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Pupillary Activity Measured by Reflected Infra-Red Light

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CASSADY, J. M., G. R. FARLEY, N. M. WEINBERGER AND L. M. KITZES. *Pupillary activity measured by reflected infra-red light*. PHYSIOL. BEHAV. **28**(5) 851–854, 1982.—A simple, reliable and inexpensive device for continuously monitoring pupillary changes is described. It is an improved version of a device first suggested by L. Stark. Infra-red light is transmitted to the iris and reflected back onto the receiver. Consequently, the device is effective only if the eye is immobile. Pupillometer ouput was related linearly to measurements made directly from motion pictures of pupil mobility. Experimental data are presented which demonstrate the stable baseline and minimal noise levels of the pupillometer in a conditioning experiment using paralyzed cats.

Pupillometer Conditioned response Pupillary behavior

MEASUREMENT of pupil size has been found useful in variety of clinical and experimental situations. Static pupillary diameter has been measured by direct observation via an operating microscope [5] and by photography [8, 11, 19]. Pupillary motility has been measured by cinematography [10], by reflected light from the iris to a photocell outside of the eye [11, 15, 21], by direct light through the lens to a photocell within the eye [17] and by using a laser device [4]. In the latter case, the iris is scanned 50 times per second, producing a continuous representation of pupil diameter. Human pupillary size has been monitored by television [6, 7, 20] and most recently this technique has been adopted for use with animals [12]. The devices used by these researchers are somewhat complex and expensive. We sought a simple, reliable and inexpensive method to continuously monitor pupillary behavior in paralyzed cats during the acquisition or performance of a Pavlovian conditioned response.

The pupillometer described here developed from the work of Lawrence Stark [14, 15, 16, 21]. It measures the area of the pupil by monitoring the amount of projected infra-red light that is reflected from the iris to a photovoltaic sensor. The DC voltage output of a circular photovoltaic sensor is proportional to the size of the iris and is, therefore, inversely proportional to the area of the pupil. Although we have not systematically studied the influence of eye color, the amount of light reflected from the iris should also be proportional to iris pigmentation. This technique may therefore be inappropriate for lightly pigmented irises. The pupillometer described in this paper is useful in preparations where one dimension of the pupil (e.g., height) is fixed and the eye is motionless with respect to the recording device. Since the measurement of pupil size depends on the amount of light reflected from the iris, any deviation of the eye relative to the pupillometer would alter both the baseline and sensitivity of the analysis. We have used the pupillometer exclusively in experiments with paralyzed cats and found it to be reliable and easily positioned in front of the eye from subject to subject. It is small and inexpensive to fabricate.

PHYSICAL DESIGN

The pupillometer has three components: (1) shell, (2) infra-red source and (3) infra-red detector. The shell is a 60 mm long piece of 36 mm poly-vinyl chloride (PVC) pipe, commonly used for home sprinkler systems (Fig. 1A). Three infra-red transmitting bulbs (wavelength at peak emmission=9400 A; wavelength range=8600–9600 A; Archer TIL-32) are placed in holes drilled in the wall at one end of the cylinder. The bulbs protrude slightly from the mouth of the cylinder and slant inwardly, orientated about 30 degrees from the long axis of the cylinder, and defining an equilateral triangle with respect to the circumference of the cylinder. A circular, insulated silicon photovoltaic cell (Silicon Sensors Inc., SSG-30), 38 mm in diameter, is secured to the back of the cylinder. The response time of this photovoltaic cell is less than 20 microseconds and it can operate in temperatures from −65°C to 175°C. The use of infra-red transmitters and receivers permits the monitoring of pupil area in a completely darkened room with minimal light feedback to the retina as the pupil expands or contracts. If this is not a critical concern of the application, super-bright LEDs (red, CM4-282B) or comparable bulbs may be substituted as the light source. The device is light (75 g) and compact. A rod screwed into the cylinder wall supports the device and is used to position the pupillometer in front of the eye.

