshift of a single trial, how robustly can we predict whether the trial was performed with a dark or bright background? The answer depends on the a priori probabilities. Assuming that bright and dark backgrounds are equally probable, the histograms in Fig 3 are transformed into the likelihood functions in Fig 4 (see the Methods section for definitions). The blue trace in Fig 4 shows the likelihood that the trial had a dark background for the upshift of a given trial. The red trace similarly shows the likelihood that the trial had a bright background. For any value of the upshift, the red and blue functions are complementary; namely, the sum of these likelihoods is 1.

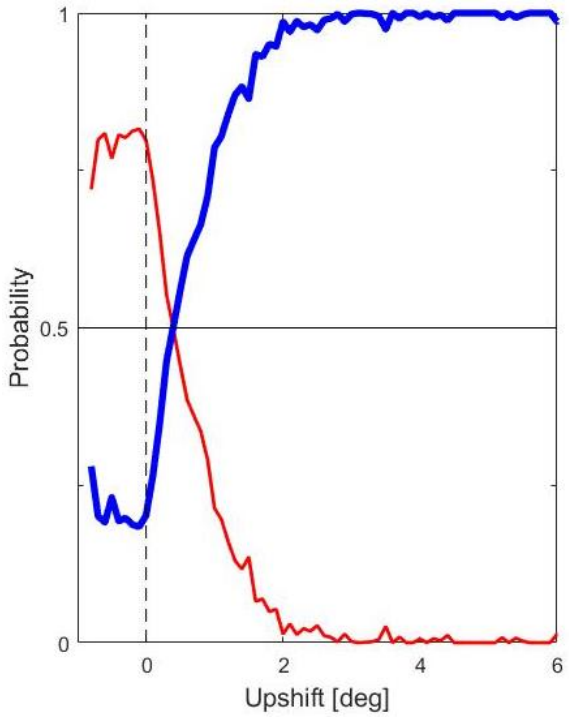

Figure 4: Likelihood functions reflecting the probability of a trial with a given upshift to have had a bright background (red trace) or a dark background (blue trace).

Trials with a negative or zero upshift are likely to have had a bright background. For increasing values of the upshift, the likelihood of the background being dark gradually decreases (red trace in Fig 4). The likelihood of the background being dark increases almost to 100% (Fig 4, blue trace). The upshift most likely to reflect a bright background was -0.1°. For this downshift, the likelihood of a bright background was 81%. A bright background and a dark background were equally likely given a trial with an upshift 0.3°. With higher upshift values, the blue trace rapidly increases, crossing 95% at an upshift of 1.8° and 99.9% at an upshift of 2.7°. In principle, beyond 3° this could be an artefact transpiring from the size of the gaze direction invisible window in bright-background trials, but this is highly unlikely to happen because gaze direction remains close to the target in most bright-background fixation trials even with large invisible windows. Thus, an upshift

well larger than the ordinary variation of bright-background fixations is highly likely to originate in a dark-background trial.

3.6 Hypothesis 1b: No sideshift

Fig 5 shows histograms of the sideshifts computed from the mixed sample of all 14 Tübingen and Rehovot monkeys. Both the blue and red histograms in Fig 5 appear symmetrically distributed around zero, with the blue histogram being slightly broader than the red histogram. Indeed, the mean is 0° for the red histogram and -0.06° for the blue histogram. The standard deviation is 0.46° for the red histogram and 0.68° for the blue histogram. The null hypothesis, stipulating that the mean sideshift in a dark background was zero, could not be rejected ($p = 0.5$), with a 95% confidence interval from -0.19 to 0.07. Thus, the histograms show that there is no systematic horizontal shift.

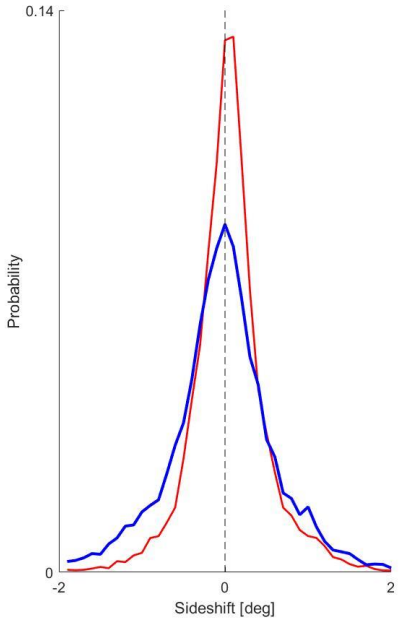

Figure 5: Histograms of the sideshifts. The histograms show no systematic sideshift.

3.7 Hypothesis 2: Habituation

3.7.1 Partitioning of the monkeys by their habituation

We divided the Tübingen monkeys into 2 ‘Tübingen cohorts’, according to the monkeys’ previous recent training. Monkeys T7, T9, and T10 were accustomed to working in dark setups on tasks such as saccadic adaptation. These monkeys were considered to be ‘dark habituated’. The other monkeys worked in setups with bright backgrounds. They were considered to be ‘bright habituated’.

According to these criteria, all the Rehovot monkeys were dark habituated. Because there were no bright-habituated Rehovot monkeys, Hypothesis 2 could not be tested independently on the Rehovot data. Nevertheless, we could define full cohorts combining the data from Tübingen and Rehovot. The full dark-habituation and bright-habituation cohorts thus consisted of all dark-habituated and bright-habituated monkeys, respectively. Because both comparisons of the Tübingen-only cohort and mixed Tübingen-Rehovot cohort yielded similar results, we surmise that the differences between the habituation cohorts do not reflect differences in results between the 2 labs but between the types of habituation.

3.7.2 Comparing the upshift in the habituation groups

Fig 3 illustrates the results of the analysis of the entire sample. We applied the same analysis to each habituation group. Fig 6 illustrates the results. Thus, there are twice the number of traces in Fig 6 than in Fig 3. For example, instead of a single trace illustrating the histogram of the upshift of the combined sample in a dark-background condition (blue trace in Fig 3), Fig 6 comprises 2 traces of the histograms of the upshift of the dark-habituated sample (thick blue trace) and the

upshift of the bright-habituated sample (thin blue trace). The same relationship holds for the red traces.

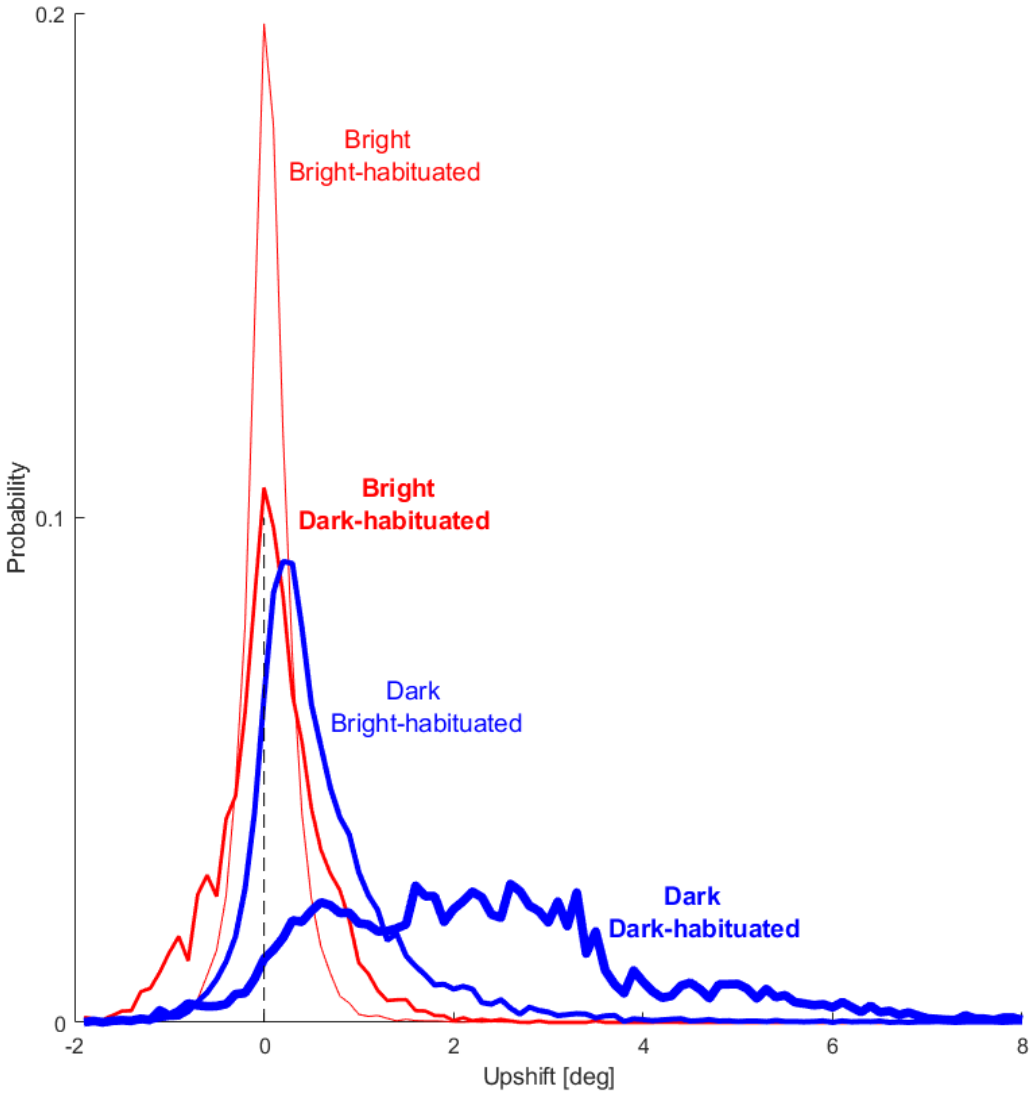

Figure 6: Cumulative histograms of the upshift of the two habituation groups. Thick traces reflect the dark-habituated group, and thin traces represent the bright-habituated group. As previously described, red reflects a bright background, and blue reflects a dark background. Each monkey contributed equally to its group. The histograms show that the dark-background upshift differs significantly between the two groups. The bright-background histograms are similar.

First, Hypothesis 1a is corroborated separately for each habituation group. The upshift histograms of both bright-background habituation groups are zero. Hypothesis 1b also holds for both habituation groups when considered separately: neither has a sideshift (not shown).

The main result of this paper is as follows. At issue is the effect of the habituation on the upshift. To clarify this effect we must compare the dark-background upshift in the dark-habituated group (thick blue trace of Fig 6) and the bright-habituated group (thin blue trace). The thick blue trace differs strikingly from the thin blue trace. Thus, in a dark background, the upshift depends on the monkey's habituation.

In more detail, the mean ± standard deviation of the thin blue and thick blue histograms is 0.5±0.8° and 2.2±1.7°, respectively. The median of the thin blue and thick blue histograms is 0.3° and 2°, respectively. The interquartile range for the thin blue and thick blue histograms was 0.8° and 2.2°, respectively. The thick-blue histogram not only shifted to higher upshift values; it is also wider than the thin blue histogram. Perhaps the most significant difference is in the fraction of trials with a positive upshift. This fraction is depicted in Fig 6 by the area under the histogram traces to the right of the dashed vertical line at upshift = 0. In the bright-habituation group (thin blue trace), 77% of the trials had a positive upshift. In the dark-habituation group, 93% of the trials had a positive upshift. Thus, a monkey's habituation dramatically influences the likelihood of a dark-background trial showing a positive upshift.

3.7.2.1 Reverse direction: Guessing the monkey's habituation from the upshift of a given trial

Given the upshift of a single dark-background trial, can we reliably estimate whether the trial was performed by a bright-habituated or a dark-habituated monkey? The answer depends on the a priori probabilities. For the sake of this discussion, we assume that the two habituation states are equally likely to occur. This is a useful assumption for the hypothetical case in which the monkey's habituation might be any possible state but is unknown.

The line of thought is similar to that illustrated for bright and dark backgrounds in Fig 4. Assuming that the bright and dark backgrounds are equally probable, the histograms in Fig 3 are transformed into the likelihood functions in Fig 7. For a given upshift, the value of the cyan function at the given upshift is the likelihood that the monkey was habituated to the dark. The brown function is the likelihood that the monkey was bright habituated. At any value of the upshift, the sum of the likelihoods is 1.

The brown likelihood function peaks at a 0° upshift, with a value of 85%. Then, the brown function descends, and the cyan function ascends. They cross each other at a 1.25° upshift. The cyan curve exceeds 95% at an upshift of 3.7°. At a 3.8° upshift, the likelihood is 97%. Thus, a single dark-background trial with an upshift of 3.8° almost certainly comes from a monkey that is habituated to working in the dark.

We can now combine the computations illustrated in Figs 4 and 7 to try to infer both the habituation group and the background of the trial in which the upshift was measured from the upshift of a single trial. We assume equal probabilities for all 14 monkeys for habituation states and visual bright and dark background levels. The black curve in Fig 7 illustrates the likelihood function. Close to a zero

upshift, the black trace is very low (3.2% at -0.1°). Thus, a trial with no upshift has an approximately 96% likelihood of not being a dark-background trial of a dark-habituated monkey. Any trial with almost no upshift almost certainly either had a bright background or was recorded from a bright-habituated monkey. At high upshift values, the very high values of the blue curve in Fig 4 have the effect of making the black trace almost identical to the cyan curve in Fig 7. Thus, a trial with a high upshift is very likely to be a dark-background trial of a dark-habituated monkey. (For example, for a 3.8° upshift, the likelihood is 93.5%, and the blue function in Fig 4 is exactly 1).

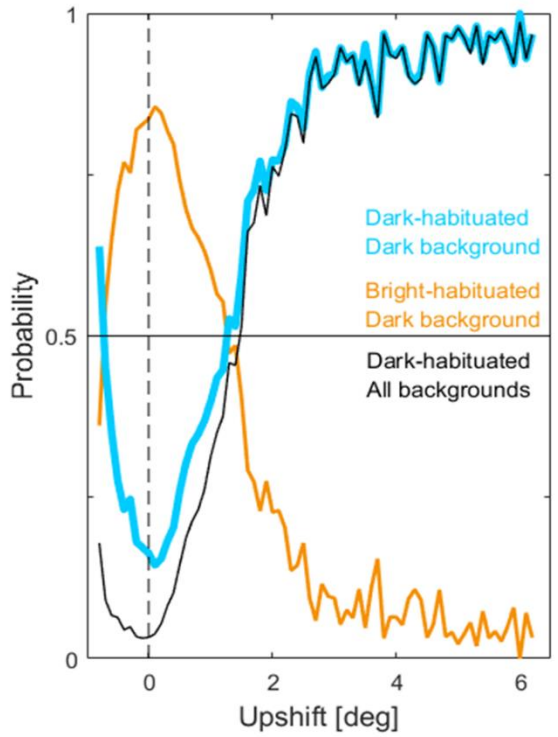

Figure 7: Likelihood functions reflecting the probability of a trial with a given upshift *and dark background* to be from a bright-habituated monkey (brown trace) or dark-habituated monkey (cyan trace). The black trace reflects the combined likelihood of a trial with a given upshift to have had a dark background *and* be from a dark-habituated monkey.

3.8 Control: analysis of the 10 Tübingen monkeys alone

Do the differences between the dark-habituated monkeys and the bright-habituated monkeys indeed reflect different habituation? Four of 7 dark-habituated monkeys came from Rehovot, but no bright-habituated monkey did. Could the difference between habituation groups reflect differences between Rehovot and Tübingen?

To investigate this possibility, we calculated the population analysis separately on the Tübingen monkeys. We compared the upshift histograms in the 3-monkey dark-adapted group to those in the 7-monkey bright-adapted group. The study could not be repeated separately on the Rehovot monkeys because there were no bright-habituated Rehovot monkeys. Nevertheless, we surmise that if there were crucial differences between the Rehovot and Tübingen cohorts, they would have been revealed by removing the Rehovot data.

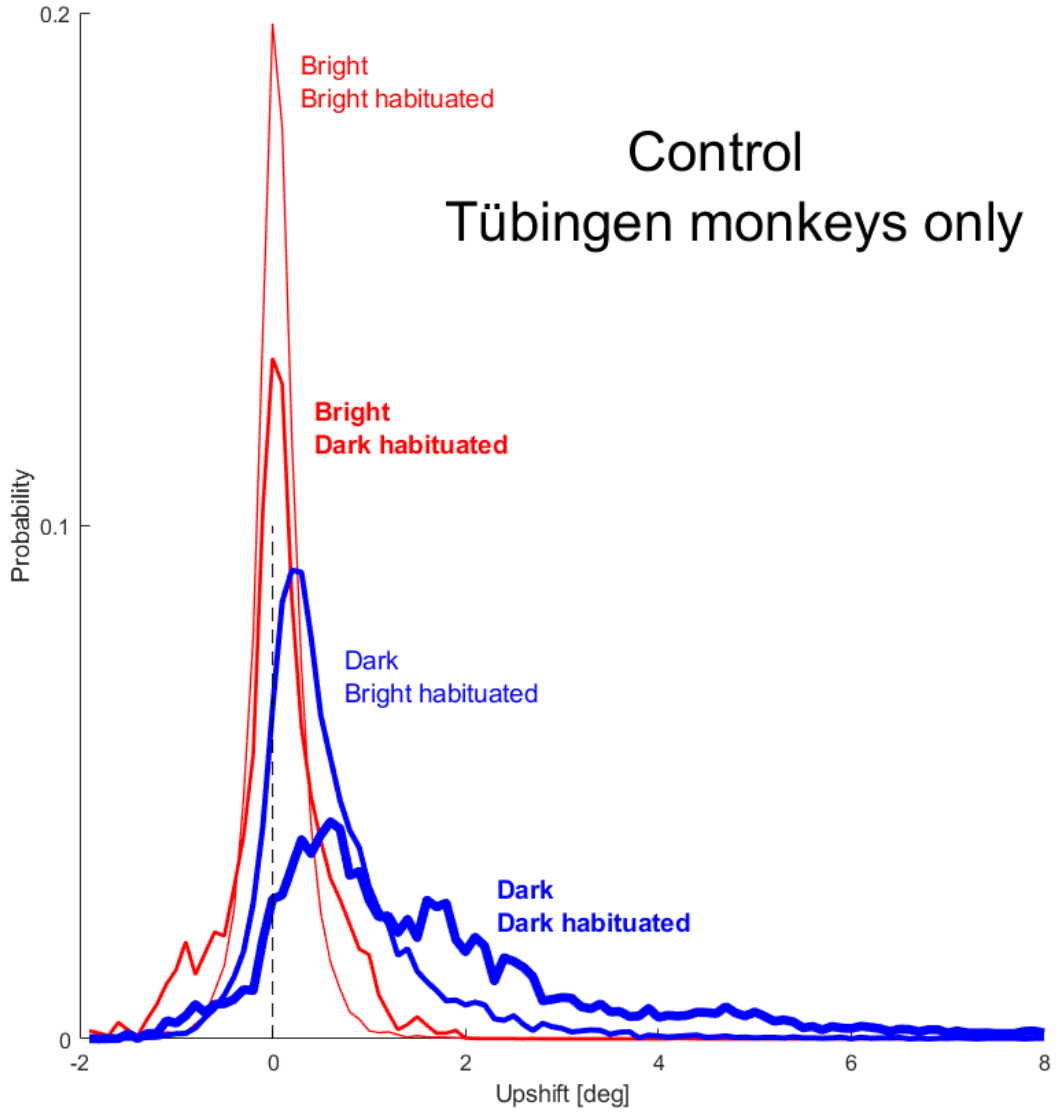

Figure 8: A control study. Same format as Figure 6, but calculated only from the data of the Tübingen monkeys. Thick traces reflect the dark-habituated Tübingen monkeys, and thin traces represent the bright-habituated Tübingen monkeys. Each monkey contributed equally to its group. The histograms show that the dark-background upshift differs significantly between the two groups. The bright-background histograms are similar.

Figure 8 shows that the hypotheses are confirmed also for the subsample containing only the Tübingen monkeys. The format of Figure 8 is identical to that of Figure 6, and all the observations made for Figure 6 hold also for Figure 8. In particular, the thick blue histogram is clearly separated from the thin blue, with the thick blue taking mostly higher values of upshift. In more detail, both parts of Hypothesis 1 were confirmed in the Tübingen only group of monkeys. For Hypothesis 1a, 'upshift is ubiquitous', the mean ± standard deviation values for the bright- and dark-background upshift are 0±0.4° and 0.83±1.27°, respectively. The population mean dark-background upshift is positive (*p=0*, t-test). For Hypothesis 1b, 'no sideshift', both bright-background and dark-background sideshift values are distributed around zero (straight ahead). A mean of 0 cannot be rejected for both the bright- and dark-background groups (for the dark-background sideshift, *p=0.4*, t-test).

The Tübingen only group results also confirmed Hypothesis 2. The bright-background upshift values are similar for the 2 habituation groups (0 ± 0.33° for the bright-habituated group and 0 ± 0.53° for the dark-habituated group); both histograms do not vary significantly from 0 (p = 0.4). A dark background evokes an upshift in both habituation groups (0.54 ± 0.85° for the bright-habituated group and 1.54 ± 1.73° for the dark-habituated group). The main result, concerning the habituation groups, is that the upshift is higher in the dark-habituated group. Indeed, the mean upshift is higher in the dark-habituated monkeys than in the bright-habituated monkeys (*p=0*, t-test). The results can be reverse analyzed, yielding likelihood functions similar to those in Fig 7.

In summary, the 2 hypotheses are confirmed in the Tübingen monkeys alone. These results strongly support the conjecture that the differences indeed stem from the different habituation, not from differences between the Rehovot or Tübingen samples.

3.9 Dividing the Tübingen monkeys to cohorts

Unfortunately, beyond the division into the dark-background and bright-background cohorts, there is not much we can say about the relationship of the Tübingen monkeys and the upshift. All the Tübingen monkeys were adult males, who, with one exception, trained for at least the 6 months prior to our testing on their tasks. The single exception was a monkey who had a break before our sessions, but before the break had worked for years. All trials incorporated at least 1 s of fixation, regardless of the particulars of the tasks. Thus, all the monkeys were experts in visual and visuomotor performance. Importantly, monkeys were never switched between dark-background tasks and bright-background tasks. This fact allowed us to divide the Tübingen monkeys into the two cohorts, according to the luminance of the background that was used with each monkey.

Thus, the question of how in more detail habituation drives the modulation of upshift size might have to wait for experiments specifically designed to clarify this specific question.

4 Discussion

4.1 Significance of the large sample used in the present study

The present study is based on data from an unusually large sample of monkeys. How important is the size of the sample for getting to the results? Could we have reached the same conclusions based on data from less monkeys? On the one hand, we show in the control study (Fig 8) that the data from the 10 Tübingen monkeys alone support the conclusions. On the other hand, the 14-monkey database, with its equal representation for both backgrounds, not only lends better support to the conclusions, but also is better suited to attempt to reach conclusions. Moreover, a 10-monkey

database is also unusually large. With the current standard sample of 2-3 monkeys, identifying differences between subpopulation similar to the one described in the current manuscript is, unfortunately, impractical.

4.2 The findings do not reflect a difference between the Rehovot and Tübingen monkeys

The 14-monkey sample consisted of 2 subsamples, including 10 monkeys evaluated in Tübingen and published data from 4 monkeys recorded in Rehovot. The monkeys in the 2 locations varied in species: the Rehovot monkeys were *Macaca fascicularis*, whereas the Tübingen monkeys were *Macaca mulatta.* In addition, the cohorts differed in the details of the procedure, laboratory equipment, previous training, living arrangements, and so on, as any 2 laboratories differ from each other. These differences can be viewed as strengthening the conclusions of our study; however, in principle, they could also be confounding factors. Differences between the habituation cohorts could theoretically reflect differences between the Rehovot and Tübingen monkeys rather than the effects of habituation. The Rehovot monkeys were all dark habituated. All the bright-habituated monkeys were located in Tübingen, but 4 out of the 7 dark-habituated monkeys came from Rehovot. If, regardless of habituation, the upshift in the Rehovot monkeys had been greater than that in the Tübingen monkeys, this could erroneously appear in our study as a difference between the habituation cohorts.

To test this concern, we performed an analysis on the Tübingen monkeys alone. Thus, we compared the upshift in the 7 Tübingen bright-habituated monkeys (‘Tübingen bright-habituation cohort’) to that in the 3 Tübingen dark-habituated monkeys (‘Tübingen dark-habituation cohort’). All the monkeys in the 2 compared Tübingen habituation cohorts were from the same laboratory, were the

same species (*Macaca mulatta*), lived in the same groups, lived in the same living conditions, had gone through the same procedures, were evaluated with the same instruments – all except for the details of the previous training, namely, the habituation. Therefore, if the differences we associated with the habituation were, in fact, differences between the Rehovot and Tübingen cohorts, the upshift in the 2 Tübingen habituation cohorts should have been the same. The results were the opposite: the difference between the two Tübingen habituation cohorts was large and statistically significant to a *p-value* of 0 (with the resolution of standard double-precision arithmetic). We conclude that the differences between the habituation cohorts are indeed related to the habituation, not the Tübingen versus Rehovot cohorts.

4.3 The upshift and its specificity are confirmed

One major result of the present study is that the existence of the upshift was precisely confirmed and delineated by the large sample. Fig 3 shows the histograms of the upshift (the vertical component of the gaze direction) in trials of the 14 monkeys. The figure shows that most trials had a positive upshift (85%); second, the values of the upshift were distributed from low values (less than 1°) to highly eccentric values (up to 6-8°). Thus, although the upshift in many individual trials was low, the distribution of all trials of the entire large sample assuredly confirms the existence of the upshift.

The large sample not only confirms the presence of the upshift for the vertical component of gaze direction but also shows that the upshift is specific in that there is no systematic sideshift. This is illustrated in Fig 5 (see also Fig 2). One reason for the specificity of the upshift is as follows: An interpretation commonly suggested for the upshift is that of an automatic attempt induced by the dark background to avoid fixation of the target by the fovea or foveola. If this were the genuine full

explanation for the upshift, one could expect that the gaze directions of the different trials would distribute approximately equally in all directions. However, the fact that the upshift is specific to the vertical and within the vertical to the upward direction, shows that this avoidance hypothesis cannot alone fully explain the upshift.

4.4 Difference between the habituation cohorts

Does the habit of working in the dark affect the upshift? Hypothesis 2 proposes that the dark-habituated monkeys had more frequent and larger upshifts than the bright-habituated monkeys. Whether a monkey trained predominantly in the dark during the months prior to our testing greatly influenced the monkey's upshift. Indeed, the mean upshift in the dark-background trials of the dark-habituated cohort was 2.2°, which was more than 4 times that in a trial from the bright-habituated cohort (0.5°). The upshift was conspicuous in the bright-habituated cohort, with the null hypothesis of the mean = 0 rejected at $p=1$ and 77% of the trials having a positive upshift. However, in the dark-habituated cohort, the upshift was not only greater but also even more prevalent, with 93% of the trials showing an upshift (see Fig 6). Thus, a monkey's habituation to darkness greatly influences the upshift.

4.5 The upshift is not explained by the rod hotspot/dorsal rod peak

The dark background that elicits the upshift may be associated with night vision. When we first studied the upshift, we were led to associate the upshift with the distribution of rods in the retina (Barash et al., 1998). Rods are denser in the dorsal retina, reaching a peak at an eccentricity similar to that of the blind. The location of peak rod density is called the 'dorsal rod peak', or 'rod hotspot' (Curcio et al., 1990; Packer et al., 1989; Wickler and Rakic, 1990; Wickler et al., 1990). We

suggested that the upshift is related to the rod hotspot/dorsal rod peak. However, it is now clear that this suggestion reflected an oversimplification.

