US-US conditioning of the rabbit’s nictitating membrane response: Emergence of a conditioned response without alpha conditioning

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Two experiments were conducted to examine whether classical conditioning of the rabbit’s nictitating membrane response (NMR) could be obtained when paraorbital electrical stimulation served as both the conditioned stimulus (CS) and the unconditioned stimulus (US). In Experiment 1, a range of stimulation intensities (0.1, 0.25, 0.5, 1, and 2 mA) and durations (10, 25, 50, and 100 msec) were presented in order to detail the frequency, latency, amplitude, and duration of unconditioned responses (URs) to the paraorbital US. On the basis of the response characteristics obtained in Experiment 1, a set of stimulation values was adopted in Experiment 2 to (1) examine whether classical conditioning could be obtained when paraorbital electrical stimulation served as both the CS and the US, and (2) determine whether conditioning was related to the intensity of the CS. The data from Experiment 1 indicated that response frequency, amplitude, duration, latency, and peak latency were a function of stimulus intensity and duration. The results of Experiment 2 revealed significant levels of conditioned responding that emerged as a function of CS-US pairings. There were no systematic changes in the UR to the CS as a function of CS-US pairings. Moreover, the levels of conditioning obtained using a paraorbital CS were a function of CS intensity. The results are discussed in terms of the relevance of US-US conditioning studies to contemporary theories of associative learning and the search for the neural substrates of learning and memory.

A major purpose of the present experiments was to examine whether classical conditioning of the rabbit’s nictitating membrane response could be obtained when paraorbital electrical stimulation served as both the conditioned stimulus (CS) and the unconditioned stimulus (US). The presentation of two USs follows a tradition of US-US conditioning experiments first described by Pavlov (1927) and examined more recently in the context of bidirectional conditioning (e.g., Asratyan, 1965; Gormezano & Tait, 1976), counterconditioning (Dearing & Dickinson, 1979), and associative transfer (Scavio, 1974; Scavio & Gormezano, 1980; Tait, Quesnel, & Ten Have, 1986). Whereas previous US-US conditioning studies have employed different USs (e.g., water and paraorbital stimulation; Dearing & Dickinson, 1979; Gormezano & Tait, 1976), in the present study we employed the same stimulus (paraorbital stimulation) as both the first and the second US.

Another major purpose of the present experiments was to examine whether an unconditioned response (UR) to the CS (i.e., alpha response; Hull, 1934) would change as a function of conditioning (see Carew, Abrams, Hawkins, & Kandel, 1984; Hull, 1934; Kandel & Spencer, 1968; Schreurs, 1989; Skelton, Mauk, & Thompson, 1988). More generally, in the present study, we attempted to (1) examine the argument that pairing-specific changes in an existing response constitute sufficient grounds for asserting that associative learning has been demonstrated when classical conditioning procedures are employed, and (2) advocate the power of the emergence of a new response as the index of associative learning.

The term “alpha response” was first coined by Hull (1934) to refer to a UR elicited by the CS and to distinguish that response from a response that emerged as a function of CS-US pairings (i.e., the conditioned response, CR). Later, Grant (e.g., Grant, 1943, 1944; Grant & Adams, 1944) examined the nature of a number of different responses, including the alpha response, that were observed to occur during human eyelid conditioning. Grant and his associates observed that during a conditioning trial, the human eyelid exhibited (1) a reflexive response to a light CS they termed alpha, (2) a second reflexive response to the light, termed beta, which occurred only after adaptation to the subdued lighting of the testing room, (3) a CR to the light that emerged as a function of CS-US pairings, and (4) a UR to an air-puff US. Each of these responses could be distinguished by the latency with which they occurred during the conditioning trial. For example, alpha responses were typically

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responses of very short latency occurring within 50–100 msec of CS onset (see also Woody & Brozek, 1969), beta responses occurred next (100–250 msec), and CRs occurred later in the CS-US interval (250–800 msec). As a result of careful latency specifications, the occurrence of CRs can be, and has been, detected from among a number of different responses that may occur on a conditioning trial (e.g., see Gormezano, 1966, 1972; Grant, 1943; Martin & Levy, 1969).

