AN APPARATUS AND PROCEDURE FOR CONDITIONING THE EYE-BLINK REFLEX IN THE SQUIRREL MONKEY¹

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Although classical conditioning of the eye-blink reflex has been studied extensively with human subjects, there appears to have been only one programmatic attempt to investigate the process in infrahuman primates (Hilgard and Marquis, 1936; Marquis and Hilgard, 1937). Inasmuch as current research in human eyelid conditioning has become increasingly concerned with the effects of various attitudinal and set variables in the conditioning process (e.g., Grant, 1964; Spence, Homzie, and Rutledge, 1964) it would be useful to extend the technique to infrahuman primates for comparative purposes. Further, the use of primates affords the opportunity to investigate physiologically the process and mechanisms of classical eyelid conditioning as they relate to brain structures as well as other types of behavior.

Two reasons lie behind this paper, which describes in detail an apparatus and procedure developed for use with the squirrel monkey: (1) Abbreviated descriptions appearing with published reports of human eyelid conditioning data have generally been insufficient to permit detailed duplication of the apparatus or general procedure without further communication with specific investigators, and (2) A convenient literature reference is needed for citation in reporting research from our laboratory.

APPARATUS

Physical plan. The conditioning laboratory is designed to insure that the subject is separated from the experimenter and the conditioning apparatus by a partition. A 23 by 17

in. one-way vision window permits observation of the subjects being conditioned. By opening the top-hinged window the experimenter, from his usual position at the Polygraph, can make minor adjustments in the recording apparatus attached to the monkey. The perimeter of the window has been treated with Scotch Pressure-Sensitive Tape insulating tape to insure maximal sound deadening characteristics of the partition when the window is closed and locked. Both walls of the partition as well as all other walls and ceilings in both rooms have been covered with sound absorbent tile; attenuation of the ambient noise level inside the experimental chamber is approximately 10 db as measured by a General Radio Type No. 1551 B sound level meter. Continuous operation of an exhaust fan masks the reliable auditory cues arising from the programming and recording equipment.

Conditioned stimuli. Thus far, only auditory and visual conditioned stimuli have been employed. Tones which vary in frequency and intensity are produced with Hewlett-Packard Model 200 AB oscillators and are delivered through a 5-in. cone-type speaker mounted on the ceiling of the chamber 35 in. directly above the subject's head.

Visual stimuli are produced by a Grason-Stadler Model E4580 multiple stimulus projector whose remote unit can be attached to the primate chair, placing the source of visual stimulation a few inches from the subject's eye. Sudden changes in total illumination of the chamber are also used as conditioned stimuli.

Air system. A brief puff of air or compressed nitrogen, delivered to the subject's cornea, is the stimulus most widely used for eliciting the unconditioned blink reflex. It is particularly with respect to the characteristics of this stimulus that much vagueness exists in published literature.

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The air supply originates with a central air compressor housed in the basement of the Medical Sciences Building. It furnishes compressed air to all laboratories in the building. The outlet is activated by a valve which, when opened, permits the air to pass through a Cuno Model P10 filter, thus removing any foreign matter such as rust, moisture, or oil which may accumulate in the air as it circulates through the building's system. After passing the filter, the air is fed through an Alemite Model G30028 air regulator valve. Outlet pressures from this valve can be regulated to the nearest ounce per square inch as read from the accompanying Hoke Model RJ-25 gauge. Upon leaving the regulator valve the air passes through 45 in. of rubber tubing, inside diameter $\frac{3}{8}$ in., and is fed into an ASCO (Automatic Switch Company) Model 826213 28 v dc solenoid valve mounted inside the partition separating the experimental chamber and control room. Activation of the solenoid opens the valve and permits the air to pass through 19 in. of rubber surgical tubing, inside diameter $\frac{5}{32}$ in., which is connected to a square-end 18-gauge hypodermic needle mounted on the apparatus attached to the subject's head (see below).

Eyelid recording apparatus. Figure 1 is a sketch of the recording device developed for use with squirrel monkeys. Essentially it consists of a circular platform which supports a Gioninni Model 85111 microtorque potentiometer. The potentiometer is held in position by a Plexiglass frame which also supports the unconditioned stimulus delivery device. The arm of the potentiometer is attached mechanically to the monkey's eyelid by a length of 5.0 surgical wire connected to a small square of cotton cloth. One end of the wire is attached with De Khotinsky cement to the arm of the potentiometer; a small hook in the other end retains the cloth which in turn is fixed to the anterior margin of the superior surface of the eyelid with a drop of flexible collodion. Tension on the return spring of the potentiometer arm is adjusted to a point that will permit the arm to track upward movements of the eyelid without undue impairment of the blink. Torque values ranging from 3.087 to 3.416 mm g have been found to satisfy this requirement since values within those limits produce smooth records without inducing unilateral habituation of the unconditioned response.

