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Memory Consolidation of Weak Training Experiences by Hormonal Treatments

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. *Memory consolidation of weak training experiences by hormonal treatments.* PHARMACOL BIOCHEM BEHAV **37**(4) 729–734, 1990.—Day-old chicks trained on a single-trial passive discrimination avoidance task using a concentrated chemical aversant, methyl anthranilate (MeA), have been shown to exhibit three stages of memory processing; short-, intermediate- and long-term. A similar learning task with the aversant diluted to 20% in ethanol leads to short- and intermediate-term memory, but no long-term memory. Subcutaneous administration of selected doses of the stress-related hormones, noradrenaline, ACTH and vasopressin in close temporal proximity to the training trial, produced long-term memory in chicks trained on the weakly reinforced task, mimicking the outcome of strongly reinforced learning and of retraining with the weakly reinforced task reported previously. These effects are shown to be associated with the production of a nonenergy-dependent phase of the intermediate memory stage, postulated to be necessary for long-term memory consolidation.

Day-old chicks Weakly reinforced training Noradrenaline Stress-related hormones Memory consolidation Intermediate memory

CONSIDERABLE evidence is available to suggest that the intensity of the conditioning stimulus in aversive learning affects the extent to which the experiences are coded into long-term memory (LTM) [e.g., (15, 17, 18)]. There is a general consensus of opinion that this effect of stimulus intensity may be mediated by nonspecific stress or arousal consequences (15, 17, 18). Indeed, Gold and McGaugh (15) have suggested that nonspecific arousal processes associated with a learning experience may determine whether or not experiences are coded into LTM and that these processes may involve alteration in the level of hormones from the pituitary-adrenal axis. This view is consistent with the one expressed by Kety (17,18). The latter suggests further that these arousal effects are mediated by catecholamine activity.

Posttraining administration of noradrenaline [NA: (22)], adrenaline [A: (13,15)], mild flurothyl (4) and pentylenetetrazol (19), have been shown to facilitate consolidation of memory following aversive training with a low intensity training stimulus. Similar effects have also been observed with adrenocorticotrophin [ACTH: (14)].

Moreover, noradrenergic agonists such as NA and amphetamine (8), as well as hormones including arginine vasopressin (AVP) and ACTH (23), have been shown to be effective in counteracting amnesia induced by agents such as ouabain and the antibiotics [see also (7,21)].

The nature of the actions of the catecholamine agonists and the hormones outlined above is as yet unclear. Recent findings from Gibbs and Ng (11,12) are of interest here. Chicks trained on a single-trial passive discriminated avoidance learning trial show a retention function consisting of three memory stages; short-term (STM), intermediate-term (ITM) and long-term memory (LTM), with the stages separated by transient deficits at 15 and 55 minutes postlearning (10,23). Gibbs and Ng (12) suggested that the ITM stage consists of two phases: phase A which is energy dependent and susceptible to blockade by the metabolic inhibitor 2,4-dinitrophenol (DNP); and phase B, following from phase A, which is not susceptible to DNP inhibition. They provide evidence consistent with the view that consolidation of the learning experience into LTM may depend on triggering mechanism operating in the transition of memory from phase A to phase B of ITM. NA, ACTH and AVP have been shown to extend phase A of ITM and to delay the crossover from ITM to LTM by some 35 minutes, from 55 minutes to about 90 minutes postlearning (23). These authors have provided evidence to suggest that these hormones overcome antibiotic inhibition of LTM by extending phase A of ITM and thus delaying the triggering of LTM formation until after the inhibitory effects of the antibiotics have dissipated (12).

We have reported that day-old chicks trained on a passive avoidance task with reduced aversiveness of the training stimulus do not show consolidation of the experience into LTM (5,6). The evidence suggests that within the Gibbs and Ng three-stage model of memory formation (10,23), the retention function under such training conditions consists of the short-term memory (STM) and the intermediate-term memory (ITM) stages, with no evidence of the LTM stage. Furthermore, the intermediate stage of this function consisted entirely of Gibbs and Ng's ITM phase A (5). Finally, consolidation of this training experience into LTM was achieved with a second training trial with the weak training stim-

[1]Requests for reprints should be addressed to Professor K. T. Ng, Department of Psychology, LaTrobe University, Bundoora, Victoria, Australia, 3083.

ulus and this occurred concomitantly with the appearance of phase B of ITM (6).