When Grant and Adams (1944) examined the effects of CS-US pairings on alpha responses, they found that, relative to a light-alone control in which the frequency of the alpha response decreased, there was a higher, constant level of alpha responding to light that did not change during the course of light-airpuff pairings. More recently, Skelton et al. (1988) have shown that a light source placed within 1 cm of the rabbit’s eye elicited an EMG response in the eyelid muscle (cf. Bruner, 1965). Skelton et al. (1988) also observed that after lesions of the cerebellum and tone-airpuff pairings, there was an increase in the area of EMG activity as a function of both unpaired and paired light and airpuff presentations. With further training, the area of EMG activity decreased as a function of unpaired CS/US presentations and increased as a function of paired CS-US presentations.

A number of other preparations, including Aplysia siphon withdrawal (e.g., Carew, Hawkins, & Kandel, 1983; Carew, Walters, & Kandel, 1981), the cat spinal preparation (Patterson, 1975; Patterson, Cegavske, & Thompson, 1973), and the cat short-latency eyelid response (Woody & Brozek, 1969; Woody, Yarowsky, Owens, Black-Cleworth, & Crow, 1974), have demonstrated pairing-specific changes in a UR (alpha) to the CS (for details, see Schreurs, 1989). As a result, Carew et al. (1984), Hawkins and Kandel (1984), and Woody and Brozek (1969) have suggested that there is no fundamental distinction between an alpha response and a CR. Carew et al. suggested that the classical conditioning procedure causes an alpha response to develop into a CR. This development is argued to occur even in preparations such as the rabbit nictitating membrane response (NMR) that do not have an alpha response, because even though there may not be a "behavioral" alpha, there is always a "neural" alpha response that becomes a CR (Carew et al., 1984). An alternative, more traditional view argues for a clear distinction between an alpha response and a CR, with the latter emerging only as a function of CS-US pairings (e.g., Gormezano, 1966; Hilgard, 1936; Hull, 1934; Lashley, 1916; see also Schreurs, 1989). In the present US-US conditioning experiment, the first US elicited a response (alpha) and, as a result, it was possible to assess the merits of the argument that alpha responses become CRs. Specifically, by monitoring the alpha response during CS-US pairings, it would be possible to detect any pairing-specific changes in the response. Moreover, with a sufficiently long CS-US interval, it would be possible to detect responses other than the alpha response that might occur before the US.

Experiments in which two USs are paired have traditionally been employed to examine the nature of associations (Asratyan, 1965; Gormezano & Tait, 1976; Pavlov, 1927) and to address questions about motivational states (Dearing & Dickinson, 1979; Scavio, 1974; Scavio & Gormezano, 1980; Tait et al., 1986). In the former case, US-US conditioning experiments have been used to ascertain whether associations are bidirectional—that is, formed both between a CS and a US and between the US and the CS (Asratyan, 1965; Gormezano & Tait, 1976). In the latter case, USs of opposing motivational states (e.g., food and shock) have been paired together and CRs resembling responses to the second US have appeared relatively quickly to the first US (Gormezano & Tait, 1976; Tait et al., 1986). In contrast, a tone CS previously paired with a US (e.g., shock) thus producing one motivational state, has been shown to take a relatively long time to become associated with a US (e.g., food or water) producing another motivational state (Scavio, 1974). Tait et al. (1986) have argued that the results of these two experiments reflect a difference in the "cuing" and "motivational" functions of the CS in each case (Dearing & Dickinson, 1979). In the present study, the CS would be expected to function as a cue with the same motivational sign as the US.

EXPERIMENT 1

The purpose of Experiment 1 was to (1) characterize the UR to paraorbital stimulation in the rabbit, and (2) determine a range of paraorbital electrical stimulation parameters that would serve as both a CS and a US in classical conditioning of the rabbit NMR. Specifically, the experiment was designed to allow manipulation of the intensity and duration of paraorbital electrical stimulation in order to detail frequency, amplitude, duration, latency, and peak latency characteristics of an unconditioned NMR to paraorbital electrical stimulation (e.g., see Schreurs, 1987).

