Factors contributing to the magnitude of conditional fear following a 24-h retention interval: The incubation effect

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Two experiments were conducted to examine the effects of the number of conditional stimulus-unconditional stimulus (CS-US) pairings and the intensity of the US on "spontaneous" changes in the magnitude of conditional fear following a 24-h retention interval. The results of the first experiment showed that the magnitude of conditional fear following the interval increased as a function of the number of CS-US pairings. The results of the second experiment showed that, with the number of pairings held constant, increasing the intensity of the US resulted in an increase in the magnitude of conditional fear following the retention interval. These data are consistent with an associatively based account of incubation.

Jacobs and Blackburn (1987) provided evidence that incubation ("spontaneous" changes in the magnitude of conditional suppression over a 24-h retention interval) requires the presence of an association between the conditional stimulus (CS) and the unconditional stimulus (US). In their study, rats in an experimental group received six pairings of 30 sec illumination of a houselight and mild (0.10 mA) electric shock, whereas rats in three control groups received the same number of presentations of (1) the shock, (2) the houselight, or (3) random presentations of both stimuli. No conditional response was observed in any group on the first conditioning day. On the second conditioning day, those rats that had received contiguous pairings of the CS and US displayed profound conditional fear on the first and all subsequent trials. Rats in the control groups displayed no conditional fear on these test trials. This increase in conditional fear following a retention interval, known as *incubation*, thus appears to depend upon previous pairings of the CS and US.

These data suggest that the incubation of conditional suppression is an associatively based phenomenon. If incubation behaves as do other associative phenomena, then we would expect the incubation effect, as evidenced by changes in the magnitude of conditional fear following a retention interval, to increase as a function of the num-

ber of pairings between the CS and US and to increase as a function of the intensity of the US (Mackintosh, 1983). The present experiments examined these predictions.

EXPERIMENT 1

Method

Subjects. Seventy locally bred, experimentally naive male Sprague-Dawley rats (100-150 g) were on a reversed 12:12-h day:night cycle. They were individually housed and given free access to food and water before the beginning of the experiment. With the exception of transportation to and from the experimental chambers, the rats remained undisturbed in their home cages from weaning until the end of the experiment. All training and conditioning sessions were conducted during the night portion of the day:night cycle.

Apparatus. The apparatus consisted of 10 Perspex chambers (25×30×18 cm; previously described by Jacobs & Blackburn, 1987). Each chamber had a hole, 1 cm in diameter, in the center of the front wall 4 cm above the floor. Two stainless steel rods (7×0.5 cm) were mounted outside the chamber on each side of the opening, 1 cm beneath it. Attached to the rods, beneath the center hole, was a Perspex block (6×2×3 cm) that could be moved and locked at various positions on the rods. A hollow stainless steel tube (through which liquid could be presented) was mounted through a hole drilled near the top of the block in such a way that the tip entered the center of the hole.

The tube was connected to a solenoid valve mounted outside the sound-attenuating chamber (44×32×30 cm) in which the experimental chamber was contained. The solenoid valve was attached to a reservoir containing a 0.1% saccharin solution. Operation of the solenoid valve released approximately 0.01 ml of the saccharin solution to the licking tube. Each tube was in circuit with a lickometer that was electrically isolated during the presentation of scrambled shock.

A 28-V houselight was centered on the front wall of the chamber, 10.7 cm from the grid floor. A speaker was mounted at the center of the chamber ceiling. The floor consisted of 3-mm-diameter stainless steel grids mounted 6 mm center to center, through which scrambled electric shock could be delivered.

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Procedure. Each rat was given 24 h exposure to a 0.1% saccharin solution 1 week before the beginning of the experiment and was deprived of water for 24 h before the first experimental session.

Preexposure Day: On the first experimental day, each rat was removed from its home cage, confined in an experimental chamber, and trained to lick the tube for brief access to the saccharin solution. Licking produced access to 0.01 ml saccharin solution on a variable-ratio 3 (VR-3) schedule for the first 20 saccharin presentations, on a VR-5 schedule for the next 20 presentations, and on a VR-10 schedule for the remainder of the 20-min experimental session.