AMPLIFICATION

The circuit for amplifying the DC signal generated by the

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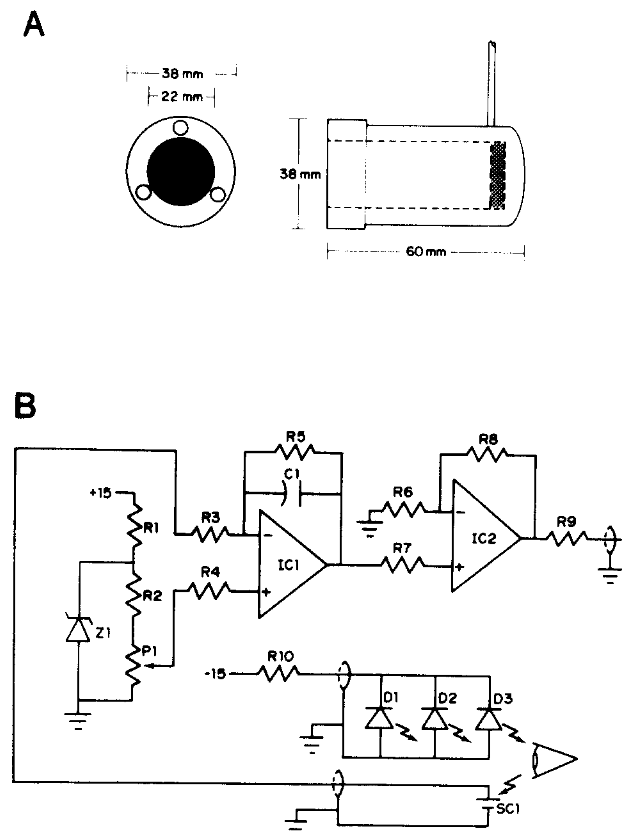

FIG. 1. A. Diagram of pupillometer. Hatched area is photo cell. Vertical rod is support for placement of pupillometer. B. Circuit diagram of the pupillometer. Parts list: R1=100 ohms, 1 watt resistor; R2=47K ohms resistor; R3, R6=1K ohms, 1% resistor; R4, R7=910 ohms, 1% resistor; R5=100K ohms, 1% resistor; R8=9.1K ohms, 1% resistor; R9=47 ohms resistor; R10=150 ohms, 5 watt resistor; C1=100 pF capacitor; P1=1K ohms, 10 turn potentiometer; Z1=Zener diode, 6.2 volt (1N4735); D1, D2, D3=Infra-red/Superbright LEDs; SC1=solar cell (SSG-30LC); IC1=operational amplifer (μA725); IC2=operational amplifier (μA741). The μA725 must be frequency compensated according to the manufacturer's specifications: a 47 ohm resistor in series with a 0.01 μF capacitor connected between pin 5 and ground.

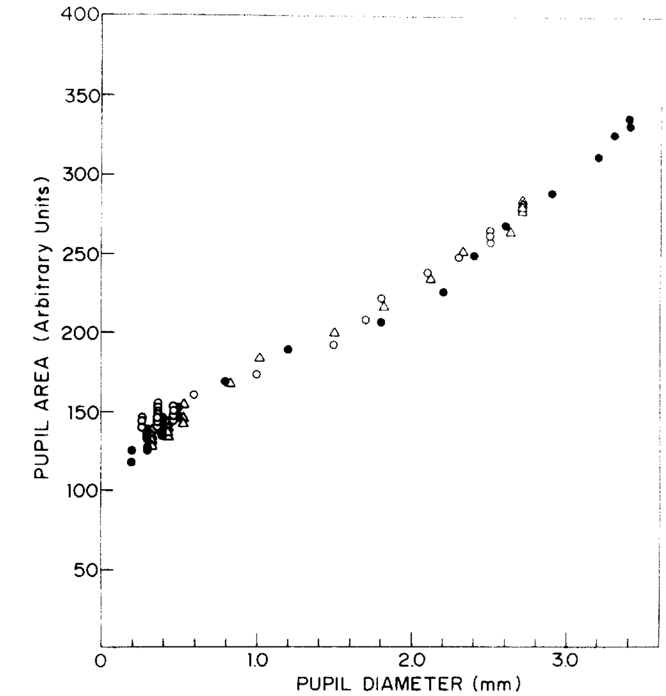

FIG. 2. Validation of pupillometer measurements. Pupil area as determined by the pupillometer (ordinate) is compared with pupil diameter measured from photographic images (abscissa). The three symbols (closed circles, open circles and open triangles) represent measurements from three mild shock trials (30 scores/trial).

photovoltaic cells is shown is Fig. 1B. The major component is a Fairchild μA725 operational amplifier featuring low offset voltage and low current drift with temperature and time, low noise, high power supply rejection, high common mode rejection, and high gain. Light sources D1, D2, and D3 generate light which is directed at the eye and is reflected from the iris back to the photovoltaic cell (SC1). The photovoltaic cell generates a voltage proportional to the amount of reflected light, which is inversely proportional to the area of the pupil. This voltage is led to IC1 which inverts, amplifies (100 gain), and serves as a low pass filter (3 dB cutoff frequency=16 kHz). The output of IC1 is then led to IC2 which further amplifies the signal by a factor of 10 and provides circuit output. Potentiometer P1 provides a DC offset voltage which allows the DC signal of the pupillometer to be centered in a voltage range appropriate for the recording equipment being used. The portion of the circuit involving Z1, R1, and R2 provides a stable reference voltage for this function. The pupillometer has low noise and almost no drift. In our laboratory the output of this circuit is led to either a low-level DC pre-amplifier or directly to the DC driver amplifier of a polygraph (Grass Model 7) where it is recorded on chart paper. Maximal responses of the pupillometer may be obtained by monitoring the signal with the polygraph while adjusting the distance between the pupillometer and the eye. Optimal separation is approximately 1–2 centimeter.