First, the variability of the upshift documented in Fig 3 makes it highly unlikely that the upshift always brings the target's image to the well-localized rod hotspot/dorsal rod peak. The rod hotspot/dorsal rod peak is not as well delineated as the fovea. Nevertheless, the wide upshift histogram (Fig 3, blue trace) makes it highly unlikely that the target image falls on the rod hotspot/dorsal rod peak in most of the dark-background trials.

Second, the distribution of the upshift shows values that probably do not reach the eccentricity of the dorsal rod peak/rod hotspot. The presently published histological data is not sufficient to state rod density as a visual angle, certainly not when considering individual variability. Nonetheless, the data in the relevant papers (Packer et al., 1989; Wickler and Rakic, 1990; Wickler et al., 1990) suggest that the rod hotspot/dorsal rod peak is more eccentric than the upshift in most or all of the trials.

Third, the difference between the size of the upshift in the 2 habituation groups varied considerably. The bright-habituated monkeys, with a less eccentric upshift, were particularly unlikely to have the target reach the dorsal rod peak/rod hotspot in most of their trials.

Finally, by definition, the rod hotspot/dorsal rod peak is related to scotopic conditions. However, as described in the next section, the upshift recorded in previous published studies as well as in the present study is not part of scotopic vision.

4.6 The upshift is not (only) part of scotopic vision

The hallmark of studies of scotopic vision is appropriate sufficiently long dark adaptation. The time course of dark adaptation has a characteristic shape, consisting of 2 exponential decreasing legs (Hecht et al., 1937; Hecht and Shlaer, 1938; Reuter, 2011, Schwartz, 2017). It is well known that during the first leg, which can last approximately 15 mins, vision is dominated by cones; rods remain saturated during this period. For full dark adaptation, the subject must remain in the dark for approximately 45 mins (Hecht and Shlaer, 1938; Normann and Werblin, 1974).

Neither the present study nor previous studies involved proper dark adaptation intervals. In the 1998 study, we tested the recovery by alternating blocks with bright and dark backgrounds. Gaze directions in the bright-background blocks almost coincided with each other, as did gaze directions in the dark-background blocks (Fig 4 of Barash et al., 1998). We also tested background switches within trials (Figs 7 and 8 of Barash et al., 1998). At least in some trials, the upshift emerged within a few seconds from the onset of the dark background.

In the present study, we also refrained from keeping the Tübingen monkeys in the dark for a long time. In the entire data collection process, we alternated between blocks of bright-background trials and blocks of dark-background trials. A block usually lasted approximately 5 minutes. Even if another minute was added for the extra time spent during the change between the blocks (clearly an overestimate), data collection is confined to the time vision is dominated by cones.

The upshift might be present also in scotopic conditions; however, the results of the present study together with those of previous studies show that the upshift is definitely not specific to scotopic conditions.

4.7 Relationship of the upshift to the previously described upshift of memory-guided saccades in the dark

The upshift we studied in the current paper is not the only one described in studies of monkeys' gaze. Another type of upshift evoked by a dark background was described in 1991 (Gnadt et al., 1991). In this study, monkeys performed memory-guided saccades to remembered visual targets in either a dark or bright background. The monkeys showed a systematic upward shift of saccadic endpoints in darkness. Post-saccadic gaze direction remained fixated well above the targets. A follow-up study (White, Sparks, & Stanford, 1994) confirmed and expanded the results: the endpoints of the memory-guided saccades were systematically deviated upwards with respect to visually guided saccades, beyond the limits expected from the trial-by-trial variability. More specifically, White et al. (1994) reported that the saccadic endpoints, which probably approximate post-saccadic fixations, are more variable in the dark; they also suggested that the error observed in memory-guided saccades in darkness had another component in addition to memory-dependent processes, which might reflect the lack of a structured visual background. Could this memory-related upshift be related to the upshift we studied in the present paper?

In 1998, we tested this possibility by recording visual- and memory-guided saccades in dark and bright backgrounds. In dark-background trials, the 2 types of upshift were both present, appearing to summate with each other (Fig 9 of Barash et al., 1998). Hence, we presumed that the 2 types of upshift reflect independent neuronal processes. However, the present study's observation that the upshift size might vary suggests the possibility that the 2 types of upshift are not as separate as we previously thought.

4.8 A possible involvement of the superior colliculus

Where in the brain is the upshift generated? Any of the multiple regions involved in fixation and saccades can be involved, but one region that appears especially pertinent is the superior colliculus (SC) because of its involvement in sensorimotor transformations for fixations and saccades (Coe & Munoz, 2017; Edelman & Goldberg, 2002; Hafed, Goffart, & Krauzlis, 2009; Krauzlis, Goffart, & Hafed, 2017; Munoz & Fecteau, 2002). Preliminary evidence illustrated how the neuronal activity in the SC might change (Spivak, Thier, & Barash, 2017).

A particular aspect of an area pertinent to the upshift might be that it would show up-down asymmetry coupled with left-right symmetry, much like our Fig 3. Hafed and Chen reported that the upper visual field is overrepresented in the SC and the lower visual field underrepresented (Hafed & Chen, 2016). The upper and lower visual fields also differed in single-unit activity parameters. The authors did not report left-right differences, certainly when the two colliculi are taken together – and this absence of lateral asymmetry is reminiscent of our Fig 5.

The superior colliculus is made pertinent also by another line of work. Stanford and sparks (Stanford & Sparks, 1994) recorded from saccade-related burst neurons in the SC, comparing discharges associated with reflexive saccades and memory-guided saccades. The activity differed in the two tasks. Based on their analysis of the SC activity, Stanford and Sparks suggested that the SC is not the source of the memory-guided saccade upshift. Rather, they suggested that the upshift results from addition or omission of signals downstream from the SC.

Whether and how Stanford and Sparks' conclusion apply to the dark-background-contingent upshift remains to be seen.

4.9 The difference between the habituation groups might suggest the function of the upshift

We described in the Introduction (section 1.4) how the rod-peak hypothesis failed to provide a function for the upshift. Nonetheless, the upshift might still have to do with vision in the dark. Since the increased upshift was observed in the dark-habituated monkeys, presumably the increased upshift has adaptive value for a function these monkeys exercise. Indeed, one such function is vision in the dark. We thus suggest that the data of the present manuscript supports the possibility that the upshift aids vision in the dark (though not necessarily scotopic vision).

4.10 Conclusions

The first conclusion of the present study is that the dark background-contingent upshift is a ubiquitous robust phenomenon. It is specific to the vertical dimension: there is no systematic sideshift. This conclusion is based on findings from a large sample of 14 monkeys, and it appears to hold true for 2 different macaque species. The second and main novel conclusion is that the size of the upshift reflects previous experience. Monkeys previously trained in the dark showed a larger upshift than monkeys trained in bright light. Thus, the upshift is a visuomotor reflexive behavior whose amplitude is modulated by the cumulative experience of the individual monkey during the months preceding the recording. Whether the monkey trained in the dark or bright light modulated the amplitude of the reflex. The extent to which monkeys were accustomed to working in the dark recalibrated the gaze direction. These findings support the underlying hypothesis that the function of the upshift is indeed in improving vision when the background is dark.

5 References

Barash, S., Melikyan, A., Sivakov, A., & Tauber, M. (1998). Shift of visual fixation dependent on background illumination. *Journal of Neurophysiology*, *79*(5), 2766–2781. doi:10.1152/jn.1998.79.5.2766

Caggiano, V., Pomper, J. K., Fleischer, F., Fogassi, L., Giese, M., & Thier, P. (2013). Mirror neurons in monkey area F5 do not adapt to the observation of repeated actions. *Nature Communications*, *4*, 1433. doi:10.1038/ncomms2419

Cherici, C., Kuang, X., Poletti, M., & Rucci, M. (2012). Precision of sustained fixation in trained and untrained observers. *Journal of vision*, 12(6), 31-31.

Coe, B. C., & Munoz, D. P. (2017). Mechanisms of saccade suppression revealed in the anti-saccade task. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, *372*(1718). doi:10.1098/rstb.2016.0192

Dash, S., Catz, N., Dicke, P. W., & Thier, P. (2012). Encoding of smooth-pursuit eye movement initiation by a population of vermal Purkinje cells. *Cerebral Cortex*, *22*(4), 877–891. doi:10.1093/cercor/bhr153

Edelman, J. A., & Goldberg, M. E. (2002). Effect of short-term saccadic adaptation on saccades evoked by electrical stimulation in the primate superior colliculus. *Journal of Neurophysiology*, *87*(4), 1915–1923. doi:10.1152/jn.00805.2000

Fuchs, A. F., & Robinson, D. A. (1966). A method for measuring horizontal and vertical eye movement chronically in the monkey. *Journal of applied physiology*, *21*(3), 1068–1070. doi:10.1152/jappl.1966.21.3.1068

Goffart, L., Quinet, J., Chavane, F., & Masson, G. S. (2006). Influence of background illumination on fixation and visually guided saccades in the rhesus monkey. *Vision Research*, *46*(1-2), 149–162. doi:10.1016/j.visres.2005.07.026

Hafed, Z. M., & Chen, C.-Y. (2016). Sharper, stronger, faster upper visual field representation in primate superior colliculus. *Current Biology*, *26*(13), 1647–1658. doi:10.1016/j.cub.2016.04.059

Hafed, Z. M., Goffart, L., & Krauzlis, R. J. (2009). A neural mechanism for microsaccade generation in the primate superior colliculus. *Science*, *323*(5916), 940–943. doi:10.1126/science.1166112

Hecht, S., Haig, C., & Chase, A. M. (1937). The influence of light adaptation on subsequent dark adaptation of the eye. *The Journal of General Physiology*, *20*(6), 831–850.

Judge, S. J., Richmond, B. J., & Chu, F. C. (1980). Implantation of magnetic search coils for measurement of eye position: an improved method. *Vision Research*, *20*(6), 535–538. doi:10.1016/0042-6989(80)90128-5

Krauzlis, R. J., Goffart, L., & Hafed, Z. M. (2017). Neuronal control of fixation and fixational eye movements. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, *372*(1718). doi:10.1098/rstb.2016.0205

Munoz, D. P., & Fecteau, J. H. (2002). Vying for dominance: dynamic interactions control visual fixation and saccadic initiation in the superior colliculus. *Progress in Brain Research*, *140*, 3–19. doi:10.1016/S0079-6123(02)40039-8

Reuter, T. (2011). Fifty years of dark adaptation 1961-2011. *Vision Research*, *51*(21-22), 2243–2262. doi:10.1016/j.visres.2011.08.021

Snodderly, D. M. (1987). Effects of light and dark environments on macaque and human fixational eye movements. *Vision Research*, *27*(3), 401–415. doi:10.1016/0042-6989(87)90089-7

Snodderly, D. M., & Kurtz, D. (1985). Eye position during fixation tasks: comparison of macaque and human. *Vision Research*, *25*(1), 83–98. doi:10.1016/0042-6989(85)90083-5

Spivak, O., Thier, P., & Barash, S. (2014). Persistence of the dark-background-contingent gaze upshift during visual fixations of rhesus monkeys. *Journal of Neurophysiology*, *112*(8), 1999–2005. doi:10.1152/jn.00666.2013

Spivak, O., Thier, P., & Barash, S. (2016). 55.01 / BB1 - Scotopic fixation eye movements dependence on eye position: separate visual modes? Presented at the Society for Neuroscience 2016, Society for Neuroscience.

Spivak, O., Thier, P., & Barash, S. (2017). Sensorimotor transformations in monkeys under scotopic and photopic conditions. Presented at the Society for Neuroscience Annual Meeting 2017, Society for Neuroscience.

Spivak, O., Thier, P., & Barash, S. (2018). Monkeys use the rod-dense retinal region rather than the fovea to visually fixate small targets in scotopic vision. *BioRxiv*. doi:10.1101/290759

Stanford, T. R., & Sparks, D. L. (1994). Systematic errors for saccades to remembered targets: evidence for a dissociation between saccade metrics and activity in the superior colliculus. *Vision Research*, *34*(1), 93–106.

White, J. M., Sparks, D. L., & Stanford, T. R. (1994). Saccades to remembered target locations: an analysis of systematic and variable errors. *Vision Research*, *34*(1), 79–92. doi:10.1016/0042-6989(94)90259-3

Appendix 3:

Barash, S., Spivak, O., & P. Thier (2020). Scotopic band: serial processing through foveal substitution in scotopic vision of monkeys. *Submitted*.

Scotopic band: serial processing through foveal substitution in scotopic vision of monkeys

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Short title:
The scotopic band

One-sentence summary:
Serial processing exists in night vision as in day vision; a 'scotopic band' substitutes for daytime high-acuity fovea.

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Abstract

High-acuity photopic (daytime) vision is serial: the eyes move so that the small, cone-dense fovea of each eye scans one target of interest after another. Specialized eye movements repeatedly shift the fovea to the next targets (saccades), and keep the fovea there (fixations). Here we describe, in monkeys, scotopic (nighttime) eye movements that shift targets not to the fovea but to a 'scotopic center' dorsal to the fovea. We further describe a 'scotopic band'. As visual conditions change within the scotopic range, the scotopic center systematically relocates along the scotopic band. Whereas the scotopic center parallels the fovea, the scotopic band has no apparent parallel in photopic vision.

Main text

High-acuity visual perception of primates is based on serial processing. Acuity is constrained by the instantaneous light-receptor density. Over the surface of the retina, the receptor density varies widely; only at the fovea, a small spot at central retina, is cone density high (*1*). Only the small image region that falls on the fovea can be analyzed at high acuity. The term 'direction of gaze' refers to foveal gaze; it is determined by the position of the eyeballs in their sockets. As the eyes move, the instantaneous gaze direction moves with the eyes. Because they determine gaze direction, eye movements are crucial for high-acuity vision. The sensory impressions for high acuity vision accumulate through a series of fixations of 'targets', with quick jumps ('saccades') leading gaze from one target to the next. Vision evolves through fixations and saccades; consequently, fixation and saccadic eye movements turn photopic (daytime) high-acuity vision into a serial processing operation. What about scotopic (nighttime) vision – does it also incorporate a serial processing scheme based on some 'scotopic center', a confined retinal region of preferred scotopic processing? If it does, what is the retinal geometry of this location? Does it relate to the fovea? In what ways is it like the fovea? In what ways do the fovea and the presumed scotopic center differ, and why?

Three previous lines of observations appear to support the possibility that a scotopic center does exist. The first observation involves the geometry of rod density. Rods are dense dorsal to the fovea. Rod density systematically increases while going dorsally, starting just dorsal to the fovea, till peaking at a spot called either Dorsal Rod Peak (DRP) or Rod Hotspot (*2*, *3*). This peak of rod density is not nearly as sharp as the foveal peak of cone density, but the rod peak/hotspot is a quite well defined region, distinct from the rest of the retina. In fact, the maximal values of cone and rod densities are similar (*2*, *3*). Thus, at least the primary necessary condition for localized preferred scotopic processing appears to be satisfied.

The second observation is that, in darkness, monkeys appear to direct their gaze above the target (*4*–*6*). Could this 'upshift' indeed reflect scotopic use of the rod peak instead of the fovea? Although the idea initially seemed promising (*6*), problems confound this proposal. First, the upshift does not match well the retinal location of the rod peak. The upshift varies largely from fixation to fixation, whereas the rod peak is fixed in its retinal place. Second, the upshift could be related to photopic vision rather than to scotopic -- an idiosyncrasy unrelated to rods. The well-established time-course of dark adaptation (*7*, *8*) shows that rods remain saturated long (> 10 min) after dark onset. But upshift was documented to emerge, at least in some monkeys, within seconds from dark onset (*6*, *9*). In any case, published upshift studies generally lacked proper dark adaptation. Only with proper dark adaptation can the upshift's putative relationship to scotopic vision be tested, so whether and how upshift relates to scotopic vision is unclear. Third, although assigning a putative function to the rod-peak is appealing, it is not a complete concept of a scotopic center adapted for scotopic serial processing.

The third observation which might support the existence of a scotopic center is that the pattern of saccades and fixations lingers on at night (*5*, *10*). Common thinking associates these movements solely with the fovea; presumably, a fixation keeps a target on the fovea, and a saccade subsequently shifts the next target to the fovea. The sensorimotor

transformation of a saccade is thus presumed to reflect target foveation. The transformation activates the extraocular muscles so that the target's image would fall on the fovea. This rationale is paradoxical for scotopic vision: cone-dense, the fovea is rod-sparse, hence insensitive in the dark. Why shift the image to an insensitive location? A putative scotopic center would constitute a solution to this paradox.

Hypothesis

The hypothesis pursued in the present manuscript is illustrated in Fig 1A. We propose the existence of a 'scotopic band', a confined, elongated retinal region extending dorsally from the immediate vicinity of the fovea. We first suggest that, at any instance, a 'scotopic center' locus in the scotopic band carries a preferred role in scotopic vision much like the fovea does in photopic vision. Second, in scotopic vision, specialized sensorimotor transformations enable fixation and saccadic movements to shift the target's image directly to the scotopic center and keep it there, much like the analogous sensorimotor transformations in photopic vision do with the fovea. Third, as visual and other conditions change, the active scotopic center moves along the scotopic band. With these components, scotopic vision incorporates serial processing based on the scotopic center, much like the fovea-based serial processing of high-acuity photopic vision.

The scheme suggested here might be valid also for mesopic vision. Mesopic vision might be based on the lower end of the scotopic band, together with the fovea. However, in this manuscript we deal only with the scotopic range.

In presenting the results we will use the previously defined notion, of upshift of the foveal line of gaze. This notion describes the results: we make a standard calibration of the foveal line of gaze in photopic conditions, and then in scotopic conditions observe upshift of this line. The significance of every such statement is that a scotopic center is activated, and its position on the scotopic center is given by the measured upshift.

Operational definitions

We emphasize that the definitions of the scotopic center and band are functional, based solely on the eye-movement behavior that we recorded. More specifically, we recorded fixations and saccades in monkeys, before and after proper (45 min) dark adaptation. We define the scotopic center by comparing the scotopic, post-adaptation gaze directions to the pre-adaptation photopic. We defer the question of how the scotopic band is related to rod density to the discussion.

In monkey studies of gaze direction, the technique of monitoring gaze direction using coils implanted over the sclera is now standard for more than 5 decades (*11*, *12*). Recordings with this technique are extremely stable and, critically, do not depend on the pupil's contraction and dilation. Video-based eye tracking devices, now popular in studies of humans, is subject to artefacts and inaccuracies with the enlarged pupil of scotopic vision (*13*–*16*). Video-based tracking is especially perilous when studying a phenomenon such as upshift, which is itself a deviation of gaze direction. In addition, non-scotopic upshift is well characterized in monkeys

(we have now recorded upshift in 14 monkeys), whereas reports in humans are not entirely consistent (see ahead).

A scotopic center substitutes for the fovea

Is there indeed a scotopic center used to fixate targets in scotopic vision? We test a first experimental prediction related to fixations and a second experimental prediction related to saccades. To clarify the situation, we start with an example.

Directing gaze to the scotopic center

Imagine a monkey looking at a bird on a tree, first during daylight, then at night (Fig 1B). What would change at night, if the scotopic center hypothesis is valid? The eye would rotate, so that the bird's image would fall on the scotopic center rather than on the fovea (dotted line, lower sketch of Fig 1B). Consequently, the fovea's line of gaze would now be directed above the bird (dashed line, lower sketch of Fig 1B). Because measurements of gaze direction refer to the foveal line of gaze, the rotation appears to be an upshift of gaze direction, much like that observed in non-scotopic conditions (*4*, *6*, *9*). However, the measured upshift does not truly reflect the apparent deviation of foveal gaze away from the bird. Rather, the upshift reflects switching of the active retinal region, from the fovea to the presumed scotopic center. The visual system's serial processing would switch from using the fovea as its preferred retinal region to using the scotopic center.

Thus, the first experimental prediction transpiring from the hypothesis is that in scotopic vision there is upshift. Because the term upshift directly describes the result, we would use it, but the connotation is always that of the implicated switching from the fovea to the scotopic center.

We studied 3 rhesus monkeys already trained in visual and oculomotor tasks. A daily session started with a standard calibration procedure, conducted in bright light. Then followed: a photopic study, a 45-min dark-adaptation interval, in complete darkness, and a scotopic study. In trials of both photopic and scotopic studies, the monkey had to fixate a central fixation spot, then follow with his eyes the spot's jump to another location, and fixate the target at its new location. The initial fixation spot and all targets were on the horizontal meridian. The horizontal meridian locations allow us to test the predictions, see ahead. We collected data in photopic and scotopic conditions; we will call data – trials, saccades, and so on – 'photopic' or 'scotopic' according to the conditions that prevailed when collected. Photopic data will be displayed in red, scotopic in blue.

We compared the mean gaze directions in photopic and scotopic trials. We actually made 2 sets of comparisons. The first set compared fixation of the central target that preceded the saccades. The second comparison encompassed fixations of the peripheral targets, during the post-saccadic fixations. The comparisons are illustrated in the left and right panels for each monkey, correspondingly. Each dot in the scatterplots reflects the mean gaze direction of one trial. The dots are positioned in the scatterplots according to the gaze direction of the individual trial. To be clear, the measurements reflect the direction of the foveal line of gaze, regardless of whether the fovea actively senses (as in photopic vision) or not (as in scotopic).

The mean gaze directions were assessed from 0.25-s intervals, in the post-saccadic data long enough after preceding correction saccades to have ended. Thus, both photopic and scotopic data reflect fixations of targets at the same locations, all on the horizontal midline of the screen. Nonetheless, foveal gaze directs upwards in scotopic vision; the clusters of blue dots are positioned above the red clusters in all 6 panels. No systematic horizontal shift is present. The Figure illustrates results of many trials (847, 594, 398 photopic trials, 1216, 611, 588 scotopic, correspondingly in M1, M2, and M3). Nonetheless, the blue and red clouds are almost perfectly separate. The only exception is a small set of scotopic trials (~60) of monkey M1, in which the foveal line-of-gaze directs to the target even though M1 is using scotopic vision. We will allude to this small set later. For statistics, it is useful to concentrate on the vertical component of gaze direction. The histograms of the vertical components reflect the almost perfect separation of photopic and scotopic gaze directions. The overlap of the histogram is very small, 0 in M1 (not counting the 60 trials mentioned above), <4% (23/594) in M2, 0 in M3. In all 3 monkeys, both at the center and in the periphery, the means of the photopic and scotopic foveal-line-of-gaze directions differ from each other ($p<10^{-264}$ in each of the monkeys, t-tests). In line with previous studies, we define upshift to be the difference of the vertical components of mean scotopic position from the mean photopic. Applying this definition, the upshift is 6.2 deg in M1, 2.1 deg in M2, and 5.6 deg in M3. Thus, the prediction stated at the start of this section is validated. There is upshift in scotopic vision.

The larger clusters in scotopic vision indicate that the scotopic center is larger than the fovea. These results are consistent with reported variation of photopic upshift (*6*, *9*).

Although we tested upshift only on the horizontal meridian, it is enough to show the presence of upshift in scotopic conditions. Moreover, because this upshift is very similar to the photopic upshift tested over a large part of oculomotor space (6, 9) it is most likely that scotopic upshift is present in all orbital positions.

Saccades targeting the Scotopic Center

Schematic Fig 1C illustrates the sensorimotor transformations of saccadic eye movements, in photopic vision (top) and scotopic (bottom). The sensorimotor transformation of a typical photopic saccade is marked by a trajectory that leads the fovea's line of gaze directly from the initial fixation spot to the target. The precision of the saccade is critical: if the line of gaze lands, at the end of the saccade, off the target, the result is, effectively, nullifying high-acuity vision. What happens in scotopic vision? The eye begins with an upshift, as seen in Fig 2A. Hence, for the line of foveal gaze, the saccade's starting point is drawn in Fig 1C above the target. Now, if saccades always aim to foveate, then we expect the saccade to end with the foveal line of gaze on the target (solid red arrow in lower part of Fig 1C); thus there will be no upshift at the end of the saccade. Presumably, upshift would then emerge (dotted line if Fig 1C). In the present study, foveation is the null prediction. Transpiring from the hypothesis that a scotopic center is used in scotopic vision much like the fovea in photopic vision is the alternate prediction – that saccades shift the target's image directly to the scotopic center (Fig 1C, blue vector). In the conditions of our experiment, because the fixation spot and all targets are positioned on the horizontal meridian, if the null prediction holds saccades must consistently have a downward component, so as to foveate the targets; whereas if the alternate prediction holds, even though scotopic centers are presumably larger than the

fovea (see above), saccades must be directed roughly horizontally, maintaining about the same upshift.

Fig 2B illustrates example trajectories of saccades in 3 monkeys, photopic (red traces) and scotopic (blue traces). All trajectories were horizontally shifted to start from the vertical meridian (this shift was applied only to the example traces of Fig 2B, not to the analysis of the entire database in Figs 2C,D). The superimposed photopic (red) trajectories remain close to the horizontal axis; these saccades took a largely straight course, ending with the fovea directed close to the relevant trial's target. The scotopic trajectories (blue) are very different: as expected, the foveal line of gaze starts directed above the central fixation spot. The vertical range of the scotopic gaze directions is larger than the vertical range of the photopic gaze directions, in line with the observation of Fig 2A, possibly reflecting that the scotopic center is indeed larger than the fovea. After the saccades start, the trajectories of the foveal line of gaze transpire in a near-horizontal direction. A few traces have a slight downward component; others go slightly up. Importantly, the saccade endpoints remain in the vertical range of the starting points, suggesting that the saccades end in the active scotopic center. Turning to the predictions sketched in Fig 1C, the example saccades are very similar to the blue arrow, representing the alternate prediction, that the saccades shift the target directly to the scotopic center. The trajectories are entirely inconsistent with the null prediction. Not even one blue trajectory ends on the target. The vertical range of the endpoints of the blue trajectories is all above the range of the endpoints of the red trajectories. Thus the example blocks suggest that the null prediction is rejected.

Figs 2C,D show the results of a systematic analysis of all saccades recorded in each monkey in this experiment. Fig 2C confirms that scotopic saccades have a main sequence similar to that of photopic saccades. The speeds of scotopic saccades in our data were somewhat lower than the speeds of photopic saccades, but we do not know if this would have generalized to all experimental conditions; in any case, the blue main sequence plots look like those of standard saccades. The main results are illustrated in Fig 2D. These results make it possible to test the null prediction and evaluate the alternate prediction systematically, based on the entire database. Fig 2D shows, for each monkey, two upshift histograms. The filled gray histograms show the upshift just before saccade onset; the unfilled histograms delimited by the solid line show the upshift briefly after saccade offset. Zero upshift, that is, no upshift, is marked as a vertical line on each panel of Fig 2D. Let's now assess the predictions.

The null prediction states that in scotopic vision, too, saccades foveate the target. Thus, at the end of the saccade there should be no upshift. In other words, the trial-by-trial upshift histogram should be centered on 0 upshift. This prediction is decisively rejected. The means of the end-saccade upshift histograms are positive for all monkeys, with p=0 (t-tests). For M2 and M3 no trials start or end close to 0; for M1, only a tiny minority (4.8 %) which probably reflects the same rare state as the ~60 fixation trials mentioned above. Thus, the null prediction is decisively rejected.

Rejecting the foveation hypothesis is the main result of this section. Nonetheless, for evaluating the alternate prediction, we compare the start-saccade and end-saccade histograms of each monkey. The start and end saccade histograms are not identical – the two-sample Kolmogorov Smirnov test gives $p<10^{-6}$, for each monkey. The means change too

– but only by little: on going from start- to end-saccade, the mean upshift values change by 8% (M1), -9% (M2), 5% (M3). This change is but a small fraction of the size of the scotopic center. Thus, the saccade directs the target quite directly to the active scotopic center.

