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Application of infrared detection in the recording of eyelid movements in rabbits

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Abstract. Classical conditioning of the eyelid reflex has been used for a long time to study associative learning in animals and humans. A new experimental procedure for rabbit's eyelid conditioning was constructed and described. A phonopneumatic stimulator generated conditioned and unconditioned stimuli and a photoelectric transducer acting in close infrared converted movements of rabbits' eyelid to electric signals. An example of acquisition and extinction training is illustrated. This method of eyelid movement monitoring is noninvasive. It may be useful for chronic studies of learning processes in rabbits when used with headpieces for microdialysis probes, electrodes or cannulas which could be implanted into brain structures.

Key words: eyelid conditioning, photoelectric transducer, phonopneumatic stimulator, rabbit

Classical or Pavlovian conditioning of the eyelid or nictitating membrane (NM) has long been used to study associative learning. The method is perhaps the most widely used paradigm in both humans and animals. A neutral tone as conditioned stimulus (CS) is paired with an unconditioned stimulus (US) consisting of a paraorbital air puff (McCormick et al. 1982) or electrotactile (Moon et al. 1994) stimulus. Initially, only the US elicits NM movement and eyelid closure – the unconditioned response (UR). The NM and eyelid behave essentially identically during conditioning (McCormick et al. 1982). However, when the CS and US are then presented repeatedly to the rabbit in a specified order and temporal spacing, a response similar to the UR develops to the CS, that is a conditioned response (CR). Classical conditioning of the rabbit NM response has been developed into a model system for studying action of drug, e.g. anaesthetics (Moon et al. 1994), in the learning process.

There are several methods of monitoring NM/eyelid movements. The first and simplest method, applies potentiometric devices transducing mechanical changes into an electric signal, modulated and then recorded for further analysis. A resistive (Moon et al. 1994) or mechanical motion transducer (Gormezano et al. 1962) may be used. The potentiometer is directly coupled to a small loop of nylon suture in the NM of the rabbit's eye. Lid movements may also be detected with the aid of search coils in a magnetic field technique (Gruart et al. 1997, Trigo et al. 1999). With a coil implanted in the upper eyelid, lid movements through the magnetic field produced a signal proportional to upper eyelid position. Conventional electromyographic (EMG) electrodes recording the electrical activity of the *orbicularis oculi muscle* are also used (Gruart et al. 1997, Trigo et.al. 1999). In humans the blink reflex can be recorded with the aid of surface EMG electrode placed on the outer edge of the lower eyelid (Silverstein and Graham 1978, Jääskeläinen 1995). In 1989 Flaten and co-workers described a microcomputer – based system for elicitation and recording of the eyeblink reflex in humans. Eyeblinks were detected by changes in the reflection of light from the eye or eyelid. Light was emitted by a diode

