

XII. COMMUNICATIONS BIOPHYSICS*

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A. EYELID RESPONSES IN THE ALBINO RAT: SOME PRELIMINARY OBSERVATIONS

There is at present only one way to determine if an animal has learned something; the animal must behave appropriately following some training or other. This fact seems inescapable unless we can either identify the neural processes that underlie the appropriate change in behavior – or until it is possible to redefine 'learning' in terms of central processes, i. e. , with no reference to behavior. It would seem to follow, then, that if we are to give an account of the neural processes that underlie learning, we are left with a change in the activity of some effector organ to explain, no matter what our view of the learning process. Or to put it another way, we must account for particular kinds of changes in the activity of neurons that innervate the effector organ, the pools of motoneurons innervating portions of the skeletal musculature,

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for example. If this fact is inescapable, it also provides us with the only unambiguous starting point from which to begin the analysis of the neural substrate of learning. In a given learning situation it should be possible, theoretically at least, to separate out from all of the influences on the motoneuron those that are specifically related to the learned change in behavior. The task certainly is formidable; but if it is viewed a step or two at a time, those who pursue it need not seem quixotic. Clearly, the fewer the motoneurons and the simpler their organization, that is, the simpler the response, the greater will be the chance of success. But there are few, if any, simple responses in the behavioral repertoire of mammals. This report concerns a class of relatively simple responses, eyelid responses, that could, perhaps, yield to analysis, even to the point where one might reasonably look "backward" into the nervous system to trace the pathways through which learning is expressed.

Eyelid responses have some desirable features. To begin with, they are accomplished primarily by two antagonistic muscles, orbicularis oculi and levator palpebrae superioris. In man, two small smooth muscles complicate the picture, Muller's muscle in the upper lid, and the inferior palpebral muscle in the lower. But this musculature and the movements it produces seem simple in comparison with nearly any of the behaviors which are mediated by the spinal cord and involve the skeletal musculature. Moreover, eyelid responses seem to be relatively independent of other behavior, in the sense that they are involved in only a few reflexes initiated by other movements. This means that there should not be too many influences related to postural and movement mechanisms to be sorted out from those reflecting "higher" processes. In spite of this apparent simplicity, eyelid responses are subject to both of the basic conditioning procedures through which animal behavior is modified, classical (Pavlovian) conditioning and operant (instrumental) conditioning. This should make this effector system more useful to the analysis of learning. There is also an important practical advantage that eyelid responses have over responses mediated by the spinal nerves. The central portions of the neural pathways mediating eyelid responses are mainly, if not entirely, within the cranium, which means that it is quite feasible to implant electrodes in any of the relevant structures for studies of neural activity in the behaving animal.

I have picked the rat as the subject for this research. This is not an obvious best choice; quite the contrary, disadvantages are apparent from the outset, most of them related to the size of this small animal. But it seemed to me that if the difficulties associated with the size of the animal could be overcome, all of those attributes which make the rat such a good laboratory subject would be a great boon to a long-range program requiring many animals, experiments of a wide variety, and appreciable learning on the part of the researcher and his students. It remains to be seen whether the choice is good. One purpose of this report is to show that

it is possible to study eyelid responses in the rat in appreciable detail. It is also shown that conditioned eyelid responses can be rather quickly established with classical conditioning techniques, thereby satisfying one important requirement for a research program concerned with conditioning.

1. Methods

The data presented here were obtained from 28 male albino rats, descendants of the Sprague-Dawley strain. The rats weighed 250-350 gm.

Eyelid responses were measured in two ways, electromyographically and by a photoelectric method similar to that devised for humans by Bluffield and Holland¹ and for rabbits by Van Dercar, et al.² The electromyogram (EMG) was recorded from two stainless-steel, teflon-insulated, stranded wires, 125 μm in diameter, implanted subcutaneously in the upper eyelid. The EMG was first filtered to eliminate the slow potential changes below 10 Hz. Then after full-wave rectification it was integrated by means of a Grass 7P3 AC preamplifier and integrator with an integration time constant of 20 ms.

To obtain a measure of eyelid position, a photodiode (Texas Instrument 1N2175) and a miniature lamp (Edmund Scientific 40690) were mounted in front of the rat's eye on 18-gauge stainless-steel tubing. The tubing, in turn, was mounted on a connector whose mate was permanently fixed to the rat's skull. Puffs of air were presented to the eye through the tubing. The entire assembly, pictured in Fig. XII-1, was rigid, and its position relative to the eye, therefore, was constant from day to day, provided there was no loosening of the connector on the skull. This advantage of being able to record eyelid position on a day-to-day basis by making a simple connection was achieved by a two-stage implanting procedure, for the connector on the rat's skull had to be put in the right place the first time. A cement base, attached to the skull by stainless-steel screws, was established in the first stage while the rat was under deep pentobarbital anesthesia. A few weeks later, following recovery from the surgery and after adaptation to a restraining device, the rat was immobilized for several minutes by the administration of ether. The rat was lightly sedated by pentobarbital (10 mg/kg) before the ether was given. While the animal was immobilized the connector was attached to the cement on the skull, but only through a short piece of 16-gauge wire. That is, the connector was attached to one end of the wire, and the other end of the wire was imbedded in the cement on the rat's skull. The position of the connector could then be adjusted by simply bending the wire while the output of the photodiode circuit was monitored. This was done after the animal had recovered sufficiently from the ether to eat in the restraining device. When an optimum position was found, the connector itself was imbedded in the cement while the rat remained awake. The optimum position was one in which

