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Effect of Retraining Trials on Memory Consolidation in Weakly Reinforced Learning

SIMON F. CROWE, KIM T. NG[1] AND MARIE E. GIBBS

Department of Psychology, Latrobe University, Bundoora, Victoria, Australia, 3083

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CROWE, S. F., K. T. NG AND M. E. GIBBS. *Effect of retraining trials on memory consolidation in weakly reinforced learning.* PHARMACOL BIOCHEM BEHAV 33(4) 889–894, 1989.—Day-old chicks given a single weakly reinforced (20% v/v methyl anthranilate in absolute ethanol) passive avoidance learning trial showed no evidence of long-term memory. A second learning trial given at 15 minutes after initial training resulted in consolidation of the learning experience into long-term memory. The retention function resulting from two learning trials is similar to that observed with a single strongly reinforced learning trial, and consists of the stages postulated by Gibbs and Ng. With a dilution of 10% methyl anthranilate in ethanol, four training trials were needed to yield unequivocal evidence of long-term memory consolidation.

Day-old chicks Weakly reinforced passive avoidance learning Repeated training Long-term memory consolidation Dinitrophenol

THERE is ample evidence to suggest that in passive avoidance learning the consolidation of memory into a relatively permanent memory trace may depend upon the intensity of the aversive stimulus used (1–3, 12).

Using a single trial taste-mediated passive avoidance task (4), we have previously demonstrated that diluting the strength of the chemical aversant (methyl anthranilate) yields high levels of retention up to 40 to 45 minutes after training, and very little or no memory for the task thereafter (2). With a concentrated aversant, Gibbs and Ng (4,5) have provided extensive evidence to suggest that formation of memory involves at least three stages: a short-term stage (STM) available for up to 10 minutes following training and susceptible to inhibition by depolarizing agents such as monosodium glutamate and potassium chloride; an intermediate stage (ITM), available between 20 and 50 minutes after learning, and susceptible to inhibition by sodium pump inhibitors such as ouabain and ethacrynic acid; and a long-term stage (LTM) available after 55 minutes following learning and inhibited by protein synthesis inhibitors, including cycloheximide and anisomycin. Furthermore, these authors have shown that the ITM stage may consist of two phases: an energy-dependent phase (phase A) which may be inhibited by the metabolic inhibitor 2,4-dinitrophenol (DNP), and an energy-independent phase (phase B) which is not blocked by DNP (6). Within the framework of this model, we showed that the memory available to chicks trained with a diluted aversant (20% v/v methyl anthranilate in absolute ethanol) consists of the STM and ITM phase A stages only (2).

It has been suggested that consolidation of LTM in avoidance learning may depend upon the arousal effects of the aversive learning experience initiated by the reinforcing effects of the aversant (8–10). Although memory is not consolidated under weakly-reinforced learning conditions, it has been argued that such a learning experience leaves a subthreshold engram which can be raised above the threshold of consolidation by reminder trials (1,7). This reminder effect has been attributed to the effects of what is essentially an additional learning trial on a subthreshold engram (7) rather than to a retrieval failure following a "weak" initial training experience (11,14), although some doubt attaches to this conclusion (13) in a situation where the "reminder" trial consists of a reduced reinforcement version of the original trial [see, for example, (1)].

In this paper we report the results of the effect of retraining on memory consolidation following training of day-old chicks with a discriminated passive avoidance task using a "weak" aversant reinforcer. The retraining trials are identical to the original training trial.

METHOD

Animals

Day-old black Australorp, white Leghorn chicks were obtained from a local hatchery on the morning of each experiment.

[1]Requests for reprints should be addressed to Dr. K. T. Ng.