VALIDATION

In order to check the response characteristics of the pupillometer, the output of the circuit was compared to motion pictures (32 frames/sec) of the contralateral pupil on a frame by frame basis. The subject was a paralyzed cat which was intubated and artificially ventilated (see [13] for a more detailed description of these procedures). The pupillometer was placed in front of the right eye of the cat and a light film of ophthalmic ointment (Terramycin) spread on the cornea to prevent it from drying. A contact lens (courtesy of Dr. Steven Downs, Coast Contact Lens Co., Huntington Beach, CA) with a 1 mm scale was fitted to the left eye of the cat. A movie camera with a close-up lens was used to photograph the left eye of the cat. Mild paw-shock was used to evoke moderate pupillary responses. Onset of shock was indicated on the film by the brief (100 msec) illumination of a small light located lateral and posterior to the left eye. Thirty frames were examined on each of three shock trials. Each frame was projected on a screen with a calibrated scale matched to projected markings from the contact lens. These markings were further subdivided into 10 equal intervals which gave a resolution of 0.1 mm. The results are seen in Fig. 2: pupil area as determined by the pupillometer focused on the right eye is compared with pupil diameter determined by measurements from the film of the left eye. Consensual pupillary responses were used because the camera and pupillometer could not be focused upon the same eye. The resulting function is linear (r=.99; N=90) from about 0.2 to 3.5 mm of pupil diameter.

EXPERIMENTAL DATA

Experimental details can be found in the following studies which used this pupillometer: [1, 2, 3, 9, 18]. In general, cats were paralyzed with gallamine triethiodide (10 mg/kg/hr), intubated, artificially ventilated and presented a series of trials consisting of auditory white noise bursts (1 second duration) and mild electrocutaneous paw shocks (0.5 second duration). Calibration of the pupillometer was obtained by having a maximal pupillary response produced by paw shock result in a maximal excursion of the polygraph pen. The minimal shock level which produced a robust pupillary response was determined in each experiment. The white noise burst was 6 dB above ambient noise level, determined at the ear canal. Measurements of pupillary activity (dilation up) for selected single trials from one subject during various phases of training are presented in Fig. 3. The pupil response to white noise followed by paw shock (i.e., Pavlovian conditioning paradigm) is shown in Figs. 3C–3F, where an increase in pupil size during the white noise indicates that conditioning of the response has occurred. The diminished pupil response to inconsequential white noise signals before conditioning is shown in Figs. 3A–3B and the diminished pupil response to white noise after conditioning is shown in Figs. 3G–3H. The pupil response to paw shock alone is shown before (Fig. 3J) and after conditioning (Fig. 3K) and demonstrates no change in the response attributable to the procedures. The stable baseline and minimal noise levels shown in these figures are typical of the pupillometer circuit.

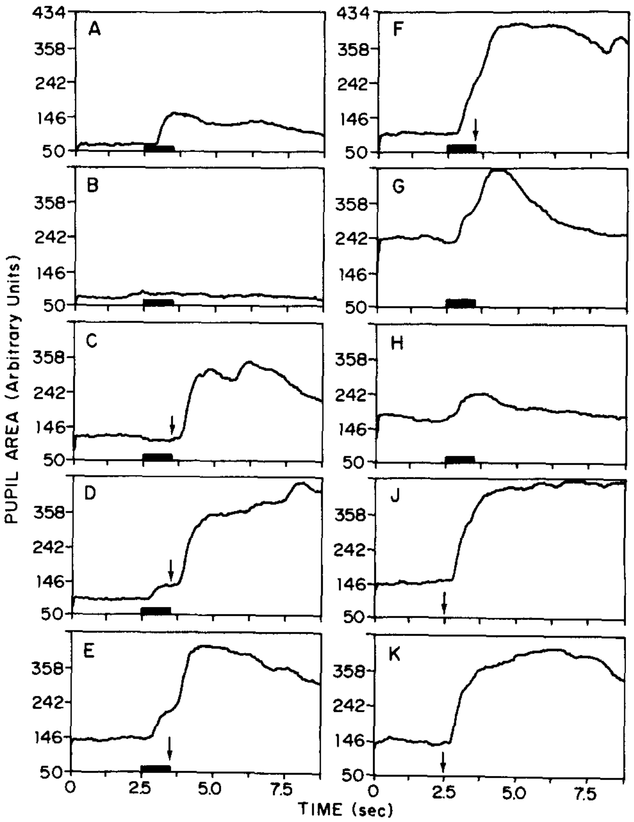

FIG. 3. Selected single trial pupillary dilation responses for a single cat under several training conditions. A rise in the curve indicates increased pupillary dilation. The black bar indicates white noise presentation; the arrow indicates paw shock presentation. (A) and (B) decreased response to white noise alone before conditioning, (C) to (F) increased response to white noise as a function of paired white noise and shock (conditioning) trials, (G) and (H) decreased response to white noise alone after conditioning, (J) response to shock alone before conditioning, and (K) response to shock alone after conditioning.