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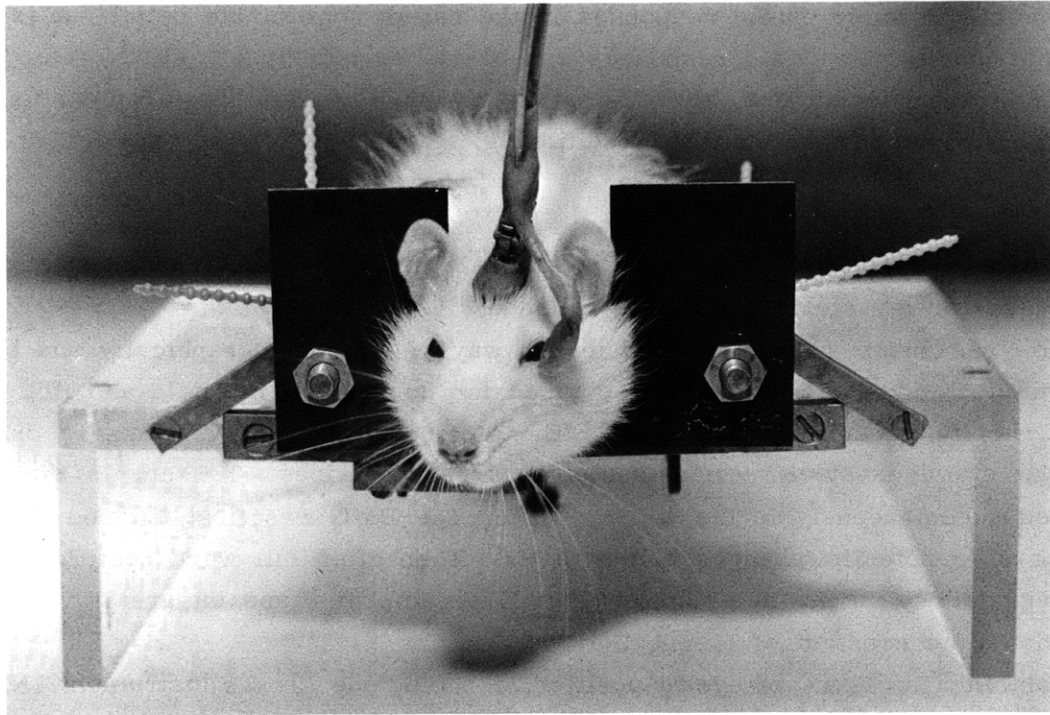


Fig. XII-1. Assembly for measuring position of eyelid shown in place on rat in restraining device with stock to prevent animal from raising forepaws to the face. Photodiode, miniature light source and tube for delivering air puffs all contained in portion of assembly in front of rat's eye.

eyelid closures gave large signals, while small movements of the eyelids or eye which were associated with other behavior, especially eating, produced relatively small ones. When the photodiode and lamp were aimed at the eye, there was an increase in the amount of reflected light when the eyelids closed.

The measures of eyelid responses were recorded on FM magnetic tape and on a Grass Model 7 polygraph. A PDP-4 computer was used to average the photodiode and integrated EMG signals and to determine peak amplitudes of individual responses. Permanent records of the average responses were made on an X-Y plotter, and some amplitude measurements were made from these plots. Data were obtained from 6 rats in which both eyelid position and the EMG were recorded simultaneously. A PDP-12 computer was used to compute correlation coefficients (Pearson's r) for peak amplitudes of the two signals and for their average waveforms. The occurrence of conditioned responses or of "spontaneous" blinks was determined from the raw polygraph records in accordance with criteria noted below.

All measurements were made while the rats were restrained in a dark, sound-attenuating box. A kind of stock was added to the restrainer described by Hall et al.³ to prevent the rats from grooming, since movements of the forepaws over the face

gave rise to photodiode responses. Rats were adapted to the restrainer for varying periods of time, but never less than a week before data were collected. A background masking noise, approximately 70 dB SPL, was present at all times and was generated by a blower which was used to ventilate the box and by a wideband noise generator.

In some experiments food pellets (Noyes, 45 mgm) were presented to the rats by means of a Gerbrands pellet dispenser and a motor-driven cup that carried the pellet to the rat. This feeding cycle required 6 s. The pellets were presented on variable interval (VI) schedules with mean values of from 0.5 min to 1.5 min. The rats were maintained at approximately 80% of their ad libitum feeding weights.

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 that had rise times and fall times of 5 ms.

A three-way solenoid valve was used to generate puffs of air by switching the flow from a high-pressure airline between tubing going to the rat and a bypass. Durations of the air puffs varied from 50 ms to 180 ms. The intensity was controlled by a regulator and monitored at the input to the valve. No attempt has been made thus far to determine the pressures at the rat's eye, and the intensities noted below refer only to the pressures at the valve. The delay between the onset of the solenoid current and the arrival of the pressure wave at the eye was measured, and adjustments were made accordingly in the stimulus marking pulses.

The procedures used in particular experiments are described below, together with the results.

2. Results

a. "Spontaneous" Blinking

One need not look very long at a rat before it becomes clear that this animal does not blink nearly as often as man does. A reasonable estimate of how frequently a rat does blink is of more than academic interest, for it is well established that the rate of conditioning is sometimes related to the rate of responding that obtains before reinforcement commences. In the case of free operant behavior, the so-called operant level becomes the baseline against which the conditioned changes in behavior are evaluated. Determinations of how frequently the rat blinks "spontaneously," i. e., when there is no stimulation of kinds known to elicit blinks, were made under two conditions. On at least two days eyelid movements, measured by the photoelectric technique, were recorded during 1-hour periods, beginning half an hour after the animal was placed in the restrainer. In these sessions food was withheld during the recording period, although pellets were presented on variable

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interval (VI) schedules, quite independent of the behavior, in the preceding half-hour. In two additional sessions, eyelid movements were recorded in the same way, except that feeding was continued on the same schedules (1 min or 1.5 min) during the recording period. Response counts were obtained from the polygraph records. Any deflection of the trace whose amplitude was equal to 40% of the maximum deflection 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 and those related to several sources of "noise," while at the same time including nearly all of the eyelid responses that one would not hesitate to call blinks.

Data from both experimental conditions are summarized in Fig. XII-2 where distributions of response rates for 26 rats are presented. Each rat is represented at

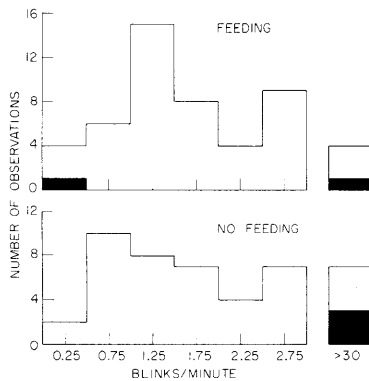


Fig. XII-2.

Distributions of rates of spontaneous blinking for 26 rats (upper histogram) and 24 rats (lower histogram) measured in 1-hour periods under two conditions. Each rat is represented at least two times in each histogram, except for two subjects not run under no-feeding condition.

least twice in each histogram by rate measures from the individual sessions. This presentation seemed preferable to some kind of average across sessions for each subject, since it was apparent that the rate for a particular subject could change markedly from one session to the next. The two shaded blocks in the upper histogram illustrate this, for they were measures from a single rat in consecutive sessions. In general, however, individual rats seem to have characteristic rates, and the variability, both within and across subjects seemed low. An apparent excess of high rates under the no-feeding condition was due largely to the performance of one subject, as indicated by the shaded area in the lower histogram.