If it is assumed that arousal associated processes mediate the formation of LTM, then it may be argued that the absence of LTM in the weakly reinforced training task that we use may be attributed to insufficient arousal of the animal. A second training trial may then be argued to produce LTM through the cumulative effects of arousal associated with both training trials [cf. (3,4)]. If the arousal effects are themselves mediated by hormonal changes, it may be expected that exogenous hormones administered contingently on a weak learning experience will lead to consolidation of LTM. In this paper we report the effects of the application of exogenous hormones on memory functions in day-old chicks trained on a weakly reinforced passive avoidance learning task.

METHOD

Animals

Day-old black Australorp, white Leghorn chicks were obtained from a local hatchery on the morning of each experiment. Approximately 16 chicks were used for each data point, depending on the number successfully trained from an initial subject pool of 20 birds.

Drugs

Subcutaneous. All drugs were made up in 154 mM NaCl. Dose-response curves and time of injection curves were completed and the optimal doses employed were: NA (l-noradrenaline bitartrate, Sigma) 150 μg/kg, ACTH 1–24 (Synthacen, Ciba Geigy) in a dose of 50 μg/chick and arginine vasopressin (Sigma) in a dose of 0.2 I.U. per chick. All drugs or saline were administered in a 100 μl dose subcutaneously in a ventral skin fold. All subcutaneous drugs were administered within 10 seconds after training on the aversive red bead, except in the case of the time of injection functions, where doses were administered as stipulated.

Intracranial. 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 (12). 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 Gibbs and Ng (10). 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. Previous studies have shown no differences in retention functions between chicks trained on the red bead and chicks trained on the blue bead (9). 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 level of discrimination memory was indexed by a discrimination ratio, 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 for all chicks pecking the blue bead on the test trial [cf. (5,6)]. Approximately 12% of all chicks avoided the blue bead, and of these, approximately 80% avoided both the red and the blue bead, while the remaining 20% avoided the blue bead and pecked the red bead. For chicks that avoided both beads, the discrimination ratio, as defined, is indeterminate. For this reason, chicks avoiding the blue bead on the retention test were not included in the analysis of the ratios. The small percentage of chicks in this category did not substantially alter the findings.

RESULTS

Experiment I: Dose-Response Function

Various doses of noradrenaline bitartrate (NA) between zero and 400 μg/kg, or ACTH 1–24 between 25 and 100 μg/chick were injected subcutaneously within ten seconds after training (see Fig. 1). Control animals received either saline or a 1.0 mg/ml dose of ACTH 4–10. Retention was tested 180 minutes posttraining. The results indicate that NA at a dose of 150 μg/kg was optimal in producing evidence of memory 180 minutes posttraining. The optimum dose of ACTH 1–24 was between 50 and 75 μg/chick. Neither saline nor ACTH 4–10 yielded evidence of memory at 180 minutes postlearning.

An unweighted means analysis of variance yielded a significant quadratic trend, $F(1,389)=4.02$, $p=0.04$, but no significant linear, $F(1,389)=2.85$, $p=0.09$, or cubic, $F(1,389)=1.61$, $p=0.20$, trend component for NA groups. A post hoc Dunnett's comparison between each NA dose and the saline control, using the harmonic mean of sample sizes, gives a critical difference of 0.30, with $\alpha=0.05$. Thus, only the 150 μg/kg is significantly different from saline. Together with the significant quadratic trend, the results suggest an inverted U-shaped function peaking at the dose of 150 μg/kg.

Similarly, an unweighted means analysis of variance of the data on ACTH 1–24 presented in Fig. 1B yielded significant linear, $F(1,67)=7.03$, $p=0.01$, and quadratic trends, $F(1,67)=5.90$, $p=0.02$. The fact that ACTH 4–10 did not facilitate retention at 180 minutes postlearning suggests that the effect of ACTH 1–24 may be mediated by corticosterone release (15).

