

METHODS & DESIGNS

Some properties of conditioned and unconditioned eyelid reflexes in the albino rat*

ROBERT D. HALL†

*Research Laboratory of Electronics
Massachusetts Institute of Technology
Cambridge, Massachusetts 02139*

Eyelid responses in the rat were recorded as electromyographic activity (EMG) or as movements detected by a photoelectric technique. Spontaneous blinks, startle responses to acoustic stimuli, unconditioned responses to air puffs, and conditioned responses based on the corneal reflex were recorded under a variety of conditions. Special attention was given to topographical and temporal characteristics of the responses. Some relationships between the EMG and eyelid movements are described, as well as changes in eyelid responses related to habituation, conditioning, and various stimulus manipulations. In addition to the substantive findings, the study suggests that the rat may yet become a useful S in the study of eyelid conditioning.

One attractive feature of eyelid reflexes that has gone almost unnoticed is a relative simplicity that might well recommend them for analysis at the neuronal level. Eyelid responses are accomplished primarily by two small muscles, orbicularis oculi and levator palpebrae superioris, and they are to a considerable degree independent of other behavior. Although clearly influenced by eye and head movements, they seem less entangled in most postural and movement activities than responses of the skeletal musculature mediated by the spinal cord. Woody and his associates (Engle & Woody, 1972; Woody, 1970; Woody & Brozek, 1969a, b, c; Woody & Engel, 1972; Woody, Vassilevsky, & Engel, 1970; Woody & Yarowsky, 1972), with their studies of the glabella reflex in cats, have been the first to recognize these advantages of eyelid reflexes and the possibilities they afford in electrophysiological inquiries into the neural substrate of conditioning. The experiments on the rat described in this paper were undertaken as part of a program initiated with the same intent, to use eyelid reflexes to study neural events underlying conditioned responses.

Except for a few early conditioning studies (Biel & Wickens, 1941; Hughes & Schlosberg, 1938) and a more recent drug investigation (Tedeschi, Fowler, Fujita, &

Miller, 1967), the rat has not been used in studies of eyelid responses. The size of this species and its reluctance to accept restraint have made the measurement of eyelid responses difficult. But it seemed that if this difficulty could be overcome, all of those attributes that make the rat such a good laboratory S would make it most valuable to a long-term research program requiring many animals and experiments of many kinds. The difficulties have not been overcome completely, but the results below are encouraging in this respect and bear on several problems in the study of eyelid responses.

GENERAL METHODS

Subjects

Male albino rats, descendants of the Sprague-Dawley strain and weighing 250-350 g, were Ss. Individual Ss, 40 in all, were sometimes used in more than one experiment.

Measurement of Eyelid Responses

The eyelid electromyogram (EMG) was recorded from two stainless steel Teflon-insulated stranded wires, 125 μ m in diam, implanted subcutaneously in the upper lid. The EMG was filtered to eliminate potential changes below 10 Hz. It was also integrated, following full-wave rectification, by a Grass 7P3 preamplifier and integrator with a time constant of 20 msec.

A photoelectric measure of eyelid position was obtained from all rats by a method similar to one described for humans by Bluffield and Holland (1963) and for rabbits by Van Dercar, Swadlow, Elster, and Schneiderman (1969). A photodiode (Texas Instruments 1N2175) and miniature lamp (Edmund Scientific 40690) were held in front of the eye on 18-ga stainless steel tubing. The tubing, through which puffs of air were presented, was mounted on a connector whose mate was permanently fixed to the rat's skull. The entire assembly was rigid, and the positions of light and diode were, therefore, constant from day to day. This advantage of recording comparable measures of position on a day-to-day basis by making a simple connection was achieved at the expense of a two-stage implanting procedure. In the first stage, a cement base was attached to the skull by stainless steel screws while the rat was deeply anesthetized with pentobarbital. Following recovery from surgery and adaptation to a restraining device, the rat was lightly sedated with pentobarbital (10 mg/kg) and immobilized by ether for a few minutes while the connector was attached to the skull through a short piece of 16-ga wire. The position of the connector and, therefore, the light and diode assembly, could then be adjusted by simply bending the wire. An optimum position was found when the rat had recovered sufficiently from the ether to eat in the restraining device, and the connector was then imbedded in the cement while the rat remained awake. The optimum position was one in which the increase in reflected light and its detection by the diode was maximal for eyelid closures and relatively small for other movements of the lid or eye, especially those related to eating.

Eyelid responses were recorded on FM magnetic tape and on a Grass Model 7 polygraph. A PDP-4 computer was used, off line, to average the photodiode and integrated EMG signals, to determine peak amplitudes and integrals of individual responses,

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†Requests for reprints should be sent to Robert D. Hall, Research Laboratory of Electronics, Room 20B-225, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139.

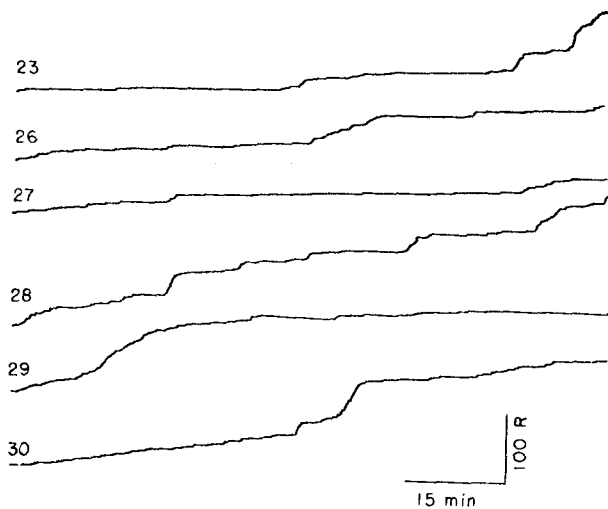


Fig. 1. Cumulative response records of spontaneous blinking for six rats.

and to compute correlation coefficients (Pearson's r) for amplitudes and waveforms of the two signals. Some amplitude and latency measurements for average responses were made on plots from an X-Y plotter.

Procedures

Rats were restrained in a dark sound-attenuating box. A "stock" was added to the restrainer described by Hall, Clayton, and Mark (1966) to prevent grooming, since movements of the forepaws across the face added greatly to the noise in the photodiode signal. Rats were adapted to the restrainer for various periods, but never less than a week, before data were collected. A masking noise from a wideband noise generator and blower, approximately 70 dB SPL, was always present.

Acoustic stimuli were presented through a speaker located 14 cm above and 20 cm behind the rat's head. Electronic switches were used to present tone bursts with rise times of 5 msec.

Air puffs were generated by a three-way solenoid valve that switched a high-pressure air line between the rat and a bypass. Durations of the air puffs varied from 50 to 180 msec; intensities ranged from approximately 0.1 to 1.5 psi.

In some experiments, rats were fed intermittently on random schedules with mean intervals of 30-90 sec, called random interval (RI) here to avoid confusion with variable interval reinforcement. Pellets (Noyes, 45 mg) were presented by a Gerbrands pellet dispenser and a motor-driven cup that carried the pellet to the rat. This feeding operation required 6 sec. Rats were maintained at approximately 80% of their ad lib feeding weights.

SPONTANEOUS BLINKING

Spontaneous blinking is a persistent feature of human behavior. Although there are wide individual differences, rates of 15-20 blinks/min appear to be average (Drinkwater & Flint, 1968; Lawson, 1948; Sidowski & Nuthmann, 1961) and must be taken into account in some conditioning experiments (e.g., Lipkin & Moore, 1966). The rate is obviously lower in the albino rat; it was the purpose of this experiment to determine how much lower.