The median rate of spontaneous blinking was 1.5/min under both feeding and no-feeding conditions. This was unexpected in view of the fact that presentation of the food pellets and their ingestion were accompanied by the blink component of the startle response on the one hand (see below) and, frequently, by many eyelid closures during the consummatory behavior. These responses were not counted, of course, in the measurements of spontaneous blinking, and their occurrence appears to have had no effect on that behavior.

Spontaneous blinking in the rat tends not to be periodic, as the cumulative response records of Fig. XII-3 indicate. They tend rather to occur in groups, and my impression is that these periods of activity are often periods of other more general movement. Although relatively small bursts of eyelid responses are not necessarily

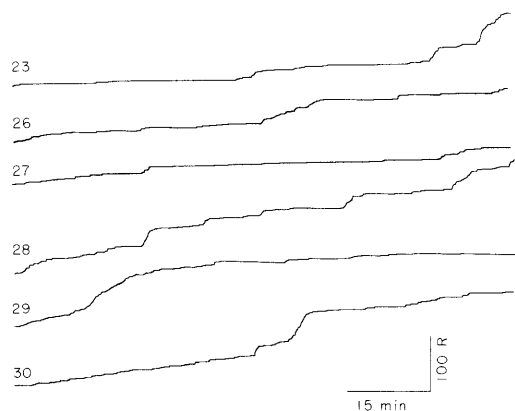


Fig. XII-3.

Cumulative response records of spontaneous blinking from 6 rats recorded during sessions of 1.5 hour duration. Voltage criterion used to define response was determined by Schmitt trigger whose input came from photodiode circuit. Criterion was determined empirically for each subject to avoid false counts attributable to signals related to other movements.

accompanied by other obvious activity, the larger bursts like those in the records for rats 29 and 30 nearly always occur during periods when there are movements of the head, trunk or limbs. It can also be seen in Fig. XII-3 that often the rat does not blink for periods of 10-20 min.

b. Blink Component of the Startle Response

It has been noted that the rats often blinked in response to activation of the feeder, a loud rotary solenoid device. The rat's startle response to strong acoustic stimuli has received a great deal of attention recently, particularly in studies of habituation. It is usually measured by stabilimeter techniques that are responsive to general skeletal movements. The blink component of this generalized response is of interest to us because of the possibility that it might confound eyelid responses of a more specific kind, particularly in aversive conditioning situations wherein frightened animals may exhibit lowered thresholds for startle responses. Startle responses to the feeder were examined, therefore, in 10 rats during the sessions in which spontaneous blinking was measured. Pulses indicating feeder activation had been recorded on magnetic tape, and it was possible thereby to compute average responses and to make a number of other measurements.

Average responses from four rats, from two different groups, are presented in Fig. XII-4, together with plots of peak amplitudes of individual responses from the same sessions. The data from rats 2 and 3 were obtained in sessions in which pellets were presented throughout on a VI 0.5 min schedule, but recording did not

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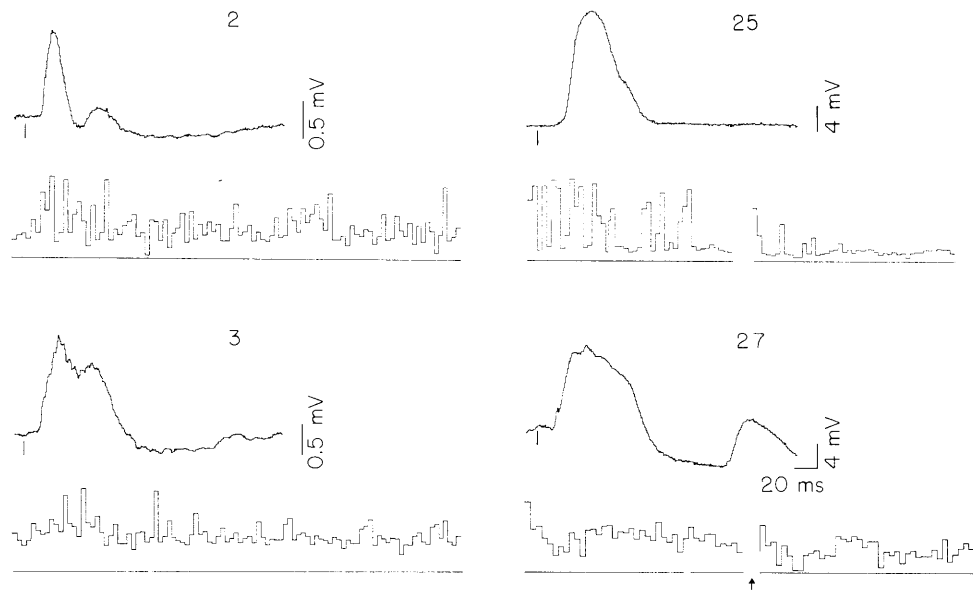


Fig. XII-4. Average startle responses from 4 rats to loud acoustic stimulus generated by activation of pellet dispenser. Averages for rats 2 and 3 for first 50 responses; for rats 25 and 27 averages are for first 5 responses. Peak amplitudes of individual responses shown below averages for each subject. For rats 2 and 3, 100 responses were measured from continuous recording period. For rats 25 and 27, 40 or 45 responses from beginning of sessions were measured and the same number after 1-hour interruptions in feeding, noted by breaks in curve at arrow.

commence until the session had been in progress for half an hour. There was some small decrement in response amplitude for both subjects, but the startle was really quite persistent in both cases. It should be pointed out that this was not the animals' initial experience with the feeder; they had been exposed to it daily for several weeks. It was not unusual for startle responses to the feeder to persist over such periods of time, although they ultimately became negligible in most animals. The data from rats 25 and 27 were obtained from sessions in which one half-hour of feeding on a VI 0.75 min schedule was followed by 1 hour of no feeding and then by another half-hour of feeding. The response decrements within the session are clearer here, although the principal part of it occurs quickly in rat 27. The decrements appear to be a genuine habituation of the startle response. Note, for example, the spontaneous recovery of the responses upon resumption of the feeding.

