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One-trial context fear conditioning with immediate shock: The roles of transport and contextual cues

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In three experiments, using a total of 120 albino rats, we assessed whether transportation cues might evoke some of the freezing (i.e., defensive immobility) that we see in a context on a day following a footshock given immediately after placement in that context. The results suggested that immediate shock could directly condition strong fear to both simulated and actual transport cues. Although conditioning to transport cues explains some of the freezing that is seen on the test day, it does not explain all of it. We also found evidence that some of the freezing is due to conditioning to permanent features of the context in which the immediate shock is given. The results support a role for transport cues in theories of context conditioning and argue against shock-processing accounts of the conditioning deficit that results from immediate shock.

A rat is placed in a box and given a single brief shock. On the next day it is returned to the box and its behavior is examined. The behavior is found to consist in part of *freezing* (i.e., defensive immobility). Such freezing is regarded as an index of fear, classically conditioned to the box or context. The amount of freezing depends critically on the time interval between placement and shock. The freezing is weak if the interval is very short (0–15 sec) and grows stronger as the interval lengthens (e.g., 45–135 sec); it weakens again as the interval increases further (Bevins & Ayres, 1995; Fanselow, 1986, 1990; Kiernan & Westbrook, 1993; Maes & Vossen, 1992; Westbrook, Good, & Kiernan, 1994). Students of Pavlovian conditioning recognize this data pattern as the inverted-U shaped interstimulus interval (ISI) function. This change in conditioned-response strength with the increase in time between the onset of the to-be-conditioned stimulus (CS) and the onset of the unconditioned stimulus (US) is a pervasive result in Pavlovian conditioning with discrete CSs (Kimble, 1961; Rescorla, 1988). Despite wide variations in the range of effective ISIs across various conditioning preparations, the qualitative similarity of the shape of ISI functions in these preparations is frequently taken as strong support for a "general process" approach to the study of learning (for discussion, see Mazur, 1994, chap. 9).

In one-trial context fear conditioning, at least one point along the ISI function is still controversial. Fanselow and his colleagues (e.g., Fanselow, 1986, 1990; Fanselow, Landeira-Fernandez, DeCola, & Kim, 1994) have reported that when shock occurs immediately upon context placement, context conditioning is completely absent. Indeed, Fanselow coined the term "immediate-shock deficit" to describe this lack of context fear. However, our laboratory (Bevins & Ayres, 1994, 1995) and the Blanchards' (Blanchard, Fukunaga, & Blanchard, 1976) have reported some context conditioning, albeit weak, with an immediate shock. More recently in our laboratory (Bevins, McPhee, Rauhut, & Ayres, 1997), we have confirmed the latter finding, using a variety of measures in addition to freezing.

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The evidence that Bevins and Ayres (1995) found for context conditioning following immediate shock consisted of freezing that occurred mainly at the start of the test session. That pattern of freezing suggests that transport, handling, and placement cues may have played a role in evoking the freezing that was measured. These cues would have preceded the immediate shock on the conditioning day. To the extent that the same cues recurred on the subsequent test day, they might have evoked at least some of the freezing that was measured during testing. The transport, handling, and placement cues and their stimulus traces would presumably prevail mainly at the start of the test session.

In the present research, we sought to determine the role of transport, handling, and placement cues, hereafter termed *transport cues*. Can these cues indeed become conditioned and, thus, contribute to the freezing that we see in the test session that follows experience with immediate shock?

Any assessment of conditioning to transport cues is difficult to make, because the measure of conditioning (i.e., freezing) cannot be taken during transport and because the very procedure of transporting, handling, and placing the rat in a test box is likely to prevent the freezing response from occurring until the animal is released into the box. Given that the measured freezing occurs only in the test box, it is hard to tell whether it is evoked in part by transport cues or entirely by the test box itself.

One possible method of assessing conditioning to transport cues might be to simulate them, using a second chamber or context. Like the transport cues, the second context would precede the immediate shock in the conditioning context; but, unlike actual transport cues, the second context would permit both the expression and the measurement of freezing in its presence. In Experiment 2 of this report, we used a second context to simulate the transport cues. The second context, termed the *transport context*, differed from the *immediate context* (where immediate shock was delivered) in terms of its visual, olfactory, spatial, and tactile attributes. We asked whether it was possible to demonstrate conditioning to the transport context. An affirmative answer would implicate transport cues in the conditioning that is sometimes seen following immediate shock.

Before we conducted Experiment 2, it was necessary to perform a preliminary experiment, Experiment 1, to demonstrate that there was minimal generalization between the contexts slated to serve as the transport context and the immediate context. Strong generalization between these contexts would preclude a clear interpretation of results (see the next section).

After finding evidence for substantial generalization decrement across our two contexts in Experiment 1 and then finding evidence for conditioning to simulated transport cues in Experiment 2, we conducted Experiment 3 in order to search for evidence of conditioning to more realistic transport cues.

EXPERIMENT 1

Bevins et al. (1997) used a two-way shuttle box with black and white sides in their multiple-measure study of the effects of immediate shock. To relate the present results as closely as possible to theirs, we sought initially to use those black and white sides as our transport and immediate contexts. Our measurements, however, indicated strong generalization between those sides. That generalization precluded a clear interpretation of any results that we might obtain in the design that we planned for Experiment 2. For example, if the transport and immediate contexts were so similar as to produce strong generalization between them, then placing the rat in the transport context would be akin to placing it in the immediate context. If the rat spent any appreciable time in the transport context before receiving immediate shock in the immediate context, the immediate shock would be tantamount to a delayed shock. It would condition strong fear to the immediate context, and that fear would generalize readily to the transport context. Such a result would be of little theoretical interest. Of much greater interest would be a failure of the immediate shock to support strong conditioning to the immediate context (because of an unfavorable ISI), coupled with a successful conditioning of strong fear to the transport context (because of a favorable ISI). Such a demonstration would be possible only if little generalization occurred across contexts. The purpose of Experiment 1 was to demonstrate that little generalization occurred between the boxes that we intended to use in Experiment 2 as the transport and immediate contexts.