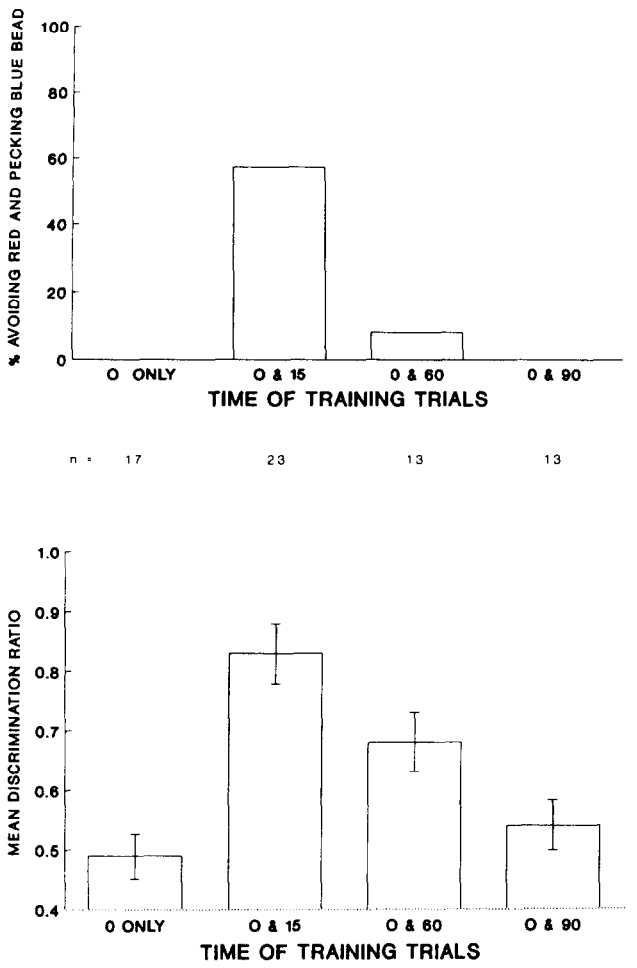

FIG. 1. Retention levels measured at 180 minutes postlearning of chicks trained on a passive avoidance learning task with a weak reinforcer (20% MeA in absolute ethanol) and given a second training trial at 15, 60 or 90 minutes after the first trial. Using planned contrasts of proportions (15), pairwise differences (top figure) greater than or equal to 0.25 would be significant at $\alpha = 0.05$.

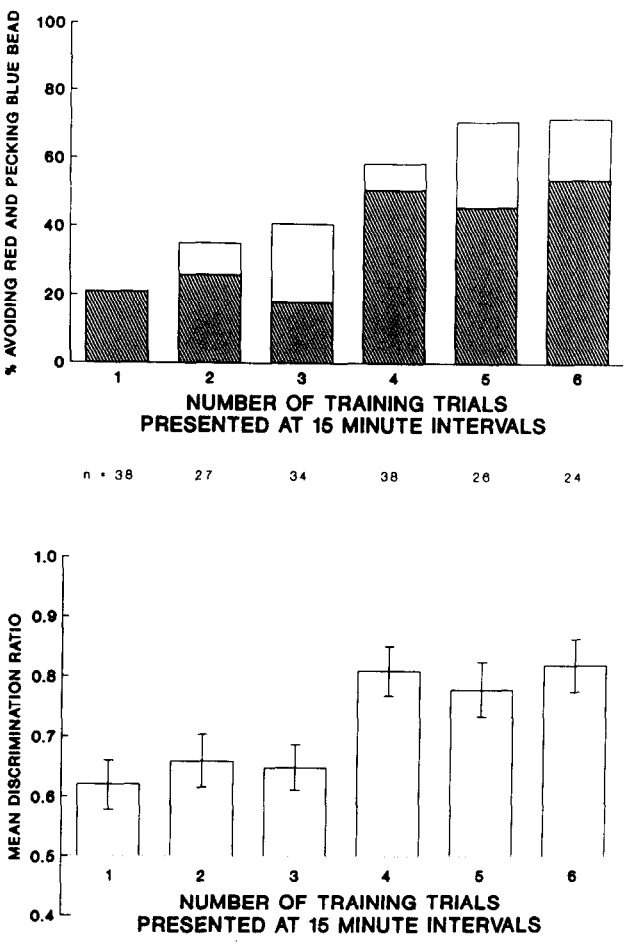

FIG. 2. Effect of varying the number of training trials (at 15 minutes following the first trial) on retention levels measured 180 minutes after initial training with a 10% v/v dilution of MeA in absolute ethanol. Unshaded areas in the top figure show the cumulated percentage of chicks in each condition avoiding the red bead, independent of their response to the blue bead or the number of completed training trials. Pairwise differences in proportion of chicks avoiding the red and pecking the blue bead (top figure, stippled) would be significant at $\alpha = 0.05$ if the difference is equal to or greater than 0.24.