Experiment II: Time of Injection Effects

A dose of 150 μg/kg NA, or 50 μg/chick ACTH 1–24 or 0.2 I.U./chick of arginine vasopressin [AVP; this dose has been shown by Gibbs and Ng (11) to be optimal in extending phase A of ITM in their model] was administered subcutaneously at various times before and after training (Fig. 2), with retention tested 180 minutes postlearning. Control groups were injected with 154 mM saline immediately after training. The results suggest that administration of hormones five minutes before or immediately after learning resulted in retention levels considerably higher than those observed with saline administered at these times. These retention levels were also higher than the levels obtained with the other times of injection of the hormones, although in the case of ACTH 1–24 and AVP, administration of the hormones five or 15 minutes after training also tended to increase retention levels relative to saline-treated controls.

A two-way unweighted means analysis of variance yielded significant treatment, $F(3,451)=2.696$, $p=0.046$, and time of injection, $F(6,451)=4.245$, $p=0.00$, main effects, but no signif-

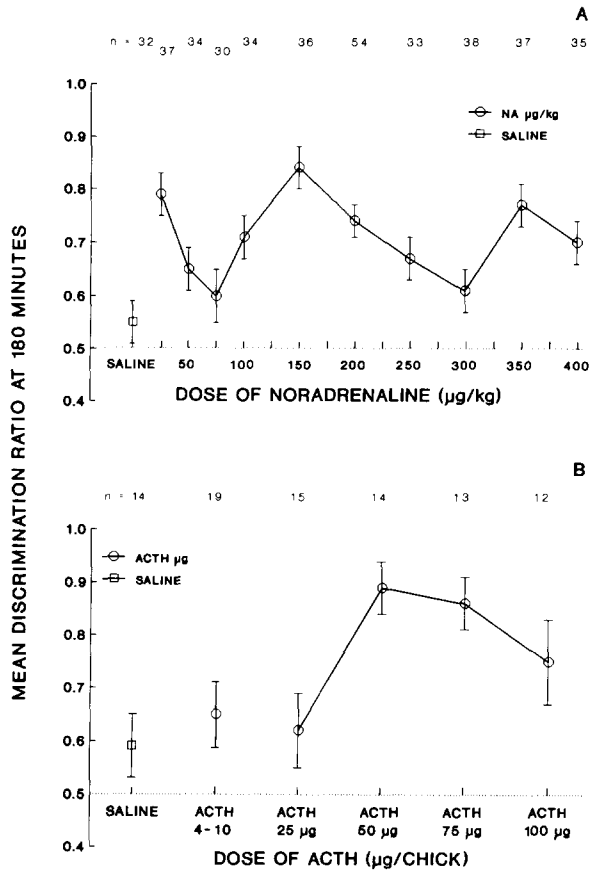

FIG. 1. Effect of varying doses of subcutaneously administered noradrenaline (A) or ACTH 1–24 (B) on retention at 180 minutes after learning, measured as mean discrimination ratio ± SEM. Drugs were given immediately postlearning.

icant treatment by time interaction effect, $F(18,451) = 1.38$, $p = 0.137$. The absence of an interaction effect may be attributed primarily to the overrepresentation of similar hormonal functions relative to the single saline function.

A Dunnett's post hoc test for the treatment main effect, comparing each drug against saline, and using the harmonic mean of sample sizes, gives a critical mean difference of 0.053 for $\alpha = 0.05$. Thus, on the average, there was a significant increase in retention levels for all the hormonal groups when compared with the saline-treated groups. Similarly, Dunnett's tests comparing the mean retention level at each training-injection interval against the mean level of retention at the +0 minute training-injection interval yields a critical mean difference of 0.108. It would appear that the −30-, −15-, and +30-minute groups showed significantly lower retention levels compared with the group given drugs immediately after learning. Despite the nonsignificant interaction effect, the results of the post hoc tests suggests that injections of hormones close to or after training may be optimal in enhancing retention levels.