After each rat had achieved the VR-10 schedule, presentations of the CS began. The CS, which consisted of 30 sec illumination of the houselight, was presented six times during the remainder of the session on a variable-interval 95-sec schedule. The rats were required to perform a minimum of five licks during the 30-sec interval that preceded stimulus presentation. If fewer than five licks occurred during this interval, the CS was withheld and the 30-sec interval was restarted. This continued until the response criterion was met.

Conditioning Day 1: The rats were treated as they had been on the preexposure day, with the following exceptions. During the 20-min conditioning session, the rats in Group 1 received one presentation of the CS paired with the US (a 0.20-mA, 0.75-sec scrambled electric shock). The rats in Group 2 received two CS-US pairings, the rats in Group 3 received three, those in Group 4 received four, those in Group 5 received five, and those in Group 6 received six. The rats in Group 0 licked the tube for access to saccharin during this session, but received no presentations of the houselight or shock. Each rat was removed from the conditioning chamber immediately after the conditioning session and was given 30 min free access to water.

Conditioning Day 2: Twenty-four hours after conditioning, each rat was returned to the experimental chamber and was treated in a manner identical to that described for Group 6 on Conditioning Day 1.

Data Analysis. Suppression ratios were calculated for each trial using the expression $A/(A+B)$, where A is the number of responses in the presence of the 30-sec CS and B is the number of responses in the 30-sec period that immediately preceded the presentation of the CS. The value of the ratio is below 0.5 if the rate of licking decreases in the presence of the CS and is equal to 0.5 if the rate of licking does not change during stimulus presentation.

Statistical analyses were carried out using Rodger's (1974, 1975a, 1975b) method. The Type I error rate was set at 0.05 for each statistical decision.

Results and Discussion

All rats learned to lick the tube for saccharin reinforcement. An analysis of variance failed to detect significant differences in the mean rate at which the rats licked the tube during the 30 sec that preceded the presentations of the CS on Conditioning Day 1 (mean rate of licking ranged from 4.1 to 5.3 lick/sec.)

Figure 1 presents the mean suppression ratios obtained from the seven experimental groups on Conditioning Day 2. The data displayed in this figure indicate that the magnitude of conditional suppression that developed after the retention interval was a function of the number of CS-US pairings that occurred on Conditioning Day 1. The result from an analysis of variance was consistent with this impression. The analysis detected significant differences among the group means [$F(6,56) = 3.47$]. A post hoc analysis of the form given in Table 1 implied the following order of population mean suppression ratios:

$$0 > 1 = 2 > 3 = 4 > 5 = 6 \text{ pairings.}$$

The data indicate that the incubation of conditional fear is a function of the number of CS-US pairings. Incubation was observed with as few as one or two pairings,

Figure 1. Mean suppression ratio obtained for each group (Groups 0, 1, 2, 3, 4, 5, and 6, grouped according to number of CS-US pairings received) in Experiment 1 on the test day.

and was at a maximal detectable level following five pairings. The data indicate that the failure to detect conditional fear on Conditioning Day 1 (e.g., Jacobs & Blackburn, 1987) cannot be attributed to an insufficient number of CS-US pairings on this day.

EXPERIMENT 2

Method

Subjects and Apparatus. Fifty-four locally bred, experimentally naive male Sprague-Dawley rats weighing approximately 100–150 g were used. They were housed and maintained as described in Experiment 1. The equipment described in Experiment 1 was used in Experiment 2.

Procedure. The adaptation and deprivation procedure described in Experiment 1 was used in Experiment 2. On the preexposure day, each rat was assigned to one of six groups, trained to lick the tube, and preexposed to the CS as described in Experiment 1.