Method

Subjects. The subjects were 8 male albino rabbits (Oryctolagus cuniculus), 80–100 days old and weighing approximately 2.0 kg upon arrival. All rabbits were individually housed, given free access to food and water, and maintained on a 12:12-h light/dark cycle in the colony for at least 1 week before the start of the experiment.

Apparatus. The apparatus and recording procedure for the NMR were modeled after those described by Coleman and Gormezano (1971) and Gormezano (1966). In brief, each subject was restrained in a Plexiglas box and trained individually in one of eight sound attenuating, ventilated chambers (Coulbourn Instruments Model E10-20). A stimulus panel containing a speaker and houselights (10-W, 120-V incandescent lamps) was mounted at a 45° angle, 15 cm anterior to and 15 cm above the subject’s head. An ambient noise level of 55 dB was provided by an exhaust fan. Paraorbital
electrical stimulation consisted of 60-Hz ac pulses delivered by a programmable two-pole shocker (Coulbourn Instruments Model E13-35). Stimulation intensities were 0.1, 0.25, 0.5, 1, and 2 mA and were presented at durations of 10, 25, 50, and 100 msec. The electrical stimulation was delivered via stainless steel Autoclip wound clips positioned 10 mm below the lower eyelid and 10 mm posterior to the dorsal canthus of the eye. The sequence and timing of stimulus events were controlled by a Compaq 286 computer system equipped with a DAS-16 analog I/O board (MetraByte Corp.), a P10-12 digital I/O interface board (MetraByte Corp.), and ASYST software (Asyst Software Technologies). The Compaq/Asyst system was modeled after the Apple II/FIRST system developed by Scandrett and Gormezano (1980).

The detailed aspects of transducing nictitating membrane movements have been described by Gormezano and Gibbs (1988). Briefly, a small hook was attached to a nylon loop sutured into, but not through, the nictitating membrane of the rabbit’s right eye. The hook was connected to one end of an L-shaped hypodermic tubing lever containing a ball-and-socket joint. The other end of the lever was connected to a low-torque rotary potentiometer (Litton Industries Model PS091-023). The signal from the potentiometer was transmitted to an analog-to-digital (A/D) converter (5-msec sampling rate; 0.05-mm resolution) on the DAS-16 analog I/O board. The individual A/D outputs were stored on a trial-to-trial basis for subsequent analysis.

Procedure. All rabbits received 1 day of preparation and adaptation, and 2 days of testing. On the preparation/adaptation day, hair surrounding the rabbit’s right eye was shaved, a small loop of surgical nylon (Ethicon 6-0) was sutured into, but not through, the nictitating membrane, and stimulation electrodes were applied. The rabbits were placed in the apparatus for a period of time corresponding to the duration of the subsequent testing sessions. On each testing day, the subjects received a total of 80 trials presented at an average intertrial interval of 60 sec (range 50–70 sec). Each trial involved the presentation of 1 of 20 possible combinations of stimulus intensity (0.1, 0.25, 0.5, 1, or 2 mA) and stimulus duration (10, 25, 50, or 100 msec). Four separately randomized sequences of the 20 stimulus combinations were presented on each testing day, with the restriction that the same value of intensity or duration could not occur on more than three consecutive trials.

A UR was defined as any extension of the nictitating membrane exceeding 0.5 mm that occurred after the onset of the paraorbital electrical stimulation and before the end of the observation interval (800 msec after stimulation onset). Amplitude of a response was scored as the maximum extension of the nictitating membrane. Onset latency of a response was identified as the point at which a response rose 0.1 mm above the baseline. Peak latency of a response was determined as the latency at the maximum extension of the nictitating membrane. Response duration was calculated from the point of stimulation onset to the point at which the response returned to within 0.1 mm of a flat baseline. Although the rabbit NMR to paraorbital stimulation is uniphasic and returns to a relatively flat baseline, the response does not always return to the pre-CS baseline during the observation interval. Accordingly, response duration was determined to be the point at which the response returned to within 0.1 mm of a “baseline” level calculated as the mean response level during the last 200 msec of the 800-msec observation interval.