Experiment III: Effects of Hormones on the Retention Function

NA at the optimum dose of 150 µg/kg, ACTH 1–24 (50 µg/chick) or AVP (0.2 I.U. per chick) was administered subcutaneously immediately (within ten seconds) after training and retention

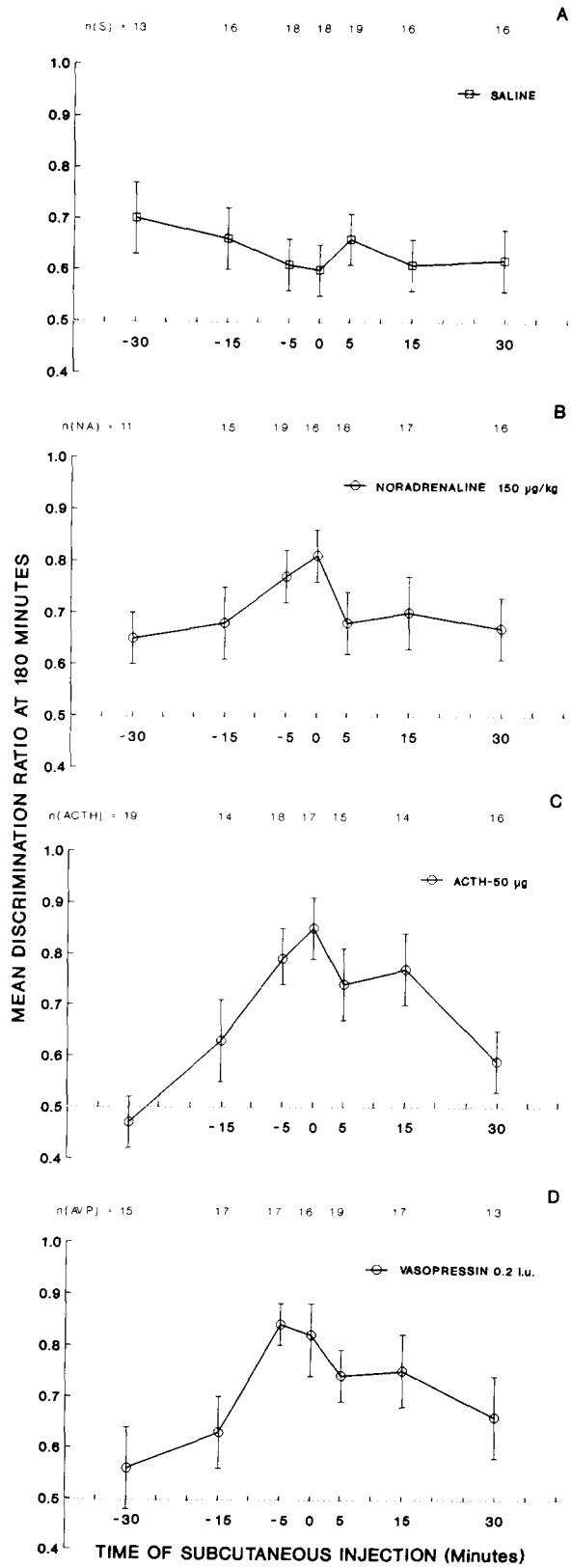

FIG. 2. Effect of varying time of subcutaneous administration of saline (A), 150 µg/kg of noradrenaline (B), ACTH 1–24 (50 µg/chick: C), or AVP (0.2 I.U./chick: D) on retention, measured 180 minutes postlearning, as indexed by mean discrimination ratio ± SEM.