We set out to study saccades over the horizontal meridian with the hope that they would provide a clear-cut, visible test of the foveation hypothesis, and they did (Fig 2B,D). We may still ask if a larger set of target positions would have yielded a different answer. With regard to the main point, of testing the foveation hypothesis, a larger set wouldn't have mattered: rejecting a prediction for a subset of all cases is enough to show that the hypothesis fails. Because the upshift extends similarly above and below the horizontal meridian, as do saccades, that saccades end close to the scotopic center most likely generalizes to a broad band below and above the horizontal meridian. Upshift far below or far above the horizontal meridian changes nonlinearly (*6*, *9*) as do saccades to these areas (*9*, *17*) so further studies will have to test whether the saccades and the upshift co-vary far above or far below the horizontal meridian; it is also possible that in these cases the post-saccadic scotopic center relocates on the scotopic band, see ahead. For most of the cases, however, the post-saccadic scotopic center is all but identical to the pre-saccadic scotopic center.

The Scotopic Band

Introduction

Having presented evidence for the existence of a scotopic center and of eye movements tailored to enable its use, we now get to testing the second part of the hypothesis guiding this manuscript. We suggest that, as visual conditions change, the position of the scotopic center changes too. We define the scotopic band as the range of positions taken by the scotopic center. The results that we will present indicate that the scotopic band is an elongated structure positioned on the vertical meridian, beginning almost immediately dorsal to the fovea and extending dorsally to an eccentricity of at least 10 - 15 deg visual angle.

Photopic vision does well with a small circular center. Why would scotopic vision require an elongated band, over which the scotopic center relocates? We speculate the following. Photopic vision remains largely unchanged over its operational range; object recognition is similar in the bright and dim ends of the photopic range. Scotopic vision at the bright end of the scotopic range is similar to photopic vision, but at the dim end of the scotopic range the required processing is very different. It is interesting to note that rod density most probably varies along the scotopic band (*2*, *3*, *18*).

Background luminance impacts the scotopic center's position on the band

We were led to the notion of a scotopic band by repeating incidental observations that suggested the background luminance affects the size of the upshift. Here we systematically test this effect. We thus started to test the scotopic band hypothesis by assessing the upshift for a range of background luminance levels. We start by illustrating the effect of background luminance on the upshift of single trials, in an example block sequence (Fig 3A). We kept the target small and bright, with fixed luminance; this approach insinuates that it is indeed the background, not the target, that might explain observed changes in upshift (*6*, *9*). The monkey was dark-adapted for 45 minutes, then testing commenced. A sequence of brief blocks

followed each other, with background luminance maintained during a block and increased slightly from block to block. Each dot reflects the upshift of a single trial. All trials of a block are drawn with the same color. The squares mark the mean shifts of all the trials in the block and drawn in the same color as the single trials of the block.

The scotopic range is covered in about 4 blocks (blue, yellow, cyan, black, in increasing luminance). Black reflects luminance at the transition between scotopic and mesopic (see Legend and Methods). Green reflects an even more intense background; red is full photopic.

The upshift varies considerably from trial to trial. Nonetheless, the mean upshift values show a robust trend: as the background luminance increases, the upshift decreases, gradually, from block to block. Although most of the decrease is in the deep scotopic range (blue to cyan), some of the decrease is present well into the mesopic range. We will not go into detail regarding mesopic vision, only note in passing that the most ventral part of the scotopic band might be involved in mesopic vision.

Guided by the example session illustrated in Fig 3A, we turn to the first experimental prediction emerging out of the scotopic band hypothesis: as the background luminance increases within the scotopic range, we suggest that the upshift decreases. Fig 3B shows the data obtained from 2 monkeys, M2 and M3. The single trials' upshift was normalized, to help compare the data of the 2 monkeys. The normalization was linear and defined so that the photopic shift was set to 0 and the full scotopic shift to 1. Importantly, the scotopic range was thoroughly sampled. Targets were small (0.25 deg radius) and bright enough to be scotopic, yet salient at all tested background luminance levels (10^{-3} cd/m^2) (see next section). Thus, Fig 3B illustrates the upshift at several luminance values throughout the scotopic luminance range. Importantly, the monkey is fully dark adapted to the level of the tested background throughout data collection.

The results are clear cut: in both monkeys, the upshift goes down with increasing background luminance. The normalized graphs of the upshift of the 2 monkeys are both monotonically decreasing and similar to each other (blue to violet dots, up to 10^{-4} cd/m^2).

This result strongly supports the notion of a scotopic band. As the background gradually stepped up throughout the scotopic range, the scotopic center relocated to a systematically more ventral location, closer to the fovea. The range of these movements of the scotopic center makes up the scotopic band.

The scotopic band near threshold

Near-threshold fixations

The existence of the scotopic band implies that using the scotopic center necessitates repeated acts of choosing where on the band to position the active scotopic center. We will call this act of choosing a desired position on the band "scotopic band setting". An effect of scotopic band setting is that the sensorimotor transformations involved in scotopic saccades and fixations are more complex than those of photopic vision, because the scotopic transformations depend on a parameter (position on the band). Moreover, the question

comes up: does scotopic band setting happen always automatically? The previous case of scotopic band setting, by the background luminance (Fig 3), can, it appears, happen as automatically as, say, photoreceptor adaptation. Are there other cases of scotopic band setting that might not appear to be automatic? Guided by this question, we turn to study threshold situations.

Large targets allow fixation accuracy to be measured only roughly, because adequate fixation can aim at any location within the large target. To avoid this caveat we have used in the studies described so far only small targets; to ensure that these small targets are salient, the targets had to be bright, high on the scotopic luminance scale. We now seek to explore fixations of dimmer, larger targets, because such target are common in scotopic vision. Does their fixation involve upshift? What part of the scotopic band is used? Of particular interest are threshold situations. Before getting at all to upshift, we will ask: what parameters determine thresholds for making saccades to scotopic targets? With respect to upshift, does a threshold situation influence scotopic band setting? That is, does upshift of fixation near threshold differ from the upshift of a physically similar target while it is salient? Such finding would suggest that while close to threshold, scotopic band setting is influenced by a non-automatic process.

The rationale of studying threshold situations is the following: Light conveys information. As luminance goes down, photons become less ubiquitous, the available information carried by the light changes. The ambient light significantly influences the image's statistics. Near threshold, noise is especially high. Does the signal to noise ratio influence the monkey's setting of the scotopic band?

Study 3 aimed at addressing these questions. Example sessions of the 2 monkeys are illustrated in Figs 4A,B, which will be described shortly; altogether we ran 5 sessions of M3, then 7 sessions of M2. The results will be illustrated by a single session for each monkey (Figs 4A,B), but all that analysis that will be presented pertain to the entire database of all sessions recorded in this study.

For creating a threshold situation that would allow assessing the effect on the upshift, we varied (a) the target's size, and (b) the target's luminance. Figs 4A,B illustrate the results of example sessions of monkeys M3 and M2, correspondingly. Each point reflects the monkey's performance in a single block. A block consisted of 16 trials. In a trial, a fixation target appeared in one of 8 possible locations, and the monkey had, typically to fixate a target – more specifically, to remain within an invisible window around the target. A hit was a trial in which the monkey fixated the vicinity of the target (see Methods for details). Crucially, if the target was too weak to be noticed, unless the eye happened to roam within the invisible window by chance, the trial ended up as an error. So the crucial result of a block was the count of how many of the block's 16 trials ended up as hits. We ran sequences of blocks. Each sequence had a fixed target size; target size did vary from sequence to sequence. Each sequence is illustrated in Figs 4A,B with a specific color. In the first block of each sequence (the leftmost point in the appropriately colored trace) the target was so dim that it went largely unnoticed. Indeed, the first blocks contain only 0-2 hits of the 16 trials, with the hits probably reflecting trials in which the eyes happened to be directed to the vicinity of the extremely weak target. The second block's target was slightly more luminous, and then, for

each subsequent block, the target's luminance was slightly stepped up. After a few blocks, more of the trials turned out to be hits; eventually, all, or almost all trials in the block were hits. At this level of performance, the target was salient. More specifically, we call the monkey's performance in a block 'full', or 'salient', if there were 14-16 hits in the block's 16 trials; 'threshold' if there were 3-13 hits. After completing a sequence, the monkey rested in the dark, allowing for refreshed dark adaptation, and then began another sequence, with smaller targets (usually with target radius being one half that of the most recently completed sequence). As targets became smaller, higher levels of luminance were needed to trace out the psychometric curve. Importantly, throughout the series of sequences, the entire range of scotopic luminance was monitored. The first sequences used very large targets (5.5 and 4 deg radius, correspondingly), and very dim lights were enough for the targets to be salient (just a little more than 10^{-6} cd/m^2, in the low range of scotopic luminance range). For the smallest targets used, with 1/8 deg radii, only with luminance at the high end of the scotopic range (near 10^{-3} cd/m^2) were the targets salient. Note how regular the psychometric curves look, even though they are based on quite few trials. Thus, these sessions track the threshold for saccades and fixation throughout the scotopic range.

To explore the monkeys' performance, let us first ask, for each target size: with targets of that size, what level of luminance is needed for reaching threshold performance? What luminance level is needed for reaching salient, full performance? We analyzed the database of Study 3 (see above). Fig 4C,E, as expected, show that the smaller the target the higher is the luminance needed, for both threshold and salience. The quotient between threshold and salience luminance levels remains close to 2 for all but the largest targets.

Next, we ask: how does a target's size interact with the required luminance, at threshold and salience? The number of photons absorbed is proportional to both target luminance and target area. Technically, the product of target luminance by target area is proportional to the target's luminous intensity (given in cd). Figs 4D,F show the monkeys' performance, the same data as in Figs 4C,E, as a function of the target's luminous intensity. For both threshold and salience, the total luminous intensity is almost fixed for small targets, with radius ≤ 1 deg (monkey M2), ≤ 2 deg (M3). The luminous intensity required for larger targets goes up, both for threshold and for salience. These findings are reminiscent of the psychophysical laws of stimulus detection, particularly of Ricco's and Piper's laws (*19*). They suggest that for small targets spatial summation is efficient, but for large target summation breaks up.

Upshift is higher near threshold than with salient targets

Let us now return to the main theme and ask whether the scotopic band setting, namely, the measured upshift, reflects the fixation's context being in salient or threshold circumstances. We ask: what is the upshift measured with the different target sizes in salient conditions and in threshold? Does the upshift depend on target size at all? If it does, is the upshift measured independent of whether the target is fixated in threshold or salient conditions?

Figs 5A,E show the mean upshift recorded in all Study 3's sessions (see above). There are 2 lines in each of Figs 5A,E, thus 4 lines altogether. The circle-shaped points connected by the thin black lines encode targets at salience. These conditions are illustrated in the example block of Figs 4A,B as the dots above the top horizontal blue line, while the psychometric curves reach full performance. Note that the dot's color in Figs 5A,E identifies the target size

and the sequence of blocks that traces that target size's psychometric curve in Figs 4A,B. The square-shaped points connected by the thick gray lines encode targets at threshold. These conditions are illustrated in Figs 4A,B as the points between (and including) the two horizontal blue lines, between near-null performance to near-full. Thus, for each target size used, we get 2 points in Figs 5A,E: one point for the mean upshift at salient performance, one point for the mean upshift at threshold performance.

The main result of study 3 now comes out. For targets of all sizes, for both monkeys, the mean upshift is higher at threshold than at salience. The scotopic band is set differently in salient performance, and near threshold.

Indeed, if we pick any target size, and look for the dots reflecting the mean upshift measured with targets of that size, the circle, signifying salient performance, lies below the square, reflecting threshold. This relationship is depicted in Figs 5A,E by the dotted yellow and bright orange lines for the two smallest-radius targets, but is evident for all sizes; the threshold upshift graph lies above the salience graph, throughout the target-size range. These observations hold for both monkeys. Not all these comparisons are statistically significant: for monkey M3, the upshift at threshold is significantly higher than at salience for all radii (paired t-test, $p<10^{-4}$ for radius < 4 deg, $p<10^{-2}$ for radius 4 deg and 5 deg). For monkey M2, whose mean upshift is smaller, this relationship is significant for small targets ($p<10^{-3}$ for radii < 1 deg, and slightly significant or insignificant for larger radii ($p=0.02, 0.3, 0.06, 0.8$ for radii 1, 2, 4, and 5.5 deg, respectively). Thus, for small targets the upshift is highly significantly larger at upshift than at salience. For larger targets, the mean upshift at threshold is higher, but less significantly or even insignificantly. Recall that the required gaze direction is less well defined for large targets, because gaze can direct to any part of the large target. This may lead to the compromised statistical significance in M2, whose upshift was lower. Indeed, in not one of these 4 target sizes was the mean upshift higher at salience than at threshold. In sum, the results support the observation that upshift is higher at threshold than at salience; a more dorsal location of the scotopic band appears to be used at threshold than at salience.

Threshold or luminance?

We have now presented evidence that upshift is greater at threshold than at salience. We set out to test upshift at threshold and salience with the hope of showing that scotopic band setting is not always an automatic reflex-modification as in the response to background luminance (Fig 3). However, the observations we made could still, in principle, be automatic responses to increasing luminance; the only difference being that here the increasing luminance is of the target, not the background. By definition, at threshold targets are less luminous than at background. Does this explain away the difference between upshift at threshold and upshift at salience?

Let us consider a concrete case, and start by validating, for that case, that the upshift is higher at threshold than at salience. Examining again Fig 5A, consider the rightmost, yellow circle and yellow square. The yellow circle shows that the mean upshift recorded while monkey M3 fixated salient targets of 1/8-deg radius was 4.37 deg. The yellow square shows that the analogous mean upshift at threshold was 7.35 deg. The histogram of all the upshift values measured while M3 fixated salient 1/8-deg-radius targets is illustrated in the top panel of Fig 5B; the histogram of the threshold values is in the panel immediately below, see markers on

each panel. The histograms of salience and threshold differ from each other; the mean upshift at salience is significantly lower than the mean upshift at threshold ($p<10^{-6}$). The yellow square and yellow circle in Fig 5A are connected to each other with a dashed yellow line. The line is vertical, as both the square and the circle reflect responses to targets of the same size, 1/8 deg. Now examine the same upshift data illustrated as a function of target luminance (Fig 5C). The yellow vertical line is now tilted leftward, illustrating that lower luminance is needed for threshold performance than for salient (see Fig 4C). This recapitulates the question: can we reject the null postulate, that the higher upshift of threshold only reflects the lower target luminance involved?

Threshold is not only lower luminance

We return to the case considered above, of threshold and salience of M3, illustrated in Figs 5A,C with the yellow dashed line, connecting the yellow square (upshift at threshold) and yellow circle (upshift at salience). Recall that these data reflect fixations of the smallest target, of radius 1/8 deg. We will consider this pair together with the corresponding pair of points, one step to the left, relating to targets with ¼ deg radius, colored bright orange; note also the dashed bright orange line connecting the points. See also upshift histograms in Fig 5B. We will similarly consider the analogous data of monkey M2 (yellow and bright orange lines, points, and histograms, in Figs 5E-G). In all these pairs, the mean upshift at threshold is significantly higher than at salience. Recall that the graphical expression of the control postulate was that with the 1/8-deg target, threshold required lower luminance than salient performance, hence in Fig 5C the dashed yellow line is tilted leftward. The same tilt holds for the yellow and bright orange lines of both monkeys.

So, is the upshift of 1/8-deg threshold (yellow squares in Figs 5A,C) higher than the upshift of 1/8-deg salience (yellow circles in Figs 5A,C) only because the 1/8-deg targets at salience more luminous than the targets at threshold? To test this alternate explanation, let us now compare again the upshift of the 1/8-deg threshold (yellow circles) with salient performance, but this time not with the same-size target; rather, with ¼-deg target (bright orange circle). Considering the ¼-deg radius target at threshold by themselves, the relationship of threshold and salience yields findings similar to those observed with the 1/8-deg targets. The bright-orange dashed line connecting the circle and square are vertical in Fig 5A and tilted leftward in Fig 5C, signifying that the targets are the same size and require less luminance at threshold than at salience.

However, when we compare the upshift at 1/8-deg threshold to ¼-deg salience, the situation reverses. The dotted gray lines in Figs 5A,C are tilted rightwards: the ¼-deg targets at salience are less luminous than the 1/8-deg targets at threshold.

This observation is inconsistent with the control postulate we sought to test. If the upshift at 1/8-deg threshold was higher than at 1/8-deg salience only because the threshold targets were dimmer, that the even dimmer targets at ¼-deg salience should have had even higher upshift than the 1/8-deg threshold targets. Therefore, the observation that the upshift at ¼-deg salience is lower than at 1/8-deg threshold strongly indicates that the observed upshift

at threshold reflects not an automatic response to target luminance; rather, it appears to be a response to the context of threshold.

The same results apply to each of the 4 pairs of threshold – salience upshift values designated above, marked by the yellow and bright-orange dotted lines in Figs 5A,C,E,G, and displayed as histograms in Figs 2B,F. Thus, target luminance by itself cannot explain the higher upshift at threshold. Scotopic band setting appears to be directly affected by the context of performing near threshold.

Not the target's luminous intensity

As discussed earlier, Figs 4D,F showed that the target luminous intensity is very similar for targets of radius 1/8-deg to 1 deg (M2), and to 2 deg (M3). Only with larger radii is a higher level of luminous intensity needed. Thus, it appears that at least with small targets, reaching threshold requires about the same number of photons captured per second, invariant of the target's size. This holds for both threshold and salient performance. Thus, the question comes up: could it be that the upshift reflects the luminous intensity of the target?

Figs 5D,H show the upshift as a function of the luminous intensity, for threshold and for salient performance. Focusing for a start on Monkey M3's salient performance (circles connected by a thin black line in Fig 5D), the graph points corresponding to the performance with the small targets are arranged in a vertical line, reflecting the value of luminous intensity common to these small targets. The points corresponding to the small targets are spread out along this line segment. The same situation applies to the small targets in the threshold graph (squares connected by thick gray line in Fig 5D), as well as to the analogous graphs of monkey M2 (Fig 5H). Thus, the upshift changes while the luminous intensity remains almost unchanged. It follows that scotopic band setting does not reflect the luminous intensity.

The graphs of luminous intensity of the 2 monkeys (gray lines in Figs 5D,H) are positioned separately from the salience graphs. This separation between the graphs of threshold and salience suggests that there is a mode change separating threshold from salience, reflecting a different region of the luminous intensity – upshift space. Thus, in terms of luminous intensity, upshift at threshold might reflect a different mode of scotopic band setting from salience.

Discussion

This manuscript pursues the hypothesis that primate scotopic vision incorporates serial processing by a confined retinal region, 'scotopic center', much like the high-acuity processing by the fovea in photopic vision. Specialized sensorimotor transformations shift the scotopic center to the pertinent next location in the scene, and keep it there for processing the input. However, in difference from photopic vision, the scotopic center is not stationary like the fovea. Rather, there is a 'scotopic band'. The scotopic center relocates on the scotopic band according to the ambient luminance and to the perceptual situation.

To test this hypothesis, we showed, first, that in scotopic darkness, a scotopic target is fixated by a confined retinal region dorsal to the fovea. We called this region the scotopic center.

Second, centrifugal horizontal saccades shift the target's image not to the fovea but directly to the scotopic center. These two points indicate that specific scotopic sensorimotor transformations enable the use of the scotopic center for scotopic serial processing. Third, persistent background scotopic luminance systematically relocates the scotopic center to a different position along the scotopic band. The darker the background is, the more dorsal is the scotopic center located on the scotopic band. Fourth, near-threshold conditions implicate a relocation of the scotopic center to a more dorsal position on the scotopic band. This scotopic band setting does not reflect only the physical characteristics of the target, specifically luminance and luminous intensity, and is likely to reflect the context of the task's near-threshold conditions.

Our study is purely functional. Our eye-movement recordings intriguingly correlate to the previous histological results on the geometry of rod-density (*2*, *3*, *18*, *20*). The scotopic band correlates to the rod-dense retinal region dorsal to the fovea. The increase of the upshift with dimmer ambient light correlates to the increased rod density found at more and more dorsal locations.

The observation of the scotopic band opens up many questions. Briefly, does specialized circuitry process signals sensed by the optic band, in the retina and in the brain? Because the notion of the scotopic band is novel, no study systematically addressed the optic band. In the mouse retina, it was recently discovered, signals generated in rods are occasionally switched to and processed by cone-based circuitry. This imaginative discovery begs the question of identifying and understanding the retinal circuitry for processing the scotopic band. What parameters vary gradually along the scotopic band? Regional specialization in the retina, the fovea in particular, is a hot issue (*1*). Furthermore, where in the brain is the input from the scotopic band processed? Do signals from the scotopic band reach visual centers devoted to photopic vision to processing input largely from the fovea?

Another question that comes up, is whether the notion of the optic band relevant also for humans. The first paper that observed upshift in monkeys (*4*) reported that humans, in contrast, do not have upshift. Interestingly, in terms of retinal anatomy, humans do show increased rod density dorsal to the fovea (*20*). Nonetheless, more thorough training and testing might yet find evidence to upshift in humans. In any case, it appears that, in human evolution, the motor program producing the upshift was inactivated or altogether erased in human evolution. It is interesting to speculate whether this postulated sensorimotor program was forever lost or can be reactivated.

Methods

Subjects and surgical procedure

The monkeys of this study were used in neurophysiological experiments, and the general methods are described in the corresponding publications in detail; see, in particular, (Dash et al., 2012; Caggiano et al., 2013, Khazali et al., 2017, Spivak et al., 2014). We used 3 *Macaca mulatta* monkeys; they are marked M1, M2, M3. All were male, 9-11 kg each. All of the monkeys were already trained in visual or oculomotor tasks. All experimental procedures are standard. In short, we used the scleral search coil method to record foveal gaze direction,

also referred to as eye position. In the same procedure they were prepared for the eye position monitoring, the monkeys were prepared for neurophysiological recordings (not part of the present study). In particular, titanium head posts were implanted, allowing to robustly yet painlessly immobilize the heads during the experiment. Proper immobilization of the head is crucial for obtaining robust, accurate recordings of gaze direction. Surgeries were performed under intubation anesthesia with isoflurane and nitrous oxide, supplemented by continuous infusion of remifentanil ($1 - 2.5\ \mu g/(kg \cdot h)$). The relevant vital parameters were fully and tightly controlled throughout the procedure. All the 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).

Setup

The experiments were performed in a completely lightproof electrophysiological setup. The monkeys were seated in front of a cathode ray tube (CRT) screen, at a distance of 35 cm from the screen center. The CRT was an Eizo Flexscan F730, 50-cm diagonal, displaying 1024x768 pixels at a frame rate of 60 Hz. The only source of light during the experiment was the CRT monitor. Gaze direction (eye position) was sampled at 1000 samples per second.
The experiments were run, and data were collected, using the open source measurement system nrec, https://nrec.neurologie.uni-tuebingen.de/nrec/nrec, created by F. Bunjes, J. Gukelberger and others, and used as standard in the Thier lab at Tübingen. The nrec output files were converted to Matlab (https://www.mathworks.com/, MathWorks, Natick, Massachusetts, USA), and post hoc data analysis was carried out in Matlab.

Studies

A session comprised the data collected in a single day. There were 3 studies, each having its trial and session structure. *Study 1* (Fig2): fixation and saccade in scotopic dark with small scotopic-bright targets (revealing the scotopic center); *study2* (Fig 3)*:* dependence of the upshift on background luminance (revealing the scotopic band); and *study 3* (Fig 4)*:* dependence of the upshift on near-threshold. We now describe the stimuli, trial and session structure in these studies.
We first ran study 1 on monkeys M1,M2,M3. Then we ran study 2, on M3 and M2. Finally we ran study 3, again on M3 and M2. Because study 3 substantially differs in procedures from studies 1 and 2, we first describe the methods of studies 1,2, and then of study 3, separately.

<u>Studies 1 and 2</u>

Visual stimuli

The CRT's background was always uniform and featureless. In photopic conditions, the background luminance was 7 cd/m^2. In scotopic conditions it was dark, less than the scotopic threshold.
In the studies 1 and 2, the targets were circular, uniformly filled. In the study 1, photopic targets were small and bright (0.02 deg radius, 60 cd/m^2). Scotopic targets were slightly larger and dimmer (0.14 deg diameter, $7*10^{-4}$ cd/m^2). Thus the scotopic targets were bright for scotopic conditions, but well within the scotopic range. The central fixation spot and peripheral saccade targets were identical (except for position and time in trial). In the first part of study 2 (Fig 3A) the targets were small (0.02 deg radius) and bright (60 cd/m^2), similar to values we previously used to study photopic upshift (1–3); whereas the background

luminance in the first part of study 2 (Fig 3A) changed from block to block, stepping from scotopic to photopic: 10^{-6} cd/m2 (blue markers), 10^{-5} cd/m2 (yellow markers), 10^{-4} cd/m2 (cyan markers), 10^{-3} cd/m2 (black markers), 10^{-2} cd/m2 (green markers), 7 cd/m2 (red markers). In the second part of study 2 target luminance was 10^{-3} cd/m2 and target radius: 0.25 deg. Background luminance differed from block to block, from scotopic to photopic: dark (blue marker), $0.4*10^{-5}$ (green marker), 10^{-5} (cyan marker), $0.2*10^{-4}$ (grey marker), $0.4*10^{-4}$ (orange marker), 10^{-4} (purple marker), $2*10^{-4}$ cd/m^2 (yellow marker).

Locations used for targets

Study 1: in a daily session we used a set of 18 or 20 target locations, spaced 2 deg away from each other, all positioned on the horizontal meridian. In order to increase the total range of saccade sizes (for aims beyond the present study), target locations were slightly shifted from day to day. The closest targets to the central fixation spot were positioned at ±1 deg, ±1.5 deg, ±2 deg, or ±2.5 deg from the central fixation spot. Thus, the maximal horizontal eccentricity was 20.5 deg. Here we pulled together all data, taking into account the target location of each individual trial, regardless of the session's target set.
Study 2: the fixation targets had three possible locations, positioned on the horizontal meridian: the center of the screen, 10 deg to the right and 10 deg to the left of the center. Every block consisted of 30 trials; every target location was used 10 times in a block.

Trial structure

Study 1: when the central fixation spot appeared, the monkey was required to direct his gaze into an invisible window around the spot within 1 s (see next paragraph for detail). Then the monkey had to maintain fixation, that is, to keep his gaze within the window, for at least 1.5 s. Next, the central fixation spot disappeared, and, simultaneously, the peripheral target appeared. The monkey had to make a saccade into an invisible window around the peripheral target within 1 s. The target remained for 2 s on the screen and the monkey was required to keep his gaze within the invisible window around the peripheral target until the end of the trial. On detection of an error, the computer aborted the trial and initiated a new trial; on correct performance ('hit') the computer rewarded the monkey with a drop of water. Total trial duration was 4.5 s.
Study 2: the monkeys had to direct their gaze into the invisible window around the target spot, and keep their gaze there as long as the target remained on. The fixation invisible window appeared 1 s after the appearance of the target. Thus, within 1 s from target onset the monkey had to shift his gaze into the invisible window around the target. The trial was counted as a hit if the monkey maintained his gaze within the invisible window centered on the peripheral target for an additional 1.5 seconds. At the end of the trial, the monkey was rewarded for performing a hit with a drop of water as reward. After the completion of the trial, the next trial immediately followed. The location of the target changed from trial to trial in pseudo-randomized order. The total duration of the trial was 2.5 seconds.

