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ELECTRORETINOGRAPHY OF EXPERIMENTAL ANIMALS*

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ELECTRORETINOGRAPHY affords the only truly objective method of assessing retinal function in animals, since active co-operation on the part of an experimental animal is not obtainable in the same way as from a human subject. Electroretinographic records of many different animals have been published (*e.g.*, human, Adrian 1945; Karpe 1945, 1948; rat, Charpentier 1936; cat, monkey, pigeon, Adrian 1946; frog, Granit and Riddell 1934; insects, Jahn and Wolff 1941). It is the purpose of this paper to discuss the method as a routine examination of experimental animals and to describe the results obtained using a simple condenser-coupled amplifier, cathode ray tube and photographic recorder. Normal guinea-pigs and pigeons and normal and vitamin A-deficient rabbits have been used.

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The main difficulty in recording the electrical response of the retina is to keep the animal quiet. It is essential that the animal be kept comfortable and the instrument described here was designed with this particular end in view. The result is that the recording of the retinal response is now a relatively simple procedure which can be carried out repeatedly on the same animal. The design of the amplifier, light stimulator and electrodes and the method of anaesthetising and handling the animal are described in some detail in the hope that the simplifications introduced may be useful to others.

A detailed account of the history of electroretinography and of the analysis of the resulting records has been given by Granit (1947). The basic principle consists in leading off the electrical potential from the eye (usually from the cornea with reference to a point on the body as near the back of the eye as possible) to be amplified and recorded on paper or on film.

METHODS

The amplifier.—The design of equipment of this kind depends entirely on the nature of the phenomena to be recorded. When using light stimuli lasting one second or longer the amplifier must be capable of maintaining the wave form faithfully for at least two seconds and direct coupling of the stages is essential, but when flash stimuli are used (0.1 second, or less) and the response from the eye does not last above 0.5 second, condenser coupling can be used, greatly simplifying construction. The magnitude of the input is important. The response from the eye, in most animals, is between 0.05 and 0.5 mV., usually about 0.2 mV. Since a cathode ray tube needs an input of 20–100 V. to produce a good deflection, the amplifier must have a gain of at least 10^5 .

This means that there is a great possibility of interference being picked up from outside, especially 50 cycle hum from mains operated equipment and the input must therefore be screened. A cage built of expanded metal fastened to a wooden framework $6\frac{1}{2} \times 5 \times 4$ ft. and fitted with a door is suitable. All joints in the expanded metal are securely soldered and the whole bonded to a cold water main. A standard amplifier rack $3\frac{1}{2}$ ft. high is fitted into this cage and this houses the batteries, pre-amplifiers and calibrator and also serves as a bench on which the animal and stimulator rest during the experiment.

A resistance capacity coupled amplifier has been built for this work and the circuit is shown in Fig. 3. To obtain sufficient gain to drive a cathode ray tube several stages are necessary. A three stage amplifier in conjunction with a compressor circuit described

by Tönnies (1938) and Saunders (1942) has been used to minimise the effect of stray pick-up from the leads and the animal and also the effect of potentials derived from heart-beats and respiration. The calibrator network is contained within a metal screen inside the compressor unit and can be switched in and out of circuit as required without disturbing the connections to the animal. The calibrator is tapped to give 0.02, 0.2, and 2.0 mV. Normally only the 0.2 mV tapping is used. Coupling condensers and grid and anode resistors are made as large as possible throughout the amplifier to maintain a high time-constant. With the values shown in Fig. 3 a constant input gives an output falling to half maximum in about 0.4 seconds.

Recordings are made on 35 mm. recording paper in a Cossor model 427 camera with a film speed of 1 cm./second. Sweep circuits are not used for recording electroretinograms. The compressor input stage and battery operated pre-amplifier stage are kept within the screening cage and are connected to the mains operated stages and recording gear outside the cage by means of screened cables. The camera and motor can be operated from within the cage by mechanical arrangement so that no interference is introduced from this source.

The light stimulator.—The arrangement is set out diagrammatically in Fig. 1. The light source is a 6v. 30w. V-filament car headlamp bulb fitted in an adapted gunsight. The current flowing

FIG. 1.