Waveforms of the startle blinks illustrated in Fig. XII-4 are quite representative of those from all of the rats. They were also plotted on more extended time bases for the purpose of making measurements of response latencies. With one exception, onset latencies ranged from 10 to 16 ms. The exception was rat 25 whose rather late response, with an onset latency of 20 ms, is shown in Fig. XII-4. The median latency

of the initial peak was 35 ms (range, 20-50 ms). These values were compared with comparable measures for unconditioned responses to air puffs of several intensities in the same subjects. There appeared to be no consistent difference in the onset latencies of the two responses. Startle blinks did peak earlier, however, and in most instances were of shorter duration than responses to corneal stimulation. Compare the durations of startle blinks in Fig. XII-4, for example, with the unconditioned responses to air puffs in Figs. XII-6, XII-7, and XII-9. (Note the different time scale used in Figs. XII-6, XII-7, and XII-9.) In three rats startle blinks did not peak earlier than responses to air puffs, but a discussion of these exceptions will be deferred until the compound nature of the response to the air-puff stimulus has been considered.

c. Eyelid EMG and Measure of Eyelid Position

Because I have relied heavily upon the photoelectric measurements of eyelid position as the measure of eyelid responses, it was desirable to know how this measure was related to the underlying muscle activity. Edwards⁴ in this laboratory had begun the investigation of the eyelid EMG in lightly anesthetized rats. In the present work 6 rats were exposed to air puffs of 4 different intensities at one of three repetition rates in one (2 rats) or two (4 rats) sessions. The data from these few subjects are still too cursory to permit an adequate description of the eyelid EMG, or its relationship to the measure of position, but several points of interest deserve mention at this time. One of these is illustrated in Fig. XII-5 where the raw EMG is shown in the upper trace and the integrated signal in the lower trace of each pair. The response in A has a brief early component with a latency of approximately 12 ms which is followed after a short pause by a component of greater amplitude and duration. The responses in B and C were recorded from the same rat during the same series of stimuli (air puffs of moderate intensity presented at a rate of 1/30 s), and the two components of the response in A are seen to be at least partly separable. Onset latencies of the early response ranged from 6-18 ms, as measured in both the raw EMG and averaged integrated responses. It was difficult to determine in either measure the range of onset latencies for the later response because the two components were not always as nicely separated as they appear to be in Fig. XII-5. When either component was strong it tended to encroach upon the other because the early one had a longer duration, while the late one had a shorter latency in such cases. The onset latency of the late response was approximately 15-30 ms. Peak latencies of the early and late components, estimated from the averaged integrated records, were approximately 30 ms and 80 ms, respectively. This apparent dual composition of the eyelid EMG in the rat was also found by Edwards;⁴ and Hicks,⁵ also in this laboratory, found response patterns in single orbicularis oculi motoneurons with

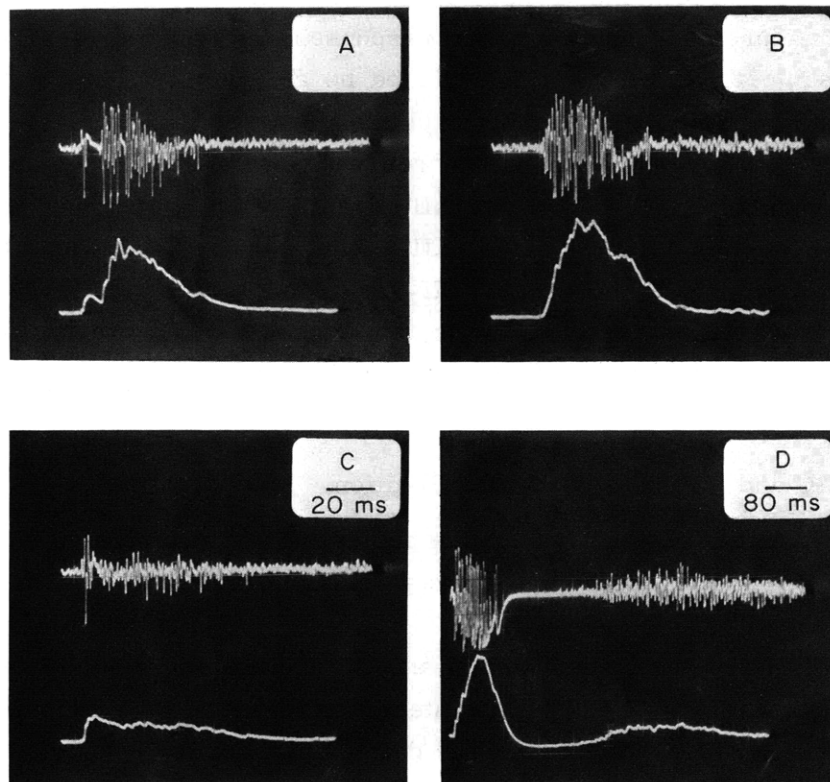


Fig. XII-5. EMG recorded from upper eyelid of rat 33 (A, B, and C) and rat CC2 (D) in response to air puff. Raw signal in upper trace and integrated signal in lower. Voltage calibration in D is 50 μ V for upper traces, and 80 μ V for integrated signal. Note different time scale for traces in D.

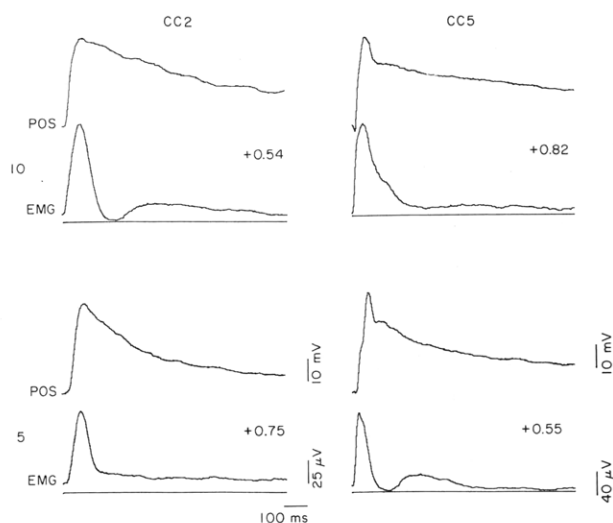


Fig. XII-6. Averages of 50 eyelid responses recorded simultaneously as photoelectric measure of position and as integrated EMG for two rats under two stimulus conditions. Relative stimulus intensities noted by numbers at left. Correlation coefficients for each pair of waveforms also indicated.

two similar components.

The relationships between the photodiode indication of eyelid position and the integrated EMG responses were examined in several ways. Note first that the mean difference in the onset latencies of the photodiode signal and the EMG was 11.0 ms, while the difference in peak latencies was 25 ms. Waveforms of the averaged responses exhibited a remarkable similarity in many cases, with correlations as high as +.95 and higher than +.70 in most instances. The most common difference between the two measures of eyelid activity was in the relative durations of the two signals. With most of the stimuli employed in these experiments the eyelid closures were quite prolonged, clearly longer than a second which was the customary analysis time. Although some remnant of the EMG response sometimes persisted for comparable periods, the duration of the major portion of the response rarely exceeded 200-300 ms. This can be seen in Fig. XII-6 where averaged responses of both types are shown for two rats. Also illustrated here is one case in which the two signals exhibit reduced correlations. Correlation coefficients for each pair of waveforms are also shown, and the lowest correlation for each subject is related to the pair in which the averaged EMG shows a period of inhibition following the initial excitatory response. That the negative-going wave in the average response is indeed an indication of an inhibition of muscular activity can be seen in the raw EMG response in Fig. XII-5D; there is a period of silence following the initial burst of activity which in turn is followed by a resumption of tonic activity. This pause in the activity of the muscle does not appear to be reflected in the position of the eyelid. The differences in the EMG responses in each rat could not be attributed directly to a difference in the stimulus because the inhibitory type of response occurred with the nominally stronger stimulus in CC2 and with the weaker one in CC5.