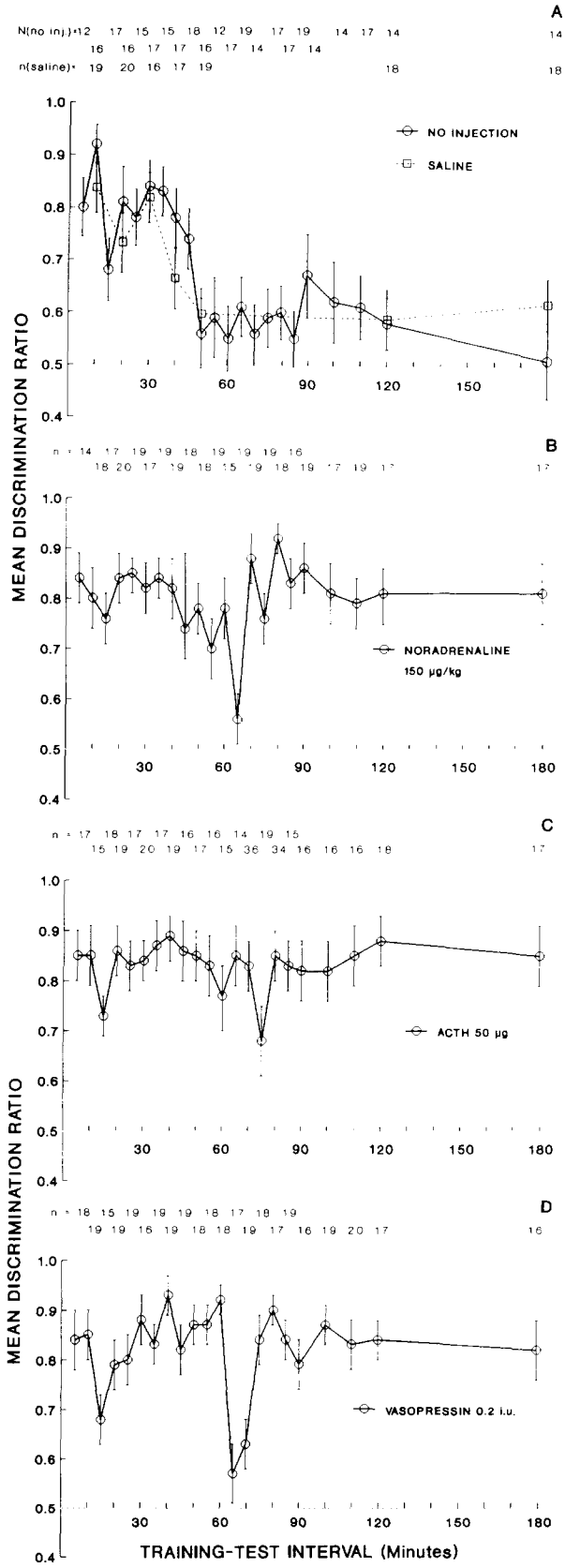

FIG. 3. Retention function immediately following postlearning subcutaneous administration of 150 µg/kg noradrenaline (B), 50 µg/chick ACTH 1–24 (C) or 0.2 I.U./chick AVP (D) as compared with no injection or saline injected controls (A). Retention was measured as mean discrimination ratio ± SEM. Pairwise significant differences in mean discrimination ratios occur when the differences between groups exceed a critical difference of 0.15, based on planned comparisons between means, using the harmonic mean of sample sizes, a pooled MS error = 0.05, and $\alpha = 0.05$.

was tested at various times after training (see Fig. 3). Control groups received either no injection or a subcutaneous injection of 154 mM saline immediately after training. The retention functions for the hormone-treated groups show high levels of retention at virtually all training-test intervals up to and including 180 minutes (Fig. 3B, C, D). This contrasts markedly with the retention functions obtained with the noninjected or saline-treated group (Fig. 3A). The retention function for hormone-treated groups also shows a marked transient deficit at 65 to 75 minutes posttraining. This may well correspond to the transient retention deficit reported by Gibbs and Ng (10) and by Crowe et al. (5) at 55 minutes after training with concentrated aversant. These authors also report an earlier transient deficit at 15 minutes postlearning. There is some evidence of this deficit in the retention functions shown in Fig. 3, but it is not substantial in any of the functions. The above results suggest that exogenous hormones at the doses used can facilitate the consolidation of long-term memory following weakly reinforced learning and the effect is optimal with the hormones applied in close temporal contiguity to the time of training.

Experiment IV: Effect of DNP on Retention Functions in the Presence of Hormones

To determine the nature of the ITM stage in the retention function produced by the hormones NA, ACTH 1–24, and AVP, DNP (0.2 mM intracranially) was administered at various times between 10 and 50 minutes after training and retention tested 10 minutes after DNP. For each hormone, control groups received 154 mM saline intracranially at each time of injection (Fig. 4). Chicks were subcutaneously administered 150 µg/kg NA, 50 µg/chick ACTH 1–24, or 0.2 I.U./chick AVP immediately after training. The results show that for all hormones, the retention function was susceptible to inhibition by DNP, when administered up to and including 40 minutes postlearning.