Approximately 16 chicks were used for each data point, depending on the number successfully trained from an initial subject pool of 20 birds. In some instances larger subject pools were employed.

Drugs

Saline (SAL, 154 mM) or 2,4-dinitrophenol (DNP, 0.2 mM, Sigma) were administered to the centre of each forebrain in 10 μl volumes by freehand injection using a Hamilton repeating dispenser syringe. A stop on the needle regulated the injection depth to approximately 3 mm, in an area of the brain where previous studies have shown amnesic effects from the drug in question (6). Drugs were injected blind and the codes were not broken until after the behavioural data for each group of chicks had been extracted.

Procedure

The experimental paradigm is essentially that described in (4). Briefly, chicks were pretrained in pairs to peck at a red and a blue glass bead, dipped in water and presented in succession for 10 seconds each. Following pretraining, a similar red bead to the one used in pretraining was coated with an aversant solution and presented to the chicks for a period of ten seconds. Several different aversant concentrations were used in the study, and these were made up as v/v solutions of methyl anthranilate in absolute ethanol. Retraining trials are essentially the same as training trials but given at selected delays after initial training. Chicks pecking at the bead typically show a disgust reaction which includes shaking their heads and wiping their beaks on the floor. The number of pecks in the 10-second period and the corresponding latencies to first peck for each bead for each chick were recorded by an on-line computer via a manual keyboard.

On the retention trials, a dry red and a dry blue bead were presented in succession for 10 seconds each, and the number of pecks in each 10-second period for each bead and the corresponding latencies to first peck were recorded for each chick. The proportion of chicks avoiding the red but pecking the blue bead at each learning-retention interval and a discrimination ratio for red

TABLE 1

ATTRITION RATES OF SUBJECTS FOR EXPERIMENT II: EFFECTS OF RETRAINING WITH A 10% DILUTION OF METHYL ANTHRANILATE

	Number of Training Trials					
	1	2	3	4	5	6
N (initial)	40	40	60	80	60	80
Avoidance on one training trial	0	9	26	37	33	55
Avoidance of blue at 180 min	2	4	0	5	1	1
N (final)	38	27	34	38	26	24
Avoidance of red and pecking blue	8	7	6	18	12	13
% Avoidance N (final)	21	26	18	47	46	54
% of N (initial) avoiding the red bead at 180 min	20	35	41	59	71	72

and blue beads were determined. The discrimination ratio was defined as the number of pecks at the blue bead on the test trial divided by the total number of pecks for each ten second trial at both the red and the blue bead [cf. (2)]. The discrimination ratio data were used in the statistical analyses. All chicks avoiding the blue bead on the test trial were excluded from final data analysis. As argued elsewhere (2) the reason for avoidance of the blue bead, which was never associated with an aversant taste, appears to be due to nonspecific effects of the treatments employed. Interpretation of this is not unequivocal. The number of birds excluded on this basis was approximately 10% for a given training test interval a similar number to that excluded as a consequence of failing to peck at the lure on the training trial.

RESULTS

Experiment I: Effects of Retraining at Various Times After the Initial Training Trial

The retention function following training of day-old chicks with either a 100% or a 20% v/v dilution of the aversant methyl anthranilate in absolute ethanol shows a transient retention deficit at 15 minutes after learning (2). At this time, chicks which would normally avoid the previously aversive red bead will tend to peck at that red bead. It is possible, therefore, at this time to give the chicks a second training trial. With a diluted aversant (i.e., weakly-reinforced learning) chicks do not tend to avoid the previously aversive red bead after 50 minutes following learning, because of absence, presumably, of LTM. A second presentation of the learning experience can also be effected at these times.

