

Non-contact eye-tracking on cats

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Abstract

The objective of visual systems neuroscience has shifted over the past few years from determining the receptive fields of cells towards the understanding of higher level cognition in awake animals viewing natural stimuli. In experiments with awake animals it is important to control the relevant aspects of behavior. Most important for vision science is the control of the direction of gaze. Here we present Dual Purkinje eye-tracking on cats, which—as a non-contact method—brings a number of advantages. Along with the presented methods for calibration and for synchronization to off-the-shelf video presentation hardware, this method allows high precision experiments to be performed on cats freely viewing videos of natural scenes. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Eyetracking; Cat; Electrophysiology; Video stimulation; Natural visual stimuli; Awake animals

1. Introduction

The visual system of higher mammals is a widely used model system for the study of sensory processing. Whereas in the early years of this research the visual system has been analysed mostly in anaesthetized and paralysed preparations (Hubel and Wiesel, 1998), current interest is shifting to the study of awake and behaving animals. Only then can mechanisms of attention (Roelfsema et al., 1998; Posner and Petersen, 1990; Desimone and Duncan, 1995; Colby and Goldberg, 1999), working and motor memory (Romo and Salinas, in press) and consciousness (Engel et al., 1999) be addressed. The drawback of these preparations is that it is difficult to control the motivation and behavior of awake animals. In the context of vision research it is of particular importance to measure the movements of the eyes, since the direction of gaze determines the mapping of the visual field onto the retina. This task is particularly challenging in the exploration of early visual areas where receptive fields are small. Due to this problem, even the properties of the cat's visual system, which have been extensively studied in the anaesthetized

preparation, are largely unknown during wakefulness. To reduce this surprising discrepancy it is necessary to utilize practical and precise methods for eye-tracking with cats.

Several techniques have been developed to measure the orientation of the eyes, which fall into two classes: (1) contact methods measure the eye movements via appliances fixed in or to the eye, e.g. the scleral search coil method (Fuchs and Robinson, 1966); (2) non contact methods measure the direction of gaze without attachments to the eye, typically using optical methods such as infrared limbus tracking or video based systems. The latter methods share the advantage that they do not require surgery and do not have disturbing or painful side effects. They are therefore widely used on human subjects and non-human primates. Of these methods the Dual Purkinje Image (DPI) eye-tracking technique is—at least when applied to humans—among the most precise. Here we apply this method, which has previously only been applied to humans, to cats, obtaining a non-contact method for eye-tracking. Together with precise calibration and synchronization to video presentation hardware this results in a complete system for controlling stimuli as well as monitoring eye movements of awake animals. This system thus allows reconstruction of the visual stimulus on parts of the retina over long time spans in awake behaving cats.

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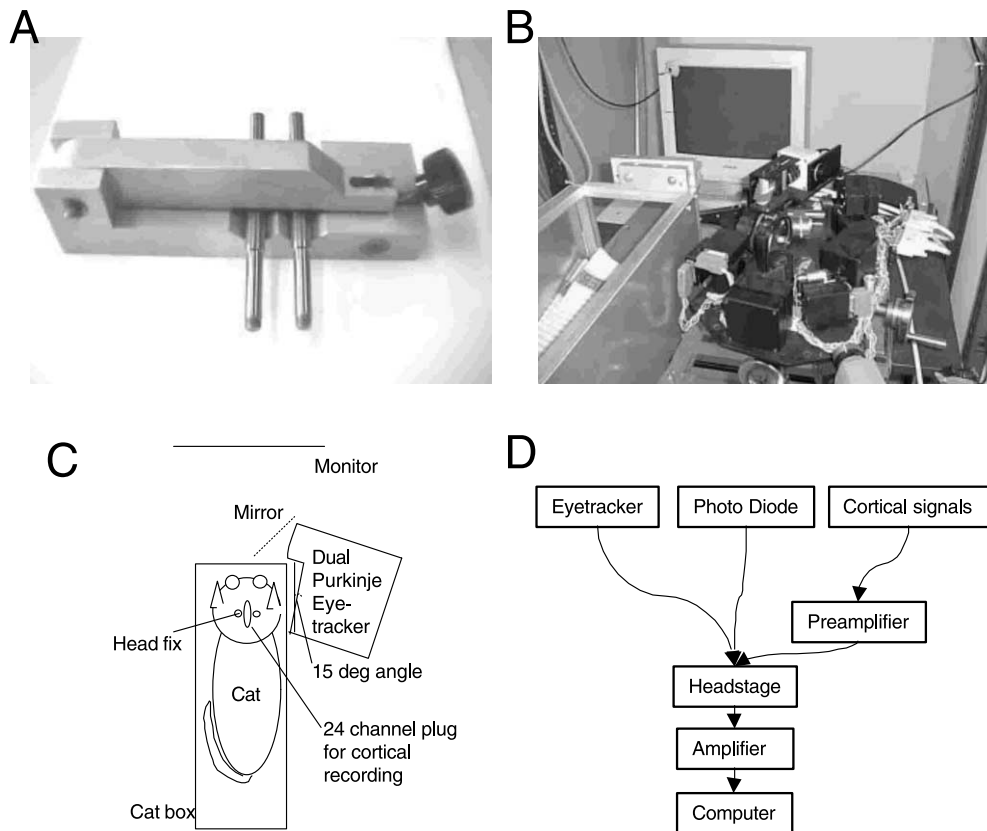