Another response factor which tends to mitigate the correlation between the measure of eyelid position and the integrated EMG is an increase in the duration of eyelid closure. This is illustrated by data from rat 33 in Fig. XII-7 where average responses obtained under three stimulus conditions are shown. Relative stimulus intensities were 10 and 5 (indicated by the first of two numbers in parentheses) and stimulus durations were 190 ms, 130 ms or 80 ms. With decreases in the intensity or duration of the stimulus the duration of eyelid closures was progressively less, the waveform of the photodiode response became simpler, and the correlation between the waveforms was correspondingly greater.

Also shown in Fig. XII-7 are plots of peak amplitudes of the two signals for the same series of 50 stimuli from which the average responses in the upper right-hand part of the figure were derived. The high correlation, +.81, is clear in this instance, indicating simply that the two measures of eyelid responses changed over time in a very similar way. Similar plots are shown in Fig. XII-8 for two other

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rats who also exhibited relatively high correlations between the two measures in longer series of stimuli, 300 presentations of the "5-lb", 80-ms air puff presented at a rate of 1/5 s. Unfortunately, the peak amplitudes were not always so highly correlated. Correlation coefficients for two other rats, for the same stimulus

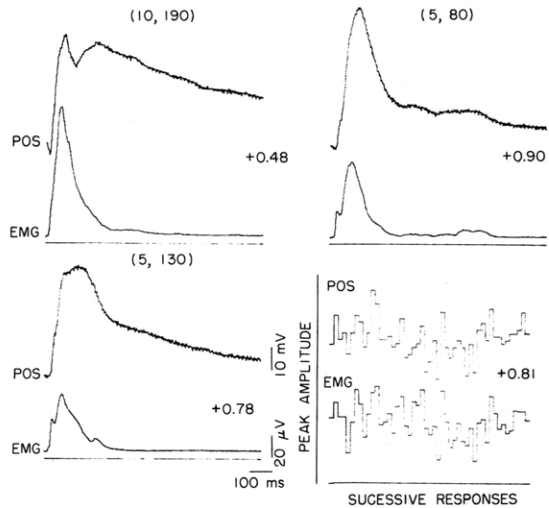


Fig. XII-7.

Averages of 50 eyelid responses recorded as change in position and as EMG for rat 33 under three stimulus conditions. First of two numbers in parentheses indicates relative stimulus intensity; the second number the duration of the air puff in ms. Correlation coefficients for pairs of waveforms shown as in Fig. XII-6. Plots in lower right-hand corner are for peak amplitudes of the two signals for the 50 responses included in the two averages for the (5, 80) stimulus condition.

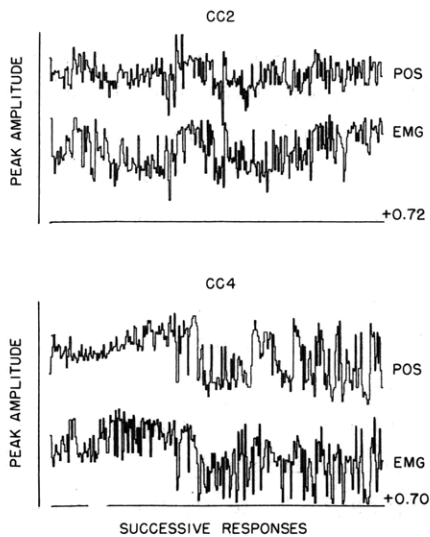


Fig. XII-8.

Peak amplitudes of 300 single eyelid responses as indicated by photoelectric measure of position and integrated EMG for two rats. Air-puff stimuli were of moderate intensity, 120 ms duration, presented at rate of 1/5 s. Correlation coefficients for pairs of traces shown at right of zero voltage line for integrated EMG. (Zero voltage reference omitted for measure of position to simplify figure.)

conditions as those described for Fig. XII-8, were only + 49 and +. 55, for example, and there were instances when the correlation coefficients were near zero for some subjects. One other feature of the curves in Fig. XII-8 should be noted, namely, the large variability in response amplitudes. Some of this variability must undoubtedly be related to the experimental conditions that from the rat's point of view can only

be disquieting at least.

d. Conditioned Eyelid Responses

Conditioned eyelid responses are usually established in human subjects with the use of air puffs as unconditional stimuli (UCS). The air puff is a convenient stimulus for at least three reasons: it is easy to administer, it does not cause tissue damage; and, in studies of human eyelid responses at least, it is painless. It seemed desirable to use it as the UCS in conditioning eyelid responses in the rat. The experiment described here was our first convincing demonstration that this would be possible.

Four rats were exposed to a classical conditioning procedure after the 4 sessions in which spontaneous blinking was measured. The conditional stimulus (CS) was a tone burst with a frequency of 3 kHz, a duration of 425 ms, and an intensity of approximately 75 dB SPL. The UCS was an intense air puff, 30 psi at the input to the valve, with a duration of 120 ms. The CS-UCS interval was 425 ms. Sixty trials were presented in each session on a VI 1-min schedule. Intervals in the schedule ranged from 15 s to 2 min. Conditioning was carried out for the first 5 days, and this was followed by a full session of extinction. On the next 2 days the trials were divided so that 30 extinction trials were followed by 30 conditioning trials (session 7) or vice versa (session 8). Two additional days of extinction terminated this part of the experiment.

Development of the conditioned eyelid response is shown in Fig. XII-9 in the average

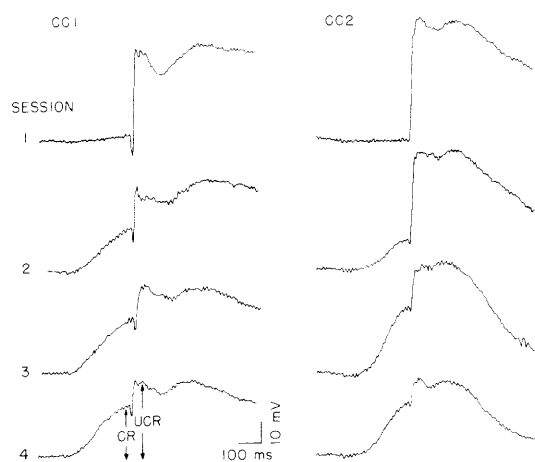


Fig. XII-9.