Results

In general, response frequency, amplitude, duration, latency, and peak latency were all lawfully related to increases in stimulus intensity and duration. The range of stimulation parameters employed was broad enough to include values too low to elicit responses and values high enough always to elicit robust responses. Because some stimulation parameters failed to elicit a response (e.g., 0.1 mA) or elicited very few responses in only 1 or 2 subjects (e.g., 0.25 mA at 10, 25, and 50 msec), measurements based on the occurrence of a response (i.e., amplitude, duration, latency, and peak latency) were collapsed across either stimulus intensity or stimulus duration to avoid empty data cells and allow for statistical analysis.

Figure 1 shows the frequency of nictitating membrane extension as a function of stimulus intensity and duration. Examination of the figure reveals that NMR frequency increased as a function of both parameters of stimulation. In particular, NMR frequency increased as a function of stimulus intensity from a value of 0% at 0.1 mA to a mean value of approximately 95% at 2.0 mA. To a lesser extent, NMR frequency increased as a function of stimulus duration from a mean of approximately 40% at 10 msec to 55% at 100 msec. Finally, Figure 1 indicates that the relationship between NMR frequency and the stimulation parameters was not a simple one. For example, the level of responding that resulted from stimulation at a duration of 10 msec was generally lower than the level of responding that resulted from stimulation at durations of 25, 50, or 100 msec across all but the lowest and highest stimulus intensities.

The foregoing observations were confirmed by an analysis of variance that yielded significant main effects of intensity [F(4, 28) = 140.06, p < .001] and duration [F(3, 21) = 37.16, p < .001], and a significant interaction of intensity * duration [F(12, 84) = 5.12, p < .001]. The panels of Figure 2 show UR amplitude, duration, latency, and peak latency as a function of values of stimulus intensity (0.5, 1.0, and 2.0 mA) and duration (10, 25, 50, and 100 msec) that reliably elicited responses. Inspection of the panels reveals, and analyses of variance confirmed, that changes in UR amplitude, duration, latency, and peak latency were a function of the parameters of

![Figure 1](image-url)
stimulation. Specifically, UR amplitude and duration increased as a function of increases in both stimulus intensity \( F(2,14) = 100.61, p < .001, \) and \( F(2,14) = 6.87, p < .01, \) respectively and stimulus duration \( F(3,21) = 5.95, p < .01, \) and \( F(3,21) = 16.23, p < .001. \) In contrast, UR latency and peak latency decreased as a function of increases in stimulus intensity \( F(2,14) = 45.70, p < .001, \) and \( F(2,14) = 6.13, p < .05, \) respectively, but increased as a function of increases in stimulus duration \( F(3,21) = 6.35, p < .01, \) and \( F(3,21) = 22.01, p < .001. \)

Examination of the UR data for individual combinations of stimulus intensity and duration that reliably elicited responses (Schreurs, 1987) revealed mean UR amplitudes that ranged from 1.22 mm (0.5 mA, 10 msec) to 5.04 mm (2.0 mA, 100 msec), mean UR durations that ranged from
173 to 352 msec, mean UR latencies that ranged from 45 to 22 msec, and mean peak latencies that ranged from 73 to 98 msec.

**Discussion**

The data from Experiment 1 clearly indicate that the rabbit’s NMR is a function of the parameters of paraorbital stimulation (Gormezano, 1972; Gormezano, Kehoe, & Marshall, 1983; Tait, Kehoe, & Gormezano, 1983). Specifically, as stimulus intensity and duration increased, the frequency of responses increased and, at the highest parameters of stimulation, responding reached a level of 100% URs. Response amplitude and duration also increased as a function of increases in stimulus intensity and duration, suggesting that as the total stimulus energy increased, the size and extent of the response increased. Changes in the timing of the response, measured as the latency and peak latency, were also related to increases in total stimulus energy, although the timing changes appeared to be more complicated. With increases in the intensity of stimulation, responses were initiated more quickly and also reached their maximum amplitude more quickly. However, as the duration of stimulation increased, there was an increase in the time at which maximum amplitude was reached. Consequently, the data suggest that the time course of the rabbit NMR changed so that the nictitating membrane reached maximum extension at or after the end of stimulation (e.g., see Millenson, Kehoe, & Gormezano, 1977). In sum, the rabbit NMR appears to be exquisitely sensitive to the different stimulus parameters employed in the present experiment (e.g., see Gormezano, 1972; Millenson et al., 1977).