Fig. 1. The experimental setup. (A) Bolts are connected to the implant on the cat's head and then fixed to the setup using a quick tightening lock; (B) The setup seen from above; (C) Sketch of the setup. Note the small oblique mirror placed between the eye and the stimulation monitor by which the infrared light from the DPI eye-tracker is projected into the eye; (D) The overall signal flow in our setup.

2. Methods

2.1. Cat preparation

Cats in our experiments carry implants used for chronic microelectrode recordings of brain activity (Siegel et al., 1999). They are of a dimension of about 1 cm^3 , made out of dental cement, and are fixed to the skull using eight titanium screws. We add two M6 nuts on top of this implant, one on each side, so that both are facing upward, perpendicular to the normal line of sight of the cat. These nuts are used as multi-purpose connectors. They can hold a micro-CCD-camera for the acquisition of videos from the cat's perspective (Betsch et al., submitted). In the present context we use them to fixate the cat's head. The body of the cat is placed in a sleeve, which is fitted using adjustable velcro fasteners. This procedure restrains movements and prevents accidental startle responses while at the same time creating a warm and pleasant environment for the animal. In the experimental setup the animal is supported by an acrylic glass tube of appropriate diameter. It takes about 1–2 weeks of daily training for the animals to become accustomed to the procedure and the setup. Training procedures last about 1 h per day,

using food as a reward for entering the sleeve and remaining in it for several minutes. Indeed, as the conduct does not contain painful or aversive stimuli, the animals relax, are calm and convenient to handle. In the setup, the head of the cat is held by two rods connected to the nuts of the implant. The rods in turn are held by a quick tightening lock (Fig. 1A). We found this approach useful to minimize disturbing manipulations of the animals and avoid fast or clumsy movements. None of the experiments cause unnecessary harm to the cats and no visible signs of stress or pain can be observed while they are in the setup. The animal treatment occurs in compliance with Swiss Federal and institutional guidelines for experimental animal care.

2.2. Dual Purkinje eye-tracking on cats

To measure the cat's direction of gaze a 'Dual Purkinje Image' (DPI) eye-tracking system is placed beside the head of the cat with its oblique mirror directly in front of the animal's head (Fig. 1B, C). This mirror is transparent to visible light and only reflects infrared light. The DPI eye-tracker (manufactured by Fourward Optical Technologies Inc., Clute, Texas, USA) measures the reflection of an infrared beam by the eye of