Our choice of techniques for demonstrating generalization decrement across contexts was guided by the following consideration. We believed that such a demonstration would be more convincing if conditioning were strong to the box in which shock occurred than if conditioning were weak. The failure of a weak fear to generalize from the conditioning box to the test box would not be particularly impressive. For this reason, we chose, in Experiment 1, to use a delayed shock (instead of an immediate shock) in the conditioning box because we knew it would condition a strong fear to that box. We did this despite the fact that, in Experiment 2, we planned to give an immediate shock in the immediate context (conditioning box) to see whether that would condition fear to the simulated transport cues (the transport context). Our assumption was that if a strong fear did not generalize from the conditioning context to the other context in Experiment 1, the weak fear conditioned to the immediate context in Experiment 2 would not generalize either. Therefore, if in Experiment 2 we found strong fear to the transport context, that strong fear must be due to the fact that the transport context preceded immediate shock in the immediate context by a favorable ISI—not that it received generalized strength from the immediate context.

METHOD

Subjects. The subjects were 40 experimentally naive male albino rats of the Holtzman strain from Harlan Sprague-Dawley, Indianapolis. About 74 days old at the start of the study, they were housed singly in wire-mesh stainless steel cages in a room that was lighted daily between 0600 and 2200 h. Experimentation occurred between 0900 and 1700 h. The rats had free access to food and water throughout the experiment. Each rat was handled for 1 min on each of 5 days before the conditioning day.

Apparatus. Two contexts were used. One context was the black side of the two-way shuttle box previously used by Bevins et al. (1997). The inside dimensions were 19.4 × 20.3 × 22.2 cm (height × width × length). The back wall (the wall farthest from our camera) and lid were Plexiglas with black cardboard mounted on the outside. The end walls were glossy black. In the center of one end wall, 14 cm above the grid floor, was a nonfunctional cue light (1.5 cm in diameter). The front wall was clear Plexiglas. The floor was made of 20 stainless steel rods (2 mm in diameter), with their centers spaced 1.2 cm apart. Under the floor was a switch whose depression signaled a computer in an adjoining room to start the session. A sheet of gray cardboard lined the litter tray. The cardboard was covered with about 125 cc of cat box litter (Tidy Cat Scoop). The cat litter provided a sand-like floor and a distinctive odor. It also allowed the box to be cleaned easily after each rat had been run, because it caused urine to clump into a ball. Before each rat was conditioned or tested, the chamber was washed with a solution of 5% distilled vinegar (5% acidity) and 95% tap water. A frosted red bulb (7.5 W, 110 V), mounted about 30 cm in front of the front wall of the chamber and about 27 cm above the grid floor, provided the lighting needed for videotaping.

The second context was a larger, square box with inside dimensions of 23.0 × 45.3 × 45.3 cm. The walls and lid were constructed of clear Plexiglas. The floor was made of 24 stainless steel rods (6 mm in diameter), with their centers spaced 2.0 cm apart. A sheet of gray cardboard lined the floor below the rods. A vanilla-scented deodorizer (Stick-Ups) was opened about 2 mm and placed on the inside of the lid of the box to provide a distinctive scent. The box was wiped with tap water before each rat was conditioned or tested. The box was placed inside a sound-attenuating cube (.61 cubic meters) with its front door left open. Lighting was provided by a white-frosted bulb (15 W, 120 V) mounted on the ceiling of the housing cube.

A window air conditioner provided masking noise of 68 dB in the conditioning room. A Grason Stadler shock source (E1064) provided a scrambled grid-shock US (2 sec, 1.3 mA). The rats were filmed with a Panasonic video camera (Model AG–180). Facing the camera was a 28-V white indicator lamp (6 mm in diameter), mounted on a metal stand (4 cm wide × 6 cm high). The lamp was shielded from the rat's direct view by a small cardboard box that surrounded the stand. The lamp was centered just outside and below the front Plexiglas wall of the shuttle box. Throughout each session, it flashed on (0.1 sec) and off (1.9 sec). Its flashing on the video tape was used to pace behavioral observations.

Procedure. On the conditioning day, each rat was carried individually on the experimenter's arm from the colony to the conditioning room. There, half the rats were placed singly in the black side (B) of the shuttle box. After an interval of 120 sec, they were given a single 2-sec, 1.3-mA grid shock. They were removed from the box 30 sec after shock termination. The other half of the rats received the same treatment in the large, clear Plexiglas (P) box. On the next day, the rats were tested for freezing in a 5-min test session. Half were tested in the box where they had previously received shock; half were tested in the other box. Thus, four groups of rats were formed (B–B, P–P, B–P, and P–B). On the test day, each rat's behavior was videotaped. Through experimenter error, the camera was not turned on for 1 rat in Group B–B and 1 in Group P–P, reducing the sample size in these two groups to 9.

Behavioral observations. In all of the experiments reported here, freezing in a 5-min test session was scored for each rat from the videotapes. Freezing was defined as the absence of movement except that of the rat's sides in breathing (e.g., Fanselow, 1980). Not freezing was defined as anything else. Each rat's behavior was sampled every 2 sec, paced by the flashing 28-V white indicator lamp previously described. A primary rater always scored the behavior of all the rats. A secondary rater independently scored the behavior of a subset of those rats (usually all the rats on an arbitrarily selected reel of videotape). Neither rater knew the rats' experimental histories. The Pearson product moment correlation between the freezing scores obtained independently by the two raters always exceeded .95.