The unconditioned stimulus delivery device shown in Fig. 1 is fabricated from aluminum and stainless steel. The variety of possible adjustments permits the air puff to strike the eye at any angle, and from whatever distance desired. When the arm supporting the delivery hose is secured to the top of the Plexiglass frame, however, the air delivery system remains firmly in place and the location of the opening of the hypodermic needle relative to the monkey's cornea is unaffected even by violent head movements.

The entire recording device weighs 30 g and is attached to the monkey's head with the aid of three plastic posts surgically fixed to the skull. These posts are inserted into three matching holes in the base of the platform and the unit is secured with set screws. Because of the rigidity of the placement of the recording unit, the spatial relationship of the potentiometer arm and the eyelid does not change during an experimental session. This eliminates a source of intrasubject variation which may be encountered in the data obtained from human subjects.

Amplification system. Movements of the potentiometer arm described above are transformed into dc voltage changes through the

Fig. 1. Drawing of recording apparatus.

circuit presented in Fig. 2. Voltage from a Burgess No. 5156 dry cell battery is impressed across the IK Gianinni potentiometer through the switch. The output of this circuit is fed into a Grass Model 5 driver amplifier and ink-written on one channel of a Grass Model 5 Polygraph such that a 3 mm pen deflection corresponds to 1 mm of movement of the eyelid. The latter unit is equipped with an event marker which records onset and termination of the conditioned and unconditioned stimuli. All stimulus durations as well as activations of the event marker are controlled by Grason-Stadler behavioral research programming equipment. Intertrial intervals are programmed with a Gerbrands Model 1A tape programmer.

Primate chair. During experimentation, the animal is restrained in a primate chair which is basically a F and H Machine Company Model 1000 Rhesus Monkey Chair with No. 1018 Kit-Adapt Chair for squirrel monkey which has been further modified. The chair is equipped with an adjustable seating platform whose sides can be tightly fitted to the animal's hips, minimizing lateral movement. Principal modifications include the addition of padded head positioners through which openings are provided for the monkey's ears, a removable Plexiglass front plate which, when in position, prevents the animal from raising its arms above the shoulders and interfering with the recording equipment. All portions of the chair in contact with the animal are readily removed for cleaning. A useful feature of the chair is that the degree of restraint can be varied. Since many monkeys have a tendency to drowse under maximal restraint, it is often useful to provide some freedom of movement to prevent the onset of sleep.

PROCEDURE

Surgical preparation. To prepare the head of the monkey for daily attachment of the recording device, a simple aseptic surgical procedure is carried out in which three vertical plastic posts are cemented to the dorsal surface of the skull several days before the first conditioning session. The monkey is placed in a primate stereotaxic head-holder to prevent movement during the operation.

After retracting a large scalp flap, a solution-sterilized template, identical to the base of the

Fig. 2. Diagram of circuit which transforms lid movements into dc voltage changes.

recording device, and with three nylon posts (6-32 threaded nylon rod) extending downward from the template for about 1 in., is placed on the skull and attached by acrylic cement. The posts are held in the template by set screws. When the cement hardens the template is removed from the posts (Fig. 3). The recording device can then be attached to the posts in the same manner as was the template.

Placement of monkey in chair. The animals are both housed and transported from the animal quarters to the laboratory in portable guillotine-door wire living cages. To facilitate transfer from living cage to primate chair, a five-sided Plexiglass cover is placed over the cage and a quantity of nitrous oxide gas sufficient to render the animal temporarily unconscious is introduced through an opening in the top of the cover. Care is taken to administer an amount of nitrous oxide which will produce unconsciousness without cyanosis.

Fig. 3. Surgical placement of posts to which the recording device is attached. (See text for detailed explanation.)

Fig. 4. Subject as it appears during conditioning. The grip bar is found to have a calming effect.

While unconscious, the animal is removed from the cage and secured in the chair. Monkeys ordinarily regain full consciousness and show no residual anesthetic effects after 30 sec; *i.e.*, pupillary reactions to light and skeleto-muscular reactions to painful stimuli are judged normal.

After the monkey has been placed in the chair, it is transferred to the experimental chamber and the recording device attached. Figure 4 is a photograph of an animal during conditioning.

Although the various pieces of apparatus and the techniques described above have been developed for use with the squirrel monkey, the same general plan can be followed with other laboratory primates. For example, with minor enlargement of the head positioning device we have obtained data from several *Cebus albifrons* and plan to employ this species in occasional future work.

REFERENCES

- Grant, D. A. Classical and operant conditioning, in: Melton, A. W. (Ed.), *Categories of Human Learning*. New York, Academic Press, 1964, pp. 1-39.
- Hilgard, E. R. and Marquis, D. G. Conditioned eyelid responses in monkeys with a comparison of dog, monkey, and man. *Psychol. Monogr.*, 1936, No. 212, 47, 186-198.
- Marquis, D. G. and Hilgard, E. R. Conditioned responses to light in monkeys after removal of the occipital lobes. *Brain*, 1937, 60, 1-12.
- Spence, K. W., Homzie, M. J., and Rutledge, E. F. Extinction of the human eyelid CR as a function of the discriminability of the change from acquisition to extinction. *J. Exp. Psychol.*, 1964, 67, 545-552.