Of particular interest to the present study were the parameters of paraorbital stimulation that would serve as the CS and the US in a conditioning experiment (e.g., Schreurs, 1988). The parameter requirements for a CS were several. First, in order to study the alpha response and the effects of conditioning upon the alpha response, parameters were needed that reliably elicited a response but were still sensitive to potential changes in frequency (e.g., values at or above 0.5 mA and 25 msec). Second, to study whether there were lawful effects of CS intensity on conditioning, at least three intensity values were necessary to describe a function (e.g., 0.5, 1.0, and 2.0 mA). Third, for any given CS-US interval, the shorter the duration of the response to the CS, the more time available to observe the emergence of any other responses before the US elicited a UR. Examination of the results showed that a stimulus duration of 50 msec yielded URs with a frequency of at least 50% but that were at or below 300 msec in duration across all stimulus intensities studied. Consequently, CS intensities of 0.5, 1.0, and 2.0 mA presented for 50 msec were chosen to elicit responses of greater than 1 mm that would return to baseline within 300 msec. A response with a duration of no more than 300 msec would allow sufficient time to detect CRs using CS-US interval values longer than 300 msec that were still capable of supporting substantial levels of conditioning (400-1,000 msec; e.g., Gormezano et al., 1983; Schneiderman & Gormezano, 1964). The parameters to be used for the US were the highest examined in the present experiment (2.0 mA, 100 msec). These same parameters have routinely yielded better than 90% CRs when paired with a tone CS for 2-3 daily sessions of 80 CS-US pairings, and as such have proven to be an effective US for conditioning.

**EXPERIMENT 2**

The purpose of Experiment 2 was to examine whether classical conditioning of the rabbit’s NMR could be obtained using a CS and a US consisting of paraorbital electrical stimulation, and to determine whether the level of conditioning is a function of CS intensity (e.g., Ashton, Bitgood, & Moore, 1969; Scavio & Gormezano, 1974). Previous US-US conditioning experiments have involved the pairing of aversive and appetitive USs (e.g., Asratyan, 1965; Gormezano & Tait, 1976; Pavlov, 1927; Tait et al., 1986). In each of these cases, an “appetitive” CR (salivation or jaw movement) emerged to an aversive CS (paraorbital stimulation; Gormezano & Tait, 1976; shock; Pavlov, 1927) and an “aversive” CR (NMR) emerged to an appetitive CS (water; Dearing & Dickinson, 1979; Gormezano & Tait, 1976; Tait et al., 1986). In the present experiment, if conditioning occurs, it would be in the form of an “aversive” CR (NMR) to an aversive CR (NMR) to an appetitive CS (paraorbital stimulation).

The use of paraorbital stimulation as a CS provided a means of assessing whether there were any changes in an alpha response to the CS that occurred as a function of conditioning (e.g., Carew et al., 1984; Skelton et al., 1988). That is, by eliciting a response to the CS from the onset of training, any changes in that response could be monitored during the course of CS-US pairings. In an effort to detect pairing-specific changes in the alpha response, response frequency, amplitude, duration, latency, and peak latency of the alpha response were measured for 5 days of CS-US pairings and compared to the same measures obtained during 5 days of explicitly unpaired presentations of the CS and US (Schreurs, 1989). If CRs are the enhancement of an alpha response (e.g., Carew et al., 1984), the present experiments would provide the opportunity to detect any such changes. If, on the other hand, associative learning is indexed by the emergence of a new response, the present experiment would also provide the opportunity to detect the emergence of responses quite separate from the alpha response (e.g., Gormezano, 1966; Schreurs, 1989). In fact, the present experiment revealed the occurrence of two responses to the CS, the alpha response and a new response, the CR, that emerged as a function of CS-US pairings.

**Method**

**Subjects.** Experiment 2 involved a total of 48 male albino rabbits maintained under the same conditions as those employed in Experiment 1.