Average eyelid responses from first 4 conditioning sessions for 2 rats. Averages include responses from all 60 trials in each session. Responses in this and succeeding figures from conditioning experiments were photoelectric measures of eyelid position in which eyelid closures are indicated by upward deflections. In lowest trace for CC1 arrows indicate how amplitudes of conditioned response (CR) and unconditioned response (UCR) were measured.

responses of 2 subjects from the first 4 sessions. The averages are for all 60 trials in each session. Only the unconditioned response (UCR) was present during the first session, but in succeeding sessions the conditioned response (CR) became prominent. Onset

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latencies of the CR in this and succeeding experiments with CS-UCS intervals of 400-450 ms ranged from 125 ms to 350 ms. The modal value was approximately 200 ms. The duration of the CR, measured in averages of 10 responses recorded during extinction when the CR was not confounded by the UCR, varied from a minimum of 325 ms to more than 800 ms.

Development of the eyelid CR and its extinction in all four subjects is clear in Fig. XII-10 where two measures of the CR are presented. One is the percentage of trials on which CR's occurred for blocks of either 30 or 60 trials. Any deflection greater than 2 mm which occurred in the interval starting 100 ms after the onset of the CS and ending with the onset of the UCS was counted as a CR, provided it was not clearly related to some on-going movements that commenced before the

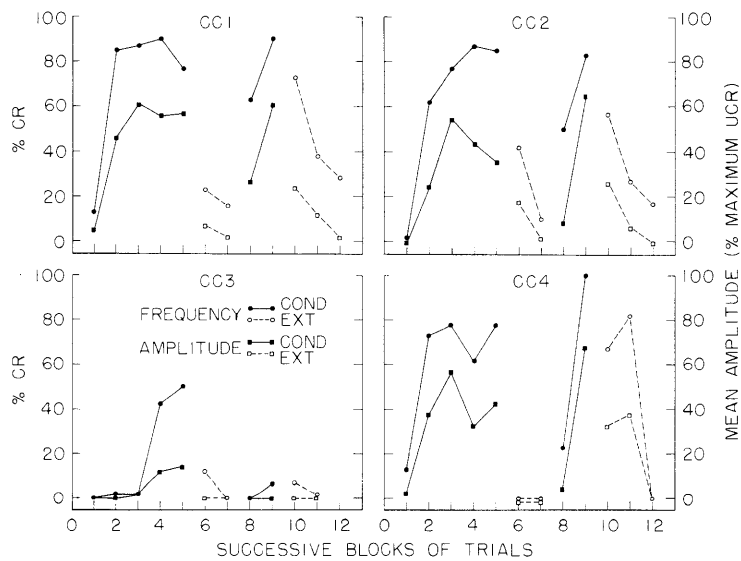


Fig. XII-10. Conditioning and extinction of eyelid responses in 4 rats shown in two measures: percentage of trials in which CR's occurred, and mean amplitude. Both measures for blocks of 60 trials, except for blocks 7-10 which contained only 30 trials each. Blocks 7 and 8 from the 7th session; blocks 9 and 10 from the 8th session in which the procedures were changed halfway through each session.

onset of the CS. The amplitude criterion was adopted as a rather conservative one after looking at many records, for deflections of 2 mm could be identified with confidence as CR's even though they were only 5-10% of the amplitude of a full blink. The other measure was the mean amplitude of the CR for the same blocks of trials. Amplitudes of CR and UCR were measured as indicated in the last trace for CC1 in Fig. XII-9. CR amplitudes have been expressed as percentages of the maximum UCR amplitude for each rat. The close correspondence between the measures of

frequency and amplitude is also clear in Fig. XII-10.

The data from subject CC3 were not very satisfactory, but they were included in Fig. XII-10 to point out a difficulty with the method used to measure eyelid position. The connector on the skull is sometimes dislodged by the infiltration of tissue between the skull and the cement. This usually takes about a month if it is to happen at all, but that can be a serious restriction on experimental designs. CC3 lost its connector on the twelfth day of conditioning, and the small responses seen during the later stages of the experiment indicate a change in the position of the assembly on the rat's head.

It remained to be shown that the changes in Fig. XII-10 were of an associative kind, and not the result of sensitization or pseudoconditioning. The three remaining rats were, therefore, exposed to a differential conditioning procedure in which a tone burst of one frequency (CS+) was always reinforced by the UCS on 30 trials in each session, while a tone burst of another frequency (CS-) was never reinforced on another 30 trials. The order of presentation of the two stimuli was irregular. Moreover, the CS+ was not the 3-kHz tone used as the CS in the preceding part of the experiment, but a 10-kHz tone. The 3-kHz tone became the CS- in the discrimination procedure. Appropriate discriminative CR's under this procedure would indicate first that the rat could perceive both stimuli and, second, that it "knew" which was followed by the air puff.

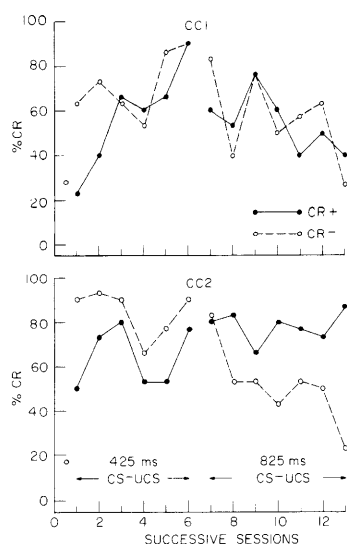


Fig. XII-11.

Differential conditioning in two subjects shown in the frequency measure for successive sessions in which the duration of the CS and CS-UCS interval were 425 ms during the first 6 sessions and 825 ms in the last 7. CS+ was a 10-kHz tone burst; CS- was a 3-kHz tone burst that was CS in preceding part of experiment shown in Fig. XII-10.

The effects of the discrimination training are presented for two of the rats in Fig. XII-11. Reintroducing the air puff led to a clear increase in the number of responses elicited by the CS-. Response levels in the last extinction session in the

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preceding part of the experiment are indicated by the unconnected points at the left of the figure. The behavior was to some extent discriminative in the first session, for there were clearly more responses to the CS- than to the CS+. In succeeding sessions there was an increase in the strength of the response to the CS+, which is clear in the data from CC1 at least, but responses to CS- gave little sign of weakening. A similar failure had been encountered in an earlier discrimination procedure with two rats conditioned with electric shock to the snout as the UCS. A study of human eyelid conditioning by Hartman and Grant⁶ indicated that performance under a differential conditioning procedure was better with relatively long CS-UCS intervals, primarily because there were fewer responses to the CS-. The CS-UCS interval was lengthened from 425 ms to 825 ms in the present experiment to see if this would lead to fewer responses to the CS-. The data of Fig. XII-11 suggest that this was so, although there were no controls for what might have happened had the interval not been lengthened. The discriminative behavior finally achieved by CC2 was at least promising, even if the poor performance of CC1 was not.