Method

Blinking in each of 26 rats was measured under two conditions: during a period of intermittent feeding on an RI

60-sec or RI 90-sec schedule or in comparable periods without feeding. On at least 2 days, photoelectric measures of eyelid movements were recorded during 1-h periods beginning 0.5 h after the rat was restrained. Food was withheld during the recording period, although pellets were presented during the first 0.5 h of restraint. In two additional sessions, eyelid responses were recorded in the same way, except that feeding was continued during the recording period on the same schedule used during the first 0.5 h of restraint. Eyelid closures that occurred during the 6-sec feeding periods were not counted.

Response counts were obtained from polygraph records. Any deflection equal to 40% or more of the maximum deflection for that S was counted as a response. This criterion was adopted after the examination of many records as one that was least likely to confound signals related to blinking with noise from several sources, while including nearly every significant eyelid closure.

Cumulative response records were obtained under a third condition for 10 of the rats. No pellets were presented in a session 1.5 h long. A response for this purpose was defined by a voltage criterion determined by a Schmitt trigger whose input was the photodiode signal. The criterion was approximately 40% of the maximum amplitude.

Results

Mean rates of spontaneous blinking, computed from mean rates of individual rats, did not differ under the feeding and no-feeding conditions. The group mean for the no-feeding condition was 1.9 blinks/min (SD = 1.0), and for the feeding condition it was 1.7 blinks/min (SD = 0.8).

Cumulative response records in Fig. 1 indicate that blinking was not periodic, but tended to occur in bursts. Observation of the rats indicated that the longer bursts, at least, were accompanied by other obvious movements of the head, trunk, or limbs. It is also evident in Fig. 1 that the intervals between blinks were frequently as long as 10-20 min.

Discussion

The low rate of spontaneous blinking in the rat suggests that it should not be a significant factor in classical eyelid conditioning studies, but a word of caution seems in order. If further study supports the observation that blinking is frequently related to other movements, then conditioning procedures that promote more widespread movement, such as struggling, should be avoided to permit unambiguous interpretations of increased responding. The finding that eyelid movements may accompany skeletal behavior of various kinds was not unexpected. Muscles of the middle ear, innervated partly by the facial nerve, also have this property in the cat (Carmel & Starr, 1963; Starr, 1964), and facial expressions in man and other animals are a conspicuous accompaniment to many types of behavior. But blinks associated with one kind of behavior do not necessarily influence other blinking. This was indicated by the finding that rates of spontaneous blinking were the same under the feeding and no-feeding conditions, even though many eyelid closures occurred during ingestion of the food and startle blinks were a common response to activation of the pellet dispenser.

EYELID COMPONENT OF THE STARTLE RESPONSE

Startle responses to acoustic stimuli are of interest because acoustic stimuli have become the preferred conditional stimuli in eyelid conditioning experiments and because the startle can be sensitized by aversive

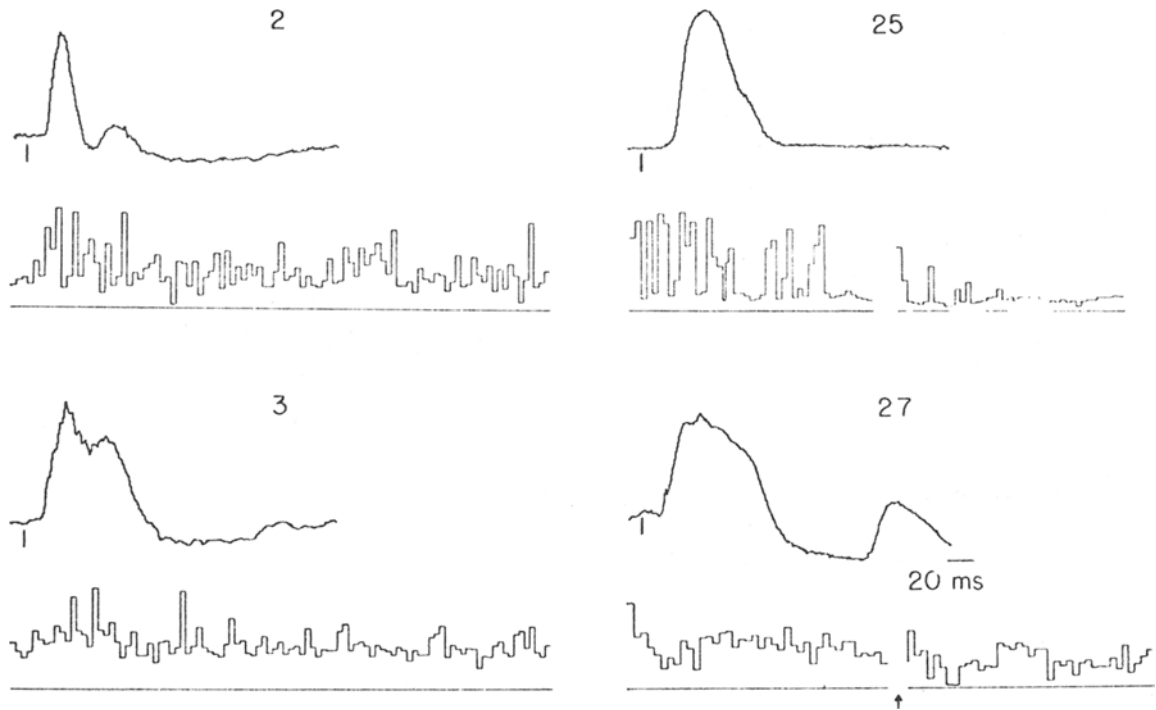


Fig. 2. Average startle responses to feeder noise from four rats and peak amplitudes of successive responses from same session. Averages for Rats 2 and 3 are for first 50 responses; for Rats 25 and 27, the averages are for the first 5 responses. One-hour interruption in sessions for 25 and 27 indicated by arrow and break in amplitude plots. In this and succeeding figures with averaged photodiode measurements of eyelid movements, or amplitude plots based on the same measure, the vertical scales are in arbitrary units. Vertical lines at left of each average indicate onset of noise.

experimental conditions (e.g., Brown, Kalish, & Farber, 1951). Although it is usually possible to avoid the complications introduced by such responses, some knowledge of their form and latency and of conditions favoring their occurrence would seem to be minimum requirements in any eyelid conditioning program.

With few exceptions (e.g., Lehner, 1941; Prosser & Hunter, 1936), startle responses in rats have been measured as "whole-organism" responses, usually by stabilimeter techniques. The eyelid component of the startle response has not been described for the rat, or for any infrahuman species as far as I know, although it has received considerable attention in people (Cobb & Sears, 1957; Gogan, 1970; Larsson, 1956, 1960; Rushworth, 1962; Sakano & Pickenhain, 1966, 1968; Wojtowicz & Gwozdz, 1968). A description of eyelid startle responses to both tone bursts and feeder noises is presented below for the rat, together with observations on their habituation.

Methods

Startle responses were investigated in three separate experiments. Responses to activation of a food-pellet dispenser were first recorded from 10 rats during sessions in which spontaneous blinking was measured in the experiment described above. Photoelectric measures of eyelid responses were recorded for 1 h beginning ½ h after the session began. Pellets were presented throughout the session on an RI 30-sec schedule. In other sessions, pellets were presented during the first half hour, but not in the following hour when spontaneous blinking was measured in the absence of feeding; and, finally, feeding was

resumed for another half hour. The presentation of pellets in these sessions did not constitute a rat's initial exposure to the feeder noise. The animals had been fed on the same intermittent schedules by the same feeder for approximately 2 h each day for 2 weeks. Strong startle responses, indicated by gross movements of the body, were infrequent when the eyelid responses were measured, and they occurred mainly at the beginning of a session.