**Apparatus and Procedure.** Unless otherwise noted, the apparatus and procedures were the same as those employed in Experiment 1.
Following 1 day of preparation/adaptation, the rabbits were randomly assigned to one of six groups (n = 8) and given 5 consecutive days of training. For half of the subjects, each of the 5 daily conditioning sessions consisted of 80 paired presentations of a CS and US and eight CS-alone test trials, with a CS-alone test trial presented after every 10th paired trial. For the other half of the subjects, each day consisted of explicitly unpaired presentations of 80 CS-alone trials and 80 US-alone trials as well as eight CS-alone test trials presented after every 20-trial block. The CS and US presentations within each 20-trial block were randomized with the restriction that no more than three CS-alone or three US-alone trials could occur consecutively. For both the paired and the unpaired groups, the CS was a 50-msec, 60-Hz paraorbital stimulation with an intensity of 0.5 (LO), 1.0 (MED), or 2.0 (HI) mA. The groups comprised the cells of a 2 x 3 factorial, with pairings and CS intensity as the factors [i.e., (Paired) LO, MED, and HI, and U(unpaired) LO, MED, and HI]. The US was a 100-msec, 60-Hz, 2.0-mA paraorbital stimulation presented 600 msec after the onset of the CS. Consequently, the CS-US interval on paired trials was 600 msec.

Response specification. A CR was defined as a 0.5-mm extension of the nictitating membrane that occurred 300 msec after onset of the CS (duration of UR to the 2-mA CS) and before the onset of the US on paired trials or before the point at which US onset would have occurred on CS-alone test trials (600 msec after CS onset). Classical conditioning of the rabbit NMR using CS-US intervals of the order of 600 msec result in high levels of responding (>90% CRs) with response latencies that move forward in time to approximately the midpoint of the CS-US interval (e.g., see Smith, Coleman, & Gormezano, 1969). Therefore, in the present experiment, if CRs are to emerge, they should occur after the UR to the CS has returned to baseline.

The data for both URs and CRs to the CS obtained on paired and CS-alone test trials were essentially the same. However, because the data from the paired and unpaired trials provided a more stable estimate of responding, the statistical analyses reported here were conducted on those data.

Results

Figure 3 shows typical response topographies for a subject that received pairings of the 2-mA CS and the US on an early trial when only the URs to the CS and US occurred (Figure 3A), a later trial when a CR to the CS began to emerge (Figure 3B), and a trial toward the end of training when the CR was fully developed (Figure 3C).

Analysis of variance confirmed these observations, yielding significant main effects of pairing [F(1, 42) = 127.18, p < .001], intensity [F(2, 42) = 22.07, p < .001], and days [F(4, 168) = 52.94, p < .001], as well as significant interactions of pairing x intensity [F(2, 42) = 21.30, p < .001], pairing x days [F(4, 168) = 53.50, p < .001], intensity x days [F(8, 168) = 8.38, p < .001], and pairing x intensity x days [F(8, 168) = 8.80, p < .001].

Examination of the UR frequency, amplitude, duration, latency, and peak latency data revealed significant effects of CS intensity across all dependent variables (Fs > 11.5, p < .001). As in Experiment 1, increases in the intensity of the paraorbital stimulation that served as the CS produced increases in UR frequency, amplitude, and duration, and decreases in UR latency and peak latency. However, with the exception of UR amplitude, there were no significant changes in URs to the CS as a function of pairings or days of training (Fs < 2.28).

Analysis of UR amplitude revealed significant interactions of pairings x days [F(4, 140) = 2.97, p < .05] and
intensity \times \text{days} \ [F(8,140) = 2.60, p < .05]. Figure 5 shows UR amplitude for paired and unpaired subjects as a function of the 5 consecutive days of training. Inspection of the figure shows a gradual increase in UR amplitude as a function of days of training, with no apparent difference between paired and unpaired subjects (mean difference = 0.23 mm) except on Day 4 of training, when unpaired subjects showed a 0.7-mm decrease in UR amplitude. There was a subsequent return to paired amplitude values (2.48 vs. 2.57 mm) on Day 5. These observations were confirmed by an analysis of trend across days of conditioning, which revealed a significant overall increase in UR amplitude \([F(1,35) = 17.17, p < .001]\) and a significant interaction of pairings \times\text{quadratic trend} \([F(1,35) = 5.68, p < .05]\), presumably reflecting the divergence of groups on Day 4 of training. These data suggest that there was no systematic effect of CS–US pairings on UR amplitude to the CS.