More convincing evidence of discriminative behavior was clearly necessary before one could accept the eyelid responses to tone bursts as indicants of genuine conditioning. To this end another group of 4 rats was conditioned under similar circumstances. The CS was a 3-kHz tone burst whose duration was 425 ms during the first 5 sessions of 60 trials each. On the sixth day, the duration of the CS and the CS-UCS interval were increased to 825 ms. This session was followed by 5 days of discrimination training in which the 3-kHz tone burst was retained as the CS+ and the 10-kHz tone burst was used as the CS-. One other new feature was employed with these rats: they were fed between trials on a VI 0.75 min schedule. It was our feeling that the discriminative behavior might be more readily established if the animals were not unduly anxious. The data presented in Fig. XII-12 seem to have confirmed this suspicion. Strong responses to the CS were established in the first 6 sessions, and discriminative behavior developed in an orderly, though hardly uniform, fashion in at least three of the four rats. Even the less orderly behavior of OP1 during the differential conditioning showed consistently more responding to the CS+ than to the CS-.

Measurements of CR amplitudes in this second conditioning experiment confirmed the finding from the first experiment, insofar as the changes in amplitude essentially paralleled the changes in response frequency. This can be seen by comparing the lower curves of Fig. XII-13 with the plots for CC5 in Fig. XII-12. Amplitudes of the UCR shown in Fig. XII-13 display 3 features of interest. The first is the decrease during the initial conditioning as the CR is established, reflecting probably a reduction in the effective stimulus due to conditioned closures, or partial closures, of the eyelids. The second is the marked increase in amplitude in the 6th session

when the CS-UCS interval was lengthened to 825 ms. The duration of the CR established with the shorter interval was such that the response was nearly ended before the UCS was presented, and the stimulus was no longer mitigated by the occurrence of that response. In all four rats the UCR was larger in this session than it was generally during conditioning, but only in CC5 and CC6 were the UCR's larger in

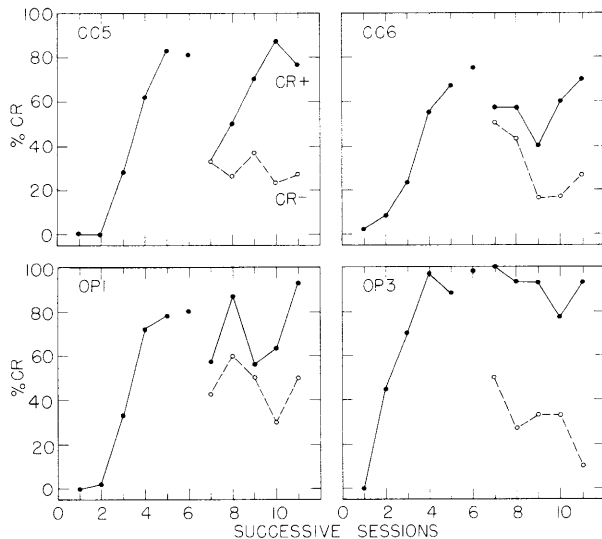


Fig. XII-12.

Conditioning of eyelid CR in 4 rats followed by differential conditioning. Measures for blocks of 60 trials in each session. CS-UCS interval 425 ms in first 5 sessions, increased to 825 ms in 6th. In differential conditioning of last 5 sessions CS+ was the same 3-kHz tone burst used as CS in first 6 sessions; CS- was a 10-kHz tone burst.

this session than in the first. The third feature is the generally higher amplitudes during the differential conditioning, which may also reflect the failure of the CR to weaken the effective stimulation at intervals as long as 825 ms.

The variability in the acquisition of discriminative CR's in the experiment of Fig. XII-12 suggested that it might be desirable to use the differential conditioning procedure from the outset of training, rather than beginning with simple conditioning and introducing the discrimination procedure at a later stage. The relative efficiencies of the two procedures for establishing a discriminative CR was also of interest. Three rats were trained under the same conditions as those used in the differential conditioning phase of the last experiment, but this procedure was in effect from the first day of conditioning to the last. Eyelid responses were measured by the

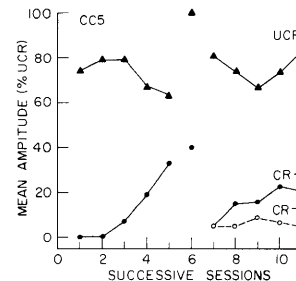


Fig. XII-13.

Mean amplitudes of eyelid responses for one rat from the same experiment shown in Fig. XII-12. Amplitudes expressed as percentage of maximum amplitude of UCR.

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photoelectric method in 2 rats, and electromyographically in another. The EMG data were disappointingly noisy in this case, and the differential conditioning did not proceed as well as it did in the other two rats whose performances are summarized in Fig. XII-14. Even in the somewhat erratic behavior of these animals it seems clear that establishing the auditory discrimination with differential conditioning from

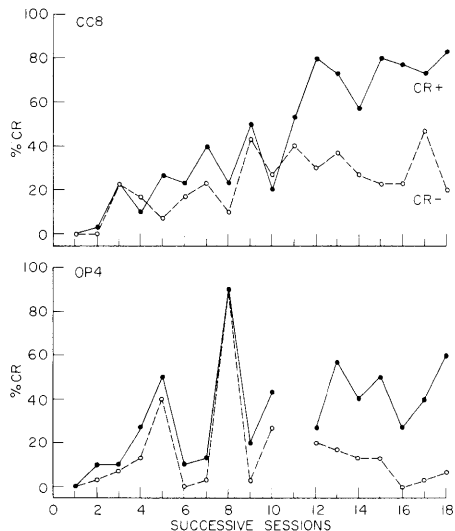


Fig. XII-14.

Differential conditioning for 2 rats. CR frequency presented as percentage of 30 CS+ and 30 CS- trials in each daily session. CR+ was a 3-kHz tone burst; CS- was a 10-kHz tone burst. Missing data for OP4 in 11th session was inadvertently erased from magnetic tape.

the outset required at least 12-14 days when 30 trials each of the CS+ and CS- were presented in each session. Rigorous comparisons with the previous group cannot be justified, but it seems likely that differential conditioning from the outset is not an efficient way to achieve the auditory discrimination. Of particular interest are the parallel changes in behavior under the two stimulus conditions before the session in which the first differential responding is evident. In any case the combined data from the three conditioning experiments provide ample evidence that the eyelid CR's are indicative of real associative processes which in part characterize adaptive discriminative behavior.