Conditioning Day 1: The rats received six CS-US pairings, as described for Group 6 in Experiment 1. The five experimental groups ($n=9$) received, respectively, 0.75-sec presentations of 0.25, 0.20, 0.15, 0.10, or 0.075 mA scrambled electric shock immediately following

Table 1
Post Hoc Contrasts of Number of Pairings Data

	Group							
Means	0	1	2	3	4	5	6	
Observed	.3746	.3164	.2674	.2276	.2124	.1005	.1022	$F_m=3.47$
	-	1	-1	-	-	-	-	0.07
	-	-	-	-	-	1	-1	0.00
	-	-	-	1	-1	-	-	0.01
	-	1	1	-	-	-1	-1	1.99
	-	1	1	-2	-2	1	1	0.04
	-6	1	1	1	1	1	1	1.36
Implied	1.01	0.49	0.49	-0.17	-0.17	-0.82	-0.82	$r^2=0.98$

Note—Observed Means represent the mean suppression ratios observed during testing for all six groups. F_m represents the maximized F ratio obtained from the analysis of variance. The column below the F_m value represents the F value obtained for each comparison in the matrix row to its left. Implied Means represent the values of the true population means as is implied by the decision matrix. r^2 represents the correlation between the implied and observed means.

presentation of the CS (30 sec illumination of the houselight). The rats in the control group ($n=9$) received six presentations of the CS unpaired with the US (Group 0.00 mA).

Conditioning Day 2: Conditioning Day 2 was identical with Conditioning Day 1.

Results and Discussion

The rats responded at approximately the same rate throughout the experimental sessions. The mean response rates ranged from 4.1 to 5.3 licks/sec. Analyses of variance failed to detect significant differences in licking rate among groups or across days.

Figure 2 illustrates the data obtained from the six groups on Conditioning Days 1 and 2. The pattern of the data suggests that suppression of licking in the presence of the CS was enhanced by the presence of the US on Conditioning Day 1 (compare data for Group 0.00 mA with that for all other groups). The pattern of the data obtained on Conditioning Day 2 indicates that the magnitude of the conditional fear that followed a retention interval increased as a function of the intensity of the electric shock used as the US on Conditioning Day 1.

A split-plot analysis of variance detected significant main effects [$F(5,54) = 5.88$], a significant trials effect [$(1,54) = 48.41$], and a significant interaction effect [$F(5,54) = 3.81$]. The significant interaction term appears to be due to the decrease in the mean suppression ratios from Conditioning Day 1 to Conditioning Day 2 for all of the groups except Group 0.00 mA. A set of randomized-blocks analyses of variance confirmed this impression. A significant change from Conditioning Day 1 to Conditioning Day 2 was detected for all groups (F ratios ranged from 5.59 to 19.67, $df=1,9$) except Group 0.00 mA.

Analyses of variance detected significant differences among the groups on both Conditioning Days 1 and 2

Figure 2. Mean overall suppression ratio obtained for each group (grouped according to intensity of the shock US in mA) on Conditioning Day 1 and Conditioning Day 2 of Experiment 2.

Table 2
Post Hoc Analysis of US Intensity Data

	Group						
Means	.25mA	.20mA	.15mA	.10mA	.075mA	0.0mA	
Conditioning Day 1							
Observed	.3360	.3577	.2993	.3388	.3587	.4338	$F_m=1.85$
	1	-1	-	-	-	-	0.04
	1	1	-2	-	-	-	0.28
	1	1	1	-3	-	-	0.01
	1	1	1	1	-4	-	0.10
	1	1	1	1	1	-5	1.42
Implied	-0.16	-0.16	-0.16	-0.16	-0.16	0.80	$r^2=0.88$
Conditioning Day 2							
Observed	.1183	.1625	.1600	.2616	.2593	.4415	$F_m=7.20$
	-	-	-	1	-1	-	0.00
	-2	-	-	1	1	-	1.42
	-	1	-1	-	-	-	0.00
	2	-3	-3	2	2	-	0.34
	1	1	1	1	1	-5	5.44
Implied	-1.17	-0.35	-0.35	0.05	0.05	1.77	$r^2=0.98$

Note—Observed Means represent the mean suppression ratios observed on Conditioning Day 1 (top panel) and Conditioning Day 2 (bottom panel). F_m represents the F ratio obtained from the analysis of variance conducted on this data. The column below the F_m value represents the F value obtained for each comparison in the matrix row to its left. Implied Means indicate the value of the true population means as is implied by the decision matrix. r^2 represents the correlation between the observed and implied means.