There is evidence to suggest that processes involved in the consolidation of LTM are initiated within the first 30 minutes or so after training with a concentrated aversant (4). It is of interest, therefore, to determine: 1) whether a second learning experience with a diluted aversant would result in consolidation of the experiences into LTM, and 2) the effective times when such retraining trials should take place.

In this experiment, chicks were trained with a dilution of 20% methyl anthranilate dissolved in absolute ethanol and given a second training trial with the same aversant solution at 15, 60 or 90 minutes after the initial training trial. A control group of chicks was given only the initial training experience. Retention was tested at 180 minutes following initial learning. The results clearly demonstrate that a second training trial at 15 minutes after initial learning produced a level of retention at 180 minutes postlearning comparable to that observed with a single learning trial using a concentrated aversant [Fig. 1: cf. (2)]. When the second training trial was given at either 60 or 90 minutes following the initial learning trial, the levels of retention observed at 180 minutes following learning were low and comparable to that observed with a single "weak" aversant training experience.

A simple analysis of variance yielded a significant between groups main effect, $F(3,62) = 11.95$, $p<0.00$. Post hoc Newman Keul's tests confirmed that chickens given a second retention trial at 60 or 90 minutes after the original training trial did not show significantly different retention levels from that observed with the control group given only one training trial. However, group two, which received a second training trial at 15 minutes after original training, demonstrated significantly higher levels of retention at 180 minutes postlearning compared to the other groups.

The above results suggest that a single additional learning trial with the diluted aversant solution is sufficient to consolidate memory into a long-term representation, provided that the second training trial is given at an appropriate time.

Experiment II: Effects of Retraining With a 10% Dilution of Methyl Anthranilate

We have previously shown (2) that not only did a dilution of 20% MeA in absolute ethanol used in the training trial result in a failure to consolidate the learning experience into long-term memory (LTM), but it also yielded retention levels lower than those observed with the use of 100% MeA. It is possible, therefore, that with varying concentrations of the aversant in the training trial, a different number of retraining trials may be needed in order to effect consolidation of LTM. This is consistent with either the view that there is a cumulative effect of retraining trials with a progressive increase in retention levels, or with the proposition of a threshold level of a memory trace (1) that is necessary for memory retrieval. To investigate this possibility, independent groups of chicks were trained using a 10% v/v concentration of methyl anthranilate in absolute ethanol. One to five additional trials were given at 15-minute intervals after the initial training trial and retention tested at 180 minutes after initial training. Chicks failing to peck the training bead in the original training or any subsequent retraining trial were eliminated from subsequent data analysis.

As indicated in Fig. 2, the proportion of chicks avoiding the red but pecking the blue bead remain low for chicks given one to three training trials while those given four to six trials showed markedly higher retention levels. A similar pattern of results is observed with the discrimination ratios. The nonshaded area on the top section of Fig. 2 represents the total percentage of the entire sample which avoided the red bead at 180 minutes, the stippled area represents those subjects which had the stipulated number of training trials. Table 1 presents details of the initial and final subject pools employed in Experiment II.

A simple analysis of variance yielded a significant between groups effect, $F(5,178) = 4.55$, $p<0.00$. Post hoc Newman-Keuls tests confirmed that the group of chicks receiving four training trials had a significantly higher mean discrimination ratio than groups one, two or three. The groups receiving one, two or three training trials did not differ significantly from one another in their mean discrimination ratios. Nor were the differences between groups four, five or six trials significant.

These results would appear to suggest that: 1) with decreasing concentration of the aversant the number of retraining trials