Results and Discussion

As can be seen in Figure 1, the rats conditioned in one chamber and tested in the other (Groups B–P and P–B) showed much weaker freezing than did the rats in the other two groups, which were conditioned and tested in the same chamber [Wilcoxon rank sums, $T(18,20) = 489$, $p < .01$]. Thus, generalization decrement was significant. The figure also suggests that the conditioning history of a chamber was much more important than were the physical characteristics of the chamber.

The strong generalization decrement found in Experiment 1 encouraged us to use its two chambers as the transport context and the immediate context in Experiment 2.

EXPERIMENT 2

In Experiment 2, we sought to assess conditioning to transport cues when those cues preceded immediate shock. In order to do so, we used one of the contexts from Experiment 1 to simulate those cues. We will term this context the *transport context*. Each rat was first placed in the transport context for 120 sec and then into a second context where it received an immediate shock. We will term the second context the *immediate context*. For half the rats, the interval that separated removal from the transport context and placement in the immediate context was short (3 sec); for the other half, it was long (24 h). On the next day, we tested half the rats for conditioning in the immediate context and half in the transport context. The groups formed were termed S–I, L–I, S–T, and L–T. Here, the letters after the dash denote the test context (immediate or transport), and the letters before the dash denote the crucial feature of the conditioning treatment; S means that there was a short gap (3 sec) between removal from the transport context and placement in the immediate context, and L means that there was a long gap (24 h). For half the rats in each gap condition (S or L), the black side of the shuttle box served as the transport context, and the large, square, Plexiglas box served as the immediate context. For the remaining rats, the reverse was true.

If an immediate shock could condition transport cues, we would expect the transport context to evoke more freez-

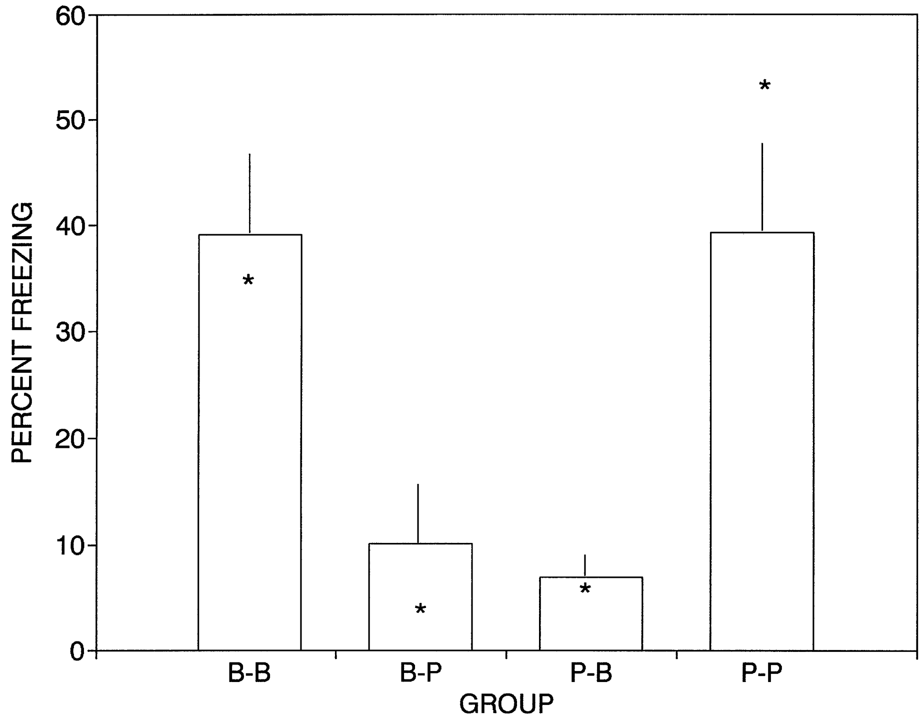

Figure 1. Results of Experiment 1. For each group, bars show the mean level of freezing; the vertical line above the bar shows +1 standard error of the mean (*SEM*); asterisks show the median. In the group names, the letter B denotes a small black box. The letter P denotes a large, square, clear Plexiglas box. The letter before the dash indicates where the rats received a shock on the conditioning day. The letter after the dash indicates where the rats were tested 24 h later.

ing in Group S–T, for which the gap between the transport context and shock was short, than in Group L–T, for which the gap between the transport context and shock was very long. Indeed, we might even expect more freezing in Group S–T than in Group L–I, even though both groups received the shock in the immediate context but not in the transport context. The basis for that prediction was that short ISIs (0–15 sec) between placement in a context and shock are detrimental to contextual conditioning, whereas longer ISIs (45–135 sec) are favorable (Bevins & Ayres, 1995; Fanselow, 1986, 1990; Kiernan & Westbrook, 1993; Maes & Vossen, 1992; Westbrook, et al., 1994). For Group S–T, the transport context enjoyed a more favorable ISI than did the immediate context in Group L–I. Note that Group L–I, rather than Group S–I, was the appropriate group to compare with Group S–T here, because some of the freezing evoked by the immediate context in Group S–I might reflect weak generalization of strong conditioning from the transport context. For Group L–I, in contrast, there should be little conditioning to the transport context that could possibly generalize to the immediate context. A third prediction of interest was this: If the cues that were a permanent part of the immediate context would be conditioned by immediate shock, we should expect more freezing in Group L–I, which was tested in the immediate context, than in Group L–T, which was tested in the transport context. In this comparison, the simulated context cues (the transport context) would presumably play no role in evoking freezing in either group because those cues were separated from shock by 24 h. Other transport cues, such as those arising from carrying the rat to the experimental chamber and placing it in that chamber would presumably be equated for these two conditions. Thus, more freezing in Group L–I than in Group L–T would presumably reflect conditioning to the permanent features of the immediate context and generalization decrement of that conditioning when testing occurs in the other context.