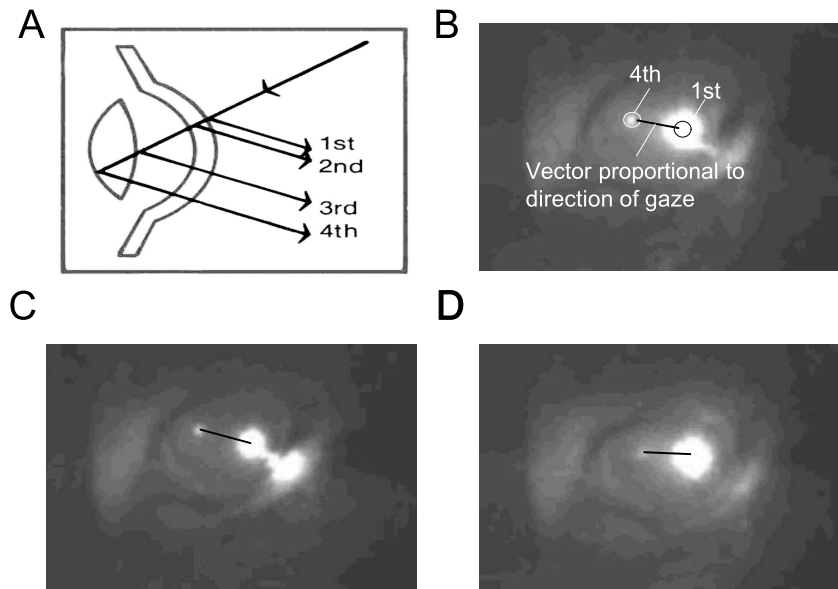


Fig. 2. The DPI eye-tracking system measures the orientation of the eye using the Purkinje images. (A) When light is shone into an eye, it is reflected at the surface of the eye (1st), at the back of the cornea (2nd) and at the front (3rd) and back (4th) of the lens; (B) The relative position of the first and fourth reflection depends on the orientation of the eye and is used to determine the direction of gaze; (C–D) Infrared images of the reflections obtained from the DPI eye-tracking system show the first and fourth Purkinje images and how their relative position changes.

the animal and thus does not disturb normal vision. When this beam is projected into an eye, it is reflected: (1) at the surface of the eye; but also (2) from the back of the cornea; (3) from the front; and (4) back of the lens (Fig. 2A). The reflection at the corneal surface is called the first Purkinje image. The reflection at the backside of the lens is called the fourth Purkinje image. These two images are captured by the eye-tracker using the same mirror that is used to shine the infrared beam into the eye. Part of the light is transmitted to a video camera that can be used to manually observe the frontal view of the eye. In these images the first as well as the fourth Purkinje image are clearly visible (Fig. 2B). When the eye turns while the light source remains fixed, the first Purkinje image moves a little due to the fact that the eye is not spherical and due to residual translations. The fourth Purkinje image moves relative to the first as the cat looks around (Fig. 2C). A sophisticated mechanism is employed by the DPI eye-tracker to track these relative movements of the fourth Purkinje image with respect to the first Purkinje image. Thus, small translations of the head do not translate into effects on the apparent direction of gaze. This property is of importance since it turns out that even with rigid steel screws it is difficult to obtain head fixation with less than 0.1 mm peak to peak movement (estimate from enlarged video images). If only the fourth image is measured, the residual movements of the head would result in an error of a few degrees, whereas the DPI eye-tracker can achieve a precision of well below a degree.

A few modifications to the human DPI eye-tracking setup are necessary before stable tracking in cats can be obtained. The fourth Purkinje image is slightly out of focus for cats. This problem is solved by moving the fourth detector all the way to the front of its housing. The position of the cat's fourth Purkinje image is much farther on the nasal side than the position of the same image in humans, and without modifications the fourth Purkinje image is not even detected. This can be partly compensated for by rotating the whole eye-tracking apparatus by about 15° outwards. Using an artificial eye (Fourward Technologies Inc.), the residual difference can be compensated for by adjusting the zero position of the eye-tracking system. After these initial setup changes, the system is usable with any cat and works reliably. Interestingly, the eye-tracker can still be used on humans without changing this setup. Before starting a recording session the eye position has to be aligned with the eye-tracker by moving the eye-tracker relative to the eye or vice versa. This is necessary since each animal has a different sized head and a slightly different implant. Proper adjustment requires some experience and is often perceived as difficult by new users. After this alignment, the system finds the fourth Purkinje image and tracks it reliably.

Correct functioning of the eye-tracker also requires darkening the room because the fourth Purkinje image is rather dim (Cleveland and Cleveland, 1992) and the rises contract in bright light decreasing the range of eye-tracking. While this may be a restriction on some kinds of experiments it seems to be a natural condition for cats, which are nocturnal animals, and furthermore

poses no problems if stimuli are presented using a monitor with low brightness settings.

The output of the eye-tracking system consists of three analog signals. The horizontal and vertical readings, ranging from -20 to $+20$ V and a TTL signal (BLINK) that indicates correct tracking. Incorrect output arises mainly during blinks of the eye, when no Purkinje images are present. Horizontal and vertical signals have a temporal bandwidth of up to 400 Hz depending on the model of the eye-tracker and thus allow measurements of fast as well as low amplitude movements.

2.3. Digitization

For the acquisition of the data obtained from the eye-tracker we use the same amplification and analog to digital (A/D) conversion setup (SynAmp amplifier manufactured by Neuro Scan Labs with Scan 4.1 used as digitization software) as we use for the physiological experiments. This guarantees that the delays from recording to A/D stage are homogenous over all signals and thus contributes to reliable data. Amplification is done in DC mode, which means that the amplifier records the unfiltered voltage traces. This is important because filtering would deteriorate the eye-tracking signal. But in DC mode it is not possible to directly record electrophysiological data from the cats. This problem is solved using a preamplifier with built in filters that replace the filter of the main amplifier (this procedure also dramatically increased our signal quality). With the overall setup (Fig. 1D) it is possible to record the eye-tracker signals as well as from up to 28 electrodes.