In a second experiment, startle responses to activation of the feeder were recorded from three new rats on the first day that the pellet dispenser was used to feed them. Pellets were presented on an RI 60-sec schedule. Responses to the first 50 presentations were recorded in both EMG and photoelectric measures. In a second session, the same measures were obtained for 100 presentations of a 3-kHz tone burst, 425 msec long, 105 dB SPL, on the same RI 60-sec schedule. There was no feeding in this session.

In a third experiment, with five more rats, the 3-kHz 105-dB tone burst was presented 200 times in one session and 100 times the following day. Stimulus conditions and response measures were the same as in the second experiment. In a third session, 100 tone bursts of 85-dB intensity were presented in the same way; in a fourth session, the intensity was raised to 95 dB. These four sessions followed 15 days devoted to a conditioning experiment in which the CS was a weaker 3-kHz tone, 75 dB SPL. In the first startle session, 50 extinction trials were presented before the stimulus intensity was raised to elicit the startle, and in succeeding sessions, the former CS was presented 10 times before stronger stimuli were introduced.

Results

The most consistent feature of eyelid responses to strong acoustic stimuli is an early brief closure with a total duration of 75-100 msec and an onset latency of

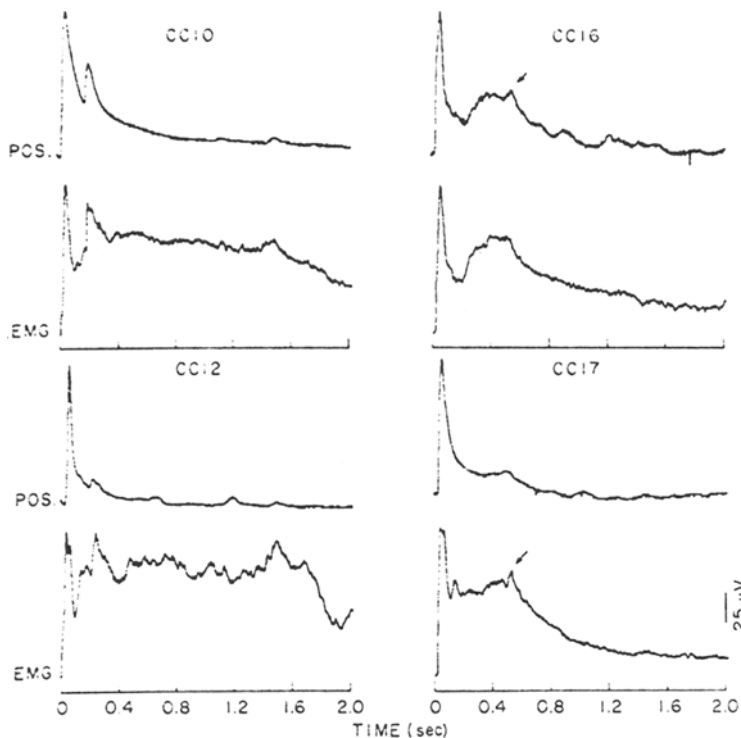


Fig. 3. Average eyelid startle responses to initial presentations of feeder noise (CC10, CC12) and 3-kHz 425-msec tone bursts (CC16, CC17). Top average of each pair is photodiode measure of position; bottom is integrated eyelid EMG. The 25-microV calibration mark is for all EMG records.

10-24 msec, when measured as a change in position of the lid. Average responses to feeder activation in Fig. 2 consist mainly of this quick closure, which, on the expanded time scale in Fig. 2, is seen to have two components, more or less distinct in individual rats, but present in all of them. For 13 rats, the median onset latency of average responses to feeder noise was 12 msec; responses to tone bursts in 7 of those rats had a median latency of 22 msec. Only 1 rat had a response to the feeder that was as late as the earliest response to tone bursts. Peak latencies of the initial closure were 20-50 msec. This variability stemmed mainly from differences in waveform like those in the averages of Fig. 2. Median peak latencies of 35 and 46 msec for responses to feeder and tone burst, respectively, were not significantly different. The median onset latency of average EMG responses to tone bursts was 12 msec, 10 msec earlier than the onset of eyelid movement. The first peak in the integrated EMG preceded the first peak of the movement record by 15-20 msec.

All of the average responses in Fig. 2 had amplitudes that were less than 50% of the amplitudes of unconditioned responses to air puffs in the same rats. These relatively small responses to the feeder noise persisted for a few weeks, during which 200 or more pellets were presented daily. Consequently, response decrements within sessions following a week or two of such exposures were generally small, like those in the peak amplitude plots for Rats 2, 3, and 27. The decrement was larger in Rat 25, who provided clear evidence of spontaneous recovery during the hour intermission. No attempt was made to trace habituation

of the startle to feeder noises across sessions, but in the second experiment, responses of long duration recorded from three rats the first time they were exposed to the feeder suggest that the responses of Fig. 3 had already undergone appreciable attrition.

Examples of the first eyelid responses to feeder activation are shown for two rats in Fig. 3, where the measure of position and the EMG are both presented. The early component is again conspicuous, but it is followed by a much longer closure that is especially clear in both records for CC10. Even the small late component in the position record for CC12 persists much longer than the brief responses illustrated in Fig. 2. (The apparent discrepancy between amplitudes of the late activity in position and EMG measures for CC12 is discussed below.)

Late components were also clear in responses to initial presentations of the tone burst, as seen in the right side of Fig. 3. There is an interesting difference between the late responses to feeder activation and tone bursts. The second peak in the feeder startle is sharper and earlier, at approximately 200 msec, than the second peak in the response to tone bursts, which peaks at approximately 400 msec. Inspection of both single and average responses disclosed that the late component of the response to tone bursts often persisted until the 425-msec tone burst ended, at which time there might be a small "off response" like those indicated by arrows in the records from CC16 and CC17. With onset of the tone, then, there was a strong early blink, characteristic of all startles, followed by a partial, more prolonged closure until the stimulus terminated. A third phase

followed, in which the eye gradually opened completely after a second or more.

Correlations between the waveforms of average EMG responses and averaged measures of movement were generally high, with a mean of $+0.77$ for seven rats in which both measures of the startle were taken. Responses from two rats, like those for CC12 in Fig. 3, were responsible for so low an average, however, because the late components were more prominent in the EMG than in the measure of position. Two factors seemed largely responsible for this; both reflect problems of measurement. First, potentials from other muscles, representing other components of the startle, contaminate the eyelid EMG. Second, poor placements of the photodiode-light assembly has in a few instances been such that the photodiode output was not a reasonably linear function of the degree of eyelid closure, but one in which partial closures were underestimated. Surprisingly low correlations between peak amplitudes of position and EMG measures, indicated by a mean r of $+0.27$, point to other complexities in the relationships between the measures.

The short latency of the eyelid startle response usually suffices to distinguish it from conditioned eyelid responses, but two other characteristics of the startle serve to enhance the distinction: the relative invariance of both latency and shape of the early component. These are illustrated in Fig. 4, where startle responses can be compared with CRs recorded from the same S in the same session. Habituation of the startle is marked by a decrease in amplitude; but the rapid closure retains its form in spite of the decrement, and the increase in latency is nominal, approximately 10 msec. In contrast, the average CR waveform, more diffuse to start with, has no constant features during extinction. Reduction of the CR is characteristically accompanied by marked increases in latency, measured in hundreds of milliseconds. The more variable late portions of the startle, however, are not so distinctively different from the eyelid CR.