Gaze direction windows

In photopic conditions, the invisible windows were square shaped, with 3 deg radius (±3 deg for each horizontal and vertical). In scotopic conditions (including the mesopic blocks in study 2), the windows were 15 deg vertical radius and 5 deg horizontal radius. These large invisible

windows reflected our wish not to artificially constraint monkey's scotopic oculomotor behavior.
Does the large scotopic invisible window allow noise into the fixation data?
This choice of parameters reflects our experience with studying the upshift. As can be seen in all the panels of Figs 2A, and in the previous studies of the upshift for photopic upshift, the gaze positions densely accumulate in a small sub-region of the large window. Fig 3 shows an example of how the clusters move upon appropriately changing the visual conditions. These clusters (and others observed in previous studies) show that the large windows allow the physiological changes in gaze direction to come through, rather than increase noise.

Daily sessions

A daily experimental session consisted of a series of blocks of trials, 100 (study 1), 30 (study 2) trials in each block, counting only hits. A session began with standard gaze direction calibration, in photopic conditions; the calibration was followed by a block or two of photopic trials. Then the monkey waited through a 45-min interval of dark adaptation. During this time, the monkey was in full darkness, and did not work. Then the scotopic blocks were recorded.

Data analysis

Study 1: to calculate the mean gaze direction during central and peripheral fixation (Fig 2 A, B) we averaged across intervals of 250 ms, one interval before the saccade started, and one after the saccade was completed. For the fixation of the central spot, the interval was sampled before the central fixation spot was turned off. For the fixation of the peripheral target, the interval was sampled at the end of the post-saccadic target fixation, shortly before the end of the trial. Therefore, the peripheral target fixation measurement was not contaminated with saccadic correction.
Study 2: Mean gaze direction during target fixation was calculated by averaging across the last 1 s fixation of the trial.
The parameters of the study 3 described in Figs 4 and 5 are explained in a separate section, ahead.

<u>Study 3</u>

Study 3 aimed at studying the upshift near threshold, using targets of different sizes. The experiment was conducted in scotopic conditions that required 45 minutes of dark adaptation prior the task. The standard gaze direction calibration was made in photopic conditions before the dark adaptation. Every trial began with an appearance of the fixation target at one of the eight locations arranged in a circle of 15 deg radius. Targets were presented in a pseudo-random order, one target per trial. A target remained on the screen for two seconds without fixation window around it. This time was intended to allow the monkey to find target location and stabilize gaze at it. If at the end of the two seconds the monkey still gazed at the target, fixation window appeared for another 0.5 seconds. Fixation of the target during the additional 0.5 seconds was rewarded with a drop of water, and the trial was labeled as correct. If the monkey broke fixation during the last 0.5 seconds of the trial or did not look at the target before the fixation window appeared, the trial was labeled as incorrect and immediately switched to the next. No reward was given in this case. Possible trial length was 2-2.5 seconds depending on monkey's performance. Two seconds trial length indicates that the monkey did

not locate the target. Trial length of 2.5 seconds indicates that the monkey detected the target and completed the trial successfully. This trial was counted as hit. Any trial length between 2 and 2.5 seconds would suggest that the monkey detected the target correctly but broke fixation during the last 0.5 seconds of the trial. In this case the trial was not counted as hit. Every block consisted of 16 trials, so that every target appeared 2 times in a block. In every session we ran several blocks. It allowed us to construct psychometric curves (Fig 4) taking into account the correct trial number and target luminosity in every block. The first block in a session contained targets at very dim luminosity that as we knew the monkey could not detect. We increased target luminosity from one block to the next by the constant range of units that was determined by the stimuli-displaying software. By increasing target luminosity from block to block we also increased the ability of the monkey to detect the target. The session was accomplished when the monkey could complete all 16 trials in a block successfully. The monkey underwent additional dark adaptation of 10-15 minutes between the sessions. The possible target radii were: 2.4, 2, 1, 0, -1, -2, -3, -4 and -5 in $\log^2$ degrees. The largest target's radius was set to 2.4 instead of 3 $\log^2$ degrees due to monitor's limitations. The size of the fixation window was 15 deg horizontal and 20 deg vertical. This size ensured that even the largest fixation target could fit the window. Target's radius decreased from session to session in a consecutive order. At every session only one target radius was used. Mean gaze direction during target fixation of the correct trial was calculated by averaging across the last 0.5 s fixation of the trial.

Bibliography

1. T. Baden, T. Euler, P. Berens, Understanding the retinal basis of vision across species. *Nat. Rev. Neurosci.* **21**, 5–20 (2020).
2. K. C. Wikler, R. W. Williams, P. Rakic, Photoreceptor mosaic: number and distribution of rods and cones in the rhesus monkey retina. *J. Comp. Neurol.* **297**, 499–508 (1990).
3. O. Packer, A. E. Hendrickson, C. A. Curcio, Photoreceptor topography of the retina in the adult pigtail macaque (Macaca nemestrina). *J. Comp. Neurol.* **288**, 165–183 (1989).
4. D. M. Snodderly, D. Kurtz, Eye position during fixation tasks: comparison of macaque and human. *Vision Res.* **25**, 83–98 (1985).
5. D. M. Snodderly, Effects of light and dark environments on macaque and human fixational eye movements. *Vision Res.* **27**, 401–415 (1987).
6. S. Barash, A. Melikyan, A. Sivakov, M. Tauber, Shift of visual fixation dependent on background illumination. *J. Neurophysiol.* **79**, 2766–2781 (1998).
7. S. Hecht, C. Haig, A. M. Chase, The influence of light adaptation on subsequent dark adaptation of the eye. *J. Gen. Physiol.* **20**, 831–850 (1937).
8. T. Reuter, Fifty years of dark adaptation 1961-2011. *Vision Res.* **51**, 2243–2262 (2011).
9. O. Spivak, P. Thier, S. Barash, Persistence of the dark-background-contingent gaze upshift during visual fixations of rhesus monkeys. *J. Neurophysiol.* **112**, 1999–2005 (2014).
10. R. M. Foerster, E. Carbone, H. Koesling, W. X. Schneider, Saccadic eye movements in the dark while performing an automatized sequential high-speed sensorimotor task. *J. Vis.* **12** (2012), doi:10.1167/12.2.8.
11. A. F. Fuchs, D. A. Robinson, A method for measuring horizontal and vertical eye movement chronically in the monkey. *J. Appl. Physiol.* **21**, 1068–1070 (1966).

12. S. J. Judge, B. J. Richmond, F. C. Chu, Implantation of magnetic search coils for measurement of eye position: an improved method. *Vision Res.* **20**, 535–538 (1980).
13. U. Wildenmann, F. Schaeffel, Variations of pupil centration and their effects on video eye tracking. *Ophthalmic Physiol Opt*. **33**, 634–641 (2013).
14. K. W. Choe, R. Blake, S.-H. Lee, Pupil size dynamics during fixation impact the accuracy and precision of video-based gaze estimation. *Vision Res.* **118**, 48–59 (2016).
15. I. Hooge, K. Holmqvist, M. Nyström, The pupil is faster than the corneal reflection (CR): Are video based pupil-CR eye trackers suitable for studying detailed dynamics of eye movements? *Vision Res.* **128**, 6–18 (2016).
16. M. Nyström, I. Hooge, R. Andersson, Pupil size influences the eye-tracker signal during saccades. *Vision Res.* **121**, 95–103 (2016).
17. L. Goffart, J. Quinet, F. Chavane, G. S. Masson, Influence of background illumination on fixation and visually guided saccades in the rhesus monkey. *Vision Res.* **46**, 149–162 (2006).
18. K. C. Wikler, P. Rakic, Distribution of photoreceptor subtypes in the retina of diurnal and nocturnal primates. *J. Neurosci.* **10**, 3390–3401 (1990).
19. P. E. Hallett, F. H. Marriott, F. C. Rodger, The relationship of visual threshold to retinal position and area. *J. Physiol. (Lond.)*. **160**, 364–373 (1962).
20. C. A. Curcio, K. R. Sloan, R. E. Kalina, A. E. Hendrickson, Human photoreceptor topography. *J. Comp. Neurol.* **292**, 497–523 (1990).

Figures

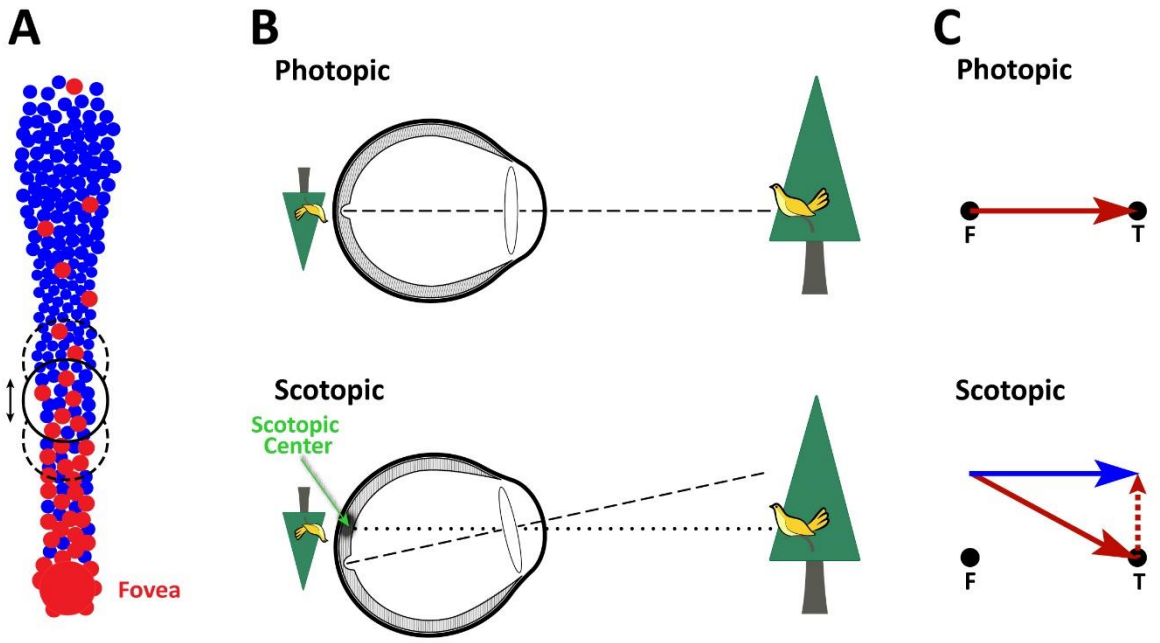

Figure 1. **(A)** Illustration of the hypothesis. Dorsal to the fovea there is a specialized 'scotopic band'. At any time, a confined region within the scotopic band functions as a 'scotopic center'. The target's image is directed to the scotopic center, much as the target's image is directed to the fovea in photopic vision. The scotopic center is the basis for scotopic serial processing, analogous to photopic high-acuity vision. The illustration schematically illustrates the photoreceptor density functions along the scotopic band, but this homology is only suggestive; our operational definition is purely functional. **(B)** Illustration of experimental prediction 1, scotopic upshift of fixation. In photopic vision, the target's image (the bird on the tree in this schematic example) is fixated by the fovea. If in scotopic vision the target's image falls on a scotopic center, an unsuspecting observer following only the foveal line of gaze would report that the subject looks above the target, not that the processing focus had switched from the fovea to the scotopic center. Such upshift was observed with dark background, but not scotopic. Dashed line, foveal line of gaze; dotted line, line of gaze to the presumed scotopic center. **(C)** Illustration of Prediction 2, saccadic sensorimotor transformations. The photopic sensorimotor transformation for saccades shifts the fovea directly from the initial fixation spot to the target. If all saccades foveate, including scotopic, then scotopic saccades too should end with the fovea on the target, even though they start with upshift (see panel B). Foveation (red arrows) is the 'null prediction'. One alternate prediction is that the scotopic band setting, the location of the center on the band, would remain similar through the saccades. Thus, the saccades would start and end with upshift, reflecting a direct shift of the scotopic center from the fixation spot to the target (blue arrow).

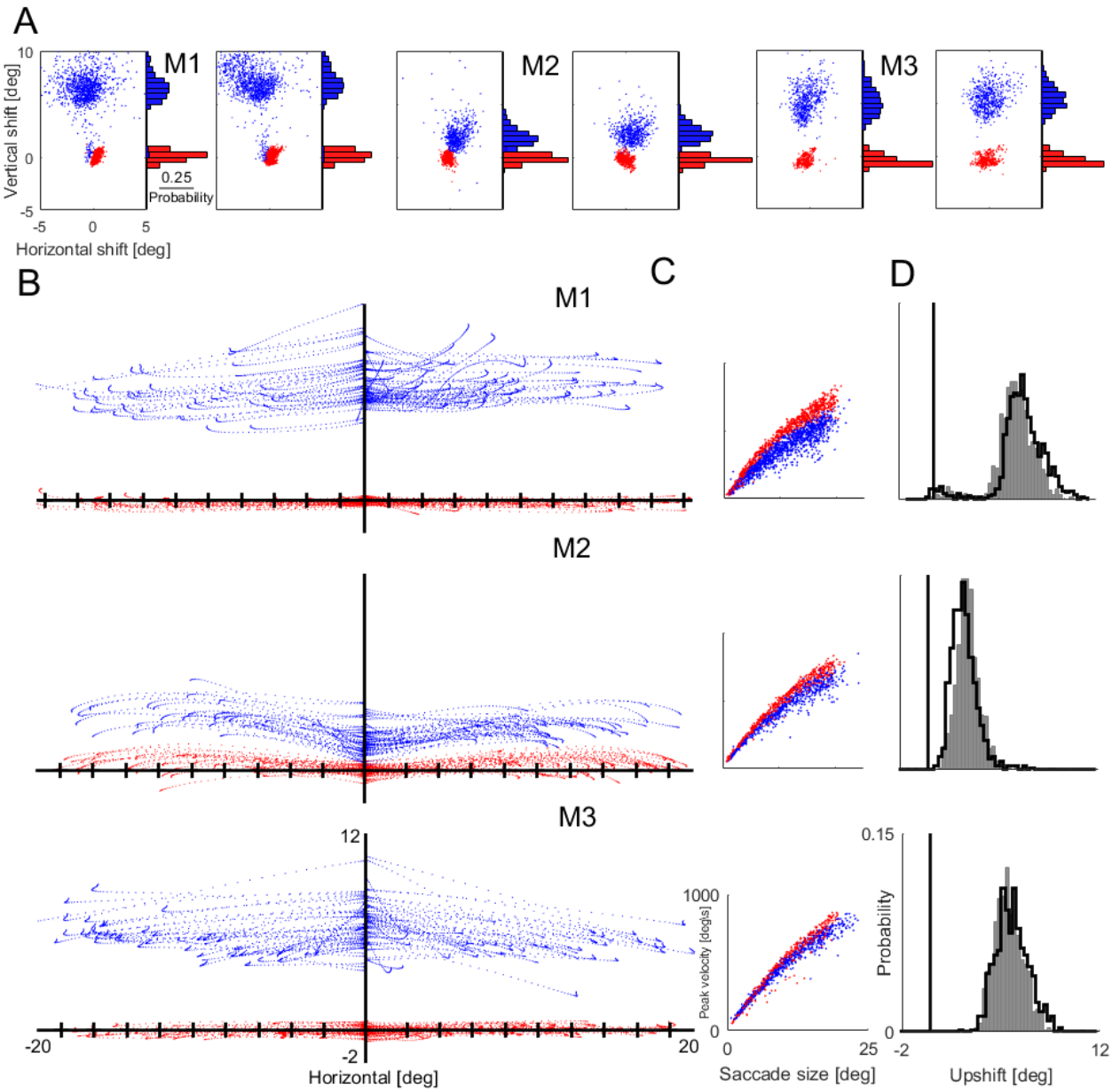

Figure 2. Scotopic fixations and saccadic eye movements shift the target's image to the scotopic center. All scotopic data is colored blue, photopic red. The 3 monkey subjects are marked M1,M2,M3. **(A)** Mean gaze direction during fixation; each dot reflects a single trial. An upshift is evident (the blue cluster is above the red). The scotopic center is defined by the blue cluster. Data are shown for central and peripheral locations (left and right panels for each monkey). **(B)** Examples of saccadic trajectories, for each monkey. For clarity, the trajectories are shifted to start from horizontal zero. **(C)** Main sequences of all saccades. Scotopic saccades have standard main sequences. **(D)** Histograms of the upshift at the start and end of the saccade (filled gray and unfilled black histograms). The vertical solid line stands for zero (no) upshift. The data reject the foveating prediction, namely, that saccades would end with no upshift.

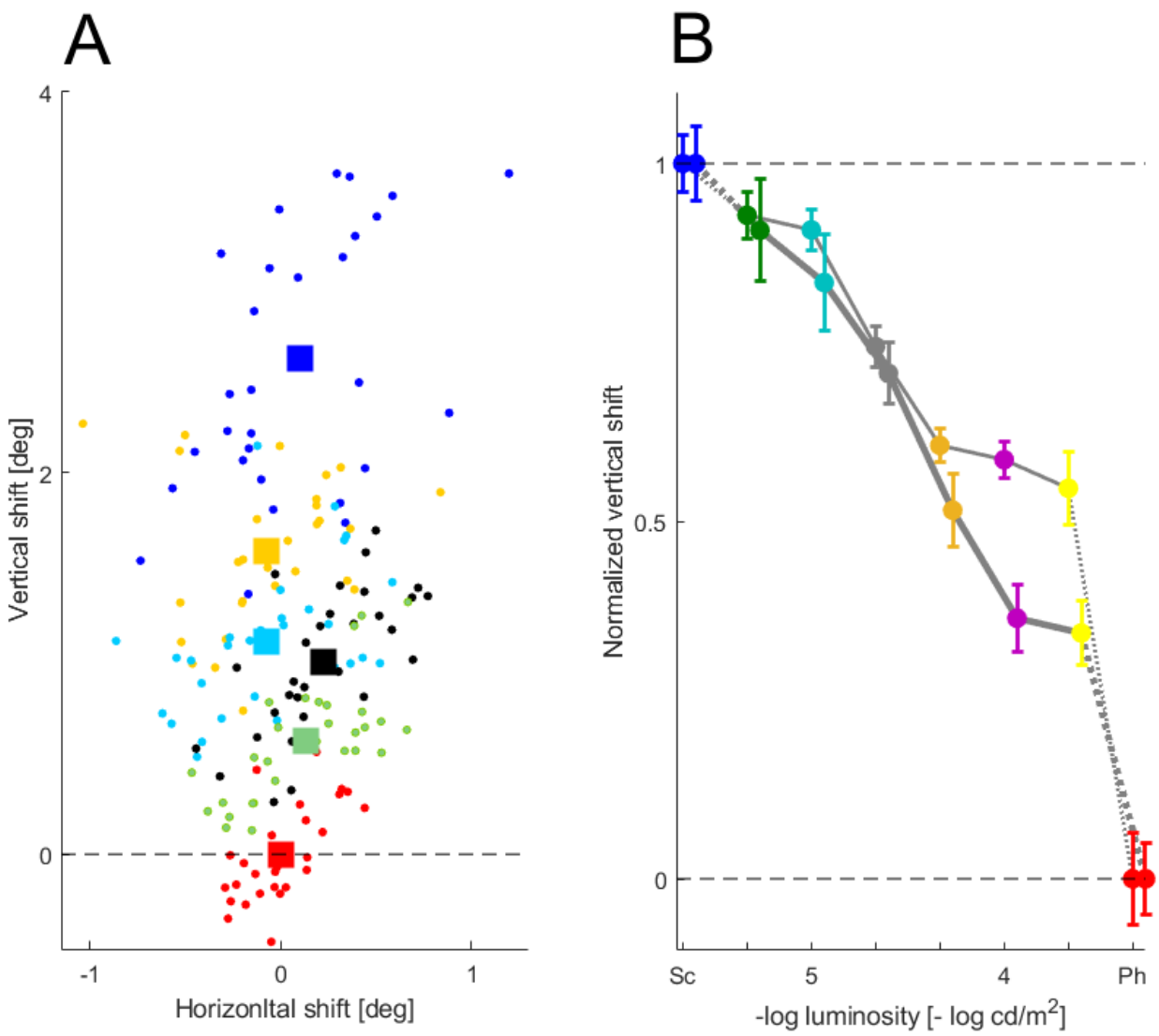

Figure 3. Evidence for the existence of the scotopic band: the darker the background, the greater the upshift is. **(A)** Example session, with trial-by-trial gaze direction data for background luminance increasing from block to block, from dark to photopic level. Every dot illustrates foveal gaze direction in one trial, while fixating small (0.02 deg radius) bright (60 cd/m^2) targets. The plot depicts six consecutive blocks, of 30 trials each. Every block had its own background illumination: 10^{-6} cd/m^2 (blue), 10^{-5} cd/m^2 (yellow), 10^{-4} cd/m^2 (cyan), 10^{-3} cd/m^2 (black), 10^{-2} cd/m^2 (green), 7 cd/m^2 (red). The squares depict the mean foveal gaze direction in each block. The dashed line marks zero upshift (no upshift). **(B)** Mean upshift for several levels of background luminance. Data were collected from 2 daily sessions for each monkey, one block from each session, and normalized to each monkey's scotopic upshift. Monkey M2's data are shown with the thick grey line, M3's as thin grey line. Each point shows the mean normalized shift of the 2 blocks with the standard error. Full scotopic shift is shown in blue, photopic (7 cd/m2 background) in red; the intermediate points show background levels in the scotopic range, green: $0.4*10^{-5}$, cyan: 10^{-5}, grey: $0.2*10^{-4}$, orange: $0.4*10^{-4}$, purple: 10^{-4}, yellow: $2*10^{-4}$ cd/m^2). Target luminance was 10^{-3} cd/m^2, target radius: 0.25 deg. The dashed vertical lines mark vertical zero (no upshift) and 1 (maximal upshift).

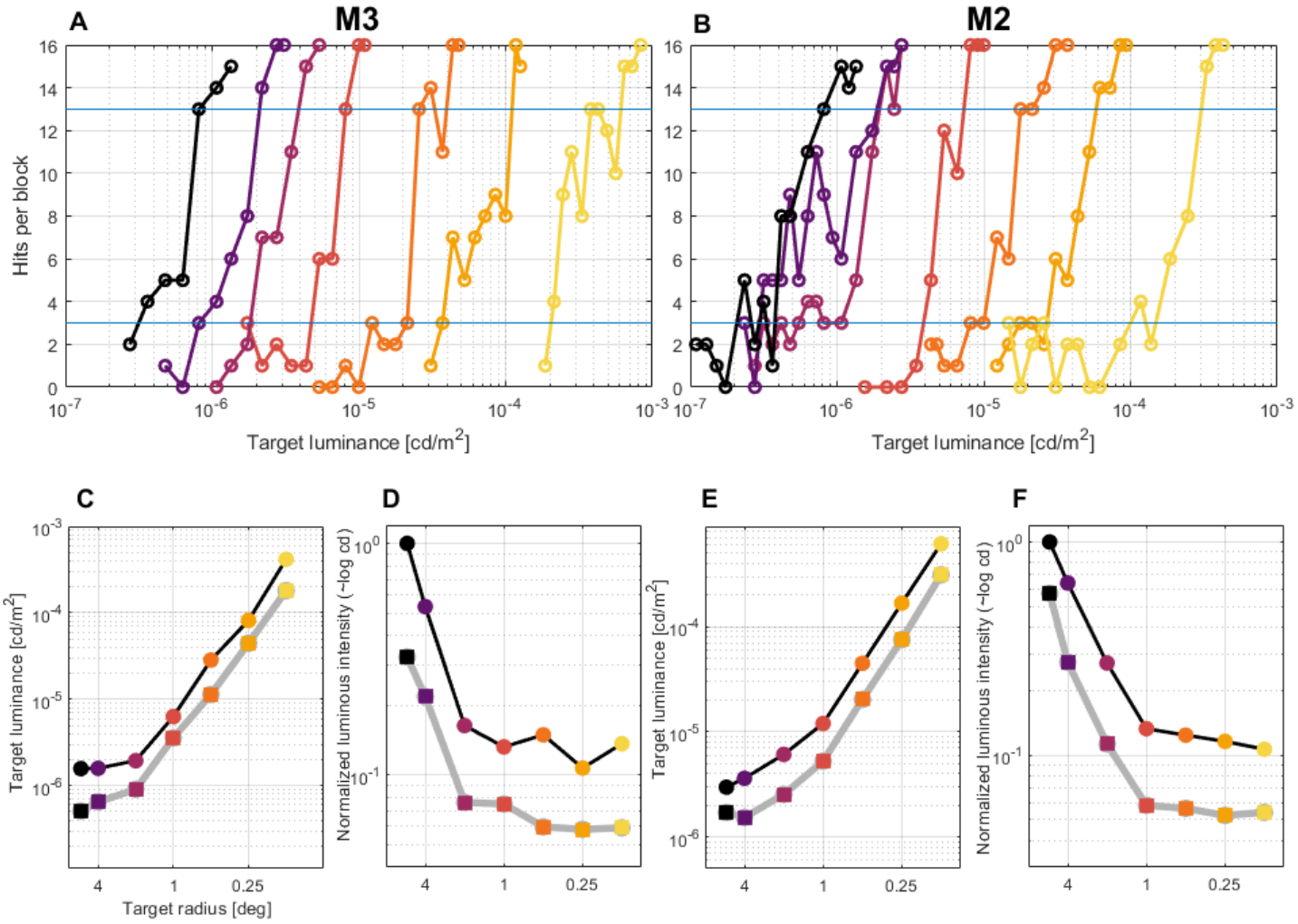

Fig 4. The monkeys' performance in a study of visual fixations in near-threshold versus salient conditions. **(A)** Example of a single session of monkey M3. Each dot reflects the number of hits in a 16-trial block using a target of the specified luminance. The session is made up of block sequences, color coded; each sequence uses a fixed target size. While keeping target size fixed, in each consecutive block the target is made slightly brighter. The resulting psychometric functions reflect the rise from no response (0-2 hits, under the lower blue horizontal line) through threshold (3-13 hits, between the 2 horizontal blue lines) to salient, full performance (14-16 hits, above higher blue line). Target radii in visual degrees, from left: 5.5, 4, 2, 1, ½, ¼, 1/8. **(B)** Same as (A), for monkey M2. **(C)** Mean target luminance required for reaching threshold (gray line) and salience (black line) for each target size, computed from all sessions of M3. The smaller the target, the more luminous the target must be for reaching performance. **(D)** M3's performance analyzed as in terms of normalized luminous intensity, proportional to target luminance times target area, also the total number of photons absorbed per unit time. Small targets need about the same level of luminous intensity to reach threshold and salience, almost invariant of target size. Large targets require graudally more luminous intensity. **(E)** and **(F)** Analysis of monkey M2's performance; same format as **(C)** and **(D)**.