2.4. False locks

In the experiments performed the apparatus occasionally picks up a false lock. In these instances the instrument confounds the border of the eye with the fourth Purkinje image. Changes in eye opening then lead to correlated changes of both horizontal and vertical apparent direction of gaze. This results in traces where the horizontal component is strictly anti-correlated with the vertical component rendering correct tracking impossible. The direction of gaze read out is also significantly shifted by as much as 15 V. These properties together make offline detection of such faulty signals easy. It would be possible to detect them online by combining a window discriminator with the eye-tracker's signals, which would be necessary for feedback systems. The faulty signals occasionally occur when the eyetracker is calibrated on the cat's eye. When the eye-tracker is calibrated using the artificial eye, false locks occur only rarely, of the order of once per recording session of about 10 min. The eye-tracker occasionally malfunctions, but careful data analysis allows the exclusion of bad data from further investigation.

2.5. Calibration

Knowledge of the direction of gaze of the animal can only be obtained after the eye-tracking system has been calibrated. The system produces analog output signals (mV) proportional to the vertical and horizontal angles of gaze. The relation between these signals and the direction of gaze, as described by gain factors and offsets, depends on the individual animal and has to be determined experimentally. Knowledge of the gain factors allows conversion of eye-tracker signals to convenient units such as degrees of visual angle. This can be used to study the size of saccades and relative eye-positions. To determine the direction of gaze the offsets must also be known. Here we mainly analyze the gain factors for the following reasons. Compared to primates cats do not have a small fovea but a large and elongated area centralis of about 5° in diameter. Thus looking at the retina the 'point of fixation' can only be defined to a few degrees. Furthermore, the absolute direction of gaze is open to subjective interpretation and is not decisive for physiological investigations of receptive field properties. Nevertheless, one possibility for estimating the offsets is to incorporate the animal's behavior by attracting its visual attention. An often employed—but experimentally very challenging—method is to gain the attention using 'interesting' objects like spoons with food or a small bell attached to a rod. We must note though that this approach relies on the assumption that the animal is really looking at the interesting object, which can only be proven reliably using another already well calibrated gaze tracking system. Thus, manual inspection of the fixation can only judge the reliability of the fixation but not its validity.

For calibration we now use a method reminiscent of the reversible ophthalmoscope (von Helmholtz, 1851). Due to the optical law of correspondence, each part of the retina is projected to the position on the tangent screen where the light would stem from that normally illuminates that specific part of the retina. From the projection image the position of salient points on the retina can be determined. This method has frequently been used in electrophysiological experiments on anaesthetized animals to determine the projection of the area centralis and to align the stimulus accordingly. Significant changes are necessary to employ this technique on awake animals. We take photographs of the back-projection of the retina onto a tangent screen: a fiber optic cable (2 mm diameter, Stoelting Physiology) is placed close to the eye (Fig. 3A). The other end is placed in front of a standard photographic flash. Since the cable is much smaller than the flash, only a small part of the light actually enters the cable. The fiber functions only to guide the light, not to focus it. The flash is coupled to a high quality SLR camera with a standard objective