2. Discussion

The results presented here are not much more than descriptions of the behavior that I intend to study, but such descriptions would seem to be a logical first step in the investigation of any behavior. I hope that they at least reflect my intention not to ignore the behavior of the organism, as is so often done in neurophysiological investigations of conditioning. It must be clear from just these preliminary results that the notion of a simple effector system is certainly a relative one, for those two, or few, little muscles of the rat's eyelid produce an assortment of behaviors

with considerable variety and more than adequate complexity. I fail to see how the investigation of eyelid conditioning can proceed with much profit in ignorance of this variety and its underlying causes.

Measurements of spontaneous blinking provided no insight into the function of blinks which are not evoked by obvious exteroceptive stimulation. The low rates of responding suggest that the blink is not very important to the rat in maintaining a moist corneal surface, although it may incidentally serve that purpose under normal conditions. In this regard one might recall that rats can be maintained under general anesthesia with their eyes open for many hours without apparent damage to the cornea.

It was somewhat disconcerting, though not surprising, to find that blinks often accompany other movements of the head and body. The muscles of the middle ear, partly innervated by the facial nerve, also show this property in the cat (Carmel and Starr⁷ and Starr⁸), and there are of course the facial expressions of man that accompany many of his activities. It is not necessarily the case that such eyelid closures in the rat are indicative of well-organized reflexes with more or less complex neural substrates. We can still hope for something simpler than that.

It was found that the eyelid response to puffs of air had two components, a very early one with latencies as short as 6 ms when measured in the EMG, and a later more prolonged response with an onset latency of approximately 15-30 ms. It is not clear at present whether these two components of the response are related to distinct reflex arcs with different afferent limbs, or whether a single reflex with complex response characteristics is involved. Hicks⁵ presented some evidence suggesting that the early component in motoneuron responses to air puffs might be related to stimulation of the eyelids, while the later component was due to stimulation of the cornea. The dual response of the rat's eyelid to air puffs resembles the orbicularis oculi reflex in humans, in that the human eyelid response to taps of the face in the orbital region also has two components. The resemblance may be superficial, but the disputed nature of the early component in the human response may point to a relevant question for the rat. Kugelberg⁹ and Rushworth¹⁰ have maintained that the early component in the human response is proprioceptive, i. e., related to stimulation of receptors in the facial musculature. Shahani and Young¹¹ and Penders and Delwaide¹² on the other hand relate the early component, as well as the late one, to cutaneous stimulation. If the adequate stimulus for the early component of the rat's eyelid response to air puffs requires stimulation of the eyelid, it will then be necessary to determine whether cutaneous or proprioceptive afferents are responsible. The fact that muscle spindle organs have been difficult to find in the superficial facial musculature of man and other species would tend to favor the hypothesis that the receptors are cutaneous, but it remains to be seen

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whether muscle afferents can be found in the rat and whether they might possibly be stimulated by the air puffs used in these experiments. The situation is complicated further by the possibility that the response to relatively strong air puffs may have a startle component. It was seen that startle blinks to strong acoustic stimulation had latencies as short as 10 ms, early enough to be confused with the early component of the response to air puffs. That a startle component may well form a part of the response to air-puff stimuli is indicated by the observation that the response frequently involves movement of the whole head and a prominent twitch of the pinna. Movements of the pinna can be observed even in the anesthetized rat. These various components will have to be separated before we can understand the reflex that is of interest in the conditioning experiments.

Relationships between the measure of eyelid position and the eyelid EMG are seen to be complex. The frequently high correlations between the two signals, some of which were illustrated here, serve, in part at least, to validate both measures of eyelid activity. The sometimes modest, but sensible, correlations simply indicate that the position of the eyelid is not always related simply to the over-all amount of activity in the muscles of the eyelid. It seems probable, for example, that prolonged closures of the eyelids are accomplished by relatively few and/or relatively small motor units whose activity does not yield large summated potential changes to be seen by gross electrodes. Abnormally low correlations between the two signals suggest, however, that errors of measurement may also be involved, and the sources of these errors will have to be found.

The discriminative behavior established in these experiments leaves little doubt that the conditioned eyelid responses are indeed just that, and not responses resulting from sensitization or pseudoconditioning. The latency of the CR was too long (125-350 ms) for the response to qualify as a sensitized startle (alpha) response to the acoustic stimulus. Such responses have been encountered in 2 rats in an experiment in which electric shocks to the snout were used as the UCS and the tone-burst CS had an intensity that was 20 dB greater than that of the CS used in the present experiments. The latencies of those responses were 20 ms, and their durations were no more than 125 ms, being very similar, then, to the startle blinks which occurred in response to feeder activation. CR's in the same animals to a less intense CS had latencies of 250-300 ms and durations of 300 ms or more.

Woody and Brozek¹³⁻¹⁵ were the first to recognize the value that eyelid reflexes might have in neurophysiological investigations of conditioning. Not long after we had begun investigating eyelid responses in this laboratory they were able to publish descriptions of their studies of the glabellar reflex in the cat and changes in the activity of the facial nucleus during conditioning. More recently Woody and his associates^{16,17} have extended their findings to include changes in the activity of

coronal-precruciate cortex under the same kind of conditioning procedure. It seems likely, however, that the changes in the very short-latency responses that they recorded were not really indicative of a conditioning process. Latencies of their CR, as measured in the facial nucleus, were 13-24 ms, which is much earlier than any response I know of that is accepted by students of conditioning as related to a true associative process. Woody and his co-workers, while believing that they do have a real conditioned response, acknowledge this problem. A more thorough review of their findings and an attempt to understand their significance should wait upon the resolution of this problem.

Eyelid responses in rats have received very little attention, undoubtedly because of difficulties in measuring the response in this small animal. Hughes and Schlosberg¹⁸ were the first to condition an eyelid reflex in the rat, and Biel and Wickens¹⁹ used the rat and eyelid CR's in an investigation of vitamin B₁ deficiency. Ebel²⁰ has more recently been concerned with eyelid CR's in the rat, but to the best of my knowledge has not yet presented much information on their development. Our techniques for measuring eyelid responses in the rat are not yet so simple that I can recommend the rat as a practicable alternative to any of the larger species used in eyelid conditioning studies, at least where large numbers of subjects are used in experiments with group designs; but there is hope that this possibility will develop in the evolution of experimental techniques.

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