Further investigation of the UR amplitude data revealed a number of interesting similarities between the present data and those obtained by researchers studying the effects of exposure to a US prior to conditioning (e.g., Mis & Moore, 1973; Saladin & Tait, 1986; Suboski, Di Lollo, & Gormezano, 1964). Specifically, although there was a significant increase in UR amplitude over the 5 days of training, there was a significant within-session decrease in response amplitude for both paired and unpaired subjects, from a mean value of 3.0 mm at the start of the session (first 10-trial block) to mean values of 2.5 and 2.4 mm, respectively, at the end of the session (last 10-trial block). A statistical analysis of within-session UR amplitudes revealed a significant effect of 10-trial blocks \([F(7,231) = 16.67, p < .001]\), but no significant main or interaction effects of pairing \((F_S < 1.10)\). In contrast, a within-session analysis of CRs on the days that CRs began to emerge revealed significant increases in CRs as a function of 10-trial blocks. A within-session examination of the emergence of CRs for all sessions revealed a consistent level of responding across the entire session (27.2%–27.4% CRs, first 10-trial block to last 10-trial block). Consequently, the present data suggest that there was a consistent within-session weakening of the UR to the CS while CRs to the CS continued to emerge.

**Discussion**

The results of Experiment 2 showed that (1) CRs to paraorbital electrical stimulation detected outside the range of URs to the CS emerged as a function of CS–US pairings (e.g., Gormezano & Tait, 1976; Grant, 1943; Tait et al., 1986), (2) the level of conditioning was a function of CS intensity, and (3) although URs to the CS varied as a function of CS intensity, there was no systematic effect of CS–US pairings on URs to the CS.

The results of the present experiment confirm and extend the results of previous US–US conditioning experiments that have shown that despite URs to the first US (e.g., jaw movement, nictitating membrane extension), animals are capable of forming an association between two USs. In each of these cases, the association formed between the two USs was indexed by the emergence of a CR that resembled the UR to the second US (e.g., membrane extension to water in the mouth, salivation to shock). In the present experiment, the CR that emerged as a function of training resembled the UR to both the CS and the US.

The manipulation of CS intensity provided a number of interesting outcomes. First, like more conventional CSs, such as tones and lights, increases in CS intensity resulted in increases in the level and rate of CR acquisition (Scavo & Gormezano, 1974). Thus, in addition to having a “motivational” role when used as a US, paraorbital electrical stimulation was an effective “signal” or “cue” when used as a CS. Second, in the case of the highest intensity CS (i.e., 2 mA), the experimental procedure consisted of the presentation of the same stimulus twice. Consequently, the rabbits were able to associate two almost identical events on the basis of their temporal sequence. However, there were a number of other potential cues in the experiment. For example, the durations of the two USs were different (50 vs. 100 msec) and any number of proprioceptive and/or kinesthetic properties of the first UR might have functioned as the effective stimulus in the association.

The results of the present experiment also showed that there were no systematic effects of CS–US pairings on URs to the CS across the five dependent variables measured (frequency, amplitude, duration, latency, and peak latency). Consequently, there was no evidence that the alpha response elicited in the present experiment was enhanced to the point of becoming a CR (cf. Carew et al., 1984). In contrast, there was strong evidence for the emergence of a CR outside the duration of the alpha response that resembled CRs elicited by more conventional CSs, such as tones and lights. In sum, the CS employed in the present experiment elicited two responses: (1) an alpha response that did not appear to change as a function of
CS-US pairings, and (2) a CR that slowly emerged as a function of CS-US pairings.

**GENERAL DISCUSSION**

Taken together, the present experiments demonstrated that (1) the rabbit NMR changed as a function of the parameters of paraorbital electrical stimulation, (2) classical conditioning of the rabbit NMR could be obtained when paraorbital electrical stimulation served as both the CS and the US, (3) the level of conditioning was a function of CS intensity, and (4) there were no systematic changes in the UR to the CS as a function of CS-US pairings.