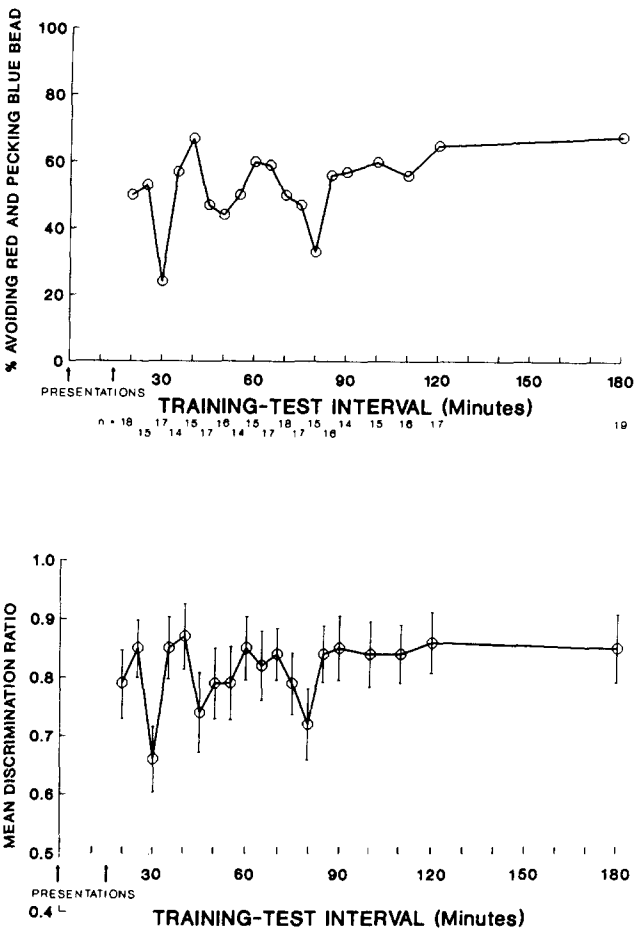

FIG. 3. Retention functions of chicks given a second retraining trial 15 minutes after initial training with 20% v/v MeA in absolute ethanol.

required for consolidation of LTM increases, and 2) the effect of repeated training trials is related to some threshold intensity of the available memory trace above which consolidation of LTM formation is "triggered."

Experiment III: Retention Function Following Two Weakly Reinforced (20% Methyl Anthranilate) Training Trials

Given that two training trials with a dilution of 20% v/v methyl anthranilate in absolute ethanol yielded memory for the task at 180 minutes following initial training, it is relevant to determine the nature of the resulting retention function. Independent groups of chicks were given a second training trial at 15 minutes following initial training and retention tested at various times between 20 minutes (i.e., five minutes after the second training trial) and 180 minutes after the initial training (see Fig. 3).

The results show that a substantial proportion of chicks avoided the previously aversant red bead and pecked the blue bead, as well as high discrimination ratios at all training-test intervals except at 30 and 80 minutes and possibly at 45 to 50 minutes after the initial training. The transient retention deficit observed 30 minutes after initial training corresponds to a delay of 15 minutes following the second training trial, and may represent the normal transient deficit observed 15 minutes after a single training trial with either a concentrated or a diluted aversant [see Fig. 4, (2)].

The transient retention deficit observed at 80 minutes following initial training may represent the second transient retention deficit observed at 55 minutes following a single concentrated methyl anthranilate training trial [see Fig. 4, (2,5)]. The apparent temporary retention loss observed between 40 and 50 minutes after the initial training trial is surprising but there is no obvious explanation for this.

Overall, these results suggest that a second training trial at 15 minutes following an initial weakly reinforced training experience produces a retention function similar in most respects to that seen with a single normal (concentrated methyl anthranilate) learning experience.

Experiment IV: Characterization of the ITM Following Two Weakly Reinforced (20% Methyl Anthranilate) Training Trials

If it is assumed that the memory observed between 15 and 80 minutes after initial training with chicks given a second training trial with the 20% aversant solution represents the ITM stage in the