Pairwise comparisons between mean discrimination ratios of NA and saline-treated animals yielded significant differences at 20, 30 and 40 minutes posttraining, $F(1,220) = 9.31$, 4.47 and 10.05, respectively; $p = 0.00$, 0.03 and 0.00, respectively. DNP was not effective 50 minutes or later after training, with no significant differences obtained at any of the subsequent retention times. A similar result is obtained with ACTH 1–24, where pairwise comparisons yielded significant differences between saline- and ACTH-treated groups at 20 and 30 minutes posttraining, $F(1,282) = 6.11$, 10.25, respectively; $p = 0.01$, 0.00, respectively. Although not significant, the difference at 40 minutes posttraining is larger than at any other subsequent training-test intervals.

Finally, pairwise comparisons between AVP and saline-treated groups yielded significant differences only at the 20-, 30- and 40-minute training-test intervals, $F(1,215) = 8.07$, 6.29 and 4.24, respectively; $p = 0.00$, 0.01 and 0.03, respectively.

The results obtained in the presence of hormones suggest that the retention functions consist of a DNP-susceptible phase between 10 and 40 minutes following training which corresponds to

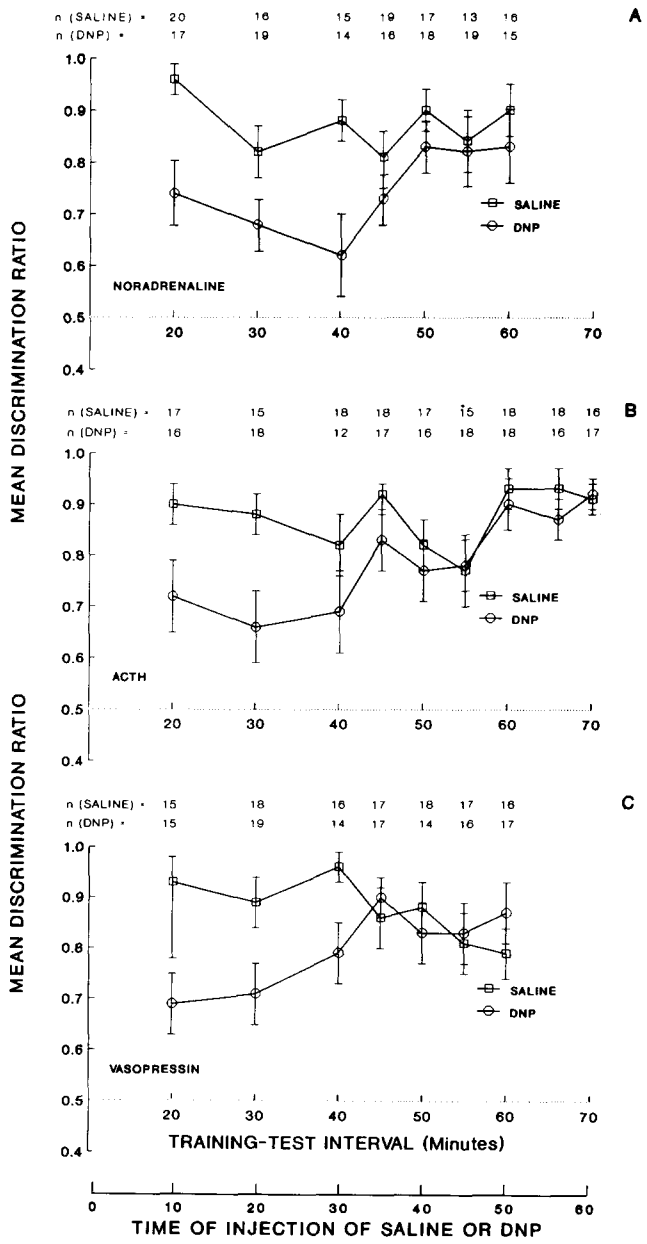

FIG. 4. Effect of DNP administered intracranially at various times after learning to chicks treated with subcutaneous noradrenaline (150 μg/kg: A), ACTH 1–24, (50 μg/chick: B) or AVP (0.2 I.U./chick: C) immediately after learning. Retention was indexed by mean discrimination ratio ± SEM, and was measured 10 minutes after DNP administration.