The light stimulator. The lamp is a 6v. 30w. car headlamp bulb with the current controlled at 5 amps. Colour temperature 2950-3000° K.

through the lamp is controlled at 5 amps., maintaining a fairly constant colour temperature of 2950-3000° K. (maker's data). Light from the bulb is focused on to a sliding neutral density optical wedge and a spring loaded shutter giving flashes lasting about 0.1 second. A second lens is used to focus the light on to the pupil of the eye being examined. Provision is also made for

arm. Contact with the eye is made by a cotton wick soaked in the solution and leading from the other arm. This arrangement is very simple and works quite well but when working in the dark trouble arises from fluid siphoning unnoticed along the wick. Care is needed in using closed electrodes because peculiar and varying potential changes may arise from air bubbles in contact with the silver rod. I have found the type of electrode shown in Fig. 2 the most satisfactory in use. Different designs are used for the corneal and indifferent electrodes to minimise the possibility of air bubbles coming into contact with the silver rods in the different positions. Rubber caps taken from vaccine phials are used to close the ends of the tubes, so that saline may be added with a hypodermic needle through the rubber while the electrode is in position. Small glass caps ground to cover the tubes holding the wicks prevent drying of the electrodes when not in use.

The silver is re-coated with silver chloride before each experiment by connecting the electrode to the positive terminal of a 4v. battery while the cotton wick dips into a dish of saline containing a platinum foil electrode connected to the negative terminal. Current is left running for about half an hour. If this is not done before each experiment, spurious potentials are likely to arise due to polarisation and these will disturb the base-line.

THE EXPERIMENTAL ANIMAL

Choice of anaesthetic.—Inhalation anaesthetics, such as ether, chloroform and anaesthetics inducing anoxia, such as nitrous oxide, depress the response and alter its character as well as being difficult to administer while a record is being taken. Chloralose has a similar effect on the response. Avertin and urethane are in some ways excellent anaesthetics, but, in our hands, the casualty rate was high and if the animals are required to survive, these anaesthetics are not recommended. Nembutal anaesthesia has given the best results for routine work where several observations have to be made on the same animal over a period of time. It may be short acting if the animal is uncomfortable and for this reason animal holding boxes of the guillotine type should not be used. A comfortable animal will remain quiet for up to an hour, sometimes even longer, under Nembutal. In cold weather the animal should lie on an electrically heated pad for greater comfort. Both eyes should be cocainised as animals sometimes become restless while lying on an opened eye under the anaesthetic.

Rabbits are given 0.5 ml. Nembutal (Abbott) per kilogram body weight into the marginal ear vein. Pigeons were found to possess

a strong blink reflex, especially from the third lid, which could not be abolished by a general anaesthetic without risk of killing the birds. These blinks give rise to large negative action currents which could swamp the electroretinogram record. They can, however, be abolished by a retrobulbar injection of procaine hydrochloride. An injection under the skin round the eye was of no use and neither was curare in conjunction with light Nembutal. For routine electroretinographic measurements pigeons were given about 0.2 ml. Nembutal (depending on weight) into the wing vein and 0.2-0.3 ml. procaine hydrochloride 4 per cent. w./v. solution with adrenalin (B.P.) into the orbit. Because of the shape of the pigeon's eye great care is needed not to puncture the globe when giving this retrobulbar injection.

Animal holder.—Each species provides its own difficulties. The method of holding varies with the animal and no universal holder has been found. Rabbits are wrapped in an electrically heated blanket (6v., 3w.) after anaesthetisation and left to lie on the bench while the stimulator and electrodes are adjusted to suit the position of the animal. One electrode is applied to a shaved spot on the top of the head, contact being aided by addition of a wetting agent, such as "Drene" shampoo, to the saline. The second electrode is applied to the cornea without a wetting agent.

A small speculum, illustrated in Fig. 4, made from 30-gauge steel wire is used to hold the lids apart during the experiment. If

FIG. 4.

Miniature speculum made from 30 s.w.g. spring steel. For pigeons these are made 1.5-2.0 cm. long, and for rabbits about 2.5 cm. long.

this speculum is earthed to the amplifier chassis stray interference can be greatly reduced. Karpe (1949) dispenses with the screening cage entirely in his clinical work, using only an earthed speculum.

A special holder was made for pigeons and is shown in Fig. 5. A block of plastic material was shaped to fit the beak and hold the head firmly with the neck supported in a hole in the top of the

FIG. 5.