Method

Subjects and apparatus. The subjects were 40 experimentally naive male albino Holtzman rats about 80 days old. Animal care and housing conditions were unchanged, as was the apparatus.

Procedure. As in Experiment 1, each rat was carried on the experimenter's arm from the colony to the conditioning room. There, each rat was placed in the transport context for 120 sec. Next, after either 3 sec (for Groups S–I and S–T) or 24 h (for Groups L–I and L–T), the rat was placed in the immediate context where, 2.5 sec later, it was shocked (1.3 mA for 2 sec). The rat was then removed from the immediate context 30 sec after shock termination and was filmed in a 5-min test session 24 h later. In the test session, rats in Groups S–T and L–T were tested in the transport context, and rats in Groups S–I and L–I were tested in the immediate context. The black side of the shuttle box and the large, square Plexiglas box of

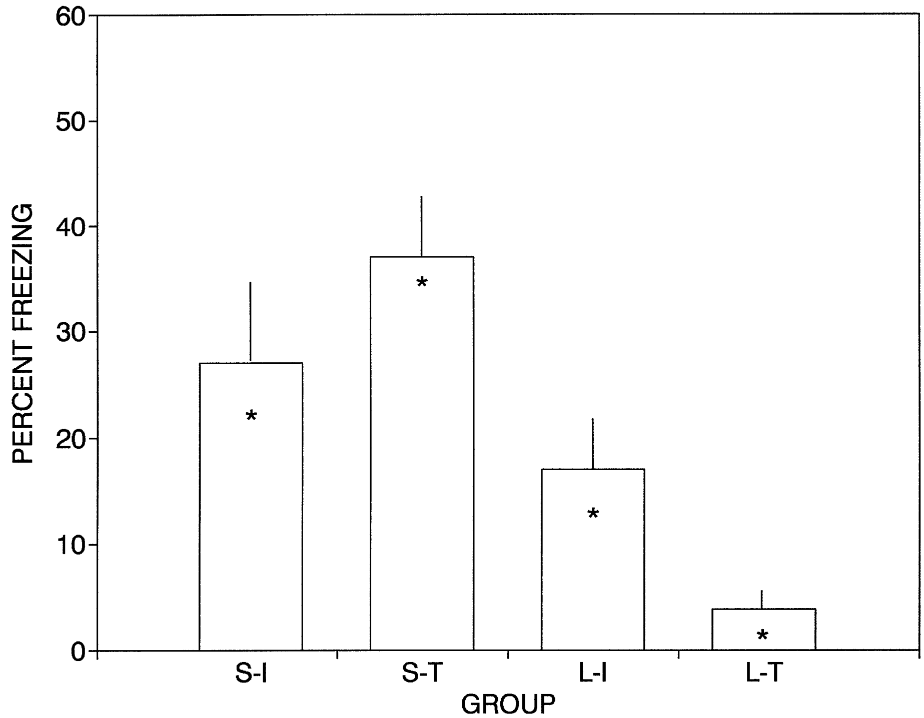

Figure 2. Results of Experiment 2. For each group, bars show the mean level of freezing; the vertical line above the bar shows +1 *SEM*; asterisks show the median. In the group names, the letter S means that there was a short (3-sec) gap between removal from a transport context and placement in the immediate context where shock was received 2.5 sec after placement. The letter L means that there was a long (24-h) gap. The letter I means that test context was the immediate context, and the letter T means that the test context was the transport context.

Experiment 1 served as the transport and immediate contexts (counterbalanced).

Results and Discussion

As is shown in Figure 2, Group S–T froze considerably more than did Group L–T [Wilcoxon rank sums, $T(10,10) = 153$, $p < .01$]. This result suggests that the transport context became highly conditioned when the gap between it and the shock in the immediate context was short. Group S–T also froze more than did Group L–I [$T(10,10) = 135.5$, $p < .05$], even though the shock occurred in the immediate context rather than the transport context. This result was expected because the transport context in Group S–T had a more favorable ISI (125.5 sec) relative to shock than did the immediate context (2.5 sec) in Group L–I. Finally, Group L–I froze more than did Group L–T [$T(10,10) = 135$, $p < .05$]. This result suggests that the permanent features of the immediate context were conditioned by the immediate shock and that this conditioning showed generalization decrement when the rats were tested in the other context. This finding is of considerable interest in its own right, but it is also interesting in comparison with results reported by Westbrook et al. (1994, Experiment 2). They found that conditioning produced by a delayed shock showed substantial generalization decrement, whereas conditioning produced by a more immediate shock showed no generalization decrement. That finding challenges the assumption we made in Experiment 1 that a strong fear conditioned by a delayed shock would be more likely to generalize to a new context than would a weak fear conditioned by an immediate shock. Our finding here of significant generalization decrement of conditioning supported by an immediate shock tends to deflect that challenge.

It might also be noted that Group S–I froze at a level that was intermediate between that of Groups S–T and L–I and did not differ significantly from either. This result is to be expected if immediate shock conditions permanent features of the immediate context and simultaneously conditions the transport context in the short-gap condition. If that is the case, the immediate context would evoke some freezing because of its own pairing with shock and some additional freezing because of the (weak) generalization from the more highly conditioned transport context.