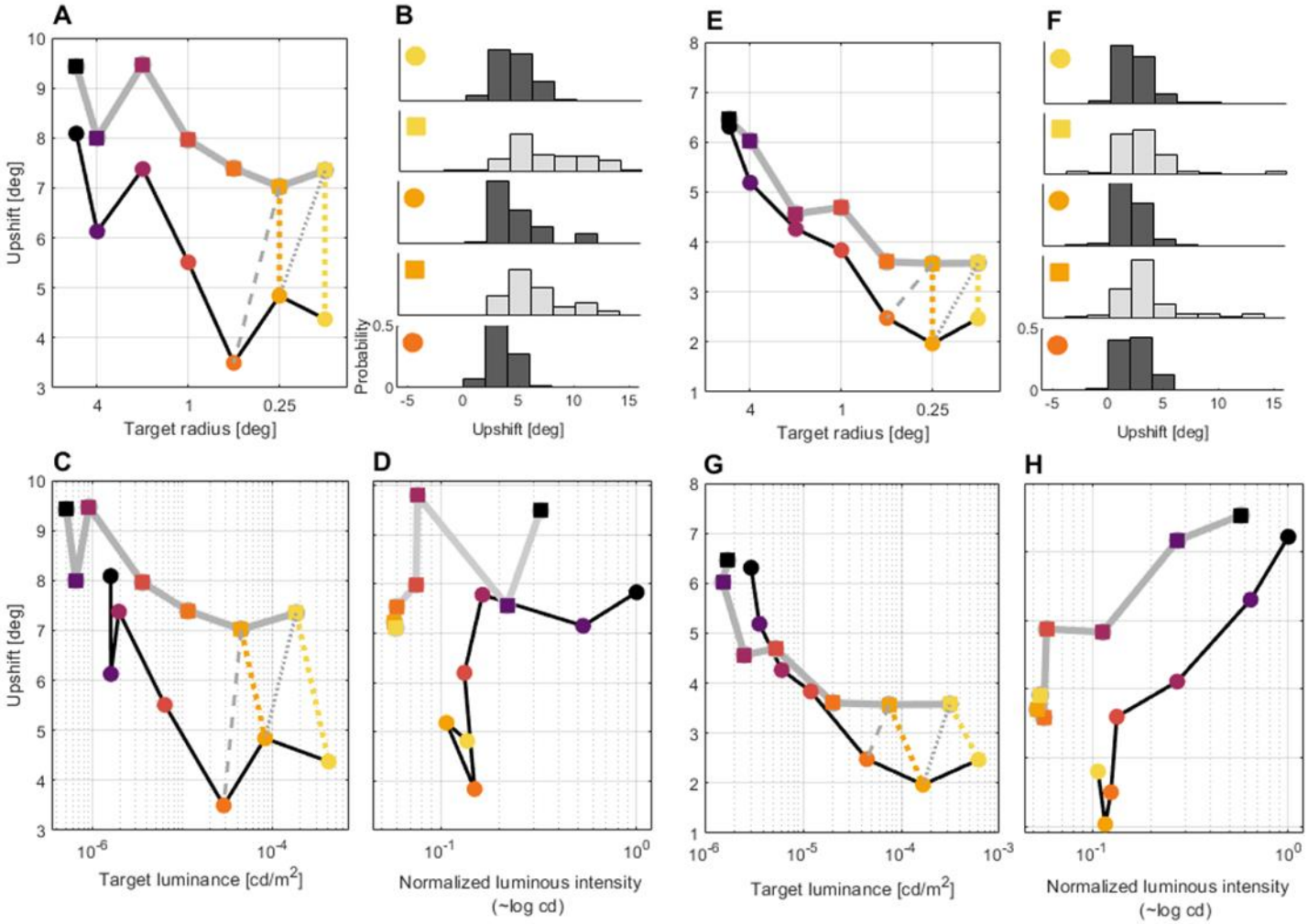

Fig 5. The scotopic center is set to more dorsal locations on the scotopic band when the task context is that of near-threshold; this effect is not an automatic response to the lower luminance, nor luminous intensity. **(A)** The upshift (position of the scotopic center on the scotopic band) at threshold (gray line) and salience (black line) plotted as a function of target radius. **(B)** Histograms of the upshift at threshold (with the 2 smallest targets, gray bins) and salience (3 smallest targets, black bins). See markers together with (A) to identify the histogram. **(C)** Same data as (A), but plotted as a function of target luminance rather than target radius. **(D)** Same data as (A), but plotted as a function of normalized luminous intensity. **(E)** to **(H)** Same format as (A) to (D), but data from monkey M2.

Appendix 4:

Spivak, O., Thier, P., & Barash, S. (2020). In mesopic and scotopic vision monkeys use the scotopic band, a retinal region dorsal to the fovea. *In final stage of preparation.*

In mesopic and scotopic vision monkeys use the scotopic band, a retinal region dorsal to the fovea

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Abstract

This study addresses eye movement mechanisms in the monkey. We recently suggested that the monkey's retina contains a scotopic center which replaces the fovea in scotopic vision. The scotopic center relocates along the scotopic band according to visual and other task conditions. Specialized eye movements shift the target's image to the scotopic center and keep it there, rather than to the fovea. Here we describe the evidence that led us to suggest the scotopic band. We studied eye movements in monkeys fixating a target in dark background. We compared 4 functional states, all with very small targets. Photopic dark, with bright targets; scotopic (meaning dark-adapted) dark, with the same bright targets; scotopic dark with scotopic targets; and mesopic background with bright targets. The results support the concept of the scotopic band. The position of the scotopic center on the scotopic band is most dorsal in scotopic vision, that is, scotopic background with scotopic target. The other states take an intermediate position on the band. Thus, the notion of the scotopic band extends also to mesopic vision.

Introduction

Fixation and saccadic eye movements are crucial for photopic vision

The evolution of a distinct, well-defined fovea is thought to be a signature of the primate and human visual system. The fovea comprises a much higher density of cones than the periphery; its boundaries are sharp; it is well defined anatomically and physiologically; and the specialized processing of foveal information goes far beyond the receptor level, through the retina's other structure and into the brain. In visual cortex, information from the fovea is vastly over-represented. The high receptor density, coupled with the special circuitry spanning retina through visual cortex, underlies the fovea's unique capacity for high-acuity vision.

Nonetheless, the fovea is small. At any one time, the fovea covers only a small patch of the visual scene. To allow for high-acuity vision of more than a small patch, the eye must be rotated in the orbit, so that the fovea covers the next location of interest. Thus, specialized eye movements have evolved in parallel to the evolution of the fovea. These movements stabilize the eyes during brief 'fixations', which allow vision to take place; and then shift the eyes, briskly yet precisely, to the next location ('saccades').

The seeming paradox of fixation and saccades in scotopic vision

In dim light, cones are desensitized. Because most of the fovea is made up of cones, in scotopic vision the fovea is largely blind. The rationale for the function of saccades breaks down. Why keep shifting the target's image to the fovea, now that the fovea's visual performance is inferior to the rest of the retina? Nonetheless, seemingly paradoxically, the pattern of fixations and saccades is sustained in scotopic vision. Why?

The scotopic band

Recently (paper submitted) we have suggested that scotopic vision also incorporates a mode of serial vision, structured around a region of preferred processing and eye movements adapted for that region. The region of relevance is the 'scotopic band'. We defined it functionally, based on eye movements. The scotopic band encompasses a long, narrow region dorsal to the fovea. At any moment, a locus within the band functions as a 'scotopic center'. Targets (locations of interest in the scene) are brought by saccadic eye movements directly to the scotopic center, and maintained at that retinal locations throughout the fixation, which allows the photoreceptors of the scotopic center to process the input image.

Although we have offered evidence supporting the notion of the scotopic band, the evidence that actually led us to this concept makes up the bulk of the present paper. So one aspect of the present paper is lending support to the existence of the scotopic band. The other aspect is extending the concept to mesopic vision. The scotopic band appears to offer gradual processing. Mesopic vision appears to involve mostly locations closer to the fovea than scotopic vision. This is a main innovation of the present paper.

The upshift

The way the setting of a location on the scotopic band is reflected in standard measurements is by a phenomenon called upshift. Snodderly (1985) and Barash et al. (1998) discovered and rediscovered the upshift by chance. When the background is dark, monkeys appear to fixate a location above the target, rather than the target itself. Snodderly reported that humans do not show upshift. Therefore it appeared to be an idiosyncrasy of monkey eye movements. However, Barash et al. (1998) suggested that the upshift might be related to some unexpected findings concerning the distribution of rods and cones on the retina, findings that were then relatively new.

Rod-dense region in the superior retina

Cones are concentrated in the monkey fovea. Extrafoveally, cone density is much lower, and it generally decreases with rising eccentricity. Rods have a more complex density map. Rods are generally denser in dorsal retina than in ventral. Dorsal to the fovea, rod density is high. On going from the fovea dorsally, gradually cone density goes down, while rod density gradually goes up. At an eccentricity reported to be close to that of the blind spot, rod density reaches a peak. Studies by the groups of Rakic (Wikler and Rakic, 1990; Wikler et al., 1990) and of Curcio (Packer et al., 1989; Curcio and Allen, 1990) found the region of high rod density in the dorsal retina. Generally, the dorsal retina was found to have more rods than the ventral retina. The rod-dense region was called 'rod hotspot' or 'dorsal rod peak'.

Early Hypothesis: in scotopic vision, the rod-dense region might replace the fovea

In light of the anatomic findings described above, Barash et al. (1998) suggested that the upshift might actually reflect a switch, when the background is dark, to a region that replaces the fovea in scotopic vision. That region was suggested to be the dorsal rod peak. Because it is dense in rods and almost lacks altogether cones, it was suggested to be the mirror-image of the fovea in scotopic vision.

The experiments outlined here were started with this hypothesis in mind. Nonetheless, even though the finding of upshift was very robust, the size of the upshift varied too much. After all, the concept of a rod hotspot replacing the fovea leads to a predicition of bimodal fixation positions; this was not reflective of the results.

The scotopic band

Eventually, we came to suggest that the entire interval connecting the fovea and, perhaps, the rod hotspot, functions in vision that involves rods. Therefore we called it the scotopic band. However the concept incorporates mesopic vision as well. Indeed, most of the region that would make the scotopic band appears to contain both rods and cones.

However, this involvement remains to be proved.

Distinction between photopic and scotopic dark

The time-course of dark adaptation is marked by two exponential phases (Normann and Werblin, 1974). Going from a bright environment to full dark, the first exponential phase is dominated by cones. It lasts typically about 15 min. The exponential rise in sensitivity reflects

the adaptation of the cones to the dark. During most of this first phase, the rods, which were initially saturated by the bright light before dark onset, also increase their sensitivity, but more slowly than the cones. Then the rate of the cones' adaptation goes down, reflecting stabilization of the cones at their maximal sensitivity. Only then do the rods start to dominate vision, as their sensitivity continues to increase beyond the maximal sensitivity of cones. Because the increase in sensitivity is exponential, theoretically it does not come to an end. However, 45 min appears to be a conservative figure.

The perception of stimuli appearing during the first and second phases of the dark adaptation time-course is likely to differ, because the first phase involves primarily cones, and the second primarily rods. Therefore, we call the first phase 'photopic-dark', and the second phase 'scotopic-dark'.

Differentiating target from background

A major point concerning the upshift is that, with a very small target over large uniform background, the upshift appears to be guided by the background, not the target itself. The primary parameter of the background is its absolute luminance, not the contrast between target and background luminosities. An important aspect of the present study was that of testing the effect of background invariant of the target. Therefore we used only very small targets, and tried to avoid changes to the target as much as possible. We could not avoid changing luminance altogether, but used only 2 levels, bright and near-scotopic. Here we call the stimulus scotopic because its luminance, 0.007 cd/m2, is borders the scotopic luminance range.

Three states to compare

One main thrust of the current work is that of comparing the upshift in 3 states. First, photopic dark background, bright small targets, duplicates the previous studies of upshift. Second, scotopic dark background and bright targets, attempts to map out the influence of the dark-adaptation 45 min interval that turns photopic dark into scotopic dark. Third, scotopic dark with scotopic target, compares the effect of the target – bright versus dim – on the upshift, following base scotopic state.

These states chart the relationship between photopic (bright target and bright background) and scotopic. We also tested mesopic conditions, where the target was bright and the background on the mesopic level.

Alternative hypothesis: the upshift might be a trait of photopic-dark

The few studies that have addressed the upshift to date have not referred to the state of dark adaptation. Recordings were started briefly after dark onset, because the upshift was thought to be more of an idiosyncrasy than a systematic switching between visual subsystems. Therefore, most of the recordings in studies of the dark-background contingent upshift were conducted in what we now call photopic-dark. If scotopic-dark had been reached in these studies, it was a rare occasion; certainly full 45-min dark adaptation was rarely if ever actuated. Therefore, even though the hypothesis relates the upshift to scotopic vision, it was not tested if there is an upshift in scotopic vision.

In our previous study, we presented evidence that scotopic vision indeed involves upshift. Nonetheless, while running these experiments we have considered an alternative hypothesis. This hypothesis could still be valid as an addition to the upshift of scotopic vision itself. What the hypothesis asserted was that upshift emerges in photopic dark; the original hypothesis suggested with time in the dark, and the passage to scotopic vision, the upshift would dissipate. There could still be processes that contribute to the upshift during photopic dark, that dissipate on going to scotopic dark.

Methods

Two rhesus monkeys were used in this experiment. All 2 monkeys were adults male, weighting 9-11 kg. All had been previously trained to perform oculomotor tasks for water reinforcement. Hence, the present project required only modest additional training for accommodating the monkeys to the present task and to dark adaptation.

All experimental procedures are standard and have been described in detail in recent publications of Thier and colleagues (Dash et al., 2012; Caggiano et al., 2013). In brief, eye position was recorded using the scleral search coil method. The monkeys were prepared for neurophysiological recordings (not part of the present study); their heads were painlessly immobilized by titanium head posts. Surgeries were performed under intubation anesthesia with isoflurane and nitrous oxide, supplemented by continuous infusion of remifentanil (1–2.5 (micro)g/kg^{-1}·h^{-1}) with full tight control of all relevant vital parameters. 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 experiments were performed in a standard electrophysiological setup. The monkeys were seated in front of a cathode ray tube (CRT) screen at the distance of 35 cm separating the monkey's eyes from the center of the screen. The CRT was an Eizo Flexscan F730, 50-cm diagonal, displaying 1,280 X 1,024 pixels at a frame rate of 72 Hz. The room was completely lightproof during the experiment, dark to the level that several human viewers could not report any impression of light after sitting in the closed room for 1 hour. Thus, the only source of light during the experiment was the monitor the monkeys were facing.

Three luminance levels were used for the background of the target. The bright, photopic background was 7 cd/m2. The dark background (both photopic and scotopic) was 0 cd/m2. For mesopic backgrounds we used luminance levels between $1*10^{-6}$ and 0.04 cd/m^2. The level of background illumination was elevated from block to block, starting with values slightly above full scotopic and finishing with values well within the mesopic range.

All targets were of the same, small size (0.02 deg). Two luminance levels were used for the fixation targets. The small bright targets had a luminance of 60 cd/m2. Dim ("scotopic") targets had a luminance of 0.007 cd/m^2. This luminance level is slightly above the scotopic range, but to be visible, we could not dim it more (without changing target size). At this luminance, monkeys successfully responded to the scotopic targets. The targets could appear in any of 24 locations, arranged in 3 concentric circles, 8 locations on each circle (Fig. 1). The fixation windows used in scotopic vision had a size of 15x15 degrees. In photopic vision the window was usually 3x3 degrees, but in some of the trials also 15x15 degrees, to verify that the smaller window size does not constrain the monkey's performance.

The experimental sessions consisted of a series of trial blocks, 120 trials in each block. Interspersed with the blocks were 45-min intervals of dark adaptation (or, some cases, light adaptation, as control, see Results). The mesopic vision condition was tested in a series of blocks that came after the baseline photopic blocks, dark adaptation interval, and scotopic blocks.

Trials lasted for a few seconds (2-5, typically 2-3 s). The last second of the trial was used for calculating the trial's median and mean eye position during visual fixation. The search coil data was sampled at a rate of 1000 samples/sec. In a previous study (Spivak et al., 2014) we showed that the mean dark-background-contingent upshift is stable after about 0.5 s from the start of fixation.

Data collection was performed using the open source measurement system nrec, https://nrec.neurologie.uni-tuebingen.de/nrec/nrec, created by F. Bunjes, J. Gukelberger and others, and used as standard in the Thier lab at Tübingen. The nrec output files were converted to Matlab (https://www.mathworks.com/, MathWorks, Natick, Massachusetts, USA), and post hoc data analysis was carried out in Matlab. The statistics used standard t-tests.

Results

The results of a session

Data collection spanned multiple daily sessions. By a 'session' we refer to the data collected from one monkey, in a single day. A session comprised a series of blocks; a block consisted of a series of trials. In all trials, the monkey's task was straightforward visual fixation: a very small target (0.02 deg radius) appeared on the screen; the monkey made a saccadic eye movement to the vicinity of the target, and remained in the vicinity of the target for as long as the target remained on the screen. At the completion of a predetermined fixation interval (typically, a few seconds) the target disappeared and the monkey was rewarded with a drop of water.

Each trial contained a single target. Target location varied between trials: targets spanned 24 locations (Fig. 1), which were presented in pseudo-random order. Each target appeared several times in a block (typically 4-5). Other than the target's location, visual parameters, such as target and background luminance levels, were maintained throughout each block. Thus, except for an inevitable minimal change of light/dark adaptation occurring throughout a block, the visual conditions were constant throughout each block. Because each block lasted only several minutes, the state of dark adaptation did not vary significantly during the duration of a block.

Fig. 2 shows the results of a typical session comprising 4 blocks: 2 in photopic and 2 in scotopic conditions. Every block consisted of 120 trials. The two photopic blocks were run first. Then the experimental chamber was totally darkened, and a 45 min dark adaptation period followed, during which no trials took place. After the dark adaptation period was completed, the 2 scotopic conditions blocks were run, one after the other.

Fig. 2A summarizes the results of the 4 blocks, superimposed. Each dot shows the mean eye position of a single trial. The red dots represent 'photopic trials'; that is, each red dot depicts the mean fixation position of a trial from the initial two blocks. The blue dots represent

'scotopic trials'. The red dots are arranged in compact, distinct clusters. The red dots in each cluster reflect trials with the same target position, illustrated in Fig. 1: the 3-circle configuration of the targets (Fig. 1) is maintained in the configuration of the clusters of the red dots (Fig. 2A). The dots in each red cluster are tight, positioned near each other – even though the trials making up a cluster were separated in time by many other trials, directed at targets in other positions, because the 24 targets were presented in a random order. That the overall target configuration (Fig. 1) is faithfully reflected by the compact red clusters (Fig. 2A), even though the trials of each cluster were scattered over the duration of a block, confirms the precision of photopic fixation.

Importantly, the tightness of the red dot clusters is not caused by external constraints on the deviation of the fixation from target position ('window size'). See Discussion for a detailed examination of this point. Rather, the tightness of the red clusters reflects the inherent accuracy and precision of photopic fixations.

The blue dots in Fig. 2A represent the 'scotopic trials'; each blue dot depicts the mean fixation position of a trial from the two blocks run after the dark adaptation, with dark background. The blue dots are also arranged in clusters, each cluster corresponding to a target location, similarly, in principle, to the red dot clusters discussed above. Nonetheless, the blue clusters differ from the red clusters, in two important ways. The most prominent difference is in the cluster positions. All blue clusters are visibly shifted to positions above those of the respective red clusters. This is the dark-background-contingent upshift previously described (see Introduction), though here first documented after an appropriate dark-adaptation interval. The second difference is that the blue clusters are visibly less tight than the red clusters. This observation

conforms with previous studies of the upshift (see Introduction). Thus, in line with previous studies, we observe in the example session dark-background-contingent upshift, and increase of trial-mean fixation position variability.

Session analysis: transforming eye position into shift

As Fig. 2A illustrates, fixation data were recorded at many target positions over a range in both horizontal and vertical dimensions. This experimental design was primarily aimed to accept results that are not specific to particular target positions. Nonetheless, this experimental design confounds the analysis because eye position cannot be directly used. A look at Fig. 2A will help: we want to characterize the relationship between the red dot clusters and the corresponding blue clusters. Evidently eye position cannot be directly used in the analysis, because it varies from one red cluster to the next. To resolve this confound, we define 'shifts', which are invariant of target positions. For the trials of the example session, Fig. 2 illustrates the transformation of the mean eye-position of all the session's trials (Fig. 2A) into shifts (Fig. 2B). Each red dot in Fig. 2A corresponds to a red dot in Fig. 2B, and each blue dot in Fig. 2A corresponds to a blue dot in Fig. 2B.

Our first aim, then, is validating the notion of shift (that is target-position-independent) and the procedure used to compute it.

The implications of the calibration procedure

We calibrated eye-position using the standard approach. At the beginning of a session the monkey looked at a series of fixation spots that appeared in preset, known screen locations. These were short fixation trials; the monkey was rewarded for each fixation. Because all monkeys were pretrained, all had looked rather precisely at the targets, resulting in tight clusters at all target positions. The experimental program sampled the voltage signal generated by the scleral search coil and computed the value at each location as the mean of the samples taken at that location. From that point on, throughout the session, all voltage signals sampled from the search coil were converted to visual degrees by interpolating the pre-sampled values. Altogether a typical calibration block consisted of about 30 trials, with the target at 9 screen positions. This is the standard approach; it gives a very good approximation to the 'true' eye positions, but errors remain. It should be noted that there are also nonlinearities, induced, in particular, by elements of the experimental hardware.

The initial photopic block that followed the calibration typically contained 120 trials, compared to the 30 of the calibration procedure, sampled at 24 positions, compared to the 9 of the calibration block. It transpires that the data of first photopic block allows for more precise calibration than that of the calibration procedure. Seeking accuracy, we decided to use the photopic block post hoc for improving the calibration.

In order to minimize the effect of the nonlinearities, we decided to use a procedure that effectively calibrates each target's location separately. The details will now be described.

Definition of shift

The rationale guiding the definition of shift is the following. The shift is to be the outcome of a calculation whose input is eye-position records. For the shift outcome to be independent of target position, the calculation itself must depend on target position. The calculation must first compare separately for each target position the scotopic and photopic eye position, or the blue dot cluster and the red dot cluster, both corresponding to the target at stake; and then generalize over all target positions.

Towards this aim we define, for each target position, a reference photopic fixation position. We define it as the mean of the red dot cluster at stake. Keeping in mind that each red dot represents a mean fixation position, of all the eye-position samples in the time-interval of the relevant trial, it transpires that the reference position is a mean of means. Each trial's mean position contributes equally to the reference position, with the horizontal and vertical dimensions calculated separately.

Given a fixation target, and the reference eye-position associated with that target, we now define the shift. The shift is the vectorial difference of the eye-position at stake and the reference eye position. This definition allows to transform both isolated eye-position samples and the mean eye-position of given trials into shifts. As long as the target is well defined, so is the transformation from eye-position samples to shifts.

With this definition, the disparate mean eye position red and blue clusters of Fig. 2A are transformed to a small cluster of red shifts all close to (0,0) and a larger cluster of blue shifts above the red cluster, illustrated in Fig. 2B. Thus, the shifts allow comparing data from all trials together, regardless of target position.

We define 'upshift' as the vertical component of the shift. This term reflects previous findings that scotopic fixation positions tend to be above photopic. Thus, upshift is defined for all entities for which shift is defined. Here we will refer mainly to mean upshifts of trials.

Verification that the shift reflects all target positions

Fig. 3 illustrates the distributions of the vertical components of the mean eye position of the trials of the example session – the vertical dimension of the dots in Fig. 2A – but with respect to their reference fixation positions. The photopic trials are centered very close to zero, in line with the definition of the reference position; the mean and standard deviation are 0.08±0.2 degrees. The scotopic trials' mean positions are shifted upwards and are more variable, at 2.72±0.8 degrees. The two distributions are almost entirely disjoint (there is one overlapping bin drawn in white, close to 0). Hence, by using each target's reference position, a global difference between all scotopic trials and all photopic trials is revealed invariant of each trial's target position. This is exemplified in Fig. 3 that shows a histogram of the means of the vertical positions for the session, whose shift data was depicted in Fig. 2B

By using the reference position as anchor, the mean upshift can be computed for each target position. The distribution of the upshifts at each of the 24 target positions is illustrated for the example session.

The justification for this lumping together comes from the observation that in each of the target locations the red and blue dots are entirely separated (Fig 2A). In each of the target positions, the difference of the blue and red means is significant (p values ranging between 9e-14 to 1.2e-5). Regarding the *horizontal* component of eye position, the difference between scotopic and photopic horizontal components was in most cases not significant; the only exceptions were the two most eccentric horizontal targets ($p = 0.037$ and $p = 0.048$ for the right and for the left most eccentric targets, respectively). Fig. 4 shows the distribution of upshift amplitudes computed separately at each of the 24 target locations, for the exemplary session. The histogram is skewed, but clearly unimodal. Not even at one of the 24 positions is the local upshift an outlier.

Therefore, we infer that both the notion of a location-independent shift and the method of computing it are valid and convey the computed upshift is indeed the relevant physiological parameter.

Modular analysis

In the following we will repeatedly compare upshifts in pairs of conditions, and will use the same format to present the results. The present section describes the format, using Fig. 5 as reference. Each Monkey's results are depicted in a row of panels; Fig. 5A,B illustrate Monkey P's results, Fig. 5C,D Monkey L's. The results of each monkey are presented as scatterplot on the left (Fig. 5A,C), and histograms on the right (Fig. 5B,D). The scatterplots show 2-dimensional shifts. Each dot stands for a single trial; the dot is placed at the coordinates corresponding the mean shift of that trials. Dots are in one of 2 or 3 colors. Red dots stand for trials in photopic conditions (bright target, bright background); blue for dark (dark background, various variations). White stands for regions of overlap between the histograms. The third color is used for additional parameters, when needed. (In Fig 5 a third color is not

used). The same number of dots are displayed for each color; the selection of trials to be displayed is random for the more populous condition. Nonetheless, because of the large number of trials the present study involves, dots often overlap each other, occluding the shapes of the distributions. Thus, presented on the right are histograms of the upshifts, the vertical components of the shifts. The histograms are plotted with respect to the vertical axis of the scatterplot. The colors of the dots are conserved in the histograms; overlapping bins are presented in white. The statistics of each comparison are presented in an associated table; for Fig. 5 it is the top part of Table 1. For each monkey, separately, the numbers of sessions and trials are presented, as well as the mean, standard deviation, and median of the upshift, in degrees of visual angle. Finally, the p-value of the t-test comparing the means of the two distribution is listed. Zero (0) indicates that the p-value is less than the smallest value representable by standard computer double-precision arithmetic.

Within-sessions versus between-sessions comparisons

Before indulging onto the body of the results we need to make a methodological distinction. Ideally, we can compare the upshifts in two conditions run one after the other, within the same session. For example, in previous studies describing the upshift a first condition was fixation of bright targets over bright background (photopic vision). Then, shortly after dark onset, fixation in a second condition was collected (bright targets over dark background, shortly after dark onset). That these conditions could be collected within the same session meant that they shared the same eye-position calibration.

When 45-min waiting periods are required, monkeys are generally willing to work after the period ends. (This holds regardless of whether the room is lighted or dark during the waiting period). However, a second waiting period is just too much. Because each condition typically involves collection of hundreds of trials, in general during a session we could record two conditions, one, baseline, before a waiting period, usually 45-min dark adaptation; the other after the waiting period.

The need to make one comparison, with one waiting period, per session, constrained the comparisons we could make within sessions. Consequently, in some cases we had to make between-session comparisons. Transforming eye-position data into shifts makes that possible. Since different sessions have different calibrations, we assume that between-session comparisons might be noisier. However, as described subsequently, all the between sessions comparisons we make are highly significant in all monkeys tested.

We always have more between-session data than within-session. Therefore, in the comparisons for which we have within-session data, we describe these comparisons. In all cases we describe also the between-session comparisons with all the available data.

Photopic-dark background evokes upshift

At issue: Does photopic-dark background evoke upshift? Previous studies, which documented the dark-background-contingent upshift, probably tested eye position mostly in photopic-dark, but this was not well controlled as the state of dark adaptation was not documented.