The mean amplitude of eyelid responses was found in each of four rats to increase as a function of stimulus intensity. Group mean amplitudes of the early component of the EMG (calculated from averages of 50 responses at each intensity for each rat) were 22, 51, and 100 microV for tone bursts of 85, 95, and 105 dB SPL, respectively.

Discussion

The eyelid component of the auditory startle response is not a simple response, but one that exhibits considerable variety. It may be large or small, a simple rapid closure or a sustained complex response lasting several seconds. The late components are variable in form and reflect some properties of the eliciting stimuli. Responses to tone bursts displayed a second component, in which orbicularis oculi actively maintained a partial closure until the stimulus ended. Only then did the eye begin to open fully with a gradual relaxation of the muscle. The second component of the startle to feeder activation may reflect a significant second component of the feeder noise, quite possibly

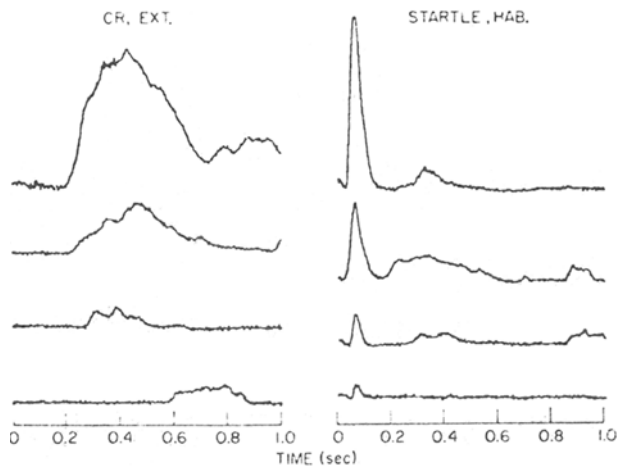


Fig. 4. Successive averages of 10 eyelid CRs during extinction and of startle responses undergoing habituation in same rat in same session. Startle averages are for first, third, fifth, and ninth blocks of 10 responses. Startle stimulus was 30 dB stronger than CS.

the return slam of the solenoid at approximately 100 msec. Late components in the human eyelid response to strong acoustic stimuli have been described by Gogan (1970) and by Larsson (1956), and other startle responses show similar late activity (Landis & Hunt, 1939; Prosser & Hunter, 1936). Gogan has regarded the late component as an orienting response, but in the rat it resembled a defensive or withdrawal reflex, with the head drawn back and the globe retracted in addition to the eyes being closed. Landis and Hunt may have been closer to the truth in holding that the late startle reactions are subject to a variety of psychological variables. The early response, strikingly consistent in form and latency, stands in contrast to the highly variable late responses and should be more amenable to analysis. The consistency of the startle latency was described by Prosser and Hunter (1936) and Hunter (1937), who appreciated that the startle differed from the CR in this respect.

EYELID POSITION AND EYELID EMG

From a functional point of view, the important aspect of eyelid responses is the movement of the lids; muscle activity responsible for the movement is of secondary importance. But it seems unlikely that we shall achieve a fundamental understanding of eyelid responses without understanding how movements of the eyelids are related to activity of the muscles. The two aspects of eyelid behavior are not equivalent, and in some cases it will be a mistake to treat electromyographic and movement records as comparable. Some relationships between the eyelid EMG and the measure of eyelid position were considered in the description of startle responses. Initial attempts to extend these observations to the corneal reflex are described next. The data, although preliminary, point to some relationships that seem clear already and emphasize the complexity of the problem.

Methods

EMG and position measures of responses to corneal stimulation were recorded in a conditioning experiment with six rats whose startle responses were recorded later, as noted above. Only 60 responses from the first session, when only two rats showed a few small CRs, were examined. Correlation coefficients

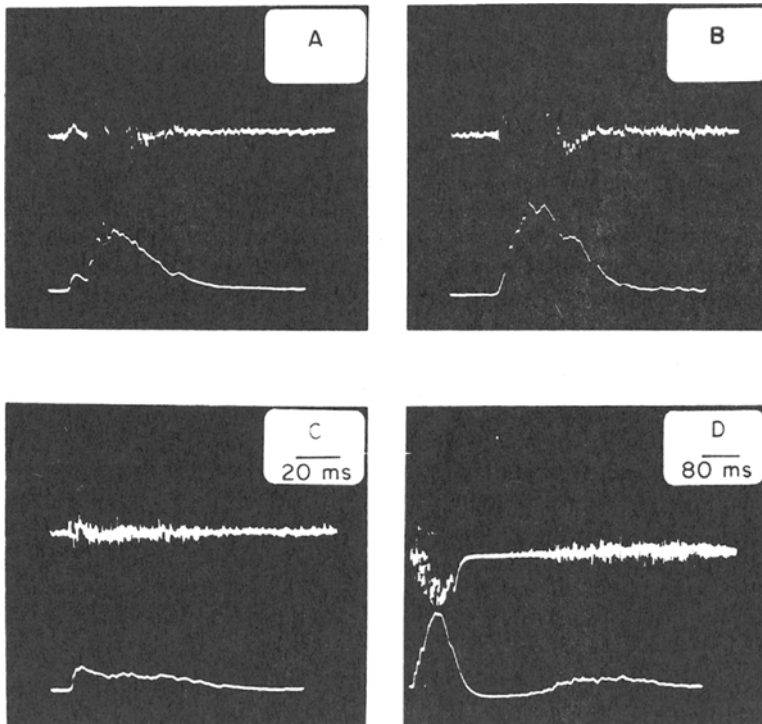


Fig. 5. EMG responses to air puffs recorded from upper eyelid of Rat 33 (A, B, C) and Rat CC2 (D). Raw signal in upper trace of each pair; integrated signal in lower. Note different time scale in D.

were computed for the average waveforms and for peak amplitudes and integrals of individual responses. The stimuli were air puffs of 1.2 psi presented on an RI 60-sec schedule.

EMG and position measures were also compared for nine other rats in one (five rats) or two (four rats) sessions, in which only air puffs were presented at intensities of 0.1-0.3 psi and rates of 1/5 sec, 1/30 sec, or on an RI 60-sec schedule.

Results

In the rat, the unconditioned response (UCR) to air puffs directed at the cornea is not always a response to just corneal stimulation. The response frequently has an earlier component whose nature was not fully appreciated until the EMG was recorded. An example of the response appears in Fig. 5A, where a very brief early component precedes a longer, larger, later component. There is often a pause between the two, typically ~10 msec in duration. The two components are separable; only the late component appears in the response of Fig. 5B, while in Fig. 5C the early component predominates. Latencies of the early component range from 5 to 15 msec, as measured in the EMG. Its duration is less than 25 msec, typically only 10 msec. The shortest latencies of the late component have been difficult to measure, because the response encroaches upon the early component, but they are estimated at 20-25 msec in the unanesthetized animal. Durations of the late activity are 50 msec or more, frequently much more. The analysis of the two components has been presented in a preliminary report (Hall & Boccard, 1972), in which it was shown that the early component is a response to stimulation of the eyelids, especially the eyelashes, and the late component is a response to stimulation of the cornea. The early

response to eyelid stimulation is not as conspicuous in the measure of eyelid position as it is in the EMG, as can be seen in Fig. 7; but its influence is quite measurable, altering the slope and reducing onset and peak latencies of the initial closure by approximately 10-15 msec and 20-30 msec, respectively.