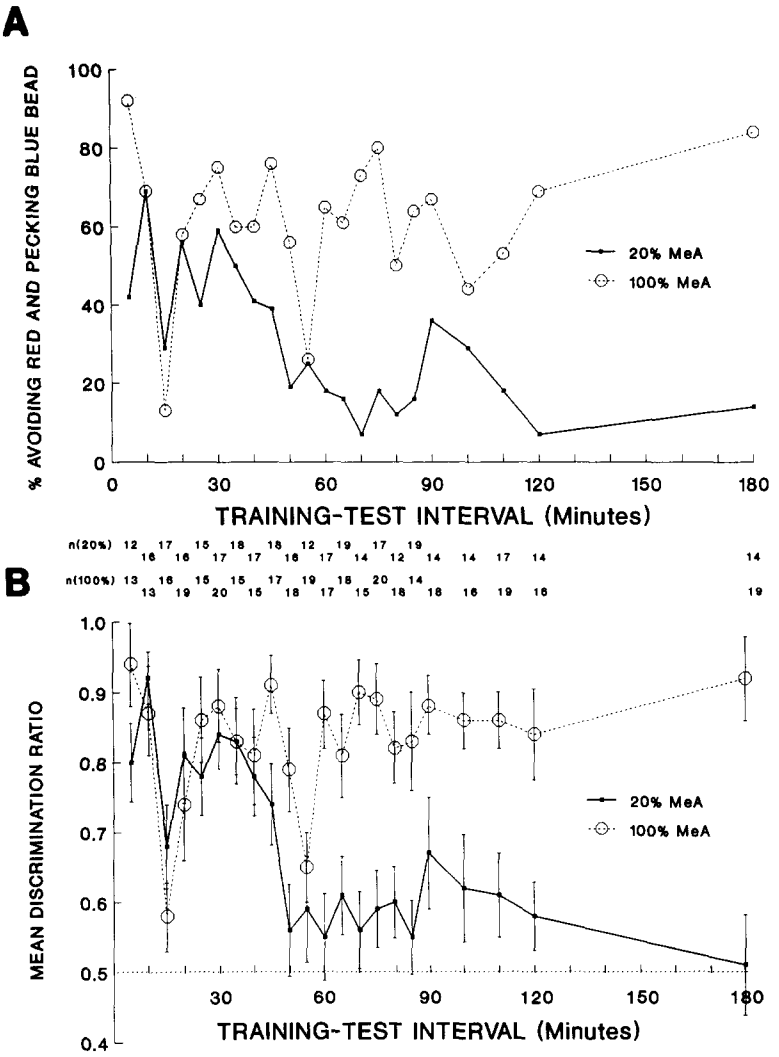

FIG. 4. Retention functions of chicks given a single training trial with 20% or 100% v/v MeA as indexed by % discrimination memory (top) and discrimination ratio (bottom). [Adapted from Crowe *et al.* (2).]

Gibbs and Ng (4,6) three-stage model, then it is interesting to determine whether this stage of memory consists of the two phases postulated for the normal retention function (6).

Independent groups of chicks given a second weakly reinforced training trial 15 minutes after initial training were administered DNP or saline intracranially at various times between 10 and 80 minutes following initial learning. Retention was tested 10 minutes after administration of DNP or saline. The results indicate that DNP is effective in inducing loss of retention when administered between 30 and 55 minutes after the initial training trial (i.e., between 15 and 40 minutes after the second training trial) but not later (Fig. 5).

An unweighted means two-way ANOVA [drugs (2) by injection time (11)] resulted in a significant drugs, $F(1,320) = 14.67$, $p<0.00$, and injection time, $F(10,320) = 5.16$, $p<0.00$, main effect and a significant interaction effect, $F(10,320) = 2.11$, $p<0.025$. Comparisons between DNP- and saline-treated chicks for each injection time showed a significant depression of retention for groups administered the drug at 30, 40, 50 and 55 minutes after training and tested 10 minutes after drug administration. Differences between the DNP- and saline-treated groups were not significant for any other time of administration. It would appear therefore that the stage of memory occurring between 40 and 75 minutes after initial training (i.e., between 25 and 60 minutes after the second training trial) consists of two phases, the first susceptible to DNP inhibition and the second not. Transient retention deficits were again apparent at 30 and 80 minutes after the initial training trial (i.e., 15 and 65 minutes after the second training trial).

DISCUSSION

The findings from this series of experiments are consistent with the observation of Cherkin (1) and others [e.g., (7)] that failure to consolidate memory for a weakly reinforced learning experience can be overcome by retraining trials. Indeed, the results also suggest that processing of memory following a successful relearning experience may follow the same sequence of stages that have been established under normal (strongly reinforced) learning (5).