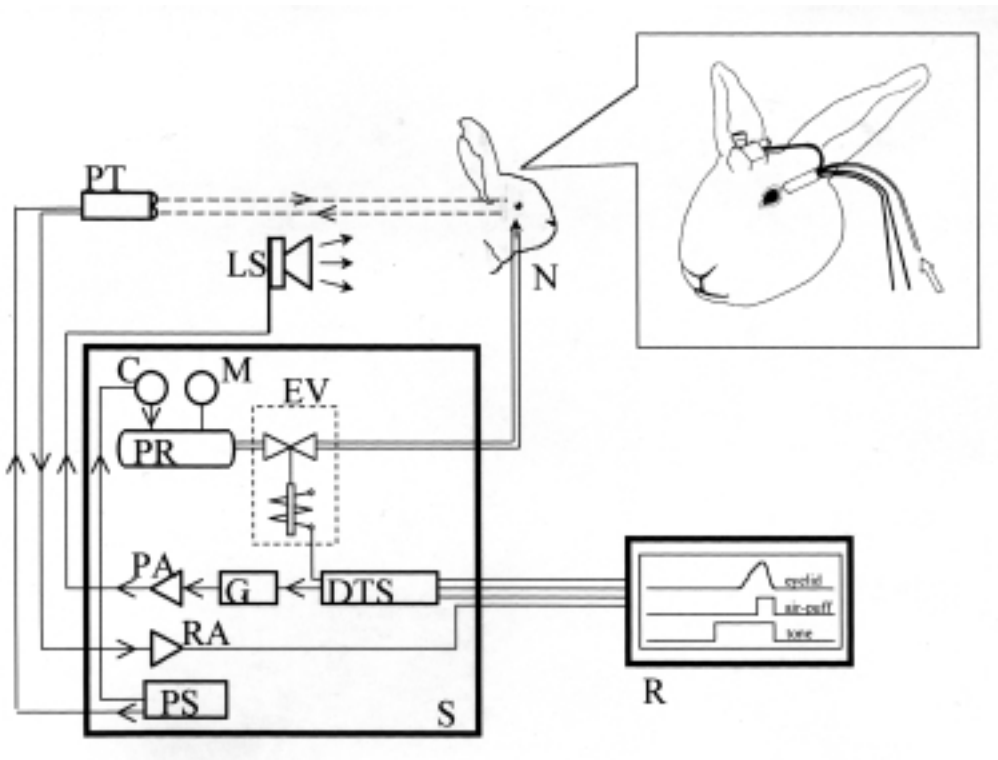

Fig.1. A scheme of the electronical and mechanical details of the experimental equipment: PT, photoelectric transducer; LS, loud-speaker; N, air puff nozzle; PR, pressure reservoir; C, compressor; M, manometer; EV, electromagnetic valve; DTS, device for time synchronization; G, generator; PA, power amplifier; PS, power supply; RA, response amplifier; S, phonopneumatic stimulator; R, recorder. Right upper corner: Design of the rabbit's head with implanted plexiglass headpiece. Air puff nozzle and photoelectric transducer fitted by a holder to the headpiece is visible.

(LED) and reflected light was detected by a photocell. As a result of the opto-electronic sensor application, the method is completely noninvasive, not requiring a nylon loop sutured in the nictitating membrane nor implantation of electrodes. A similar hardware interface system for eliciting, training and quantifying eyeblink or nictitating membrane responses for humans and animals was described some years later (Thompson et al. 1994). The opto-electronic sensor, based on an infrared emitting diode (LED), was used for transduction of the behavioral response. Infrared has been used in ophtalmology since 1982 when an infrared video instrument was developed to observe pupils in darkness (Verdick and Thompson 1991).

The present paper describes a similar method, with a photoelectric transducer and an additional special phonopneumatic stimulator (S) (Fig.1) designed for acquisition and extinction of eyelid reflex and recording. Figure 1 shows all details of the experimental apparatus. The system generates periodic air puffs and tones and controlles the recorder. The phonopneumatic stimulator is a metallic box 44 cm long, 24.5 cm wide and 13 cm high, built from parts mentioned in Figure 1. The recorder was geared 0.7 s before each trial and stopped 0.7 s after the ending of the trial. We used a modified three-channel electrocardiograph as the recorder (Fig.1). A photoelectric transducer (PT) detecting in close infrared converted the eyelid movements into electric signals. It contains an emitter of infrared light impulses, a receiver of reflected light, an amplifier and a synchronic detector, attached to a controlling generator. The infrared light emitter, supplied with the controlling generator, consists of a light emitting diode (LED) CQWP13 (CEMI, Poland). The receiver of reflected light is built from a phototransistor BPRP25 (CEMI, Poland). The output signal of the detector does not contain a background lightening component as the result of the use of impulse modulation of the lightening as well as synchronic detection of reflected light. The amplitude of the response is proportional to the degree of closure of the eyelid. When the eye is completely closed, maximum light is reflected and the maximal amplitude of the response is observed. Figure 2 (lower panel) shows the diminishing amplitude of eyelid response during extinction.

Adult male and female New Zealand white rabbits were housed in single cages in a temperature-controlled and light-regulated (lights on 06.00 h and off 20.00 h) room for at least 30 days prior to any surgical intervention. Rabbits were stereotaxically implanted with a plexiglass headpiece with guide cannulae for microdialysis study using a stereotaxic frame according to Sawyer (Sawyer et al. 1954). All procedures were carried out in accordance with NIH guidelines for the care and use of the laboratory animals. In this paper we describe only the conditioning procedure (acquisition and extinction) when the headpiece was used to fix the PT and the air puff nozzle on the rabbit's head. The surgical procedure for implantation was described in detail in Traczyk et al. (1997). In brief: a deep surgical anaesthesia was induced by subcutaneous injections of atropine sulphate (1.0 mg per animal) followed by slow i.v. injection of pentobarbital (Vetbutal, Biowet - Pulawy, 30 mg/kg). After reaching deep anaesthesia the animal was mounted on the stereotaxic frame. Five holes were made in the skull bones using a dental drill according to the rabbit stereotaxic atlas (Sawyer et al. 1954). Four holes were for the guide cannulae leading to the brain structures and the fifth hole around the Bregma was for the cannula leading to the 3rd cerebral ventricle. The tip of the 3rd cerebral ventricle cannula was used as a reference point. It was lowered stereotaxically with the whole headpiece