Holder for pigeons. The bird's neck and head protrude through the hole in the lid while its beak is held lightly in the plastic block to the right. The box is 23 cm. X 11 cm. X 9 cm. and is open at both ends.

box. The pigeon's body is held wrapped in an electrically heated blanket inside the box. The holder was designed to suit a natural position of the bird.

Production of vitamin A deficiency. — Rabbits were fed the vitamin A-deficient diet described by Mann, Pirie, Tansley and Wood (1946). The course of the deficiency was assessed by weight records, slit lamp examination of the eyes and estimation of liver vitamin A levels.

RESULTS

Specimen records from different animal species are given to show the performance of the instrument described. These results confirm and expand those of previous workers.

I. *The response to white light*

The records from a normal pigeon, guinea pig and rabbit are shown in Fig. 6. The guinea pig record shows also the signal

FIG. 6.

Top. Record from normal guinea pig. Middle. Record from normal pigeon. Bottom. Record from normal rabbit. Before the records were taken the animals had been exposed to normal room lighting. Stimulus was about ten times threshold.

from the light flash. In the others the light stimulus was too feeble to elicit a response from the photo-electric cell and no signal is seen. There is an interesting difference in the responses given by these species, perhaps the most interesting is the negative wave given by the guinea pig, also noticed by Boehm, Sigg and Monnier (1944). This kind of response was never obtained from either rabbits or pigeons. The overall amplitude of the response in each case was about 0.3 mV.

II. *The response to light of different wavelengths*

The records in Fig. 7 show quite clearly the difference in response to light of different wavelengths between the pigeon which is diurnal and has a retina composed mainly of cones and the rabbit which is nocturnal and has a largely rod retina. The stimulator was adjusted so that the same amount of light energy entered the eye in each case. The neutral wedge setting was calculated from the energy emitted by the lamp at the particular wavelength (Wein equation) and that transmitted by the colour filters (maker's data). The pigeon's eye was much more sensitive to the longer than to the shorter wavelengths, whereas the converse was true of the rabbit. These results are in agreement with those of Adrian (1946).

FIG. 7.

Response to different wavelengths. Upper pigeon, lower rabbit. Left to right. Calibration $200\mu\text{v}$, red, blue, white, equal energy.

III. *Dark adaptation curves*

In addition to recording and measuring the response to a light stimulus well above the threshold, it has frequently been useful to determine the minimum stimulus required to elicit a response. In this case a particularly quiet background was necessary but distortion of the shape of the wave was of little consequence. All

FIG. 8.

Dark adaptation curves of pigeon (upper) and rabbit (lower).

that was required was to know whether or not a response was obtained. Much of the background noise, especially high frequency valve noise, could be by-passed by means of a small condenser (say $0.002 \mu\text{F}$) connected between the input to the oscilloscope amplifier and earth. Examples of dark adaptation curves of a pigeon and a rabbit plotted on the same scale using this technique are given in Fig. 8. The animal was light adapted by exposure to an open 36-watt bulb six inches from the eye for five minutes. Dark adaptation time was measured from the instant this adapting light was switched off. At intervals the minimum stimulus required to elicit a barely discernible response was measured and the wedge reading then gave the relative sensitivity of the retina expressed in arbitrary log units.

The rabbit showed a very rapid adaptation so that after five minutes or so the sensitivity remained more or less constant. The overall rise in sensitivity was about a hundredfold after the first half minute. The adaptation of the pigeon was much slower, little change being noticed until after thirty minutes adaptation. There was, however, a definite adaptation represented by an increase in sensitivity of 1.5 log units.

VITAMIN A DEFICIENCY

Seven rabbits were made vitamin A-deficient and dark adaptation curves plotted at intervals using the threshold technique. The sensitivity of the retina gradually decreased as the deficiency progressed. Expressed in arbitrary logarithmic units, this decrease

FIG. 9.

Dark adaptation curves in vitamin A deficiency in the rabbit (see text).

was generally from about 3.6, the normal value, to about 1.5 in the deficient state. The rabbits used in these investigations all showed the same general effect although frequently the variation found between one animal and another was as great as the differences found in any one animal during the course of the vitamin A deficiency. A typical set of curves obtained from one animal is shown in Fig. 9. The "normal" curve was obtained from a weanling rabbit at the time the deficient diet was started. Curve 1 was plotted five months later when slit-lamp observation showed slight migration of the limbal pigment, but no corneal change. Curve 2 was plotted five months after Curve 1. By this time the corneal lesions had become well advanced and the animal rather weak. Immediately after this curve was taken the animal was given 10,000 I.U. vitamin A concentrate; the dose was repeated twice more and on the third day Curve 3 was obtained. The corneae were again quite clear. Treatment with vitamin A concentrate was repeated at weekly intervals for two months longer and Curve 4 obtained.