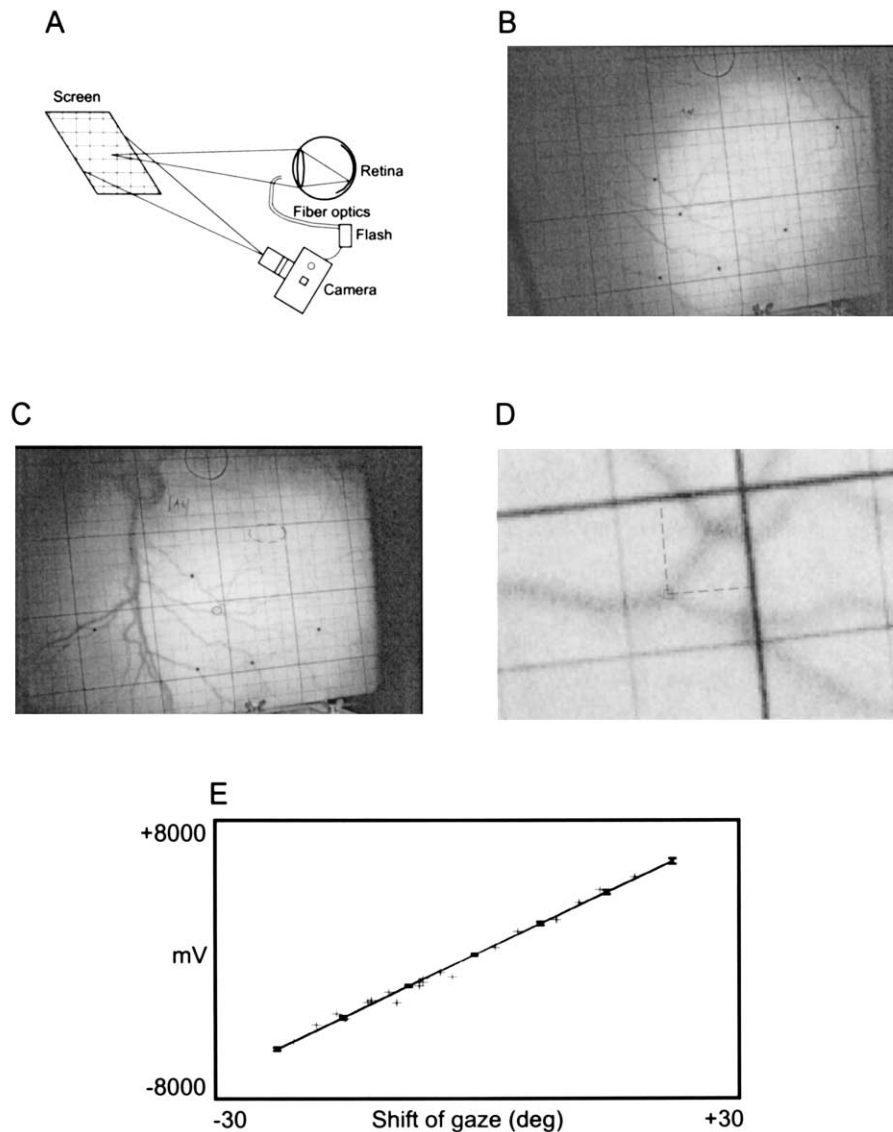


Fig. 3. Calibration of the eye-tracker. (A) Sketch of the calibration setup. An optical fibre optics guides light from a flash into the eye where it is reflected from the tapetum. This reflection is projected onto a tangent screen that bears a rectangular grid. A photo of the image on the screen is taken using highly sensitive film; (B and C) Photos taken at different directions of gaze. A grid drawn on the tangent screen is visible. A small note attached to the screen was used to identify the photos. The black dots mark points chosen for computation of the shifts between two images. In C the area centralis is marked by a dashed ellipse; (D) Enlargement of the area around point marked in C by an open circle. The chosen point was the center of a branch-point that can be determined with an accuracy indicated by the size of the small rectangle. The coordinates of this point with respect to the grid were measured in pixels and converted to degrees (dashed lines); (E) Calibration of the eye-tracker gain for horizontal shifts of the eye. For each pair of photos the vertical shift of the eye is computed and compared to the shift indicated by the eye-tracker (crosses). The slope of the fitted line gives the conversion of eye-tracker output to degrees of visual angle (in this case $302 \text{ mV} \equiv 1^\circ$). The errorbars denote the position dependent standard error of the regression line, which varies between 0.26 (near 0 mV) and 0.56 (at 6000 mV).

(55 mm, aperture 1.8) and equipped with a 1600 ASA film (Fujicolor). To obtain useful photos the room has to be completely darkened. The light flash illuminates the retina and a photo of the reflection on the tangent screen is taken. The flash is so fast (synchronization time of $1/125 \text{ s}$) that the eyes do not close before the picture is taken. We first developed the method with a set of pilot experiments on human volunteers who reported no significant disturbance by the flash. When this method is applied to cats they do not show any

indication of pain or discomfort. Indeed, this approach works especially well on cats, where the tapetum reflects a large part of the light falling on the retina. The flash is coupled by a photodiode to the recording setup and recorded simultaneously with the eye-tracker data. In this way the time-stamp when a photo is taken is recorded along with the position indicated by the eye-tracker.