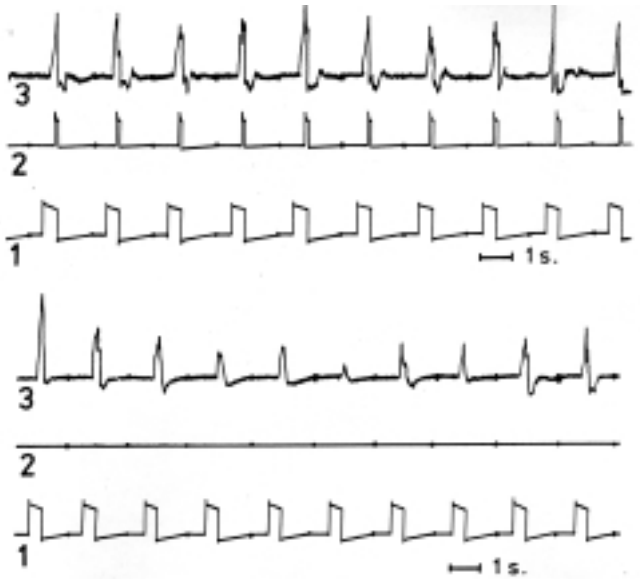

Fig. 2. An example of peristimulus eyelid responses from several trials of rabbit's eyelid conditioning during acquisition (upper panel) and extinction (lower panel). 1, tone (CS); 2, air puff (US); 3, eyelid responses (CRs). During the extinction training the amplitude of response was initially unstable, then diminished and at the end disappeared.

10 mm deep from the surface of the dura mater exactly in the middle between the cerebral hemispheres. Four stainless steel screws were screwed into the skull bones near the headpiece. The screws and the lower part of the headpiece were covered with dental cement (Duracril, Spofa). After hardening of the dental cement a few sutures were put in the skin. Each rabbit received intramuscular injections of 100,000 IU of benzylpenicillin potassium (Polfa-Tarchomin) and 0.5 g of streptomycin (Polfa-Tarchomin) daily for five consecutive days after the surgery. One month after the implantation surgery rabbits were accustomed to a special experimental cage half an hour daily, one week before the onset of training. The cage restricted body rotation but allowed free access to food and water. A rabbit in the cage was placed in a sound-attenuated large box, illuminated overhead (25 W bulb) and ventillated. A holder for the air puff nozzle and the PT were mounted with the screw on the rabbit's headpiece about 2 cm from the cornea (Fig. 1). Standard procedures of paired training for classical conditioning of the rabbit nictitating membrane and eyelid response were used (Berger and Thompson 1978). The unconditioned stimulus (US) was always preceded by the conditioned stimulus (CS) thus allowing conditioned responses to develop. The US was a 100-ms corneal air puff exerting a pressure of 0.2 kg/cm^2 measured at the steel end of the delivery plastic tube, tip diameter of 1 mm, located about 2 cm from the cornea together with photoelectric transducer. The CS was a 70 dB, 450 ms, 1 kHz tone that began 350 ms prior to the air puff onset and finished simultaneously with it. The CS and the US co-terminated, resulting in a delay conditioning paradigm. The tones were delivered through a loudspeaker mounted in the wall of the large box ipsilaterally with air puffs. The intertrial interval averaged 22 s. Each daily session of training consisted of 120 trials. During the extinction sessions only 120 conditioned stimuli (tones) were applied. Responses in trials with paired presentation of stimuli (CS + US) during acquisition training initiated in the time interval between CS onset and US onset, with amplitude exceeding 1 mm, were classified as conditioned responses (CRs). Responses elicited by CS alone during extinction were classified as CRs if they initiated during the CS and their amplitudes exceeded 1 mm. Conditioned responses were calculated as percentage of all 120 conditioned stimuli applied during one day session.

About seventy rabbits were subjected to this conditioning procedure in experiments testing the effects of vasopressin, oxytocin and tyroliberin on the learning processes. The exact methods and data processing are provided elsewhere (Orłowska-Majdak et al., Kołodziejski et al., in preparation). All animals adapted easily to the conditions of the experimental equipment. One daily session of the acquisition or extinction procedure lasted about 45 min. Rabbits attained a level of greater than 80% CRs in one acquisition session but they were over-trained in these experiments. Figure 2 illustrates the record of responses on the 8th day of repeated acquisition (upper panel) and on the 1st day of extinction (lower panel).