Another complexity that is obvious in the EMG but is not apparent in the measure of eyelid position is a "silent" period following the initial contraction of the muscle. This is seen in the records from two Ss in Fig. 6 as a drop in the EMG curves to the zero baseline for approximately 100 msec. That this drop may signify an inhibitory process is suggested by the raw EMG in Fig. 5D. Following the initial burst of activity, the muscle is almost completely silent until spontaneous activity returns, 150 msec or more later. The spontaneous activity points to one condition in which the silent periods occur, the presence of tonic muscle activity. These silent periods weaken the correlation between the measure of position and the integrated EMG, as indicated by the reduced correlation coefficients for each S in Fig. 6, where responses showing the pause are compared with responses that do not show it.

Average waveforms of the integrated EMG and photoelectric measure of position were generally similar for responses to air puffs. Of the most highly correlated pairs for each of 15 animals, 11 had correlations of +.75 to +.95. The others were all around +.45. But, as indicated in the description of the silent period, such correlations could be markedly reduced without apparent changes in the movements of the lids. Another factor associated with a reduced correspondence

between EMG and position waveforms is the duration of the closure, as illustrated in Fig. 7. Long closures can be associated with very little late muscle activity, too little to contribute significantly to the average EMG waveform. The responses in Fig. 7 became shorter as both intensity and duration of the air puffs were reduced; correlations between the EMG and position waveforms became correspondingly greater. A reduced correspondence between EMG and position measures was not, however, an invariable accompaniment of prolonged responses. In the description of startle responses, it was noted that both measures sometimes revealed responses of long duration, and this was seen frequently in the unconditioned responses to air puffs. The notion that the UCR to air puffs is a brief event needs qualification for the rat, whose response frequently lasts 3 or 4 sec, even when the air-puff duration does not exceed 200 msec.

In Fig. 7, peak amplitudes of the EMG and position measures have been plotted for a series of 50 air puffs. The correlation between these two measures of response amplitude is $+0.81$, but this apparently reasonable correlation was more the exception than the rule. For 34 sets of data from 15 rats that were presented with air puffs of several intensities and durations, the mean r was only $+0.34$. When areas under the response curves were used as the measure of the amplitude, the correlations were no better, averaging $+0.23$ in 9 rats.

Discussion

The early component in the rat's response to air puffs is due mainly to stimulation of the eyelashes or common hair of the eyelids. It is not yet clear whether it can be elicited by simple cutaneous stimulation of the lids. It must be of concern in conditioning experiments with the rat if there is a chance that it might confound the UCR or the CR under particular conditions, e.g., when conditions favor the development of a tonic partial closure of the lids. Such closures would increase the probability

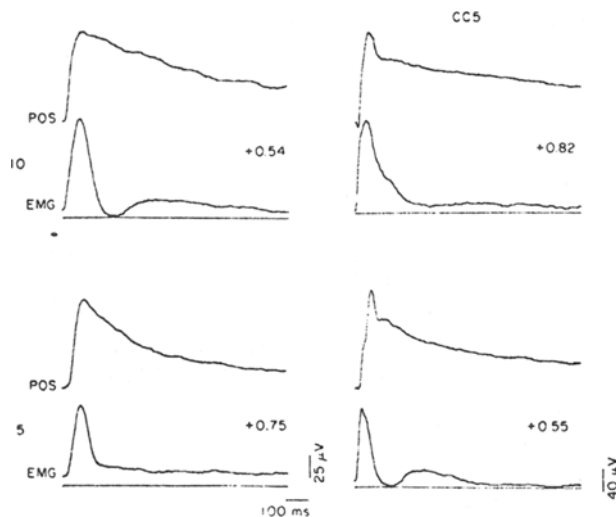


Fig. 6. Average eyelid responses to air puffs of two intensities expressed in relative units (10, 5) for two rats (CC2, CC5). Photodiode measure of position and EMG recorded simultaneously. Each average for 50 responses. Correlation coefficients (Pearson's r) indicated for each pair of waveforms.

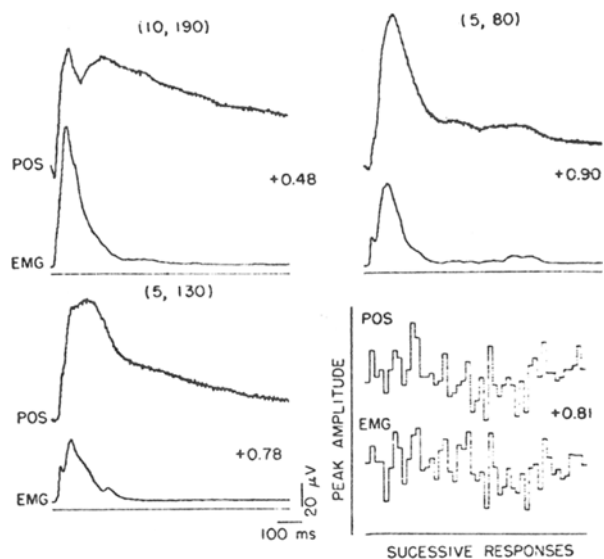


Fig. 7. Averages of 50 eyelid responses recorded simultaneously as change in position and EMG, and peak amplitudes of successive individual responses for the two measures. First number in parentheses indicates relative stimulus intensity; second number is duration in milliseconds. Amplitude plots for (5, 80) stimulus condition. Correlation coefficients for pairs of waveforms and amplitude curves. Rat 33.

of stimulating the lids with the subsequent development of the early component. Moreover, a UCS that elicits the early component may be more or less effective than one that does not. Lacking empirical evidence, one might argue the case either way.

The relationships between movement and muscle activity are not nearly as straightforward as one might expect for a relatively simple effector system. Some of the apparent discrepancies between the average waveforms of EMG and position indicators can be reasonably accounted for in terms of motor mechanisms. For example, prolonged closures associated with little EMG activity may reflect differences in muscles and their functions like those described for cat skeletal muscles (Henneman, Somjen, & Carpenter, 1956; Henneman & Olson, 1965) and human eyelid muscles (Gordon, 1951). Gordon found three types of motor units: those with the highest discharge rates that responded only during blinks, those that were active during blinks and sustained closures, and those that responded only during sustained closures. It seems reasonable to suggest, then, that the initial rapid closures are accomplished by large motor units discharging at relatively high rates and by smaller units as well, thereby yielding considerable electromyographic activity. The sustained closures, however, are maintained by only the smaller units whose summed activity is appreciably less. But this is not likely to be the whole story. Retraction of the globe must be an important factor in the rat's capacity to keep its eyes closed with minimal muscle activity, as less tension is required with the globe retracted. Indeed, we have often seen a completely silent EMG in the anesthetized rat with its eyes completely closed and the globe retracted.

Retraction of the globe during eyelid responses is a complicating factor on several other counts. To begin with, it complicates both measures of the behavior. Tonic retractions lead to baseline shifts in the photoelectric measure of position that cannot always be distinguished from closures due to contractions in orbicularis oculi. Such retractions of the globe must also reduce the maximum excursion that the lids can make and complicate the measure of movement in that way. To what extent the electrical activity of retractor bulbi influences the EMG as we have recorded it from the eyelid we don't know.