Gibbs and Ng's (12) phase A of ITM, and a DNP-insensitive phase between 45 and 60 minutes posttraining corresponding to Gibbs and Ng's phase B. More importantly, these results contrast markedly with those obtained with chicks not treated with hormones (Fig. 3A), where there appears to be no evidence of phase B [see Fig. 7: (5)]. Thus, it would appear that the capacity of various hormones which are able to facilitate consolidation of LTM following a weakly reinforced learning trial may be associated with their capacity to initiate phase B of ITM.

DISCUSSION

The findings obtained in the four experiments reported above support the notion that hormones are capable of facilitating consolidation of memory for a weakly reinforced learning experience in day-old chicks. This facilitative effect of hormones was demonstrated when the drugs were administered immediately after learning. These findings are consistent with results reported in the literature (1, 13, 20) with other species and with other tasks.

The results obtained with NA in the present study are particularly significant in view of earlier reports that NA given immediately after learning can overcome cycloheximide-induced amnesia (8). The author attributed this action of NA to the extension of phase A of ITM until such time as the initiation of protein synthetic processes associated with LTM formation are no longer affected by CXM, this initiation corresponding with the time of appearance of phase B. The present results are consistent with this interpretation. However, it may be noted that the effective dose of NA used in the Gibbs and Ng studies (12) was 50 μg/kg, while the optimum dose in the present study was 150 μg/kg. This difference may be due to the relative strength of the underlying memory trace available under the two reinforcement conditions.

While the precise mechanisms underlying this action of hormones are yet to be determined, it is possible to conclude support for the modulatory role assigned to arousal and hormones by Gold and McGaugh (15) and by Kety (17,18). Of interest to the issue of possible mechanisms is the suggestion in the present studies that the action of these hormones may involve the initiation of phase B of Gibbs and Ng's (12) ITM stage. This is consistent with Gibbs and Ng's suggestion that the triggering of LTM formation may be initiated by some process occurring in the transition from phase A to phase B of ITM, and that this triggering mechanism may involve noradrenergic processes, possibly associated with cyclic AMP activity.

In this context it is interesting to note that the β-adrenergic antagonist, sotalol, has been shown to yield amnesia, sometime after 40 minutes postlearning, in chicks trained under a similar paradigm (24). This effect of sotalol was only observed when the drug was given between 10 and 25 minutes after training and a similar effect was not obtained with two other β-antagonists, nadolol and timolol. With the latter two β-antagonists, memory loss was rapid, occurring within five minutes of administration independent of the time of administration relative to training. Furthermore, sotalol injected five minutes before training resulted in amnesia developing within 10 minutes posttraining.

These authors attributed the differences in action between the β-antagonists to possible differences in their effect on subtypes of β_2-receptors in chick brain, and/or the possibility that one type of β-antagonist might act predominantly centrally and one peripherally. Of particular concern to the present paper is the conclusion by these authors that the effect of sotalol administered sometime after training provided evidence of a sharp transition in memory processing at about 30 minutes after training. Indeed, they concluded that sotalol given at these times prevents the establishment of long-term memory, possibly through an as yet unknown effect of sotalol on protein synthesis. This conclusion was based primarily on the earlier finding from our laboratories that antibiotics such as cycloheximide yielded amnesia sometime after 30 minutes posttraining if given immediately after or before training. When CXM was given five minutes or later after training, memory deficits did not appear until 60 minutes posttraining (10).

It is now clear that cycloheximide may have a dual action on memory formation (12) and that its inhibitory effect on memory between 30 and 60 minutes posttraining may be attributed to inhibition of an energy-independent phase of memory. It would appear that the effect of sotalol obtained by Stephenson and Andrew (24) may in fact have been on phase B of intermediate-term memory. A similar result to that observed by Stephenson and Andrew (24) has been obtained in our laboratories with another β-antagonist, propranolol (unpublished data).

If the memory-enhancing effects of NA and the pressor hormones reported in this study are in fact due to the initiation of phase B of ITM under weakly reinforced learning conditions it would be useful to investigate changes in the level of catecholamine activity under varying levels of reinforcement, given the results from studies with noradrenergic blockers. This is presently under investigation.