Vitamin A concentration (Davies, 1933) in the livers of three animals killed during deficiency had fallen to 31 I.U. per gram (normal value 400 I.U. per gram $\pm$ 95; 11 observations).

DISCUSSION

General.—The technique described here was chosen after consideration of the published alternatives and a discussion of the reasons governing the choice may be useful.

The electrical method of assessing visual function can only provide information about retinal activity. *It does not tell us what the animal actually sees.* For example, it has been shown (Granit, 1945) that the cat retina contains hue-discriminating elements, whereas the animal as a whole is almost certainly colour blind. The retina is only one link in the visual system so that training experiments alone can provide information as to what the animal sees, since "seeing" involves not only the production of impulses in the optic nerve, but also an interpretation of these impulses by the animal's higher centres. Such training methods are, however, extremely tedious and time-consuming and, unless in expert hands, of little value. Where one's attention is confined to the physiology of the retina, electroretinography is the method of choice. It is rapid, a number of observations may be carried out on the same animal, the personal factor is practically eliminated and the method does give a truly objective indication of retinal activity independent of psychic or other sensory mechanisms.

Amplifiers.—A direct coupled amplifier is necessary if prolonged

stimuli are to be used. Several circuits have been published which are claimed to give good results (*e.g.*, Leksell, 1939; Matthews, 1938; Goodwin, 1941). If the recording device is capable of being operated by a push-pull circuit this type is recommended because it seems to be less susceptible to base-line drift than is the asymmetrical type. A double-beam oscilloscope needs a single-ended input so that in this case it would be better to use a compressor stage (Matthews, 1938). Capacitance in the batteries used for coupling may be troublesome if rapid changes are to be recorded, as in the case of nerve action potentials, but this can be reduced somewhat by the use of small batteries of the "deaf aid" type. These batteries are quite suitable in these positions since no current is taken from them and their life in the amplifier is almost equal to their shelf-life.

For general work, however, the resistance capacity coupled amplifier is much easier to handle and is the type I use. There are many designs and circuits published (Parnum, 1945; Parr and Walter, 1943), some of which employ push-pull operation and others single-ended circuits. My experience is that the amplifier should be kept as simple as possible—sliding contact gain controls and variable coupling and by-pass condensers should be avoided as far as possible because sooner or later the contacts give trouble. Each section should be built as a separate screened unit connected by short screened leads to avoid feed-back, which would cause the amplifier to oscillate, and to avoid interference pick-up.

Interference may arise within the instrument itself, though with a carefully constructed amplifier properly screened little trouble should arise from this source. A source of interference which arose in the early part of this work and proved difficult to locate at the time was an increase in internal resistance of the H.T. batteries in the pre-amplifier stages. Although the voltage had not decreased appreciably when tested with a meter, considerable noise appeared when the calibrator was not in use. For some time the noise was attributed to the electrodes, but when the batteries were replaced clear records were again obtained.

Light stimulator.—Both Karpe (1945) and Adrian (1946) used a large illuminated opal screen as a light stimulus rather than a focused light. This arrangement illuminates a larger area of the retina more evenly and does not need critical adjustment, but I prefer the focused light because small battery operated bulbs can be used and this eliminates the risk of introducing mains hum from lamp cables. The apparatus is also more compact and can easily be adusted to suit the position of the animal.

Electrodes.—Karpe (1945, 1948) uses contact lenses containing the corneal electrode for his clinical work. I have used lens shells

made from perspex on rabbits. These had the silver wire sealed through the perspex to prevent drainage of saline. One advantage of this electrode over the wick type is that small movements of the animal do not displace it. But in general wick electrodes have been used because so many different sizes of contact glass would be needed to fit different animals. If records are being taken from one animal species only and all animals used are about the same size, contact glass electrodes may be preferable, but for general work wick electrodes of the type described are satisfactory.