Examples of pictures obtained under these conditions are shown in Fig. 3. The photos show the prominent

structures of the cat retina. Depending on the direction of gaze at the moment the picture was taken, the blind spot, large blood vessels or the area centralis are visible (Fig. 3B, C). To compute the conversion of eye-tracker output to degrees of visual angle, about six photos are taken during one session and compared to the eye-tracker data. Salient landmarks common to several pictures are selected, usually crossing or branching points of blood vessels (Fig. 3B, C black dots). For each such point common to two photos the relative shift is measured. To allow comparison of different photos we found it useful to draw a rectangular coordinate system on the tangent screen. The position of every chosen point is measured on the photo relative to a fixed point in the coordinates system on the screen and converted to degrees of visual angle. The relative shift between two photos is computed as the mean of the shifts of all common points. This method reduces the error introduced by reading off the positions of each point. This is done for all pairs of photos, which have at least one salient point in common. Each shift is compared with the corresponding shift derived from the eye-tracker. Fig. 3E shows the measured horizontal shifts versus the eye-tracker output. As can be seen all shifts obtained lie close to the fitted line. This shows that the eye-tracking system is indeed linear over the whole range. The slope of the fitted lines gives the conversion from the eye-tracker output to degrees of visual angle for horizontal and vertical eye movements.

To accurately determine the position of each point we used digitized versions of the photos at a resolution of 1536×1024 pixels. Pictures were processed using standard graphics and image processing software (Canvas, Deneba Systems Inc.). The selected points were defined to be the middle of the corresponding crossing or branching of the blood vessels. We estimate the error of the point actually selected to be within 3 pixels of the ideal point, as indicated by the box in Fig. 3D. This corresponds to an error of less than 0.1° for the horizontal and vertical component of each point, respectively. The errors of the shift vectors between different photos therefore have a theoretical value of about 0.2° . Indeed, the standard error of the regression line fitted to the points in Fig. 3E is between 0.28° around 0 mV and 0.56° around ± 6000 mV. This incorporates both the error of the slope and of the offset. The standard error of the offset alone is 0.21° . If only the gain factor of the eye-tracker is of interest the error is reduced to an average of 0.15° over a range of more than 30° .

The procedure so far provides a highly accurate calibration of the eye-tracker gain. However, the exact direction of gaze at each instant remains unknown. A common technique used in physiological experiments with anaesthetized animals is to map the position of the area centralis and to identify this with the direction of gaze. The same procedure can also be applied to our

photos already used to determine the gains. Following standard criteria (Bishop et al., 1962) the position of the center of the area centralis can be determined with a precision of up to about 2° (marked in Fig. 3B as dashed ellipse).

From the procedure it is clear that this method of calibration can be applied to other systems that monitor eye position as well. The procedure described above works especially well on cats where the tapetum reflects most of the incoming light. Because its error arises exclusively from known sources it is a very dependable method. Furthermore, it has the advantage that not requiring training or cooperation of the animal, which is of special interest when working with cats.

2.6. Video codes

The information about the direction of gaze is especially interesting if combined with knowledge of the stimulus presented at the same time. Presenting video sequences to our animals we need to assign times to each video frame shown, because this is necessary to link the frame to the corresponding directions of gaze. Since we analyze neuronal signals on the millisecond level we also need to know the times of stimulus frame onsets with the same precision. Off the shelf solutions to this problem often use software approaches that initiate a timestamp signal whenever a new frame is shown. Such a system, however, depends on the workload of the computer and is thus subject to jitter. Modern graphics hardware designed for scientific use supports timestamp generation to interface to recording software (e.g. Cambridge Research Systems, Cambridge, UK). However, such hardware is expensive and due to limited bandwidth sometimes not the best choice for the presentation of videos.

We decided to measure the onset time and identity of each frame employing the following mechanism: the brightness of a 24×24 patch of pixels in the upper left corner of the videos is set to a uniform value that changes over time and is measured by a photodiode connected to the recording system (Fig. 1D). The patch is never black thus enabling us to measure the time of each vertical retrace. The brightness value always changes from one frame to the next. Thus it is straightforward to detect when a new frame is shown. The temporal trace of brightness from 30 consecutive frames transmits the number of the video and the number of the frame that is currently shown. The first value of the code is always 0.25 (the start code signal) whereas the others are of 0.5, 0.75 or 1. The difference in brightness values from one frame to the next can take two different values since the value must always change. Thus we obtain one bit of information for each frame shown and 30 frames together transmit frame and video number. Software routines for MATLAB

(Mathworks Inc.) accomplish this task. One function generates the brightness code for the video frames. Another function returns for given photodiode recordings the onset time of each frame, the frame number, whether frames were skipped, and the video number. This software is available from the authors.¹

We thus designed a system with the following properties: (1) it measures the time of each vertical monitor retrace; (2) it determines the time of onset of each video frame; (3) it automatically detects and reports missing frames; (4) it detects the identity of the video. The system is fault tolerant, it tolerates missing video frames, noise on the photo diode traces, and is largely tolerant of changes in brightness and contrast values. Together with the DPI method for eye-tracking it allows the image as present on the retina to be determined.