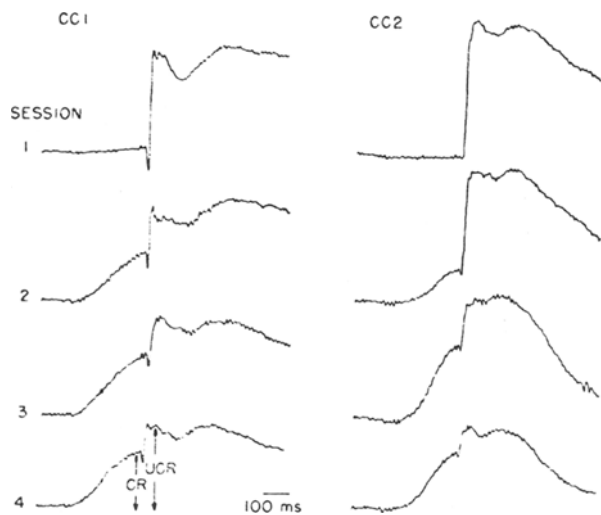


Fig. 8. Average eyelid responses from first four conditioning sessions for two rats (CC1, CC2). Averages of 60 responses. Traces start with CS onset. UCS onset is apparent in small downward deflections of traces.

CONDITIONED EYELID RESPONSES

A high priority for any program seeking a "model behavior" for the study of conditioning is to determine if the response can be readily conditioned, with ease and regularity in the species of choice. Although the studies by Hughes and Schlosberg (1938) and Biel and Wickens (1941) established that it was possible to condition eyelid responses in the rat, they provided little information about the requisite conditions and the nature of the conditioned response. The following experiments were undertaken to obtain additional information. The emphasis is on conditioning in the individual S. This emphasis derives from some practical limitations and from the conviction that credible relationships between neural activity and behavior will

depend upon convincing demonstrations in individual organisms.

Methods

The corneal reflex was conditioned in five daily sessions of 60 trials each. These were followed by a full session of extinction and then by two more sessions in which trials were divided so that 30 conditioning trials preceded 30 extinction trials (Day 7), or vice versa (Day 8). There were two additional days of extinction. Interest in this experiment centered on the relationships between frequency and amplitude measures of conditioned responses.

The CS was a tone burst with a frequency of 3 kHz, a duration of 425 msec, and an intensity of 75 dB SPL. The UCS was an air puff, 120 msec long, with an intensity of 1.5 psi. The interstimulus interval (ISI) was 425 msec. The intertrial interval (ITI) was 60 sec with intervals of 15-120 sec.

The corneal reflex was conditioned in four more rats and made discriminative with the following procedures: Conditioning in the first 5 days was like that for the first group, except that rats were fed between trials on an RI 45-sec schedule, as they were throughout the experiment. The procedure was used in the belief that differential responding might be more easily established in rats that were not unduly anxious and that feeding between trials would help to reduce anxiety in restrained animals. On the sixth day, the CS and ISI were lengthened to 825 msec. Five days of differential conditioning followed in which the 3-kHz tone was retained as the CS+ and a 10-kHz tone was used as the CS-. The ISI remained at 825 msec. Trials of CS+ and CS-, 30 of each per session, were presented in an irregular order.

Results

Figure 8 shows the form and development of the eyelid CR in average responses from 2 rats. The few small CRs that occurred on the first day in CC1 hardly affected the average, so that only the UCR is clear in responses from both rats in Session 1. Onset latencies of the CR, measured in 20 rats in this and succeeding experiments with ISIs of 425 msec, ranged from 125 to 350 msec. The modal value was approximately 200 msec. Durations of the CR were measured in averages of 10 responses recorded during extinction and

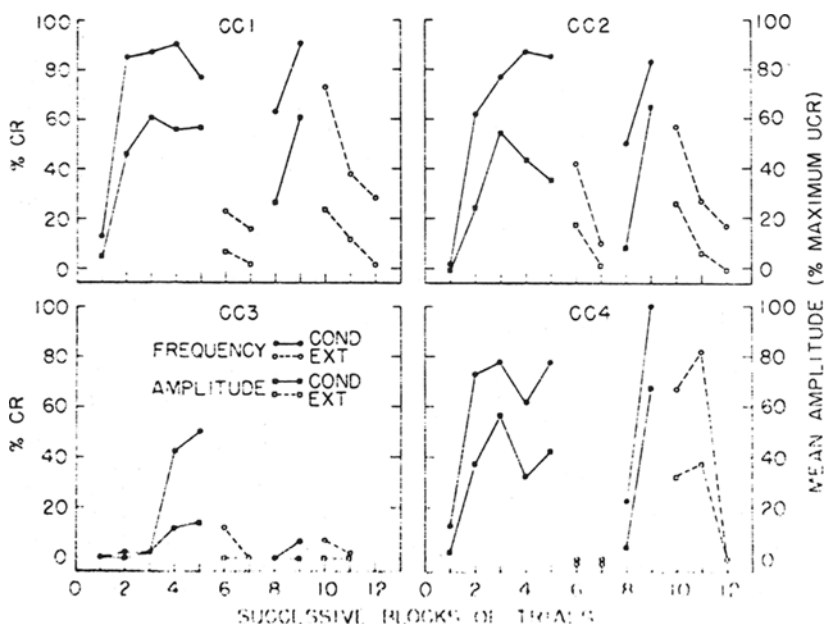
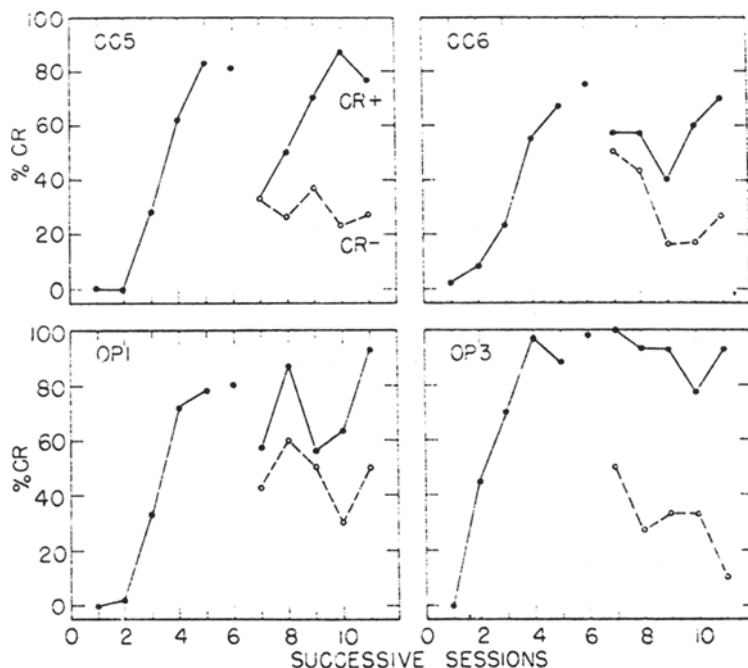


Fig. 9. Conditioning and extinction of eyelid CRs in four rats plotted as change in %CR and mean peak amplitude. Blocks 7-10 had only 30 trials each, all other blocks had 60 trials. Blocks 7 and 8 both in seventh session; Blocks 9 and 10 in eighth session.

Fig. 10. Acquisition of eyelid CR in four rats followed by differential conditioning. Sixty trials per session. ISI was 425 msec in first five sessions, 825 msec thereafter.



ranged from 325 msec to more than 800 msec. The UCR is cut short in Fig. 8, where the time scale is appropriate for examination of the CR.

The course of conditioning and extinction is shown in Fig. 9 in two measures for all four rats of the first group. The measure of mean amplitude is indicated in the bottom trace for CC1 in Fig. 8. It has been transformed to a percentage of the largest UCR, which is taken as a measure of full closure. There is a close correspondence between the two measures in all four rats. The weak CR in CC3 indicates a problem with the measurement technique, not an unsatisfactory variability in the behavior of individual Ss. The connector is sometimes dislodged from the skull. CC3 lost its connector on the 12th day of conditioning, and the small responses in preceding sessions reflect a shift in the connector's position. These data from CC3 are presented simply to indicate the measurement problem.