The complete system offers very good control of stimulus presentation and recording of conditioned responses. As a result of LED application, instead of a potentiometer or electrodes being implanted into the eyelid or *orbicularis oculi* muscle, no physical attachments to the face or eyelid of the rabbit was required. The phonopneumatic stimulator of our own construction presented assigned stimuli consecutively and accurately so the data were artefact-free and usable during all days of initial training and the following days of pharmacological testing. Conditioned responses were counted from records without any additional measurement equipment so the counting procedure was not as quick as the microcomputer hardware and software procedure described by Thompson (Thompson et al. 1994) and Akase (Akase et al. 1994) but it was thorough and precise. Moreover the photoelectric transducer is much smaller than the opto-electronic sensor applied by Thompson. It may be positioned at any distance from the eye, while the opto-electronic sensor has to be positioned exactly 4 mm from the corneal surface (Thompson et al. 1994). The proximity of the relatively large device must cause some discomfort which is a matter of great importance in conditioning experiments. Light emitted by the opto-electronic sensor positioned 4 mm from the corneal surface illuminates only a small focal point. Therefore, the device provides a maximal voltage output when the eyelid crosses the focal point, and a null voltage when a transparent surface occupies the focal point. So positioned the sensor does not "see" a partially covered corneal surface but detects only almost totally closed eyes. Our transducer positioned 20 mm from the corneal surface illuminates the complete corneal surface by a divergent beam of light. It can "see" a partially covered surface and provides voltage output proportional to the degree of eye closure.

The method reported here may be useful for chronic pharmacological studies of the effects of drugs on acqui-

sition or extinction and in other physiological studies of learning processes.

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Akase E., Thompson L.T., Disterhoft J.F. (1994) A system for quantitative analysis of associative learning. Part 2. Real-time software for MS-DOS microcomputers. J. Neurosci. Methods 54: 119-130.

Berger T.W., Thompson R.F. (1978) Neuronal plasticity in the limbic system during classical conditioning of the rabbit nictitating membrane response. I. The hippocampus. Brain Res. 145: 323-346.

Flaten M.A., Vaksdal A., Hugdahl K. (1989) An IBM-PC and Commodore 64 microcomputer-based system for elicitation and recording of eyeblink reflexes. Biol. Psychol. 29: 291-298.

Gruart A., Pastor A.M., Armengol J.A., Delgado-Garcia J.M. (1997) Involvement of cerebral cortex and nuclei in the genesis and control of unconditioned and conditioned eyelid motor responses. In: Progress in brain research (Eds. C.I.de Zecuw, P.Strata and J.Voogd). Elsevier Science B.V. 114: 511-528.

Jääskeläinen S.K. (1995) Electrophysiological study of blink reflex in humans: differences in mental and supraorbital nerves. Acta Physiol. Scand. 154: 143-150.

McCormick D.A., Clark G.A., Lavond D.G., Thompson R.F. (1982) Initial localization of the memory trace for a basic form of learning. Proc. Natl. Acad. Sci. USA 79: 2731-2735.

Moon Y., Ghoneim M.M., Gormezano I. (1994) Nitrous oxide: Sensory, motor, associative and behavioral tolerance effects in classical conditioning of the rabbit nictitating membrane response. Pharmacol. Biochem. Behav. 47: 3, 523-529.

Sawyer C.H., Everett J.W., Green J.D. (1954) The rabbit diencephalon in stereotaxic coordinates. J. Comp. Neurol. 101: 801-824.

Silverstein L.D., Graham F.K. (1978) Eyeblink EMG: a miniature eyelid electrode for recording from *orbicularis oculi*. Psychophysiology 15: 4, 377-379.

Thompson L.T., Moyer J.R., Jr., Akase E., Disterhoft J.F. (1994) A system for quantitative analysis of associative learning. Part 1. Hardware interfaces with cross-species applications. J. Neurosci. Methods 54: 109-117.

Traczyk W.Z., Orłowska-Majdak M., Walczewska A., Dziedzic B. (1997) Microdialysis of subcortical structures in conscious chronic rabbits. In: Drug transport across the blood – brain barrier: *in vitro* and *in vivo* techniques (Eds. A.Bert G.de Boer and W.Sutanto). Harwood Academic Publishers, Singapore, p.173-184.

Trigo J.A., Gruart A., Delgado-Garcia J.M. (1999) Role of proprioception in the control of lid position during reflex and conditioned blink responses in the alert behaving cat. Neuroscience 90: 4, 1515-1528.

Verdick R.E., Thompson H.S. (1991) Infrared videography of the eyes. J. Ophtalmic Photog. 13: 19-21.

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