Figure 3 shows the temporal distribution of freezing across 30-sec intervals (bins) of the 5-min test session. These data are of interest for two main reasons.

First, as mentioned above, Westbrook et al. (1994) found no evidence for generalization decrement follow-

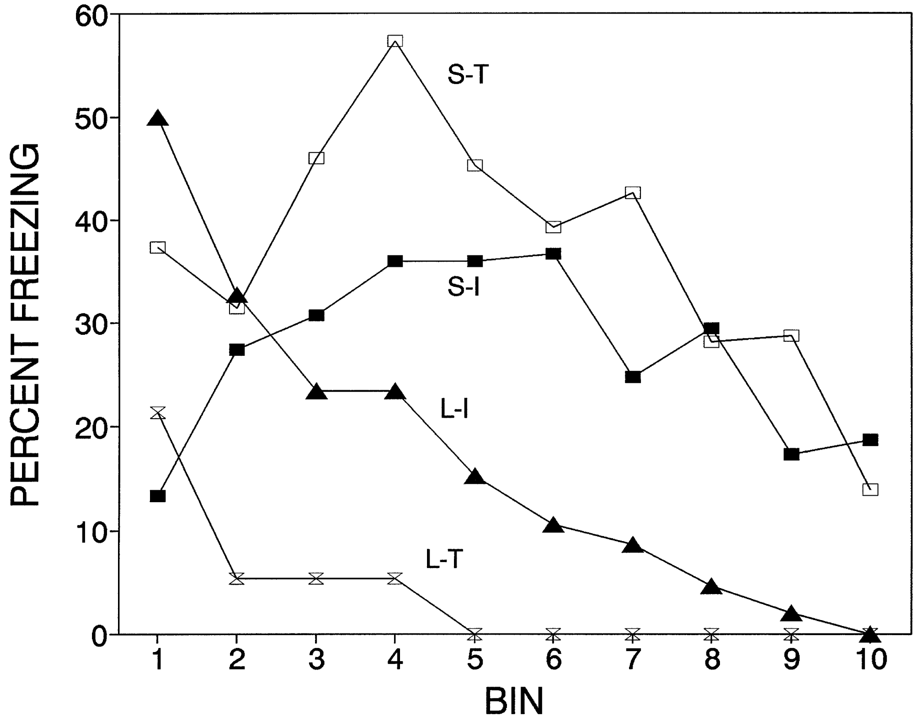

Figure 3. Temporal distribution of freezing in Experiment 2. The mean percent freezing for each group is plotted for each 30-sec interval (bin) of the 5-min test session.

ing immediate shock when rats were tested in a chamber distinctively different from the conditioning chamber. We, however, did find such evidence (compare Groups L–I and L–T in Figure 2). One possible reason for the discrepancy between laboratories is that Westbrook et al. used a 2-min test session, whereas we used a 5-min test session. Perhaps we too would have found no evidence of generalization decrement had we looked only at the first 2 min of testing. A comparison of Groups L–I and L–T in Figure 3, however, shows that in our experiment the evidence of generalization decrement following immediate shock in the immediate context was stronger in the first 2 min than at any other time.

Second, our assumptions led us to predict differently shaped distributions for different groups. For example, for Group S–T, we assumed that the transport context had a more favorable (125.5-sec) ISI relative to the immediate shock given in the immediate context. That is, the transport context was essentially paired with a delayed shock. Following a delayed shock, Bevins and Ayres (1995, Figure 5, 120-sec ISI) found an inverted U-shaped temporal distribution of freezing. That distribution peaked at roughly 2.5–3.5 min into the session, approximating the time of shock delivery on the conditioning day. By comparison, Group S–T in Experiment 2 also showed an inverted U-shaped gradient, which peaked about 2 min into the session, again approximating the time of shock delivery on the conditioning day. Likewise, we assumed that for Group L–I, the 24-h gap between exposure to the transport context and immediate shock in the immediate context was too long to support direct conditioning to the transport context. For Group L–I, then, the experience with shock should be similar to that of groups given immediate shock in Bevins and Ayres (1995). We would therefore expect the temporal distribution of freezing to be highly similar to the distribution found by Bevins and Ayres (1995) in their immediate shock groups; and, indeed, that was the case (see their Figures 2 and 5, 2.5-sec ISI). We assume, furthermore, that our Group L–T was procedurally similar to the US-alone control group used by Bevins and Ayres (1995). Both groups received an immediate shock on the conditioning day and were subsequently tested in a distinctively different box. It is therefore of interest that the temporal distribution for Group L–T is virtually identical in form and magnitude to that found by Bevins and Ayres (1995) for their US-alone control group (see their Figure 2). In contrast to the distributions for Groups S–T, L–I, and L–T, the inverted U-shaped distribution for Group S–I has no precedent that we know of. It resembles a distribution found after delayed shock, yet these rats received immediate shock in the immediate context and were subsequently tested in that context. On the conditioning day, however, the immediate shock was indeed a delayed shock with respect to the transport context. Thus, the distribution for Group S–I appears to reflect a blend of the group's experiences in the

transport and immediate contexts. The peak of the distribution approximates the time of shock delivery relative to placement in the transport context on the conditioning day. Placement in the immediate context on the test day must have resembled placement into the transport context on the conditioning day. Such placement must have triggered the temporal cues that controlled the distribution of freezing in the test session.