Acquisition of the CR and the discrimination by rats in the second group are shown in Fig. 10. Acquisition of the CR was similar in all four rats, but the differential responding developed differently in individual animals. Changes in CR amplitudes were again similar to changes in CR frequency.

Decrements in the amplitudes of average UCRs were a consistent finding. They are apparent in the average responses of Fig. 8, and averaged 24% (mean) for that group, 16% for the second group, and 27% for still another group of 6 animals over the first 5 days of conditioning. They appear with development of the CR, but another finding indicates that occurrence of the CR was not alone responsible for the decrease. Average UCRs from the first 10 and last 10 trials of the first conditioning session were measured in 14 rats. In the almost complete absence of CRs, UCRs showed a mean reduction of 18%, all but 2 rats exhibiting a decrease.

The increase in ISI in the sixth session for the second group of rats led to an increase in UCR amplitude (mean = 20%) and changes in the CR like those illustrated in Fig. 11. Immediately after the shift, the CR was "inappropriately placed," being essentially over before the delayed onset of the UCS. In succeeding sessions, onset latencies shifted from approximately 200 to 400 msec. The increase in peak latencies was longer, from about 450 to 800 msec, indicating an increase in rise times of the CR as well.

Discussion

The few data presented here serve primarily to describe the eyelid CR in rats under a restricted set of conditions, but they show that even in a few Ss one can see consistent relationships whose lawfulness has been established in more conventional conditioning experiments. The emphasis on individual data is not intended to reopen old arguments about the relative merits of experiments using many Ss and group statistics, on the one hand, and those that use few Ss and few statistics, on the other. The emphasis stems partly from a practical limitation; it is not yet very easy to do eyelid studies with the rat, and the species cannot be recommended now as an alternative to larger animals, at least for studies requiring group statistics. But we are encouraged by a recent improvement in the technique for measuring eyelid responses, and the recommendation may be forthcoming.

Acquisition of eyelid CRs, simple and discriminative, appeared similar in measures of CR frequency or peak amplitudes of average CRs. Amplitudes of average responses were presented above to illustrate another potentially useful aspect of the average response. With the increasing availability of laboratory computers, the reduction of conditioning data is greatly facilitated by averaging. As interest increases in the form and temporal properties of CRs and UCRs (e.g., Kimmel, 1965; Martin & Levey, 1969; Prokasy, 1965), some such reduction seems almost mandatory.

The decrease in average UCR amplitudes during conditioning may be related to the UCR decrements described by Kimble and Ost (1961) and Kimmel and Pennypacker (1962) in human eyelid and GSR conditioning. Eyelid UCR decrements found by

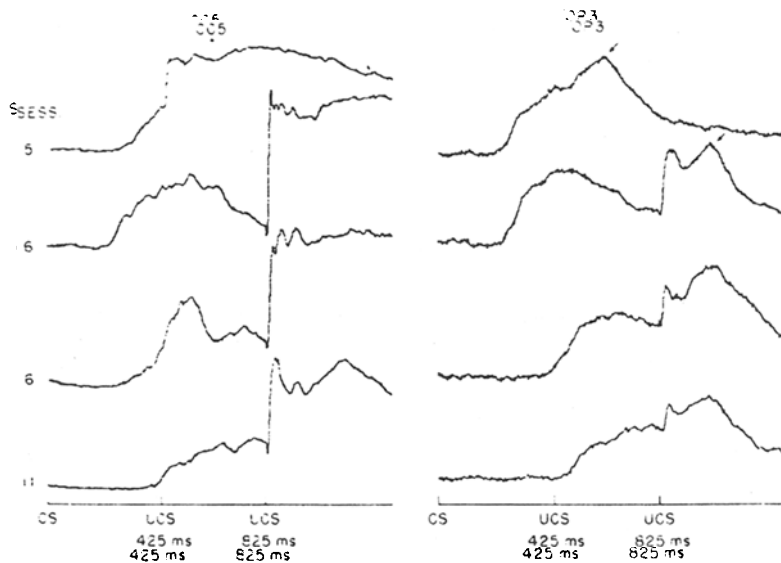


Fig. 11. Average responses from two rats (CC5, OP3) from same experiment as in Fig. 10. Each average for 10 responses. Last 10 responses in Session 5 before ISI increased to 825 msec. First and last 10 responses in Session 6 when ISI was increased. Last 10 responses from Session 11 after 5 days of differential conditioning with long ISI. Arrows in OP3 records indicate late component of UCR that was partially independent of the initial response. The latter is difficult to discern in top record where it blends with CR.

Kimble and Ost were not related to occurrence of the CR (and a subsequent reduction in UCS intensity), because they measured only UCRs that were not preceded by a CR. In the present experiments, any effects of the CR were confounded in average responses, except in data from the first conditioning sessions when very few CRs occurred. UCR decrements in that session might indicate a habituation of the response, but other factors cannot be ruled out. Lengthening the ISI appears to have been functionally equivalent to omission of the CS in the Kimble and Ost study, both operations leading to the larger UCRs. If the UCR decrements resulted only from acquired inhibitory properties of the CS, of the kind proposed by Kimble and Ost (1961) and Kimmel (1966), the inhibitory process must have been well localized in time and have terminated by the time the CR terminated. The UCR increase with the shift in ISI would not otherwise be accounted for by the release from such a process.

Changes in onset and peak latencies of the CR following an increase in ISI were generally similar to those found with ISI increases in human eyelid conditioning (Boneau, 1958; Ebel & Prokasy, 1963; Prokasy & Ebel, 1963) and for rabbit nictitating membrane CRs (Coleman & Gormezano, 1971). Movement of the CR peak towards the time of UCS onset, which seems to require a change in CR topography as well, makes it difficult to reject the view that the CR is a dynamic, adaptive response shaped by reinforcement contingencies. The view has become even more palatable in contemporary theory (Kimmel, 1965; Prokasy, 1965; Martin & Levey, 1969), where, it seems, it no longer implies a simplistic equation of classical and instrumental conditioning. The issue, however, has become too complex to be settled by some seemingly sensible changes in the behavior of a few rodents. It is at least clear that rodents, rats and rabbits, share those sensible changes with man.

REFERENCES

Biel, W. C., & Wickens, D. D. The effects of vitamin B₁ deficiency on the conditioning of eyelid responses in the rat. *Journal of Comparative Psychology*, 1941, 32, 329-340.

Bluffield, R., & Holland, H. C. The eyeblink conditioning apparatus. In H. J. Eysenck (Ed.), *Experiments in motivation*. Oxford: Pergamon Press, 1963.

Boneau, C. A. The interstimulus interval and the latency of the conditioned eyelid response. *Journal of Experimental Psychology*, 1958, 56, 464-476.

Brown, J. S., Kalish, H. I., & Farber, I. E. Conditioned fear as revealed by magnitude of startle response to an auditory stimulus. *Journal of Experimental Psychology*, 1951, 41, 317-327.

Carmel, P., & Starr, A. Acoustic and non-acoustic factors modifying middle-ear muscle activity in waking cats. *Journal of Neurophysiology*, 1963, 26, 598-616.

Cobb, W. A., & Sears, T. A. The blink reflex and its possible

relationship to the photomyoclonic response in man. *Journal of Electroencephalography & Clinical Neurophysiology*, 1957, 9, 373-374.