It is clear from the present experiments that paraorbital electrical stimulation can serve as an effective cue or signal for classical conditioning of the rabbit NMR. Even when the CS and the US were of the same intensity, an association was formed between the two events. Indeed, the association was indexed by the emergence of a response that resembled the UR to the US, an important criterion for the observation of associative learning (e.g., see Gormezano, 1966, 1972, 1984; Lederhendler, Gart, & Alkon, 1986; Schreurs, 1989). In contrast to previous US-US experiments (e.g., Dearing & Dickinson, 1979; Gormezano & Tait, 1976; Pavlov, 1927) in which the cuing and motivational roles of the first US were different (Tait et al., 1986), in the present experiment, the cuing and motivational roles of the CS were the same. In other words, the CS signaled the occurrence of the US and, presumably, also elicited the same motivational state as the US. However, because a response was elicited to the CS, any of a number of elements may have entered into the association that was formed. The question remains as to which of the stimulus properties of the CS and/or components of the response to the CS became associated with the US/UR. Interestingly, in the case of the low-intensity CS, conditioning occurred even though a response was elicited much less frequently than in the case of the medium- and high-intensity CSs. In fact, in one case, high levels of conditioning (72% CRs) emerged even when the CS rarely elicited a UR (1.3%).

The emergence of a CR in the same effector system as the UR to the US has historically been used as the index of associative learning when classical conditioning procedures have been employed (Hilgard, 1936; Hull, 1934; Lashley, 1916; Pavlov, 1927). A more contemporary conceptualization of classical conditioning is based on the view that modification of any response that results from exposure to the paired relationship between the CS and the US constitutes an example of associative learning (Rescorla, 1988). Thus, pairing-specific modification of URs to the CS in the absence of any other changes would qualify as an example of associative learning (e.g., Carew et al., 1984; Kandel & Spencer, 1968). However, such changes can be accounted for by a number of processes other than associative learning. For example, pairing-specific sensitization, dishabitation, or protection from habituation may also account for changes in an alpha response (see Schreurs, 1989). Nevertheless, Carew et al. (1984) and Hawkins and Kandel (1984) have argued that there is no fundamental difference between pairing-specific changes in an alpha response and a CR. Even if a preparation does not exhibit a behavioral alpha response, it will always exhibit a neural alpha response (e.g., see Skelton et al., 1988). It has been argued that classical conditioning strengthens a neural alpha response until it reaches threshold and becomes a CR (Hawkins & Kandel, 1984). However, the rabbit NMR preparation does not normally display an alpha response (Gormezano, 1966, 1984; Schreurs, 1989), nor has a neural alpha been detected in the motor neurons now known to control the response (Disterhoff, Quinn, Weiss, & Shipley, 1985). In the present experiments, an alpha response was deliberately elicited, in part to examine whether pairing-specific changes in the alpha response could be observed. Despite the observation of CRs, no systematic, pairing-specific changes in response frequency, amplitude, duration, latency, or peak latency could be detected. Although CRs to a CS such as a tone might result from the enhancement of a heretofore undetected behaviorally subthreshold "neural" alpha response, the elicitation of a "behavioral" alpha response that did not change as a function of CS-US pairings makes the notion of neural alpha enhancement less likely.

Finally, it is interesting to speculate about the neural circuitry that may have been involved in the conditioning observed in the present experiments. A tone CS and an airpuff or shock US have been postulated to converge at a number of sites, including the deep cerebellar nuclei and the cerebellar cortex (e.g., see Thompson, 1986). Specifically, CS inputs arrive at the cerebellar cortex via mossy and then parallel fibers, and US inputs arrive via climbing fibers. Conditioning is purported to involve long-term depression at the synapses between parallel fibers and their target Purkinje cell dendrites (e.g., Ito, 1984; Thompson, 1986). In the present experiments, US-US pairings would result in a double activation of the climbing fiber inputs to the cerebellum, which, presumably, would not produce long-term depression. Consequently, processing of CSs and USs may involve not only "cuing" circuitry such as that found in the cerebellum, but also "associative" circuitry that is functionally and/or physically separate.

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