In summary, the present results suggest that both the permanent features of the immediate context and the (simulated) transport cues that preceded the immediate shock were conditioned by that shock. Our evidence for conditioning to simulated transport cues is similar to evidence presented by Hammond (1995). He showed that conditioning to what we call a transport context could indeed occur following multiple-trial procedures in mice. Our own work, in which we used a single shock in rats and measured freezing in both the transport context and the immediate context, may be viewed as confirming and extending Hammond's findings in such a way as to enhance our understanding of the effects of immediate shock.

To the extent that we could generalize between our simulated transport cues and real transport cues, it appeared that conditioned transport cues could have contributed to some of the freezing found in tests of the effects of immediate shock (Bevins & Ayres, 1994, 1995; Bevins et al., 1997). Our evidence for conditioning to transport cues, however, was indirect because we manipulated only *simulated* transport cues. We could not yet make a definitive statement about whether actual transport and handling cues are conditioned during the immediate shock procedure. In Experiment 3, we attempted to manipulate more realistic transport cues in order to search for evidence of conditioning to them.

EXPERIMENT 3

If immediate shock conditions transport cues, we would expect those cues to contribute to freezing on the test day, provided they were the same on the test day as they were on the conditioning day. If, however, those cues were changed drastically from the conditioning day to the test day, we should see less freezing on the test day. In Experiment 3, we tested this idea by using two very different means of transporting the rats to the conditioning and test chambers. In the *arm* method, a male experimenter carried the rat from the colony to the appropriate chamber on his arm. In the *cart* method, a female experimenter pushed the rat to the chamber in a plastic tub resting on a stainless steel cart. We studied four conditions: arm–arm, cart–cart, arm–cart, and cart–arm. Here, the term before the dash indicates the method used to transport the rat to the conditioning box, and the term after the dash indicates the method used to transport the rat to the same box for testing on the next day.

Method

Subjects. The subjects were 40 experimentally naive male Holtzman-descended albino rats, bred in our colony at the University of Massachusetts. They were about 90 days old at the start of the study. Animal care and housing conditions were unchanged from Experiments 1 and 2.

Apparatus. The conditioning and testing apparatus was the black side of the shuttle box that was used in Experiments 1 and 2. In addition, a white plastic tub (20.0 × 40.0 × 50.5 cm) was used to transport the rats assigned to the cart transport. The tub was filled with wood shavings to a depth of 2 cm. It was placed on a stainless steel cart that stood 78 cm high and had a 39.5 × 61 cm carrying surface. The cart had small, hard rubber wheels (7.5 cm in diameter) and, when pushed, gave the rats a noisy and jostling ride.

Procedure. Each rat was assigned to one of four groups (*n*s = 10; arm–arm, A–A; arm–cart, A–C; cart–cart, C–C; or cart–arm, C–A). Here, the term before the dash denotes the method of transport on the conditioning day, and the term after the dash denotes the method of transport on the test day. On the test day, the data for one rat in Group C–C were invalidated because of a disruption in the laboratory. The sample size for that group was thus reduced from 10 to 9.

Using the arm method, a male experimenter, wearing a white lab coat and white vinyl gloves, entered the colony, picked the rat up by the body with his right hand and placed it on his left arm so that the rat's nose was between the arm and the experimenter's left side. The experimenter then walked the rat down a hallway to the experimental room and placed it in the conditioning context by grasping its body and facing it toward the front of the box.

Using the cart method, a female experimenter, dressed in dark gray and wearing brown cotton gloves, entered the colony, picked the rat up by the base of its tail, and placed it in the tub that was resting on the cart. She then pushed the cart down the hallway to the experimental room and placed the rat in the conditioning context by grasping the base of its tail and facing it toward the back of the box.

The conditioning procedures resembled those used in Experiments 1 and 2. On the conditioning day, the rat was placed in the black side of the two-way shuttle box and, 2.5 sec later, was given a single 1.3-mA shock for 2 sec. It was then removed from the box 30 sec later and returned to the colony. On the next day, the rat was filmed in a 5-min test session in the conditioning box.

Results and Discussion

As is shown in Figure 4, the method of transporting the rat to the test context was important. The rats clearly froze more on the test day when they were transported on that day by the cart method than when they were transported by the arm method [$T(19,20) = 480.5$, $p < .01$]. This high level of freezing probably reflects the summation of conditioned fear together with a nonassociative arousal effect produced by the cart transport method. More importantly, for our purposes, the history of the transport cues was also critical. If the method of transport was the same on the test day as on the conditioning day, the rats froze more than they did if the methods of transport on the two days were different [$T(19,20) = 497.5$, $p < .01$]. This result clearly indicates that actual transport cues can be conditioned by immediate shock and that those conditioned cues can contribute to the freezing that is seen on the test day.

Figure 5 shows the temporal distribution of freezing in the four groups. The distributions for Groups A–A and A–C resemble distributions described before for groups receiving immediate shock (Bevins & Ayres, 1995; present Experiment 2). The distribution for Group C–C, however, shows much more persistent freezing, perhaps again reflecting the summation of context conditioning