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REFERENCES

1. Ashe, J. H. and C. L. Cooper. Multifiber efferent activity in postganglionic sympathetic and parasympathetic nerves related to the latency of spontaneous and evoked pupillary dilation. *Expl Neurol.* **59:** 413–434, 1978.
2. Ashe, J. H., C. L. Cooper and N. M. Weinberger. Role of the parasympathetic pupillomotor system in classically conditioned pupillary dilation of the cat. *Behav. Biol.* **23:** 1–13, 1978.
3. Ashe, J. H., C. L. Cooper and N. M. Weinberger. Mesencephalic multiple unit activity during acquisition of conditioned pupillary dilation. *Brain Res. Bull.* **3:** 143–154, 1978.
4. Borgdorff, P. and C. F. van Mill. A low cost laser pupillometer. *IEEE Trans. biomed. Eng.* **BME-26:** 100–103, 1979.
5. Brodie, D. A. A comparison of anticholinergic drugs on gastric secretion, gastric emptying, and pupil diameter in the rat. *Gastroenterology* **50:** 45–50, 1966.
6. Green, D. G. and F. Maaseidvaag. Closed-circuit television pupillometer. *J. opt. Soc. Am.* **57:** 830–833, 1967.
7. Ishikawa, S., M. Naito and K. Inaba. A new video pupillography. *Opthalmologica* **160:** 248–259, 1970.
8. Jasinski, D. R. and W. R. Martin. Evaluation of a new photographic method for assessing pupil diameters. *Clin. Pharmac. Ther.* **8:** 271–272, 1967.
9. Kitzes, L. M., G. R. Farley and A. Starr. Modulation of auditory cortex during the performance of a conditioned response. *Expl Neurol.* **62:** 678–697, 1978.
10. Lowenstein, O. and E. D. Friedman. Pupillographic studies: I. Present state of pupillography; its method and diagnostic significance. *Archs Opthal., Chicago* **27:** 969–993, 1942.
11. Lowenstein, O. and I. E. Loewenfeld. Electronic pupillography. *A. M. A. Archs Ophthal.* **59:** 352–363, 1958.

12. Murray, R. B. and M. H. Loughnane. Infrared video pupillometry: A method used to measure the pupillary effects of drugs in small laboratory animals in real time. *J. Neurosci. Meth.* **3:** 365–375, 1981.
13. Oleson, T. D., I. S. Westenberg and N. M. Weinberger. Characteristics of the pupillary dilation response during Pavlovian conditioning in paralyzed cats. *Behav. Biol.* **7:** 829–840, 1972.
14. Stark, L. Stability, oscillations and noise in the human pupil servomechanism. *Proc. Inst. Radio Engrs.* **47:** 1925–1939, 1959.
15. Stark, L. and P. M. Sherman. A servoanalytic study of consensual pupil reflex to light. *J. Neurophysiol.* **20:** 17–26, 1957.
16. Stark, L., G. Vossius, and L. R. Young. Predictive control of eye tracking movements. *IEEE Trans. human Factors Electron.* **HFE-3:** 52–57, 1961.
17. Ueki, S. and S. Oono. A new method for continuous recording of the pupil size by means of a phototransistor. *Archs int. Pharmacodyn.* **142:** 339–348, 1963.
18. Weinberger, N. M. Neurophysiological studies of learning in association with the pupillary dilation conditioned reflexes. In: *Neural Mechanisms of Goal Directed Behavior and Learning*, edited by R. F. Thompson, L. H. Hicks and V. B. Shvyrkov. New York: Academic Press, 1980, pp. 241–261.
19. Weisse, I., H. Ganz and F. Knappen. Pupillometric studies on the beagle dog. *Naunyn-Schmiedebergs' Arch. Pharmac.* **291:** 385–394, 1975.
20. Young, L. R. and D. Sheena. Survey of eye movement recording methods. *Behav. Res. Meth. Instrum.* **7:** 397–429, 1975.
21. Zuber, B. L., and D. Miller. A simple, inexpensive electronic pupillometer. *Vision Res.* **5:** 695–696, 1965.