Recording device. — Many workers have used ink-writers for recording instead of cathode ray tubes. Ink-writers have both advantages and disadvantages. A permanent record is produced at once; the cost of recording paper is much less than film; the instruments occupy very little space and multiple instruments are available which will produce several records simultaneously on the same paper. The disadvantages are that the movements of the pen must inevitably possess considerable inertia, and even with modern instruments accurate reproduction of frequencies greater than 100 cs./second is not possible. If rapid changes are to be measured, for example the initial rise of the b-wave of the electroretinogram, there is always some over-swing of the pen and arc distortion is also always present. Cathode ray tubes, on the other hand, are, for our purpose without inertia and accurate reproduction of wave forms can be expected from D.C. to very high frequencies. The chief drawback to the cathode ray tube is that it is difficult to observe the wave form and to produce a permanent record simultaneously. This may be achieved by using a single stroke time base coupled to the shutter of the stimulator and exposing one frame at a time with a long persistence tube, but I find it more convenient to dispense with the time base and use continuously moving film with a short persistence tube, even though the exact shape of the wave cannot be seen clearly until the film is developed.

RESULTS

The electroretinograms recorded from normal guinea pigs, rabbits and pigeons confirm the results of other workers. Adrian (1946), using a different machine, showed that the pigeon was far more sensitive to red light than to blue and that the reverse held for the rabbit. My results have confirmed this. It is difficult to reconcile this large response to red light found in the pigeon with Granit's (1947) view that there is a particularly close association between the b-wave and rod activity.

The difference in sensitivity between the rabbit and the pigeon shown by the dark adaptation curves is difficult to explain. From the behaviour of the creatures in their natural environment one

would expect the rabbit's eye to be much more sensitive to dim light. From the response shown by the electroretinogram, however, the reverse would appear to be the case. It seems unlikely that the retina of the rabbit is, in fact, less sensitive than that of the pigeon, it is more probable that the difference lies in the pick-up of potential by the electrodes. The anatomy of the pigeon's is such that a more favourable positioning of the electrodes is possible than with the rabbit. There may also be differences in the conductivity of the tissues and some shunting may take place in the rabbit's eye.

Charpentier (1936) determined the effect of vitamin A deficiency in the rat on its electroretinogram. He measured the curve of dark adaptation in normal and deficient animals by using a constant light stimulus and plotting the height of the b-wave at intervals after a preliminary light adaptation. His results were obtained with a small number of animals but showed that there was probably some reduction in size of b-wave in vitamin A deficiency. His animals did not survive the experiment, so that repeated electroretinograms from the same animal in different stages of the deficiency were unobtainable.

By careful choice of the intensity of the stimulating and adapting light it has been shown (Karpe and Tansley, 1948) that increase in size of the b-wave does correspond to increase in subjective sensitivity during dark adaptation in man.

Another method of plotting the dark adaptation curves of normal and vitamin A deficient rabbits has been used here. After the preliminary light adaptation the minimum stimulus needed to elicit a barely discernible response was measured over a period of 30-60 minutes. This method seems to me theoretically more sound than that used by Charpentier. It is the ultimate limit of sensitivity that we are trying to measure in assessing the dark adaptation curve. By using minimal stimuli there is less risk of light adapting the retina while making the observation and the range of sensitivity is greater.

Dark adaptation curves of rabbits plotted by this method follow the expected course. It is interesting to note that in the vitamin A-deficient rabbits some lowering of retinal sensitivity, judged by the electroretinogram, took place before changes in the eye could be seen with the slit lamp, although the maximum recorded depression of the electroretinogram did not occur until the corneal lesions were well advanced. This is in accordance with human experience where change in dark adaptation occurs well before conjunctival and corneal change (Kitasima, 1939, Yudkin, 1945).

A single dose of vitamin A concentrate containing about 10,000 I.U. was sufficient in some cases to restore the sensitivity to its

original level. Where a doe was fed a deficient diet when the litter was born and the young were continued on the same diet, the dark adaptation curves obtained after the young had been cured by giving vitamin A had to be taken as "normal." Variations from one animal to another made it impossible to decide whether or not a particular curve was indicative of night-blindness without reference to a record taken from the same animal when normal. This variation from animal to animal emphasises the importance of a technique which makes it possible to use a single animal a number of times.

SUMMARY

1. An apparatus and technique for routine electroretinography of experimental animals are described.
2. Examples of animal electroretinograms using this technique are presented.
3. Typical dark adaptation curves of normal rabbit and pigeon obtained by a threshold method are shown.
4. Course of dark adaptation curves plotted during vitamin A deficiency and after cure with vitamin A concentrate are described.

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