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REFERENCES

1. Andrew, R. J. The functional organization of phases of memory consolidation. Adv. Stud. Behav. 11:337–367; 1980.
2. Cherkin, A. Biphasic time course of performance after one-trial avoidance training in the chick. Commun. Behav. Biol. 5:379–381; 1971.
3. Cherkin, A. Retrograde amnesia in the chick: Resistance to the reminder effect. Physiol. Behav. 8:949–955; 1972.
4. Cherkin, A.; Meinecke, R. O.; Garman, M. W. Retrograde enhancement of memory by mild flurothyl treatment in the chick. Physiol. Behav. 14:151–158; 1975.
5. Crowe, S. F.; Ng, K. T.; Gibbs, M. E. Memory formation processes in weakly reinforced learning. Pharmacol. Biochem. Behav. 33:881–887; 1989.
6. Crowe, S. F.; Ng, K. T.; Gibbs, M. E. Effect of retraining trials on memory consolidation in weakly reinforced learning. Pharmacol. Biochem. Behav. 33:889–894; 1989.
7. Dunn, A. J. Neurochemistry of learning and memory: An evaluation of recent data. Annu. Rev. Psychol. 31:343–390; 1980.
8. Gibbs, M. E. Modulation of cycloheximide resistant memory by sympathomimetic agents. Pharmacol. Biochem. Behav. 4:703–707; 1976.
9. Gibbs, M. E.; Barnett, J. M. Drug effects on successive discrimination learning in young chickens. Brain Res. Bull. 1:295–299; 1976.
10. Gibbs, M. E.; Ng, K. T. Psychobiology of memory: Towards a model of memory formation. Biobehav. Rev. 1:113–136; 1977.
11. Gibbs, M. E.; Ng, K. T. Hormonal influences on the duration of short-term and intermediate stages of memory. Behav. Brain Res. 11:103–108; 1984.
12. Gibbs, M. E.; Ng, K. T. Dual action of cycloheximide on memory formation in day old chicks. Behav. Brain Res. 12:21–27; 1984.
13. Gold, P. E.; van Buskirk, R. Post-training brain norepinephrine concentrations: Correlation with retention performance of avoidance training and with peripheral epinephrine modulation of memory processing. Behav. Biol. 23:509–520; 1978.
14. Gold, P. E.; van Buskirk, R. Enhancement and impairment of memory processes with posttrial injections of adrenocorticotrophic hormone. Behav. Biol. 16:387–400; 1976.
15. Gold, P. E.; McGaugh, J. L. A single-trace, two-process view of storage processes. In: Deutsch, D.; Deutsch, J. A., eds. Short term memory. New York: Academic Press; 1975:355–378.
16. Jones, M. T.; Hillhouse, E. W.; Burden, J. L. Dynamics and mechanics of corticosteroid feedback of the hypothalamus and anterior pituitary gland. J. Endocrinol.73:405–417; 1977.
17. Kety, S. S. The biogenic amines in the central nervous system: Their possible role in arousal, emotion and learning. In: Quarton, G. C.; Melnechuk, T.; Aldeman, G., eds. The neurosciences second study program. New York: Plenum Press; 1970.
18. Kety, S. S. Brain catecholamines, affective states and memory. In: McGaugh, J. L., ed. Chemistry of mood, motivation and memory. New York: Plenum Press; 1972.
19. Krivanek, J. Facilitation of avoidance learning by pentylenetetrazol as a function of task difficulty, deprivation and shock level. Psychopharmacologia 20:213–229; 1971.
20. McGaugh, J. L. Hormonal influences on memory. Annu. Rev. Psychol. 34:297–323; 1983.
21. McGaugh, J. L.; Gold, P. E. Conceptual and neurobiological issues in studies of treatments affecting memory storage. Psychol. Learn. Motiv. 8:233–264; 1974.
22. Meligeni, J. A.; Lederberger, S. A.; McGaugh, J. L. Norepinephrine attenuation of amnesia produced by diethyldithiocarbamate. Brain Res. 149:155–164; 1978.
23. Ng, K. T.; Gibbs, M. E. A biological model of memory formation. In: Markowitsch, H. J., ed. Information processing in the brain. Bern: Hans Huber Publishers; 1988:151–178.
24. Stephenson, R. M.; Andrew, R. J. Amnesia due to β-antagonists in a passive avoidance task in the chick. Pharmacol. Biochem. Behav. 15:597–604; 1981.