Design: We compare two conditions. Condition 1 comprises ‘photopic’ trials. The target is very small (0.02° radius), only a few pixels on the computer monitor, but bright enough to be salient over the bright background (see Methods of parameters). Condition 2 comprises

'photopic-dark background' trials. The same target, size and luminance as in condition 1, but the background is dark. The entire photopic-dark data is collected during the photopic leg of the dark adaptation curve, during the first 10 min after dark onset.

The null hypothesis states that the distributions of upshifts in photopic and in photopic-dark background conditions would be the same; hence, their means would not differ significantly. With the target kept unchanged between the conditions, any difference in upshift would reflect the changing background, not the target.

Results: Data were collected from 2 monkeys. Fig. 5 illustrates the within-session results, Fig. 6, the between-sessions. Table 1 specifies the statistics. The Figures follow the pattern described in the previous sections.

In both monkeys, the photopic-dark (blue) and photopic (red) trials make up two largely distinct clusters of dots in the scatterplots, and distributions in the histograms. Monkey P has a greater upshift than Monkey L, but in Monkey L, as well as in Monkey P, the means of the upshifts in the red and blue trials are significantly different, with a *p-value* of zero (less than the smallest value representable by standard computer arithmetic).

In Fig. 6 the separation of blue and red is slightly noisier than in Fig. 5. This is as expected, the comparisons of different sessions are likely to introduce some variability. That the difference is so small shows that the results are consistent in all sessions.

Conclusion: The null hypothesis is rejected. Thus, fixation positions in photopic-dark are shifted above those in photopic vision.

Scotopic-dark background evokes upshift even with bright targets

At issue: In the previous section, we observed that photopic-dark background evokes upshift. Does scotopic-dark background at all evoke upshift, too? The working hypothesis of this study contends that it will; an alternate hypothesis could state that the upshift phenomenon is limited in time to the photopic leg (first 10-15 min) of darkness; subsequently, the upshift would dissipate.

Design: The compared conditions are almost identical to those in the previous section. Condition 1 is photopic, bright target over bright background; condition 2 is bright target over dark-background, as in condition 1 – but with one difference: after the monkey completes the photopic condition blocks, the experimental chamber is completely darkened for a 45-min interval of dark adaptation. Only then the monkey is presented with, and performs, the dark background blocks. We call this condition 'scotopic-dark background'. Inevitably, the use of a bright target, however small, compromises the state of dark adaptation. Nonetheless, because the target is very small, this compromising effect is not very large. The advantage of using this combination, of a very small bright target over scotopic-dark background, is that we can compare directly the effect of the dark adaptation specifically of the background, in isolation from the target. This is explored in the next section.

Thus, our working hypothesis for this section is that there is upshift in the scotopic-dark background conditions. The null hypothesis states that the distributions of upshifts in the two conditions are the same, hence their means are also the same.

Results: Data were collected from 2 monkeys. Fig. 7 illustrates the within-session results; Fig. 8, the between-sessions. Table 2 specifies the statistics. The Figures follow the pattern described in the previous sections. In both Figures, red stands for photopic trials (bright target over bright background); blue stands for scotopic-dark background (bright targets over dark background, after 45-min dark-adaptation interval).

In both monkeys, blue and red dots in the scatterplots are visibly almost totally separate, with the blue dots clusters centered almost precisely above the red dot clusters. The blue clusters of Monkey P are elongated in the vertical direction, in both Figs 7 and 8; this is reflected in the higher standard deviation (see Table 2). The mean and median upshift values are greater in monkey P than in Monkey L. Nonetheless, in both monkeys, the means are significantly different, with a *p*-value of 0.

Conclusion: The null hypothesis is rejected. There is dark-background-contingent upshift after the 45-minute dark adaptation interval, which converts the dark to scotopic. Thus, the upshift is not specific to photopic-dark.

A greater upshift is evoked by scotopic-dark background than by photopic dark background

At issue: We now know that there is upshift with both photopic-dark and scotopic-dark backgrounds. Is the upshift equal in both conditions? If not, how do the two relate? At stake is the question whether the upshift is associated primarily with one of these phases in the process of dark adaptation, photopic or scotopic. A larger upshift in one of the two would suggest a special relationship of that phase to the upshift.

Design: The shifts of the photopic-dark trials cannot be directly compared with the shifts of the scotopic-dark trials. There are no sessions containing both photopic-dark background and scotopic-dark background trials. (Had we done that, we would have broken the standard structure of the sessions, possibly confounding the results in other ways). Therefore, the comparison is possible only in the between-sessions mode. Accordingly, the working hypothesis is that the upshift is greater in the scotopic-dark background than in the photopic-dark background trials.

The null hypothesis states that the two states of darkness lead to the same distribution of upshifts; or, more specifically, that photopic-dark evokes the same or greater upshift than scotopic-dark.

Results: The data compared here overlaps that presented in Figs 5-8. Hence, the data are from the same 2 monkeys. Fig. 9 illustrates the between-sessions comparison. Table 3 specifies the statistics. The Figure follows the pattern described in the previous sections. Green stands for photopic-background trials (bright target over dark background, without dark adaptation); blue stands for scotopic-dark background (bright targets over dark background, after 45-min dark adaptation interval). Recall that white in the histogram stands for overlap of the two distributions.

In both monkeys, there is large overlap between the green and blue data. This is easily visible in both scatterplots (Figs 9A,C) and histograms (figs 9B,D). The overlap between the blue and

green histograms is much larger than the overlap between the red and blue histograms in Figs 5-8.

Nonetheless, especially in the histograms but also in the scatterplots, it is apparent that the blue dots and bins tend to lie above the green dots and histogram bins. That holds for both monkeys. The means of the distributions are very significantly different, with *p-value* less than 10^{-41} in both cases. Because the *p-value* is so small, both variations of the null hypothesis are rejected.

Conclusion: The null hypotheses are rejected. In scotopic-dark background, a larger upshift is evoked than in photopic-background.

Scotopic vision evokes upshift

At issue: Is there upshift in scotopic vision? We know now that bright targets over scotopic dark background evoke upshift; could the bright targets be necessary for evoking the upshift? The combination of a bright target over scotopic-dark background is an ecologically rare stimulus. Is the upshift but an idiosyncratic response to this ecological rarity?

Design: Condition 1 is photopic vision, bright target over bright background. Condition 2 is scotopic vision, dim target over dark background, after 45-min dark adaptation. Both the scotopic-background condition, described in the previous 2 sections, and the current full scotopic-vision condition, involved the same 45-min dark-adaptation interval, and the same dark background, but differed in the target luminance. In the dark-background condition, the target was bright; in the current, scotopic-vision condition, the target was dim, 0.007 cd/m2.

The null hypothesis is that scotopic vision does not evoke upshift; thus, both photopic and scotopic vision conditions would involve the same distribution of upshifts, with the same means.

Results: Data were collected from 2 monkeys. Fig. 10 illustrates the within-session results; Fig. 11, the between-sessions. Table 4 specifies the statistics. The Figures follow the pattern described in the previous sections. In both Figures, red stands for photopic trials (bright target over bright background); blue stands for scotopic (dim targets over dark background, after 45-min dark-adaptation interval).

In both monkeys, in both within-sessions and between-sessions analysis, the scotopic vision fixations lie above the photopic fixation positions, with very small overlap. The values of the scotopic upshifts vary considerably, within monkey and between monkeys. Almost all scotopic fixations are above the range of photopic fixations. The size of the shift varies between trials and between monkeys. The trial-by-trial variability of the size of the upshift is a major aspect of the results and will be resorted to in the Discussion. As for the null hypothesis: the means of the scotopic and photopic distributions are significantly different, with p-value 0.

Conclusion: The null hypothesis is rejected. In scotopic vision, there is upshift.

A greater upshift is evoked by full scotopic vision than by scotopic-dark background

At issue: We know now the upshift is present in scotopic-dark background, with a bright small target, and in scotopic vision proper, with scotopic-dark background and dim target. Thus, the

alternate hypotheses that related the upshift to other factors than scotopic vision – photopic dark, the ecological rarity of a bright target over scotopic background – failed to explain the data. The hypothesis that the upshift is associated with scotopic vision because it reflects a tendency to fixate by the rod-dense region in superior retina, is still intact. To give this hypothesis greater strength, the prediction that full scotopic vision involves a greater upshift than scotopic-dark background should hold.

Design: This comparison is possible only between sessions, because the two conditions follow 45-min dark adaptation intervals. Condition 1 is scotopic-dark background (bright target, dark background, after 45-min dark adaptation). Condition 2 is full scotopic vision (dim target, dark background, after 45-min dark adaptation). The null hypothesis states that the distributions of upshifts in the two conditions are the same; hence, their means are equal.

Results: Fig. 12 shows the results, and Table 5 details the statistics. The format is the same as in previous Figures. Green stands for scotopic-dark background, blue for full scotopic vision. White histogram bins represent the overlap between the blue and green histograms.

The amount of overlap varies between monkeys. It is large in Monkey P and intermediate in Monkey L. Interestingly, the monkey with the largest upshift (P) has the greatest overlap. We will refer to this observation in the Discussion.

In both monkeys, the blue dots and histogram bins are evidently overall positioned above the green ones. The means are very significantly not equal, with *p-value* of 10^{-54} or less in both monkeys (see Table 5).

Conclusion: Scotopic vision evokes upshifts that are higher than those evoked by scotopic-dark background, consistent with the central tenet of this study.

Mesopic vision evokes upshift

At issue: An important aspect of the results, presented in the previous sections, is that in many conditions there is an upshift, but its size is not maximal. Bearing in mind that some of these conditions comprise ecologically questionable visual stimuli (the combination of a bright target and completely dark background), we ask if an ecologically common stimulus can also evoke upshift, but whose level is less than maximal. Mesopic-background stimuli are called for. Common in nature, intermediate between scotopic and photopic, we could expect the upshift to be sub-maximal. In the present section we test if there is upshift in the mesopic blocks.

Design: Condition 1 is photopic vision, bright target over bright background, as used before. After the photopic vision blocks, the monkeys went through a 45-min dark adaptation interval. After some testing in scotopic-background conditions, the background was gradually elevated from scotopic to a mesopic level (see Methods for details). A standard small, bright target and mesopic background were used in the 'mesopic' blocks of trials that followed.

The null hypothesis predicts there is no change in upshift between photopic and mesopic blocks.

Results: Data were collected from 2 monkeys. Fig. 13 illustrates the within-session results; Fig. 14, the between-sessions. Table 6 specifies the statistics. The Figures follow the pattern described in the previous sections. In both Figures, red stands for photopic trials (bright target

over bright background); cyan stands for mesopic (bright target over mesopic background, as described above).

In both within- and between-sessions comparisons, the cyan dots and histogram bins are above the red ones. The overlap between the red and cyan is not large. As for the null hypothesis, the p-values are less than 10^{-10} in all cases (0 in 3 of the 4 comparisons).

Conclusion: The null hypothesis is rejected. Mesopic background evokes upshift.

Mesopic vision induces an intermediate level of upshift

At issue: Continuing the previous section, we now aim to test if the upshift is intermediate in value, compared to scotopic-dark background. Recall that the mesopic background follows an interval of dark adaptation, and then testing with scotopic-dark background. Hence, scotopic dark background is an appropriate comparison for testing if the mesopic upshift is of intermediate values.

Design: Condition 1 is mesopic (bright target, mesopic background); condition 2 is scotopic-dark background. Note that the data of condition 1 were always collected after data of condition 2. The null hypothesis predicts there is no change in upshift between scotopic-dark background and mesopic blocks.

Results: Data were collected from 2 monkeys. Fig. 15 illustrates the within-session results; Fig. 16, the between-sessions. Table 7 specifies the statistics. The Figures follow the pattern described in the previous sections. In both Figures, cyan stands for mesopic trials; blue for scotopic-dark background.

There is overlap between the histograms in both within and between session analyses. In all cases, the mean of the mesopic upshifts is less than the means of the scotopic-dark background. The null hypothesis is rejected with high significance (p-value less than 10-92).

Conclusion: Mesopic background evokes upshift, but its size is intermediate, between no upshift (photopic vision) and high upshift (scotopic-dark background).

No apparent photopic shift on bright adaptation

At issue: Dark adaptation takes a lot of time. Could the 45-min by itself lead to upshift?

Design: 'Bright adaptation' consisted of a 45-min waiting interval in bright light. The computer monitor the monkeys faced was set at a level of 7 cd/m2, well within the photopic range; more intense background caused discomfort to human observers trying the 45-min interval. In addition, the experimental chamber was illuminated by overhead lights. Condition 1 is standard photopic vision (bright target and background), condition 2 is again photopic vision, but recorded after the bright adaptation interval.

Upshift is computed in the standard fashion. The null hypothesis predicts no systematic change in upshift following bright adaptation.

Results: Fig. 17 shows the within-session comparison, Fig. 18 the between-session. Table 8 shows the statistics.

The within-sessions differences are not significant in Monkey L, and very small in both monkeys – in Monkey P the mean shifts up by 0.08°, which is only 23% of the photopic upshift's standard deviation. The between-session distributions appear more noisy (scatterplots in Fig. 18), and Monkey P's difference is not significant. Monkey L's mean difference shifts down by 0.090, which is 26% of the photopic standard deviation. Thus, 2 of 4 conditions are not statistically significant, and the other 2 are very small and in opposite directions.

Conclusion: Bright adaptation does not bring about systematic upshift or downshift.

Discussion

Summary of Results

We studied the eye position with which rhesus monkeys fixate targets in the dark, as compared to bright, photopic illumination. In photopic conditions, the eyes were directed so that the fovea was close to the target. We studied fixation shortly after dark onset, while cones dominate vision ('photopic dark'), and after 45-min dark adaptation ('scotopic dark'). In all variations of dark background, the eyes were directed so that the fovea was above the target; thus, the image of the target fell on the scotopic band. The closer to full scotopic the conditions were, the higher was the upshift, that is, the more dorsal position the scotopic center was located on the scotopic band. In particular, mesopic vision involved locations on the band that were relatively close to the fovea. Dark adaptation (45-min in dark) increased the upshift, that is, shifted the scotopic center dorsally. There is no analogous photopic effect: 45-min wait in bright, photopic light leads to neither upshift nor downshift.

The upshift appears to be a trait of scotopic vision

At the outset of the present study we considered the possibility that upshift was not related to scotopic vision, but transient during the photopic dark stage. Some evidence pointed against this possibility in a previous publication (submitted). With the current, solid evidence, this alternate hypothesis is put to rest.

Support for the scotopic band hypothesis

The present study lends solid support to the scotopic band hypothesis. All the intermediate forms of adaptation, whether explicit mesopic background, or scotopic background with bright targets, resulted in intermediate positions of the scotopic center on the scotopic band. Thus, we started out expecting fixations to have a bimodal distribution, one mode being the fovea, the other the rod hotspot/dorsal rod peak. We ended with upshift having a continuous distribution. This is a subtler notion, reflecting the gradual change in receptor densities along the scotopic band. This gradually changing anatomical structure is more appropriate for processing the ecological input, which is also gradual.

Mesopic vision

Another important finding of the current study is that mesopic vision is integrated into the notion of the scotopic center. Although it was clear that the notion can encompass mesopic

vision, only with the current evidence can we suggest that indeed mesopic vision involves the more ventral positions of the scotopic band.

A switch of sensorimotor transformation occurring in nature at least twice a day

Twice a day, it has been known for long, there are transitions occurring between the two visual subsystems, the photopic visual system and the scotopic visual system. For many hours, continuously during daytime, cones mediate vision and at least most rods are just saturated. Then, after a transition period, rods mediate vision and at least most cones are just too insensitive to be useful. The transition periods, between full scotopic and full photopic states, are rather long themselves.

Now we know that, in rhesus monkeys, together with the sensory switching described above, another switching takes place: switching of the sensorimotor transformations mediating visual fixation. Because the sensory switching is slow, taking more than an hour, the sensorimotor transformation also switches slowly. This might be the ecological setting in which the graded values of upshift have evolved.

Switching of sensorimotor transformations had been studied and one criticism such studies sometimes meet is that they reflect an artificial situation that can be produced in a lab but does not reflect nature. Here we have behaviors that occur spontaneously (recall that the upshift was never rewarded), and they switch on and off in a repeating, fully predictable manner.

References

Barash S, Melikyan A, Sivakov A, Tauber M. Shift of visual fixation dependent on background illumination. *J Neurophysiol* 79: 2766–2781, 1998.

Caggiano V, Pomper JK, Fleischer F, Fogassi L, Giese M, Thier P. Mirror neurons in monkey area F5 do not adapt to the observation of repeated actions. *Nat Commun* 4: 1433, 2013.

Curcio CA, Allen KA. Topography of ganglion cells in human retina. *J Comp Neurol* 300: 5–25, 1990.

Dash S, Catz N, Dicke PW, Thier P. Encoding of smooth-pursuit eye movement initiation by a population of vermal Purkinje cells. *Cereb Cortex* 22: 877–891, 2012.

Findlay JM. Global visual processing for saccadic eye movements. *Vision Res* 22: 1033–1045, 1982.

Normann R, Werblin F. CONTROL OF RETINAL SENSITIVITY .1. LIGHT AND DARK-ADAPTATION OF VERTEBRATE RODS AND CONES. 63: 37–61, 1974.

Packer O, Hendrickson AE, Curcio CA. Photoreceptor topography of the retina in the adult pigtail macaque (Macaca nemestrina). *J Comp Neurol* 288: 165–183, 1989.

Paeye C, Collins T, Cavanagh P, Herwig A. Calibration of peripheral perception of shape with and without saccadic eye movements. *Atten Percept Psychophys* 80: 723–737, 2018.

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.

Wikler KC, Rakic P. Distribution of photoreceptor subtypes in the retina of diurnal and nocturnal primates. *J Neurosci* 10: 3390–3401, 1990.

Wikler KC, Williams RW, Rakic P. Photoreceptor mosaic: number and distribution of rods and cones in the rhesus monkey retina. *J Comp Neurol* 297: 499–508, 1990.

Within sessions

Monkey	Condition	# sessions	# trials	Upshift Median	Upshift Mean	Upshift STD	p-value
P	Photopic	20	3251	-0.03	0.04	2.13	0
	Photopic-dark		2329	3.67	3.81	2.45	
L	Photopic	8	1320	-0.07	-0.004	1.6	$<10^{-74}$
	Photopic-dark		1008	0.95	1	0.54	

Between sessions

P	Photopic	44	6985	-0.01	0,07	0,61	0
	Photopic-dark	20	2329	3.67	3.81	2.45	
L	Photopic	13	2057	-0.03	-0.02	0.34	0
	Photopic-dark	8	1008	0.95	1	0.54	

Table 1

Within sessions

Monkey	Condition	# sessions	# trials	Upshift			p-value
				Median	Mean	STD	
P	Photopic	35	6127	-0.006	0.09	2.08	0
	Scotopic-dark background		3754	4.34	4.68	2.4	
L	Photopic	12	1973	-0.03	-0.002	2.56	$<10^{-184}$
	Scotopic-dark background		2437	1.56	1.66	0.75	

Between sessions

P	Photopic	44	6985	-0.01	0.07	0.61	0
	Scotopic-dark background	35	3754	4.34	4.68	2.4	
L	Photopic	13	2057	-0.03	-0.02	0.34	0
	Scotopic-dark background	12	2437	1.56	1.66	0.75	

Table 2

Between sessions

Monkey	Condition	# sessions	# trials	Upshift Median	Upshift Mean	Upshift STD	p-value
P	Scotopic-dark background	35	3754	4.34	4.68	2.39	$<10^{-41}$
	Photopic-dark	20	2329	3.67	3.81	2.45	
L	Scotopic-dark background	12	2437	1.56	1.66	0.75	$<10^{-126}$
	Photopic-dark	8	1008	0.95	1	0.53	

Table 3

Within sessions

Monkey	Condition	# sessions	# trials	Upshift Median	Upshift Mean	Upshift STD	p-value
P	Scotopic-dark, dim target	5	388	6.02	5.78	3.08	0
	Photopic		1272	-0.02	0.2	3.38	
L	Scotopic-dark, dim target	1	237	2.67	2.78	0.83	0
	Photopic		240	-0.08	0.03	1.8	

Between sessions

P	Scotopic-dark, dim target	5	388	6.02	5.8	3.08	0
	Photopic	44	6985	-0.01	0.07	0.61	
L	Scotopic-dark, dim target	1	237	2.67	2.78	0.83	0
	Photopic	13	2057	-0.03	-0.02	0.34	

Table 4

Between sessions

Monkey	Condition	# sessions	# trials	Upshift Median	Upshift Mean	Upshift STD	p-value
P	Scotopic-dark, dim target	5	388	6.02	5.78	3.08	$<10^{-54}$
	Scotopic-dark background	35	3754	4.34	4.68	2.39	
L	Scotopic-dark, dim target	1	237	2.67	2.78	0.83	$<10^{-95}$
	Scotopic-dark background	12	2437	1.56	1.66	0.75	

Table 5

Within sessions

Monkey	Condition	# sessions	# trials	Upshift Median	Upshift Mean	Upshift STD	p-value
P	Mesopic	19	5820	1.01	1.64	1.83	0
	Photopic		2503	-0.08	-0.18	2.04	
L	Mesopic	4	1253	0.68	0.75	0.71	$<10^{-10}$
	Photopic		612	0.14	-0.03	3.9	

Between sessions

P	Mesopic	19	5820	1.01	1.64	1.83	0
	Photopic	44	6985	-0.01	0.07	0.61	
L	Mesopic	4	1253	0.68	0.75	0.71	0
	Photopic	13	2057	-0.03	-0.02	0.34	

Table 6

Within sessions

Monkey	Condition	# sessions	# trials	Upshift Median	Upshift Mean	Upshift STD	p-value
P	Scotopic-dark background	19	1750	4.7	5.3	2.8	0
	Mesopic		5820	1.01	1.64	1.83	
L	Scotopic-dark background	4	360	1.9	1.9	1.3	$<10^{-92}$
	Mesopic		1253	0.68	0.75	0.71	

Between sessions

P	Scotopic-dark background	35	3754	4.34	4.68	2.39	0
	Mesopic	19	5820	1.01	1.64	1.83	
L	Scotopic-dark background	12	2437	1.56	1.66	0.75	$<10^{-236}$
	Mesopic	4	1253	0.68	0.75	0.71	

Table 7

Within sessions

Monkey	Condition	# sessions	# trials	Upshift Median	Upshift Mean	Upshift STD	p-value
P	Light adapted	8	720	-0.02	0.05	0.39	$<10^{-4}$
	Photopic		720	-0.04	-0.03	0.35	
L	Light adapted	6	695	-0.12	-0.13	0.32	0.35
	Photopic		813	-0.09	-0.09	1.16	

Between sessions

P	Light adapted	8	720	-0.02	0.05	0.39	0.3227
	Photopic	44	6985	-0.01	0.07	0.61	
L	Light adapted	6	695	-0.12	-0.13	0.31	$<10^{-12}$
	Photopic	13	2057	-0.03	-0.02	0.34	

Table 8

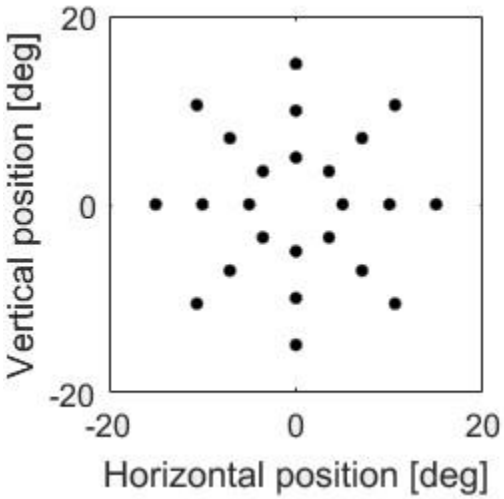
20
0
-20
Vertical position [deg]
-20
0
20
Horizontal position [deg]

Figure 1

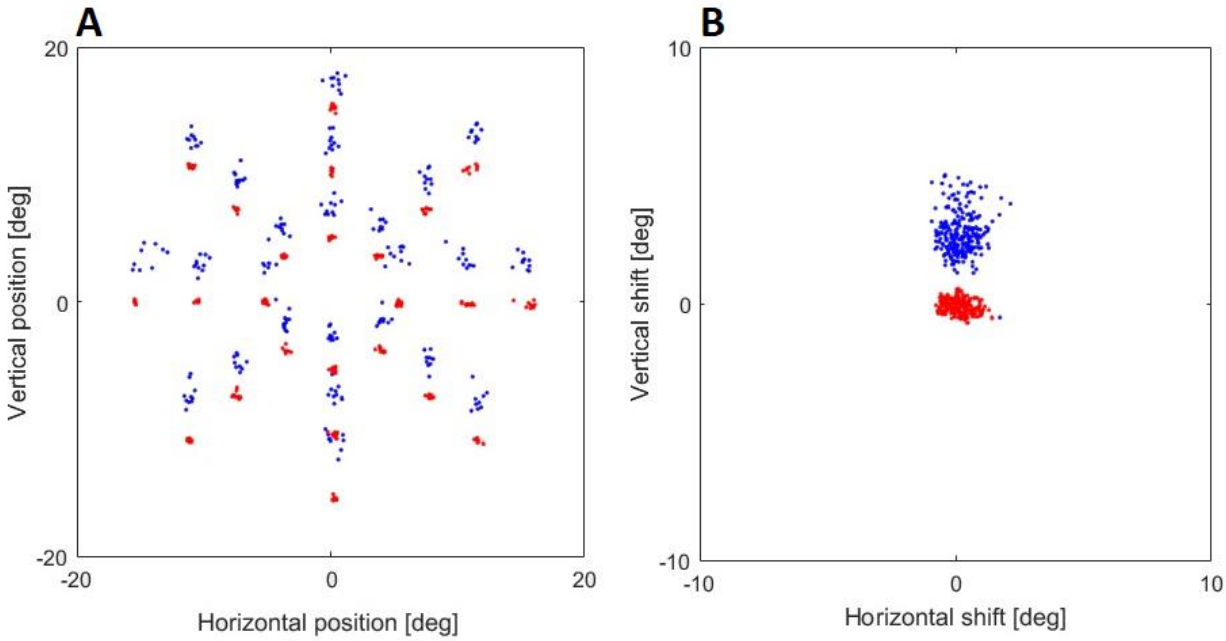
A
20
0
-20
-20
0
20
Vertical position [deg]
Horizontal position [deg]
B
10
0
-10
-10
0
10
Vertical shift [deg]
Horizontal shift [deg]

Figure 2

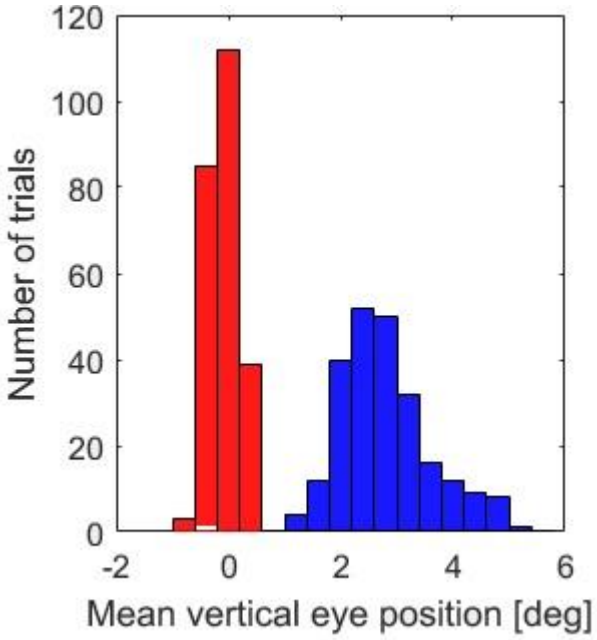
120
100
80
60
40
20
0
Number of trials
-2
0
2
4
6
Mean vertical eye position [deg]