2.7. Statistics of eye movements

Since establishing this technology, our lab has been using the DPI eye-tracking system routinely to study eye movements of cats viewing various visual stimuli. We are interested in the eye movements made while the animal is watching videos which are recorded from a camera mounted on the head of a cat which was exploring its natural habitat. Fig. 4 shows an example recording of eye-movements of a cat watching such a video. Analysis of the eye-tracker data proceeds as follows: saccade onset and end are determined by choosing a velocity threshold and detecting velocities above this threshold. Usually this threshold is determined to be above the range of the high frequency oscillations from micro-saccades and noise in the system. For a fixed threshold, distributions of saccade amplitude and duration can be obtained (Fig. 4B and C, with a threshold of 50°/s). Fig. 4D–G show frames from a video used for stimulation along with the roughly estimated direction of gaze.

3. Discussion

We have reported Dual Purkinje Image eye-tracking on cats along with a method for calibration. We further described a mechanism to synchronize the eye-tracker traces with presentation of visual stimuli such as videos. This system proves to be helpful for electrophysiological experiments on awake cats.

The selection of an appropriate eye-tracker is crucial for the results obtained in physiological experiments. There are a number of different eye-tracking methods currently used. These methods show considerable dif-

ferences with respect to precision, speed, the need for head restraint, output in head and/or world coordinates, the need for surgery, compatibility with different calibration methods and last but not least, price. An up to date overview is maintained at the University of Derby at <http://ibs.derby.ac.uk/emed>. In the following discussion please keep in mind that often theoretical limits on precision or speed are stated, which might be difficult to reach in practice or simultaneously with identical parameter settings. A summary of the characteristics of the different systems can be found in table 1, compiled from the emed database and the pages linked to from there.

The Electro-Oculogram (EOG) uses four electrodes glued to the nasal, temporal, upper and lower boundaries of the eyelids. The eye represents a dipole and thus generates a signal from the four electrodes related to the eye-movements. It has the problem of having a constant drift in the baseline, which makes it almost impossible to determine eye positions. The constant need for recalibration makes it unsuitable for electrophysiological studies that have a higher demand on precision. The precision further can be impaired by muscle artifacts and body movements. Since the electrodes are glued to the eyelids the method is inherently translation invariant and gives results in head coordinates. It is an affordable method for the detection of eye movements, e.g. for studies of the optokinetic nystagmus.

The infrared oculogram (IROG) uses a set of infrared diodes to illuminate the eye and measures the reflectance with a number of detectors. Its precision reaches values that are required for physiological experiments in higher visual areas. It has a significant amount of non-linearity and cross-talk between the horizontal and vertical channels making it more difficult to calibrate. This is why calibration on large grids of fixation spots is typically necessary. This property severely limits its use on cats. Its advantage is the reasonable precision, affordable price and convenient handling.

The video oculogram (VOG) uses a video of the eye taken from a camera typically placed near to the head. Using sophisticated computer vision algorithms makes it possible to determine the direction of gaze for both humans and monkeys. The non-linearity inherent to the method again makes fixation grids and nonlinear correction necessary. As above the method would be difficult to adapt to cats whereas for primates it is well established. Depending on the requirements on speed and precision, the costs range from affordable to pretty expensive.

The scleral search coil method ranks among the most precise methods for eye-tracking. Large field coils produce homogenous magnetic fields that change at radio frequencies and thus typically do not interfere with

¹ <http://www.ini.unizh.ch/~koerding>.

physiological recordings. Depending on the angle between the magnetic field and the scleral coils a current is induced that indicates the direction of gaze. It allows eye-tracking over a wide angular range. It allows head free operations and gives a result in world coordinates i.e. not relative to the head but relative to the field coils. It also allows the direction of gaze to be measured while the eyes are closed (e.g. for sleep research). The disadvantage of the method is the insertion of a contact lens (for humans) or the necessary

surgery for animals. Depending on the number of animals involved this surgery significantly increase the overall cost of the method.

The DPI eye-tracking system introduced here for cats excels with even higher precision than the other methods. Combined with the calibration procedure that does not require training of the animal and the synchronization to video stimulation we obtain a versatile set-up for vision research on awake behaving cats.