Of particular interest is the finding that the second stage of

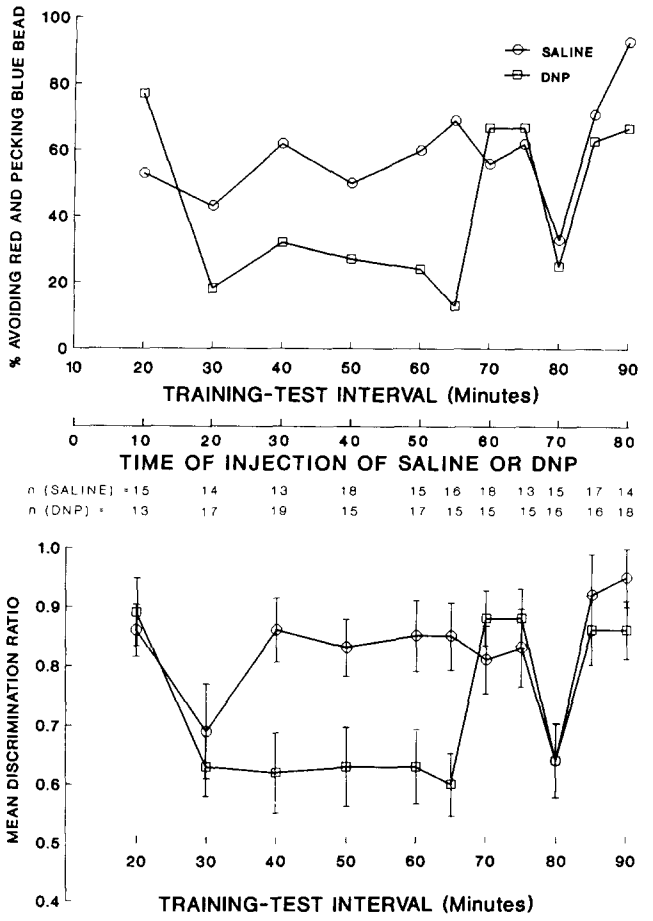

FIG. 5. Effects of dinitrophenol administered at various times after the first training, on retention levels of chicks given a second training trial 15 minutes after initial training. Pairwise differences in proportions (top figure) if greater than or equal to 0.35 would be significant at $\alpha = 0.05$.

memory formation arising from the retraining experience may share the same temporal characteristics and cellular bases as the ITM stage reported by (6). Thus, this stage of memory appears to consist of two phases: phase A, susceptible to inhibition by a metabolic inhibitor, and phase B, not susceptible to such inhibition. What is important about this finding is that the second stage of memory processing associated with one weakly-reinforced learning trial has been shown not to contain a phase B (2). This supports the argument that consolidation of LTM may depend upon mechanisms involved in the transition from phase A to phase B of ITM (6).

Although there is broad support for the view that consolidation of LTM may depend upon arousal processes associated with the learning experience (8–10), the mechanisms by which such arousal factors determine consolidation are unclear. In the case of retraining effects, the present results support the view that retraining yields consolidation as a result of cumulative arousal reaching some threshold for consolidation. The number of retraining trials required for this to occur may depend on the strength of the training stimulus. Just how such cumulation would take place is also unclear. At least two possibilities present themselves: 1) arousal effects from subsequent training trials may augment those effects generated by preceding trials, or 2) the arousal effects of a given retraining trial may augment the arousal generated by the reminder consequences of the stimulus complex at the time of that retraining trial. In this context it is interesting to note that in the present experiments, the frequency of pecking at the training bead in the retraining trials appears to be somewhat lower than that observed in the original training trial ($n = 305$, mean number of pecks during training trial $1 = 1.708$, sd 1.006; mean number of pecks during training trial $2 = 1.476$, sd 0.770). Clearly more work needs to be done on this issue.

It is also generally accepted that arousal effects in an aversive learning situation may be mediated by changes in hormonal levels associated with the stress of the aversive learning experience (8). If this is the case, it may be expected that increasing hormonal levels by contingent application of stress-related hormones such as the adrenocorticotrophic hormone, the glucocorticoids or the pressor hormones may serve to produce LTM consolidation following a single weakly reinforced training trial in the same manner that retraining has been shown to do. This possibility is presently under investigation.

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