[$F(5,54) = 1.85$ and 7.20, respectively]. The post hoc analysis presented in Table 2 revealed that the mean suppression ratios obtained on Conditioning Day 1 were ordered as follows:

0.25mA = 0.20mA = 0.15mA = 0.10mA = 0.075mA < 0.00mA.

On Conditioning Day 2, however, the order of these means was:

0.25mA < 0.20mA = 0.15mA < 0.10mA = 0.075mA < 0.00mA.

Thus, the magnitude of conditional suppression obtained on Conditioning Day 2 increased with increasing intensity of the US. This was true even though no detectable differences in conditional responding were found either among the conditioned groups or across the six trials in each group [largest $F(2,18) = 1.92$] on Conditioning Day 1. We can conclude, on the basis of these data, that the magnitude of conditional fear following a 24-h retention interval is a simple and direct function of the strength of the US used during conditioning.

GENERAL DISCUSSION

These experiments demonstrate that the incubation of fear, as shown by changes in the magnitude of conditional fear following a 24-h retention interval, increases as a function of the number of initial pairings between the CS and US, and as a function of the intensity of the US. These findings are consistent with our earlier suggestion that incubation is an associatively based phenomenon (Jacobs & Blackburn, 1987).

Although the association was behaviorally silent on Conditioning Day 1 in both the present and the earlier experiments, the associative effects were evident on Conditioning Day 2.

This finding raises both behavioral and physiological questions. At a behavioral level, the change from a silent to an articulated association could be due to an increase in associative strength that occurs during the retention interval. Alternatively, this behavioral change could be dependent upon changes in the representation of the US or its neural representation (described by Konorski, 1967, as its "gnostic unit"). In addition, such a change could result from changes in the representation of the CS or from changes in the motivational state of the organism.

At the physiological level, these associative, representational, or motivational changes could be related to several classes of neurochemical events, including those described by McGaugh (1983) and those outlined by Jacobs and Nadel (1985). Moreover, the events that are responsible for such neurochemical release may be spontaneous (Randich & Rescorla, 1981) and internally mediated processes, or may be mediated by identifiable environmental events that occur during the retention interval. These hypotheses will be more fully explored in forthcoming investigations.

REFERENCES

- JACOBS, W. J., & BLACKBURN, J. R. (1987). Incubation is an associatively-based phenomenon. *Pavlovian Journal of Biological Science*, **22**, 118-121.
- JACOBS, W. J., & NADEL, L. (1985). Stress induced recovery of fears and phobias. *Psychological Review*, **92**, 512-531.
- KONORSKI, J. (1967). *Integrative activity of the brain*. Chicago: University of Chicago Press.
- MACKINTOSH, N. J. (1983). *The psychology of animal learning*. Oxford: Oxford University Press.
- MCGAUGH, J. L. (1983). Preserving the presence of the past: Hormonal influences on memory storage. *American Psychologist*, **38**, 161-174.
- RANDICH, A., & RESCORLA, R. A. (1981). The effects of separate presentations of the US on conditioned suppression. *Animal Learning & Behavior*, **9**, 56-64.
- RODGER, R. S. (1974). Multiple contrasts, factors, error rate and power. *British Journal of Mathematical & Statistical Psychology*, **27**, 127-198.
- RODGER, R. S. (1975a). The number of non-zero, post-hoc contrasts from ANOVA and error-rate, 1. *British Journal of Mathematical & Statistical Psychology*, **28**, 71-78.
- RODGER, R. S. (1975b). Setting rejection rate for contrasts selected post-hoc when some nulls are false. *British Journal of Mathematical & Statistical Psychology*, **28**, 213-232.

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