Figure 3

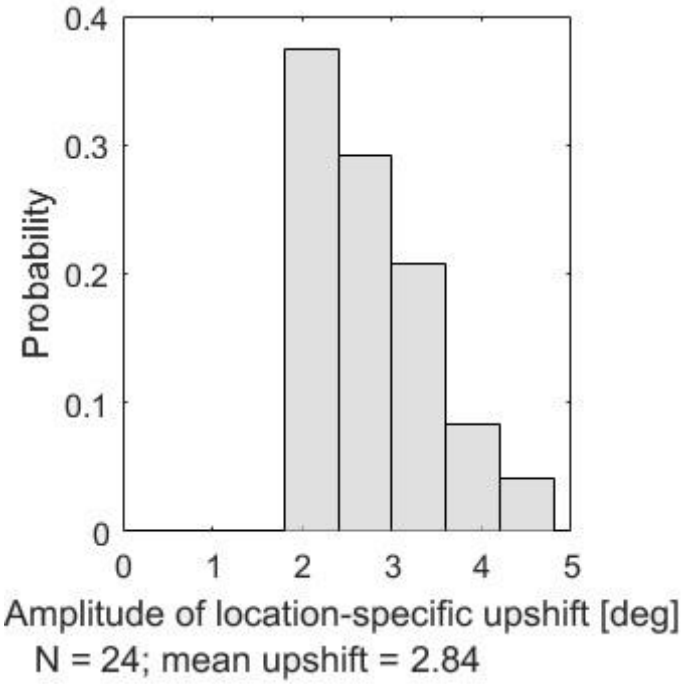
0.4
0.3
0.2
0.1
0
Probability
0
1
2
3
4
5
Amplitude of location-specific upshift [deg]
N = 24; mean upshift = 2.84

Figure 4

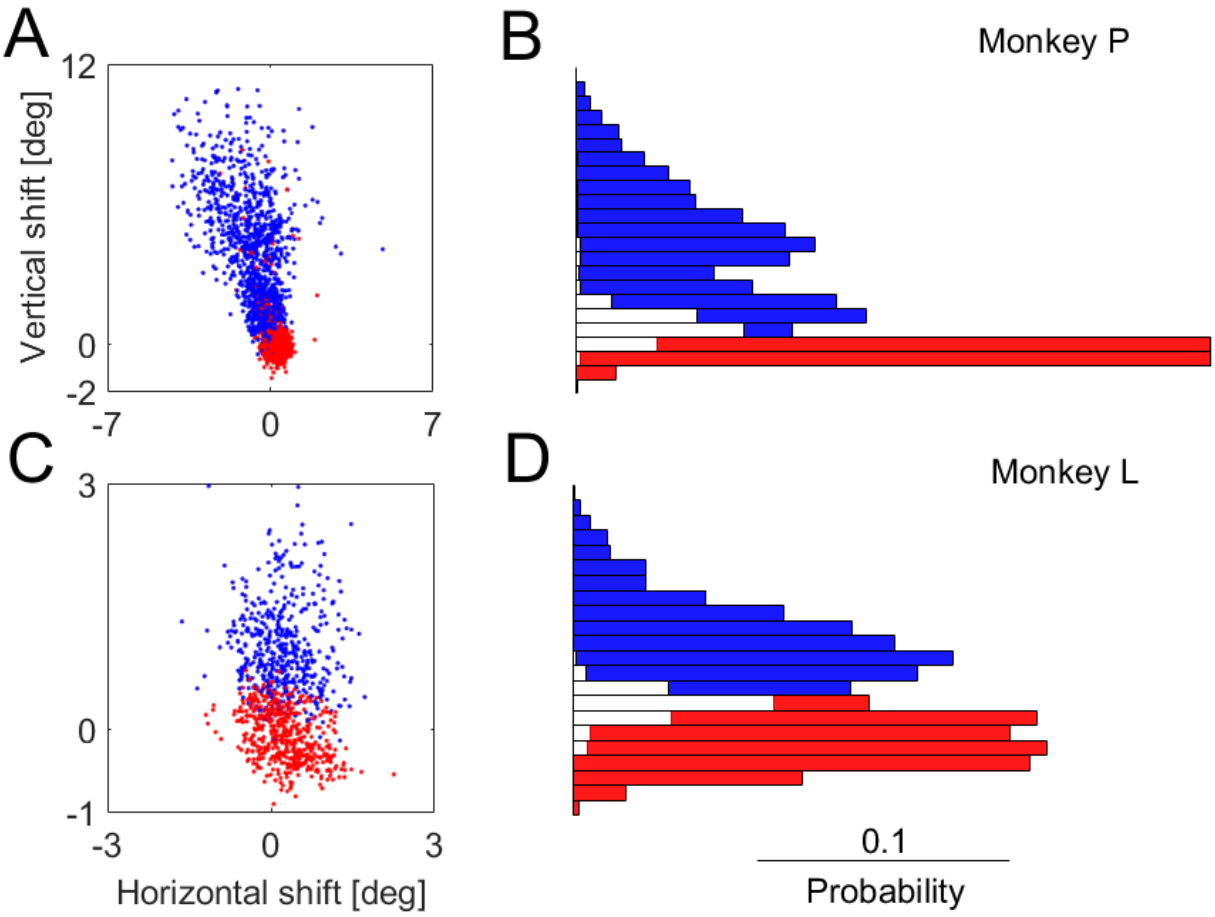
A
B
C
D
Monkey P
Monkey L
Vertical shift [deg]
Horizontal shift [deg]
12
0
-2
-7
0
7
3
0
-1
-3
0
3
0.1
Probability

Figure 5

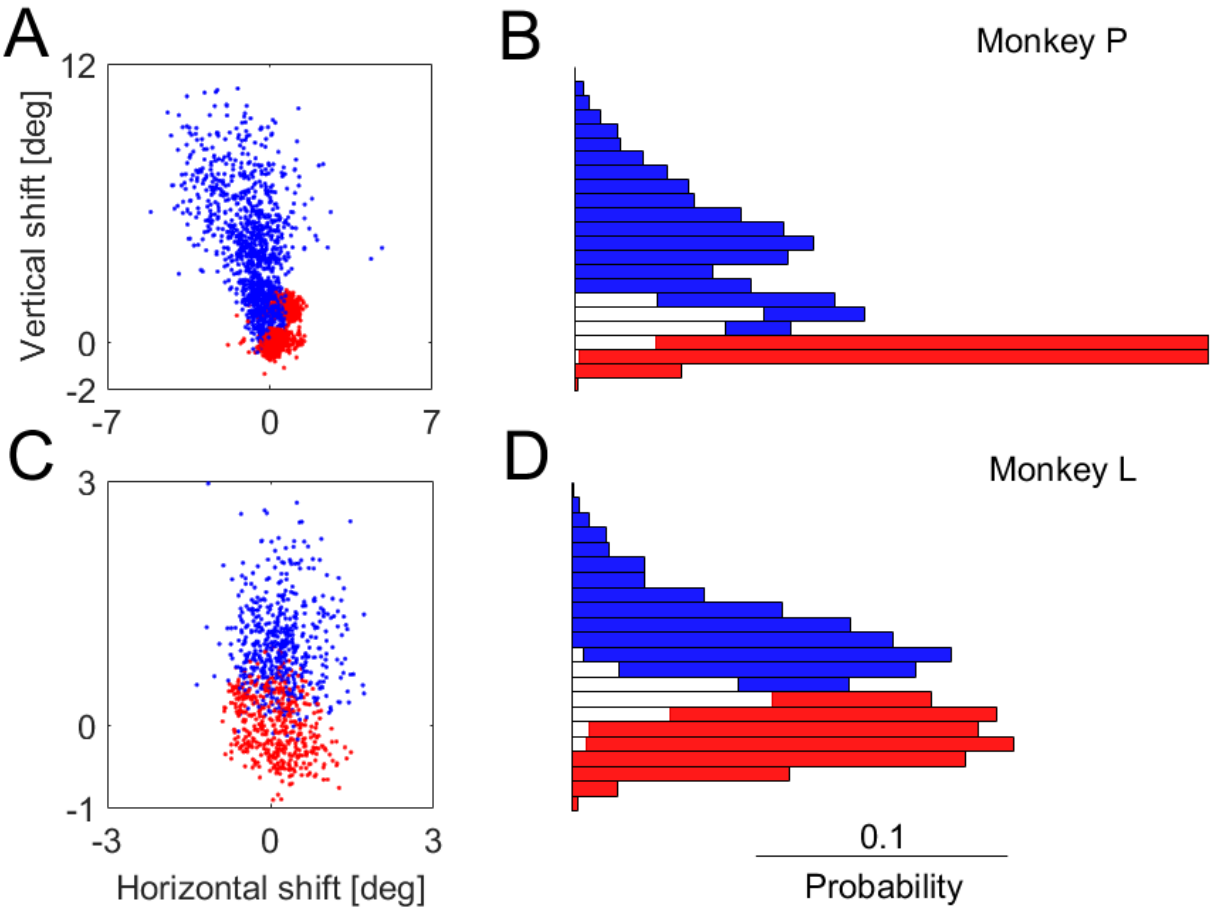
A
B
C
D
Monkey P
Monkey L
Vertical shift [deg]
Horizontal shift [deg]
12
0
-2
-7
0
7
3
0
-1
-3
0
3
0.1
Probability

Figure 6

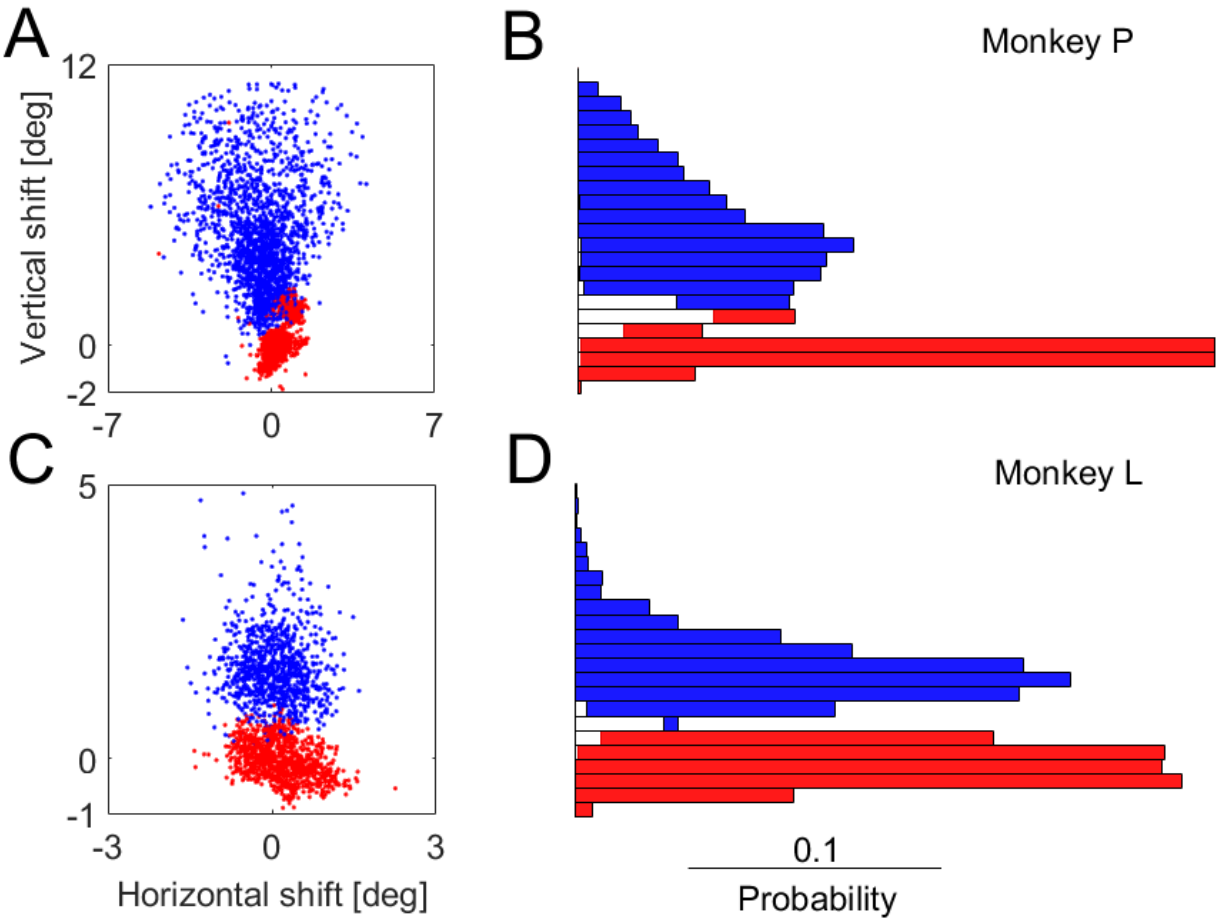
A
B
C
D
Monkey P
Monkey L
Vertical shift [deg]
Horizontal shift [deg]
12
0
-2
-7
0
7
5
0
-1
-3
0
3
0.1
Probability

Figure 7

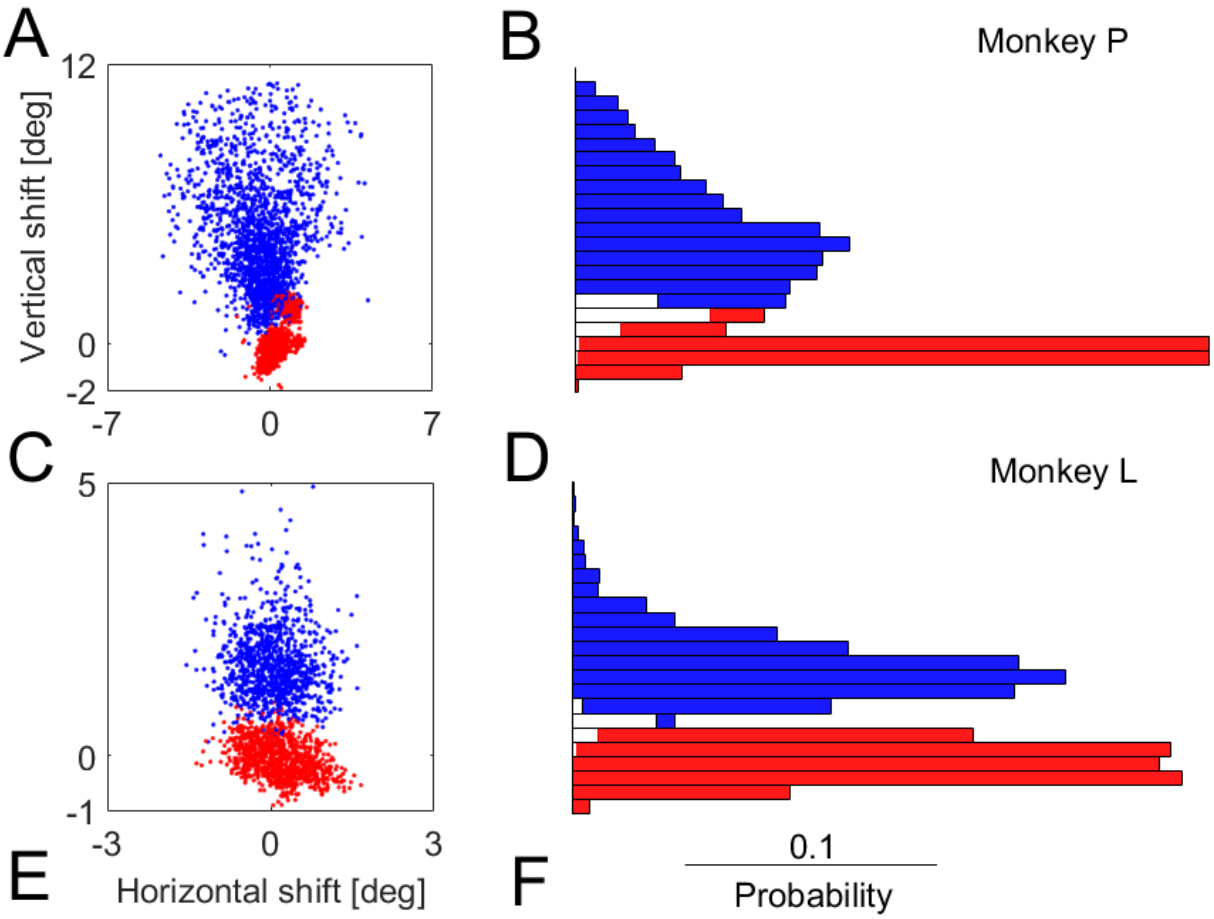
A
B
Monkey P
12
0
-2
Vertical shift [deg]
-7
0
7
C
D
Monkey L
5
0
-1
-3
0
3
E
Horizontal shift [deg]
F
0.1
Probability

Figure 8

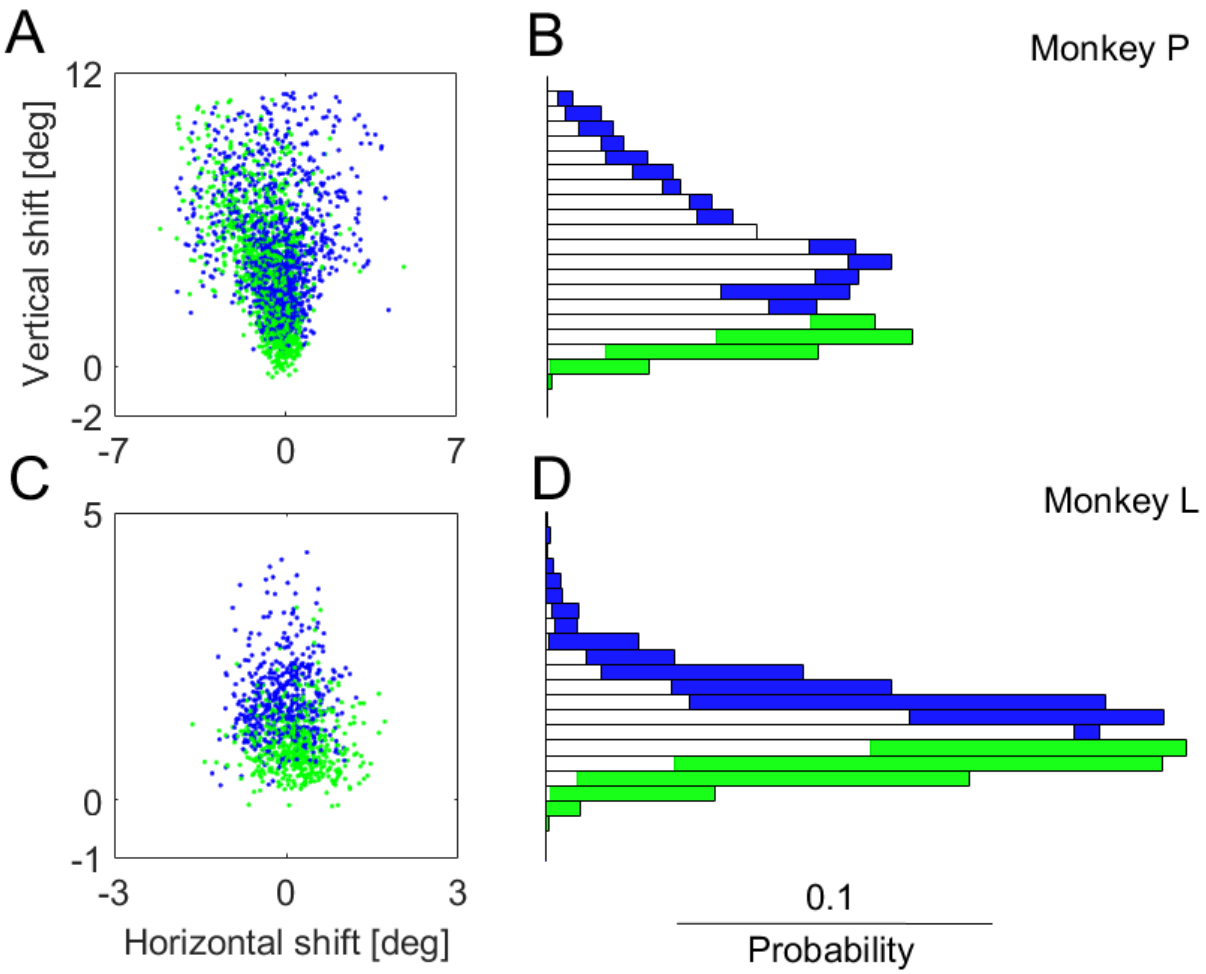
A
B
Monkey P
C
D
Monkey L
Vertical shift [deg]
12
0
-2
-7
0
7
5
0
-1
-3
0
3
Horizontal shift [deg]
0.1
Probability

Figure 9

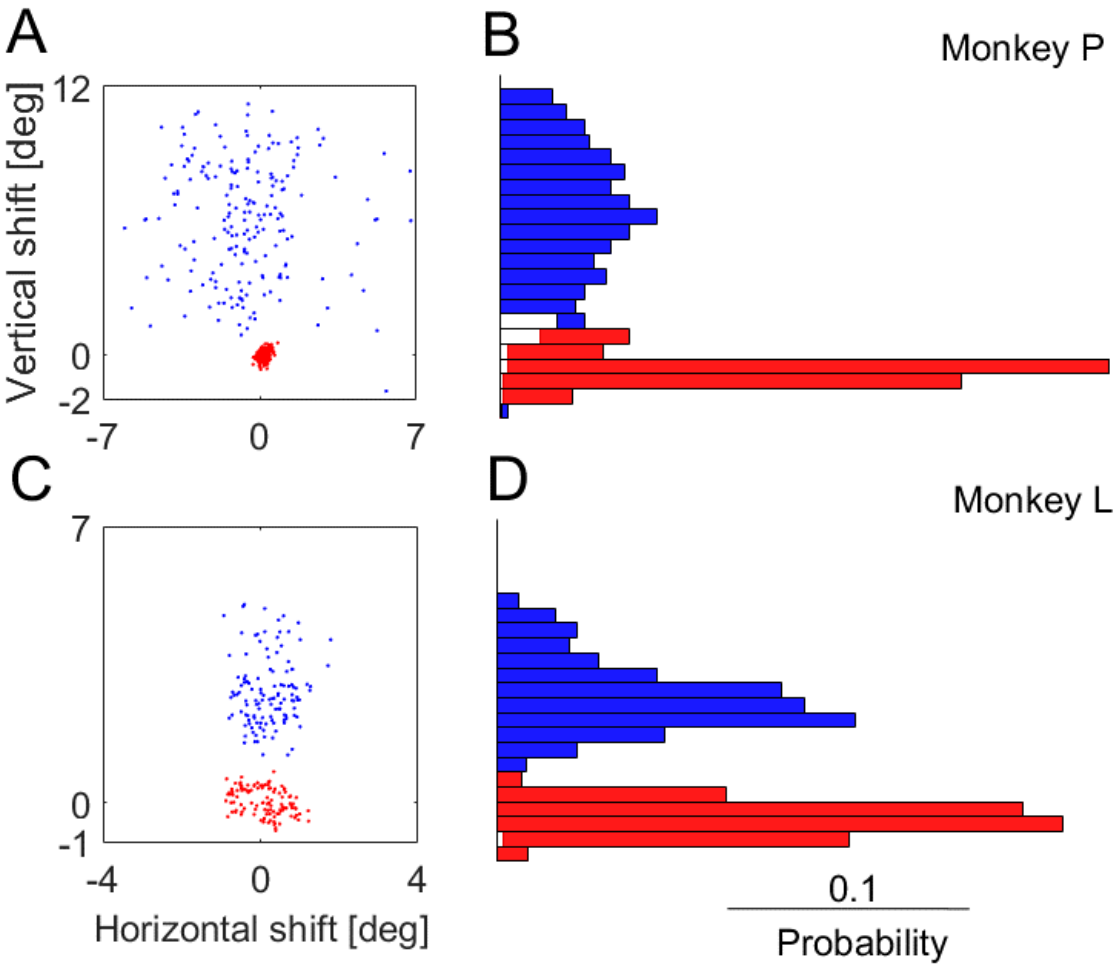
A
B
Monkey P
Vertical shift [deg]
12
0
-2
-7
0
7
C
D
Monkey L
7
0
-1
-4
0
4
Horizontal shift [deg]
0.1
Probability

Figure 10

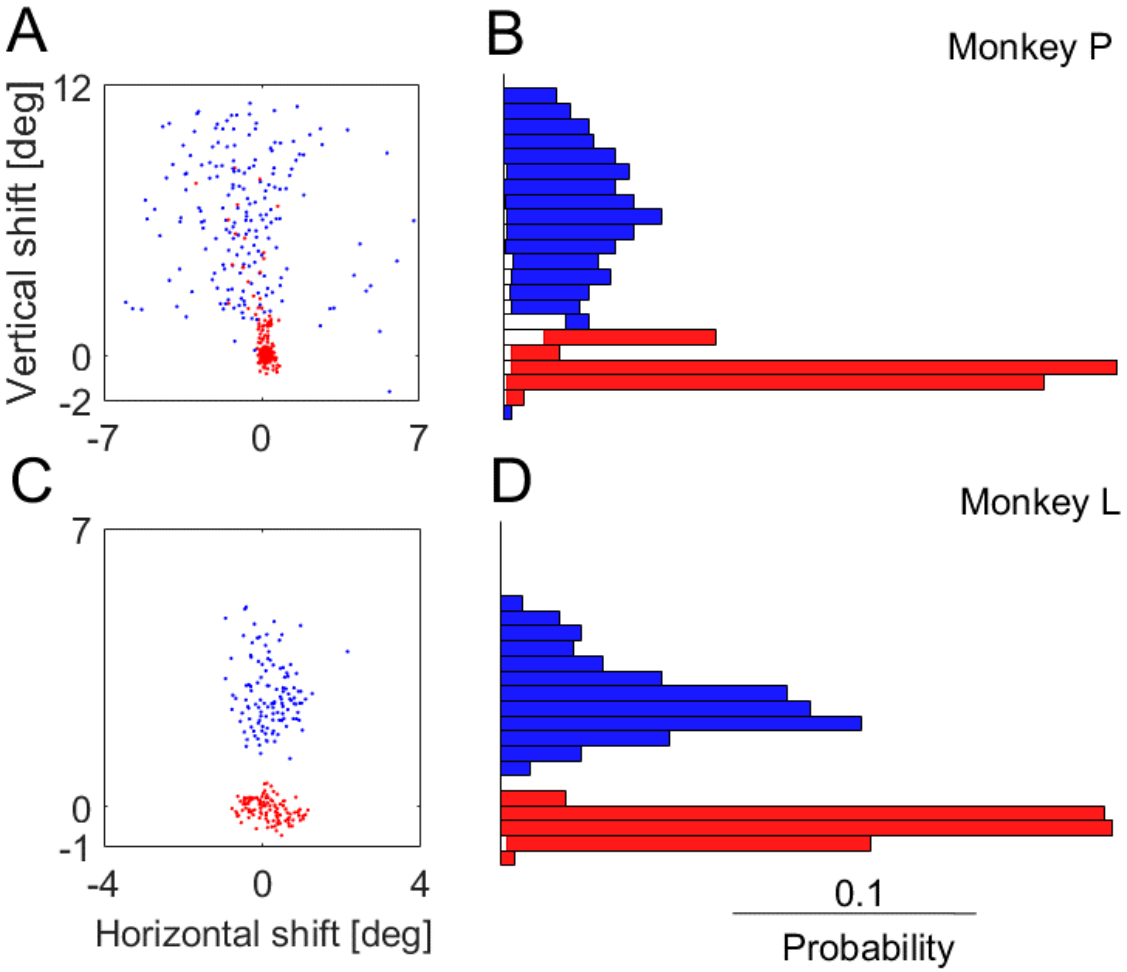
A
B
Monkey P
Vertical shift [deg]
12
0
-2
-7
0
7
C
D
Monkey L
7
0
-1
-4
0
4
Horizontal shift [deg]
0.1
Probability