Coleman, S. R., & Gormezano, I. Classical conditioning of the rabbit's (*Oryctolagus cuniculus*) nictitating membrane response under symmetrical CS-US interval shifts. *Journal of Comparative & Physiological Psychology*, 1971, 77, 447-455.

Drinkwater, B. L., & Flint, M. M. Telemetric monitoring of the reflex blink rate. *Perceptual & Motor Skills*, 1968, 26, 303-307.

Ebel, H. C., & Prokasy, W. F. Classical eyelid conditioning as a function of sustained and shifted interstimulus intervals. *Journal of Experimental Psychology*, 1963, 65, 52-58.

Engel, J., Jr., & Woody, C. D. Effects of character and significance on unit activity at coronal-pericruciate cortex of cat during performance of conditioned motor response. *Journal of Neurophysiology*, 1972, 35, 220-229.

Gogan, P. The startle and orienting reactions in man. A study of their characteristics and habituation. *Brain Research*, 1970, 18, 117-135.

Gordon, G. Observations upon the movement of the eyelids. *British Journal of Ophthalmology*, 1951, 35, 339-351.

Hall, R. D., & Boecard, D. M. Two somatic eyelid reflexes in the albino rat. Paper presented at the meeting of the Eastern Psychological Association, Boston, April 1972.

Hall, R. D., Clayton, R. J., & Mark, R. G. A device for the partial restraint of rats in operant conditioning studies. *Journal of the Experimental Analysis of Behavior*, 1966, 9, 143-145.

Henneman, E., & Olson, C. B. Relations between structure and function in the design of skeletal muscles. *Journal of Neurophysiology*, 1965, 28, 581-598.

Henneman, E., Somjen, G., & Carpenter, D. O. Functional significance of cell size in spinal motoneurons. *Journal of Neurophysiology*, 1965, 28, 560-580.

Hughes, B., & Schlosberg, H. Conditioning in the white rat. IV. The conditioned lid reflex. *Journal of Experimental Psychology*, 1938, 23, 641-650.

Hunter, W. S. Muscle potentials and conditioning in the rat. *Journal of Experimental Psychology*, 1937, 21, 611-624.

Kimble, G. A., & Ost, J. W. P. A conditioned inhibitory process in eyelid conditioning. *Journal of Experimental Psychology*, 1961, 61, 150-156.

Kimmel, H. D. Instrumental inhibitory factors in classical conditioning. In W. F. Prokasy (Ed.), *Classical conditioning*. New York: Appleton-Century-Crofts, 1965.

Kimmel, H. D. Inhibition of the unconditioned response in classical conditioning. *Psychological Review*, 1966, 73, 232-240.

Kimmel, H. D., & Pennypacker, H. S. Conditioned diminution of the unconditioned response as a function of the number of reinforcements. *Journal of Experimental Psychology*, 1962, 64, 20-23.

Landis, C., & Hunt, W. A. *The startle pattern*. New York: Farrar & Rinehart, 1939. (Reprinted by Johnson Reprint Corporation, New York, 1968.)

Larsson, L. E. The relation between the startle reaction and the non-specific EEG response to sudden stimuli with a discussion on the mechanism of arousal. *Journal of Electroencephalography & Clinical Neurophysiology*, 1956, 8, 631-644.

Larsson, L. E. Sensitization of the startle blink and non-specific electroencephalographic response. *Journal of Electroencephalography & Clinical Neurophysiology*, 1960, 12, 727-733.

- Lawson, R. W. Blinking: Its role in physical measurement. *Nature*, 1948, 161, 154-157.
- Lehner, G. F. J. A study of the extinction of unconditioned reflexes. *Journal of Experimental Psychology*, 1941, 29, 435-456.
- Lipkin, S. G., & Moore, J. W. Eyelid trace conditioning, CS intensity, CS-UCS interval, and a correction for "spontaneous" blinking. *Journal of Experimental Psychology*, 1966, 72, 216-220.
- Martin, I., & Levey, A. B. *The genesis of the classical conditioned response*. Oxford: Pergamon Press, 1969.
- Prokasy, W. F. Classical eyelid conditioning: Experimental operations, task demands, and response shaping. In W. F. Prokasy (Ed.), *Classical conditioning*. New York: Appleton-Century-Crofts, 1965.
- Prokasy, W. F., Ebel, H. C., & Thompson, D. D. Response shaping at long interstimulus intervals in classical eyelid conditioning. *Journal of Experimental Psychology*, 1963, 66, 138-142.
- Prosser, C. L., & Hunter, W. S. The extinction of startle responses and spinal reflexes in the white rat. *American Journal of Physiology*, 1936, 117, 609-618.
- Rushworth, G. Observations on blink reflexes. *Journal of Neurology, Neurosurgery & Psychiatry*, 1962, 25, 93-108.
- Sakano, N., & Pickenhain, L. Relation between auditory evoked response and startle reflex in man. *Physiology & Behavior*, 1966, 1, 215-217.
- Sakano, N., & Pickenhain, L. Evoked response and startle blink to strong acoustic stimuli of different signal meaning. *Psychophysiology*, 1968, 5, 1-14.
- Sidowski, J. B., & Nuthmann, C. Induced muscular tension, incentive, and blink rate in a verbal learning task. *Journal of Experimental Psychology*, 1961, 61, 295-299.
- Starr, A. Influence of motor activity on click-evoked responses in the auditory pathway of waking cats. *Experimental Neurology*, 1964, 10, 191-204.
- Tedeschi, D. M., Fowler, P. J., Fujita, T., & Miller, R. B. Mechanisms underlying reserpine induced ptosis and blepharospasm: Evidence that reserpine decreases central sympathetic outflow in rats. *Life Sciences*, 1967, 6, 515-523.
- VanDercar, D. H., Swadlow, H. A., Elster, A., & Schneiderman, N. Nictitating membrane and corneo-retinal transducers for conditioning in rabbits. *American Psychologist*, 1969, 24, 262-264.
- Wojtowicz, S., & Gwozdz, E. Protective function of the orbicularis oculi muscle in electromyographic investigations. *Polish Medical Journal*, 1968, 7, 30-35.
- Woody, C. D. Conditioned eye blink: Gross potential activity at coronal-precruciate cortex of the cat. *Journal of Neurophysiology*, 1970, 33, 838-850.
- Woody, C. D., & Brozek, G. Conditioned eye blink in the cat: Evoked responses of short latency. *Brain Research*, 1969a, 12, 257-260.
- Woody, C. D., & Brozek, G. Gross potential from facial nucleus of cat as an index of neural activity to glabella tap. *Journal of Neurophysiology*, 1969b, 32, 704-716.
- Woody, C. D., & Brozek, G. Changes in evoked responses from facial nucleus of cat with conditioning and extinction of an eye blink. *Journal of Neurophysiology*, 1969c, 32, 717-726.
- Woody, C. D., & Engel, J., Jr. Changes in unit activity and thresholds to electrical microstimulation at coronal-precruciate cortex of cat with classical conditioning of different facial movements. *Journal of Neurophysiology*, 1972, 35, 230-241.
- Woody, C. D., Vassilevsky, N. N., & Engel, J., Jr. Conditioned eye blink: Unit activity at coronal-precruciate cortex of the cat. *Journal of Neurophysiology*, 1970, 33, 851-864.
- Woody, C. D., & Yarowsky, P. J. Conditioned eye blink using electrical stimulation of coronal-precruciate cortex as conditioned stimulus. *Journal of Neurophysiology*, 1972, 35, 242-252.

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