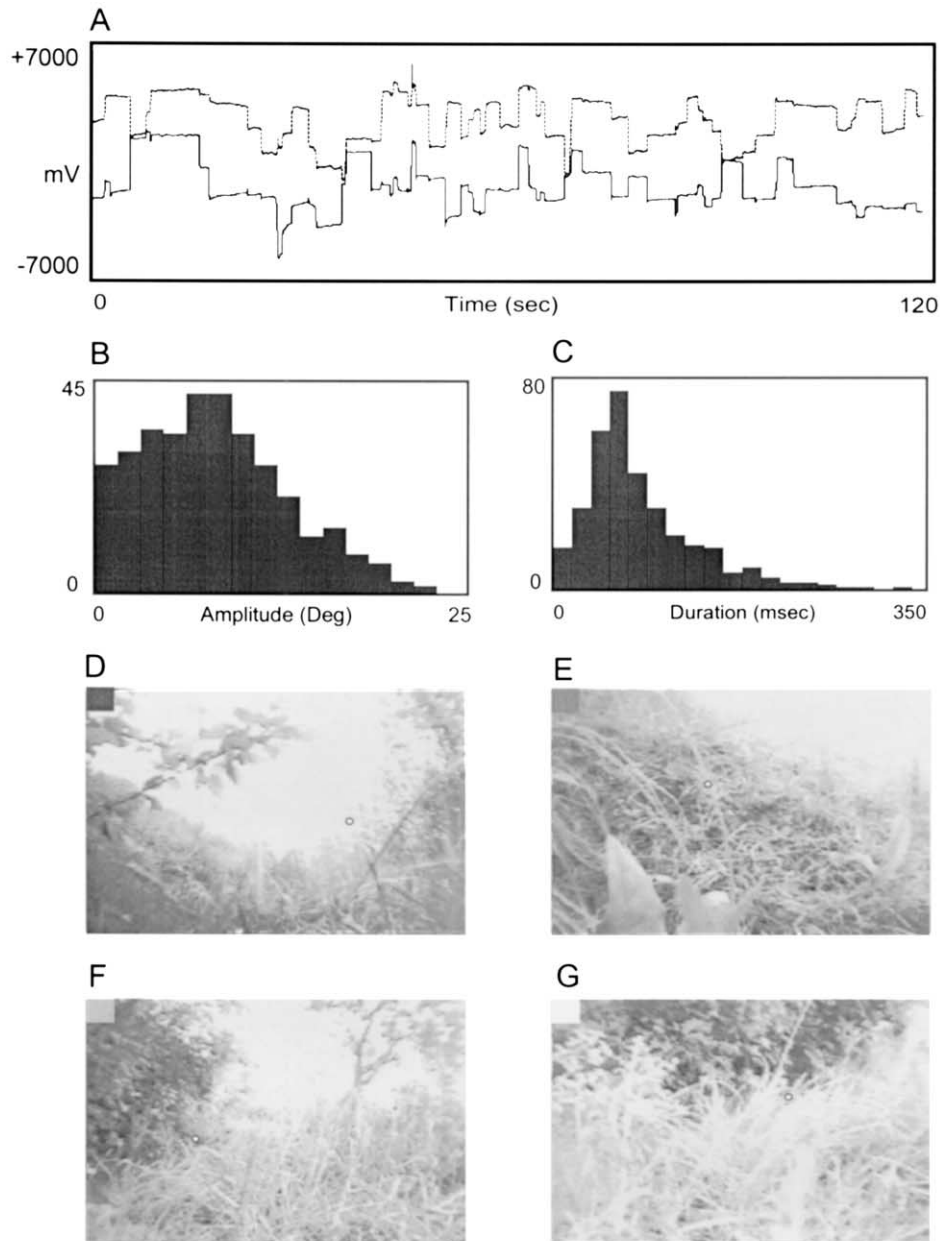


Fig. 4. Eye-tracking using videos of natural scenes as stimuli. (A) The vertical and horizontal output signals in mV from the eye-tracker are shown as dashed and solid lines, respectively (The vertical signal was shifted by +2000 mV for illustration purposes); (B) Histogram of the amplitude of eye movements during a 21 min presentation of a video sequence; (C) Histogram of the duration of the eye movements for the same video presentation; (D–G) Four images out of the video sequence together with the estimated direction of gaze (small circles).

4. Uncited references

Evinger and Fuchs, 1978; Crane and Steele, 1985; Rapaport and Stone, 1984; Müller et al., 1993

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