Figure 11

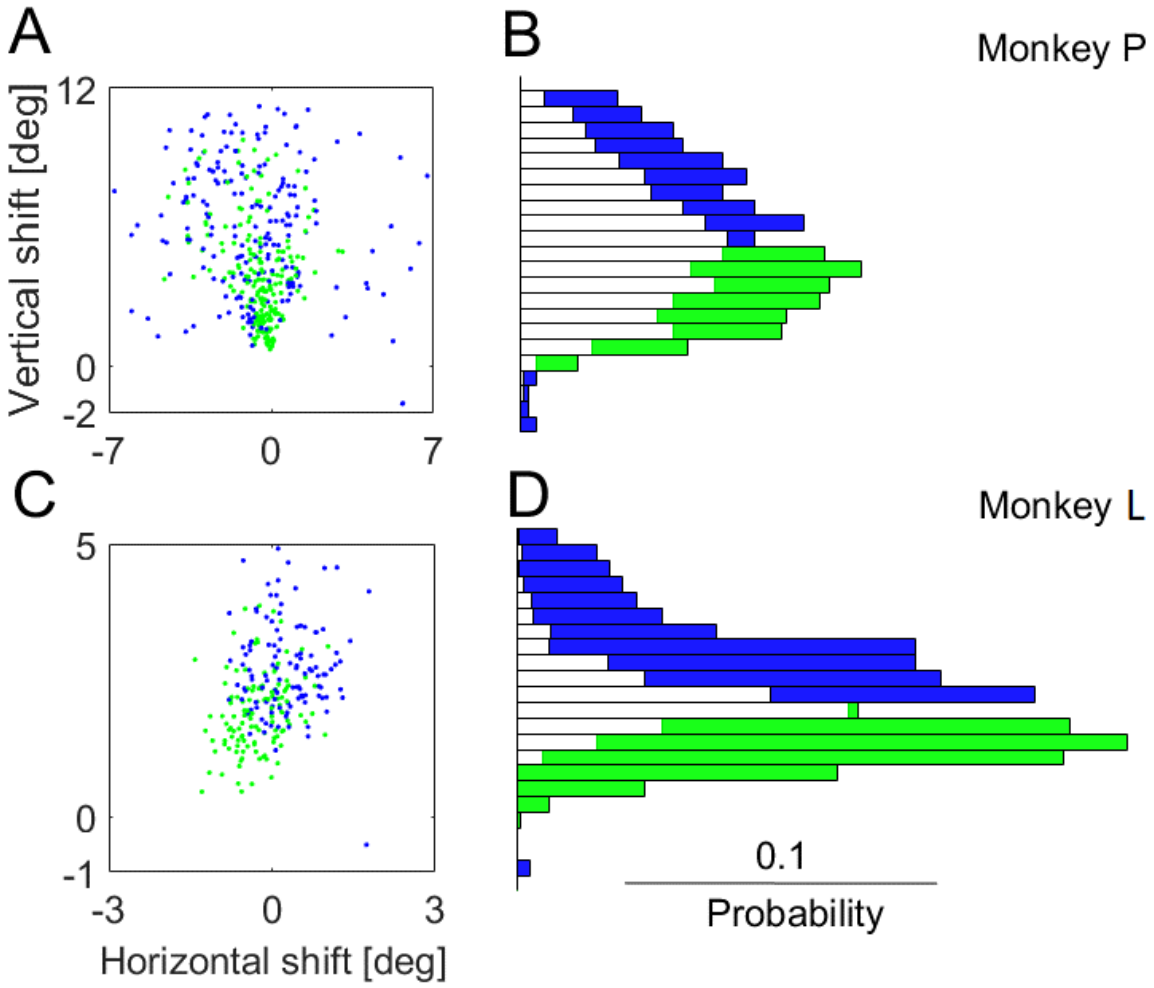
A
B
Monkey P
Vertical shift [deg]
12
0
-2
-7
0
7
C
D
Monkey L
5
0
-1
-3
0
3
Horizontal shift [deg]
0.1
Probability

Figure 12

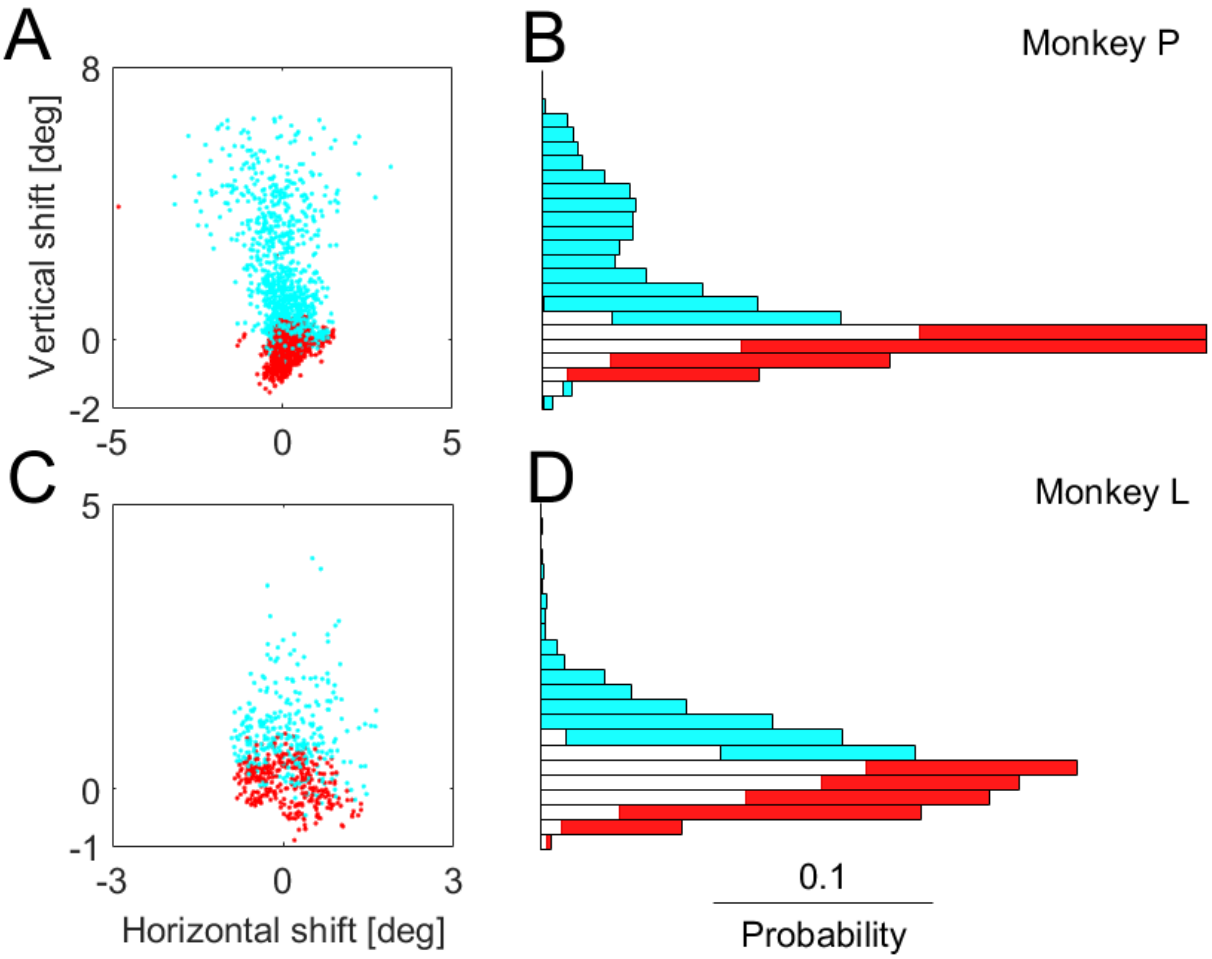
A
B
Monkey P
C
D
Monkey L
Vertical shift [deg]
8
0
-2
-5
0
5
5
0
-1
-3
0
3
Horizontal shift [deg]
0.1
Probability

Figure 13

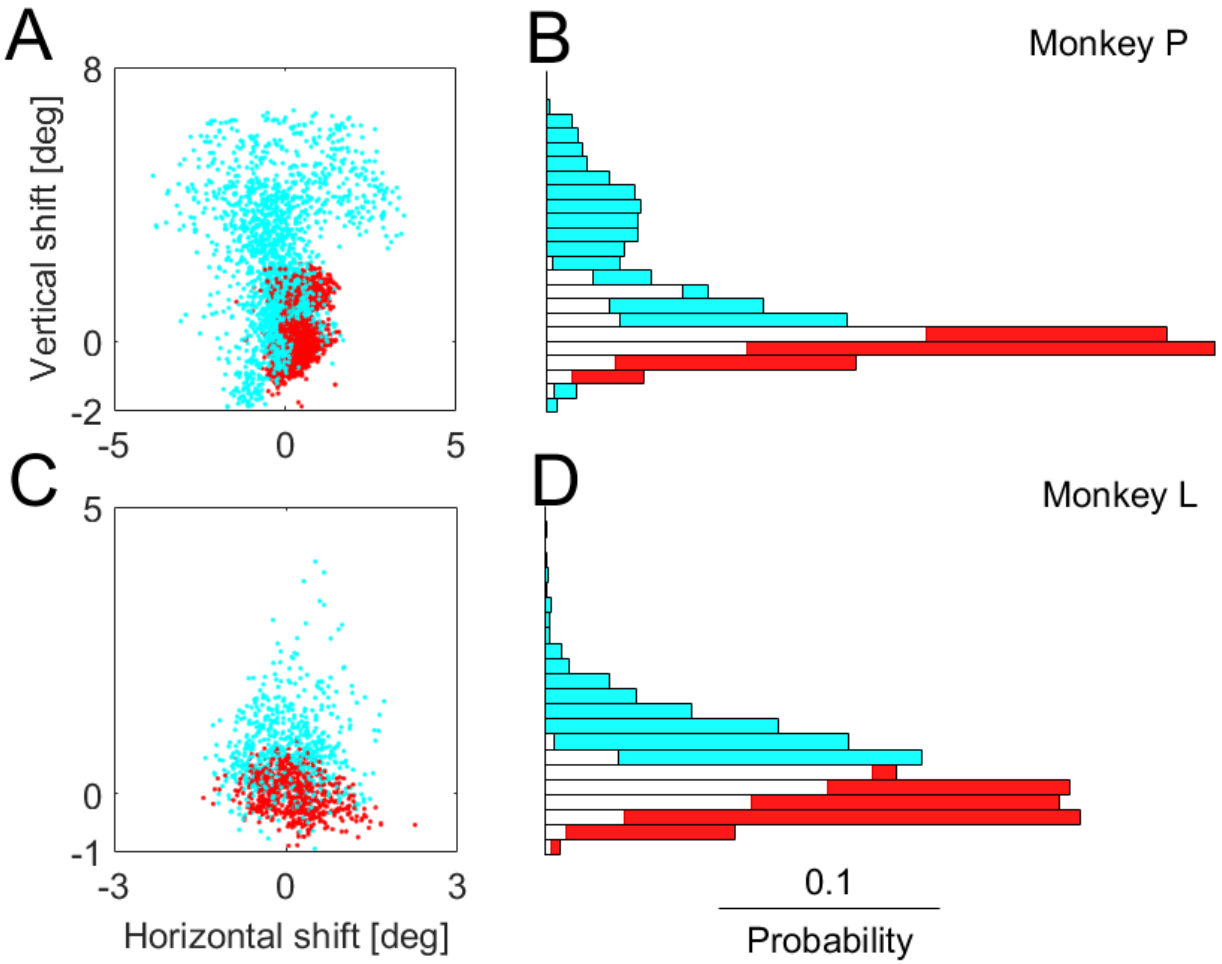
A
B
Monkey P
C
D
Monkey L
Vertical shift [deg]
8
0
-2
-5
0
5
5
0
-1
-3
0
3
Horizontal shift [deg]
0.1
Probability

Figure 14

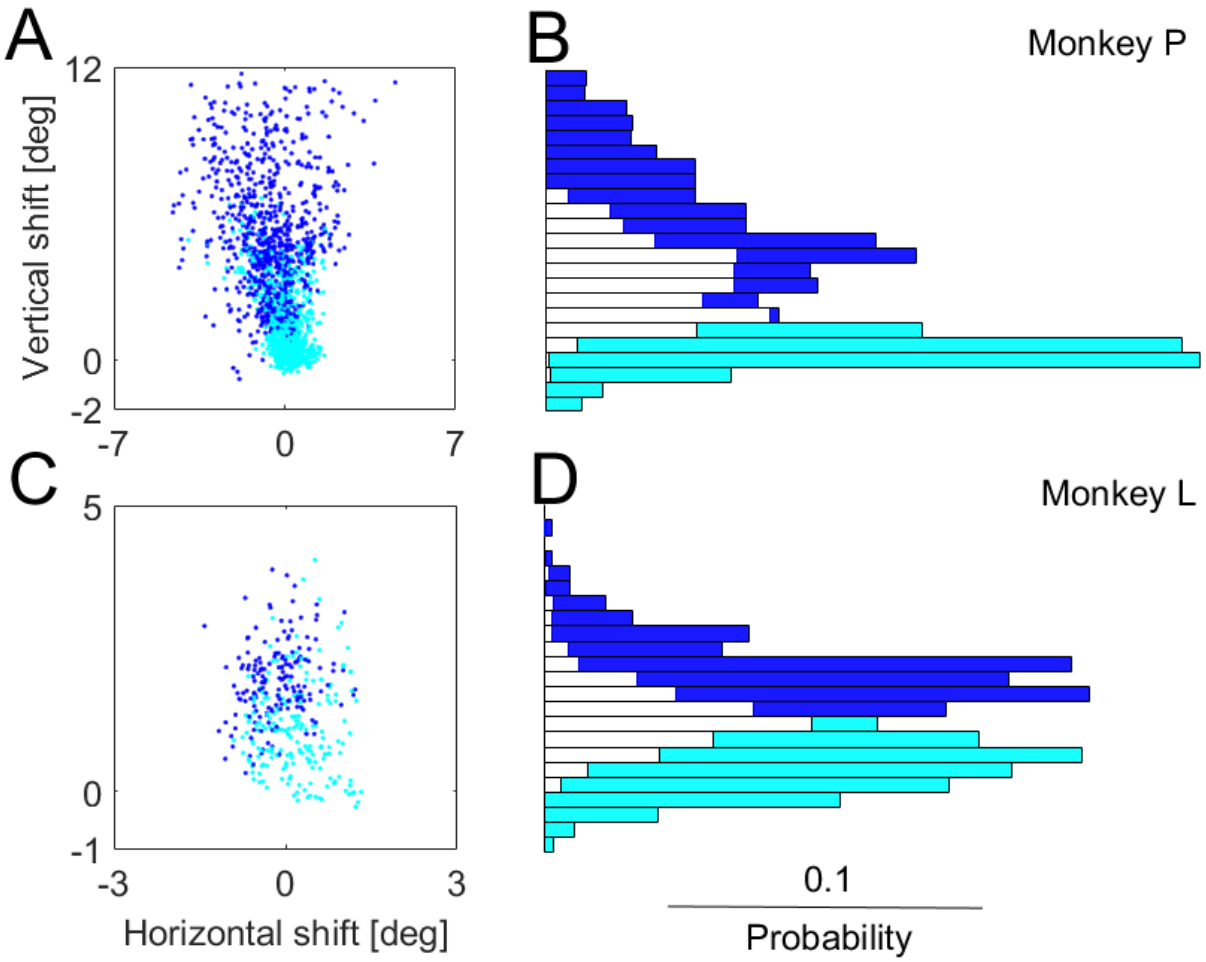
A
12
0
-2
Vertical shift [deg]
-7
0
7
B
Monkey P
C
5
0
-1
-3
0
3
Horizontal shift [deg]
D
Monkey L
0.1
Probability

Figure 15

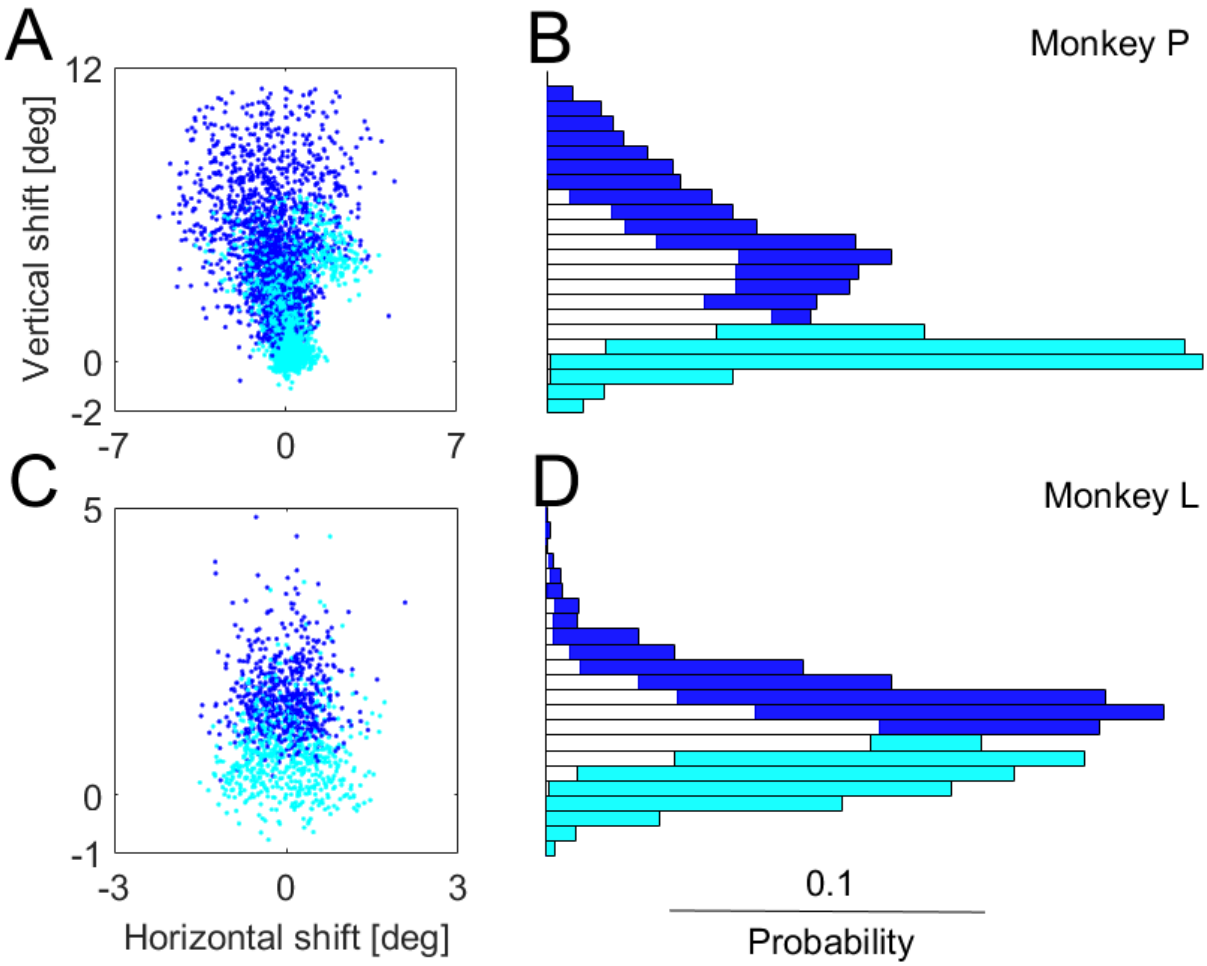
A
B
Monkey P
C
D
Monkey L
Vertical shift [deg]
12
0
-2
-7
0
7
5
0
-1
-3
0
3
Horizontal shift [deg]
0.1
Probability

Figure 16

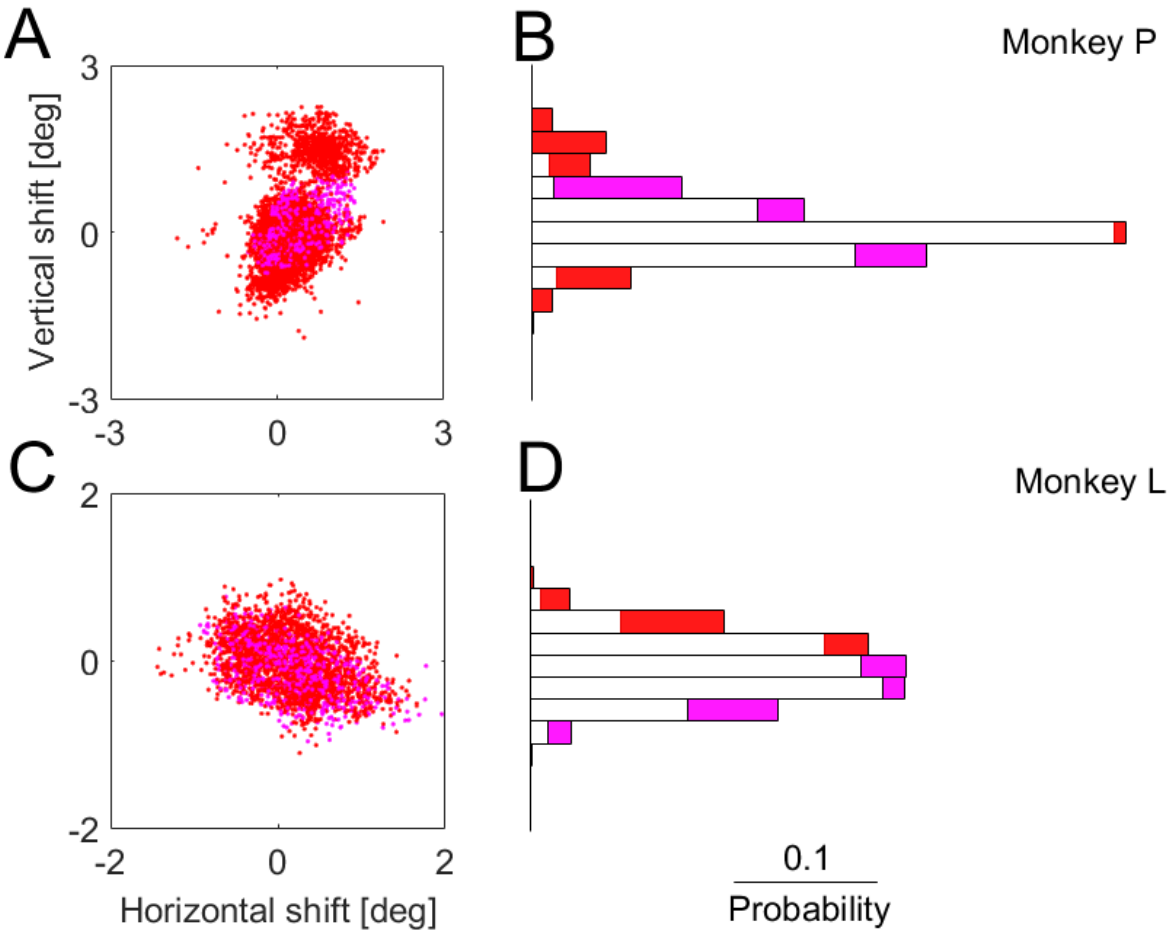
A
B
Monkey P
C
D
Monkey L
Vertical shift [deg]
Horizontal shift [deg]
Probability
0.1
3
0
-3
2
-2

Figure 17

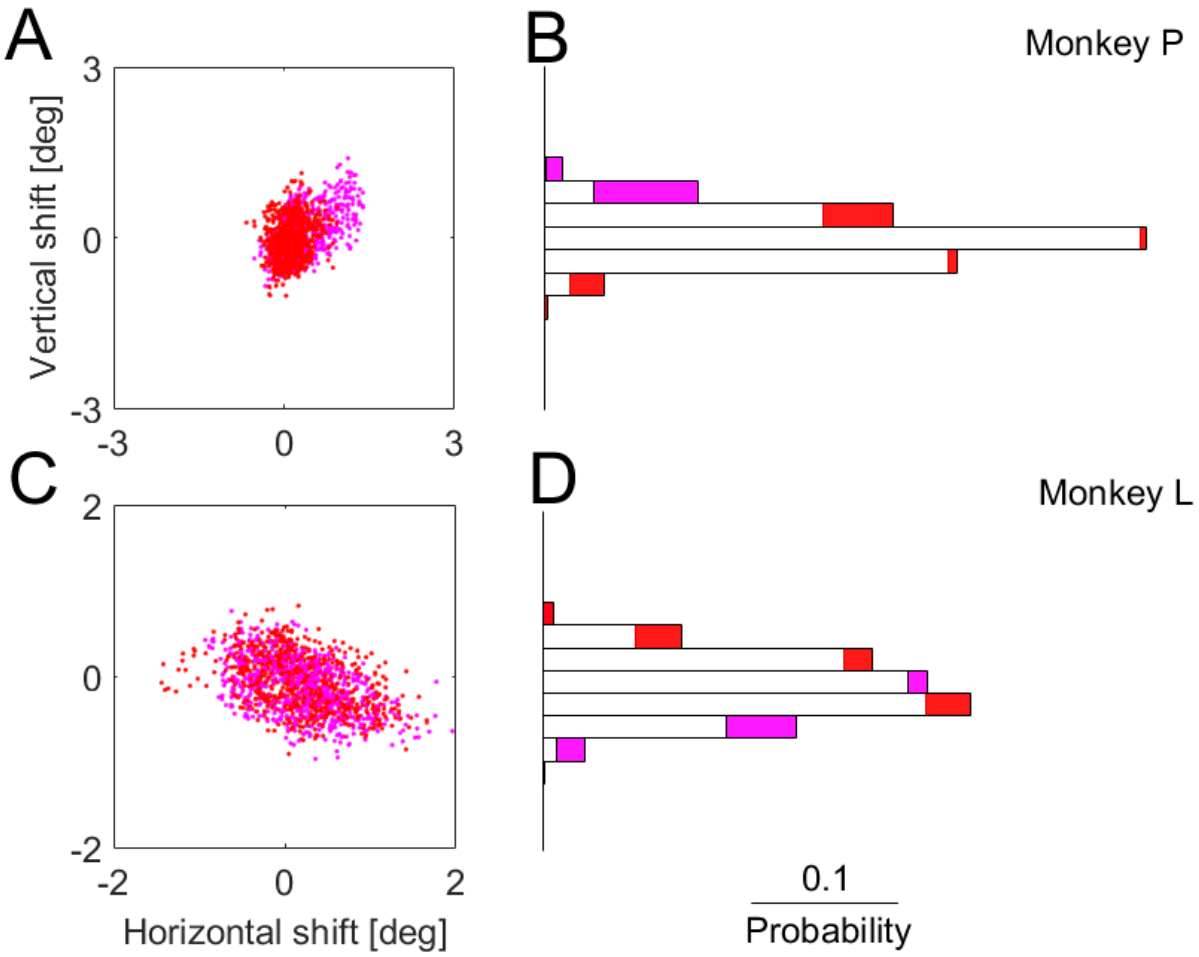
A
B
Monkey P
C
D
Monkey L
Vertical shift [deg]
3
0
-3
-3
0
3
2
0
-2
-2
0
2
Horizontal shift [deg]
0.1
Probability

Figure 18