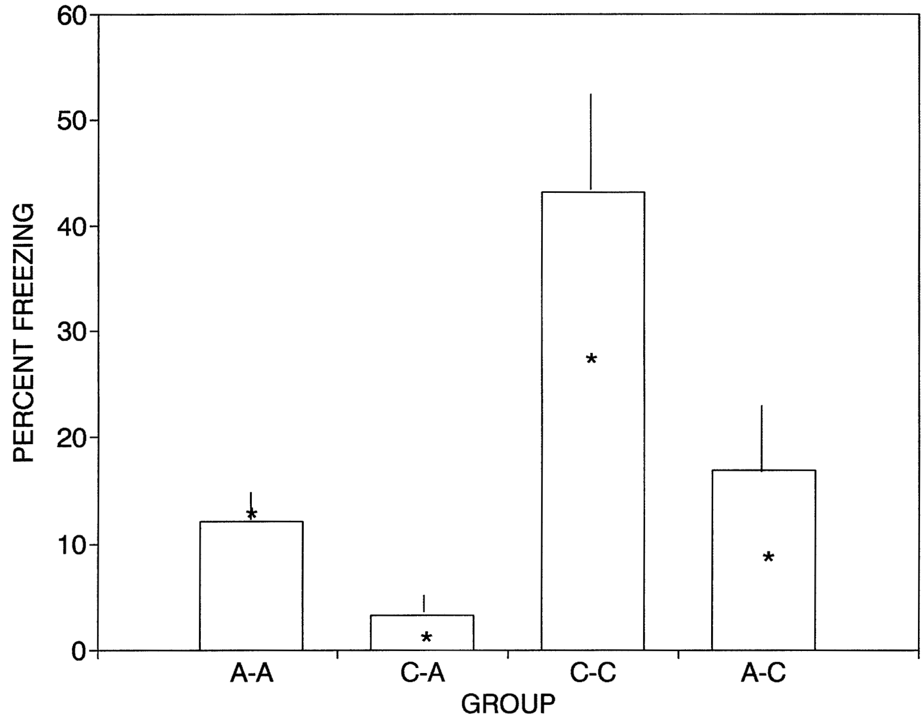

Figure 4. Results of Experiment 3. For each group, bars show the mean level of freezing; the vertical line above the bar shows +1 *SEM*; asterisks show the median. The first letter in each group name refers to the method of transporting the rat to the chamber on the conditioning day (arm or cart) and the second letter refers to the method of transporting the rat on the test day.

and a rather prolonged arousal effect of the cart method of transport. In contrast, the distribution for Group C–A looks like one that typically follows a US-alone procedure (Bevins & Ayres, 1995, Figure 2), which will be discussed below.

GENERAL DISCUSSION

The present results demonstrate that transport cues (transport, handling, and box placement) are conditioned by a single immediate shock in a context and that they can contribute to the freezing that is measured on the next day in that context. This is not to say that *all* of the freezing that is observed on the test day is due to conditioned transport cues. Some of the freezing is likely due to conditioning to the permanent features of the context in which immediate shock is received. Indeed, the results from Experiment 2 provide evidence for that possibility. In that experiment, Group L–I, which was tested in the immediate context, froze significantly more than did Group L–T, which was tested in the transport context. For both groups, a long gap (24 h) separated placement in the transport context and placement in the immediate context where immediate shock was experienced on the conditioning day. Because the gap was so long, it is unlikely that the transport context could have been directly conditioned by the immediate shock. Therefore, the transport context presumably played no role in the freezing of these two groups. There were other transportation cues (handling and box placement) that could have become conditioned and could have contributed to the freezing in the two groups, but these cues were presumably the same for both. Therefore, it is unlikely that these cues were responsible for the greater freezing in Group L–I. The most reasonable interpretation of the difference between Groups L–I and L–T, then, is that the immediate shock conditioned some permanent features of the immediate context and the conditioning showed a generalization decrement when the rats were tested in the other (transport) context.

It is well established that a short interval (0–15 sec) between box placement and shock is detrimental to context conditioning and that a longer interval (45–135 sec) is much more favorable (Bevins & Ayres, 1995; Fanselow, 1986, 1990; Kiernan & Westbrook, 1993; Maes & Vossen, 1992; Westbrook et al., 1994). Therefore, in Experiment 2, when a short gap separated removal from the transport context and placement in the immediate context, the timing favored conditioning to the transport context and did not favor conditioning to the immediate

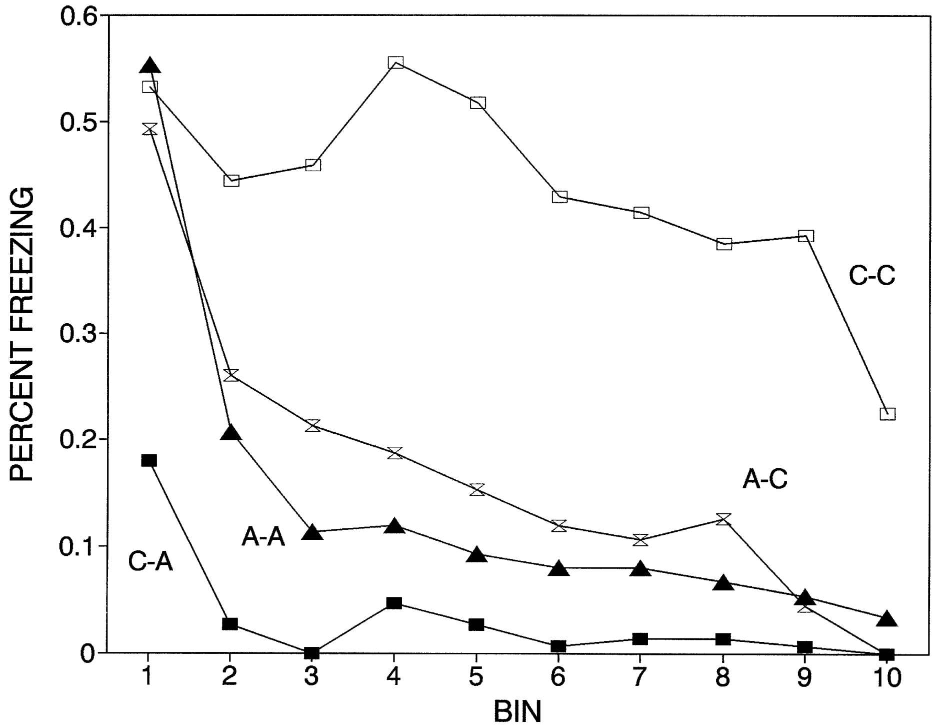

Figure 5. Temporal distribution of freezing in Experiment 3. The mean percent freezing for each group is plotted for each 30-sec interval (bin) of the 5-min test session.

context. Accordingly, under our short-gap condition, we saw more freezing in rats tested in the transport context than in those tested in the immediate context (see Figure 2).

This result bears on the hypothesis that the immediate-shock deficit is caused by a failure to process the US. Such a failure might arise if, for example, transportation and handling were to lead to a release of endogenous opioids, resulting in an opioid analgesia. More opioids might be present at the time of immediate shock than at the time of delayed shock. Bevins and Ayres (1994) tested this hypothesis by administering the opioid antagonist naloxone to rats that received immediate shock. They found that naloxone did *not* enhance the effectiveness of immediate shock and concluded that an opioid-mediated analgesia did *not* seem responsible for the immediate-shock deficit. However, they could not completely rule out the involvement of a *nonopioid* analgesia. The results of our Experiment 2 seem to rule out that involvement. That is, the same US that was ineffective in conditioning the immediate context was quite effective at conditioning the transport context (see Figure 2). It appears, therefore, that the US was well processed even though little conditioning to the immediate context occurred. Our finding, then, suggests that the immediate-shock deficit reflects a failure of CS processing and not a failure of US processing. (For other evidence against a US-processing account see Kiernan & Cranney, 1992; Kiernan & Westbrook, 1993; Kiernan, Westbrook, & Cranney, 1995.)

One theory that emphasizes a failure of CS processing to explain the immediate-shock deficit is that of Fanselow (1990; Fanselow, DeCola, & Young, 1993). According to that theory, a context is considered a polymodal stimulus made up of individual stimulus elements from different sensory modalities. The context can be conditioned only after these separate elements are processed and then integrated into a "unified representation" that may include within-element associations. According to this formulation, the immediate shock occurs before the unified representation can be formed. Hence, the immediate shock is ineffective at conditioning context fear.

A different CS-processing theory has been presented by Bevins and Ayres (1995; see also Bevins et al., 1997). According to that theory, the context comprises many elements but the animal can sample only a fraction of the available elements in any moment. Those elements that are sampled in reasonable temporal contiguity to shock are conditioned. The immediate-shock deficit is predicted by this theory to the extent that the elements sampled in the test session differ from those sampled prior to shock. The deficit need not be complete, however. Any factor that causes the rat to sample the same elements prior to shock and again during the test session will foster conditioned responding. Such elements include transport and box-placement cues. Those cues must precede immediate shock and must be present at the start of the test session. Consistent with that idea were the results of our

Experiment 3. More freezing was evoked when the transport cues were consistent across conditioning and test days than when they were inconsistent (see Figure 4). Also consistent with that idea was the extremely weak level of freezing in Group C–A of Experiment 3. That weak freezing and the temporal distribution of that freezing (see Figure 5) resemble the freezing that was found in US-alone control groups in previous work (e.g., Bevins & Ayres, 1995; Bevins et al., 1997), and, thus, it looks like a *complete* immediate-shock deficit. Reasons for such weak freezing probably include the following factors: First, the rats' transport cues differed on the conditioning day and test day. Second, the rats did not experience the arousing cart ride prior to the test. Third, they were placed facing the front wall of the box after being carried by the arm method and were placed facing the back wall after being carried by the cart method. The different methods of placement would tend to reduce the probability that they would sample the permanent features of the conditioning context on the test day in the same way that they did on the conditioning day just prior to shock.

If a context contains some particularly salient element, that element might be especially prone to being sampled both before shock and during the test session. Consistent with that idea, Fanselow (1990) found that a loud tone that was present during both the conditioning session and the test session tended to enhance conditioned responding in the test that followed a briefly delayed shock.

Another result anticipated by this stimulus sampling theory is that individual differences in context conditioning for animals that receive the same experimental protocol should depend on the animal's behavior prior to shock. Thus, the environmental stimuli that are sampled at any moment will depend on such factors as where the animal is in the environment and what it is doing at that moment. Bevins and Ayres (1995) found support for this notion. They observed each rat's behavior just before the onset of shock in a one-trial context conditioning procedure. In general, rats that were engaged in behaviors directed at specific stimuli, such as the grid or the wall, subsequently froze less than rats classified as engaging in general exploratory behaviors. Bevins and Ayres (1995) suggested that rats engaging in directed behaviors would be sampling only a very narrow subset of the stimuli that make up the environment at the moment of shock. Thus, on the test day, freezing is weak because only a small subset of stimuli were conditioned, and the likelihood of sampling these conditioned elements on the test day is relatively low. In contrast, rats that engaged in general exploratory behavior were more likely to be sampling a wide range of stimulus elements at the moment of shock. Thus, with a wider range of elements acquiring conditioned strength, the probability that the rat will sample conditioned stimuli on the test day (and, in turn, freeze) is high.

To summarize, a US-processing account of the immediate-shock deficit has not been well supported either here or elsewhere (Kiernan & Cranney, 1992; Kiernan & Westbrook, 1993; Kiernan, et al., 1995). In contrast, a CS-processing account, particularly a stimulus sampling version of that account, seems able to explain a wide range of results in one-trial context-conditioning situations, including the results of the present research.

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