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# Effect of Testosterone on Intermediate Memory in Day-Old Chicks

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GIBBS, M. E., K. T. NG AND R. J. ANDREW. *Effect of testosterone on intermediate memory in day-old chicks.* PHARMACOL BIOCHEM BEHAV 25(4) 823–826, 1986.—Day-old chicks trained on a single trial passive avoidance discrimination task show three well-defined behavioural stages in memory formation: short-term, intermediate and long-term memory. Testosterone (2 mg), given subcutaneously, yielded results which may be tentatively interpreted as an extension of the time of availability of recall from the intermediate stage by between 20 and 50 min, provided the hormone is administered no earlier than 90 min before learning. A higher dose (12.5 mg) of testosterone administered 30 min before learning had a similar effect. The findings with testosterone are comparable to those reported for pituitary-adrenal hormones.

Testosterone Intermediate memory Day-old chicks Consolidation Memory extension Hormones

CONSIDERABLE evidence has been gathered demonstrating that pituitary and adrenal hormones are capable of enhancing the learning and consolidation of memory for a wide range of learning tasks in a wide range of species of animals (for reviews see [6,14]). These findings lend substantial weight to the argument of Gold and McGaugh [12] that hormones may be important modulators of memory consolidation. While the general modulatory effect of hormones on memory formation is undeniable, there has not been sufficient information gathered about the precise nature of these modulatory effects. In a recent paper we provided evidence to suggest that stress-related hormones such as arginine vasopressin (AVP), arginine vasotocin (AVT), ACTH and corticosterone can both alter the level of retention at various stages in the memory formation process and prolong the duration of maximum availability of shorter-term stages of memory in day-old chicks trained on a single trial passive discriminated avoidance task [11]. Similar effects were observed with stress brought about in chicks by isolation [9]. Preliminary evidence from our laboratories also suggest that some of these hormones are capable of overcoming retrograde amnesia induced by a number of agents, and that this effect may be associated with the action of the hormones in prolonging the life of stages of memory available prior to long-term memory consolidation.

In the behavioural paradigm that we use, chicks are pretrained to peck at a coloured bead dipped in water prior to the learning experience. On a single learning trial, the same bead is made aversive by a chemical aversant [8]. In a series of papers Andrew *et al.* [3], Clifton *et al.* [5] and Andrew [1,2] have shown that the gonadal steroid, testosterone, administered at and around the time of pretraining is capable of interfering with subsequent acquisition of the aversion to the training stimulus. These findings were interpreted in terms of testosterone enhancing the information contained in the pretraining trial and protecting this information from the effects of the learning trial. If the pretraining trial is taken to be itself a learning experience, the possibility arises that testosterone may be a potent modulator of memory formation.

In this paper we report evidence on the effects of testosterone on the formation of memory for the single trial passive avoidance discrimination task. The findings are examined within the context of the three stage model of memory proposed by Gibbs and Ng [9]; a short-term stage lasting up to 10 minutes post-learning, followed by an intermediate stage available between 20 minutes and 50 minutes after learning, and a long-term memory stage available 60 minutes or later after learning. In particular, we have isolated two points of temporary reduction in retention level: at 15 and 55 minutes following learning. The second dip in retention level has been interpreted as possibly representing the cross-over in availability of recall from the intermediate to the long-term stage of memory. The studies reported here were directed specifically at the modulatory effects of testosterone on the duration of availability of the intermediate memory stage.

## METHOD

### *Animals*

Day-old black Leghorn white Australorp cockerels were obtained from a local hatchery on the morning of each experiment. Chicks were housed in pairs in wooden boxes at a temperature of 26–29°C provided by red light globes suspended over the boxes. Food was available ad lib. Twenty different chicks were used for each data point.

### *Drugs and Administration*

Two mg of testosterone oenanthate were administered

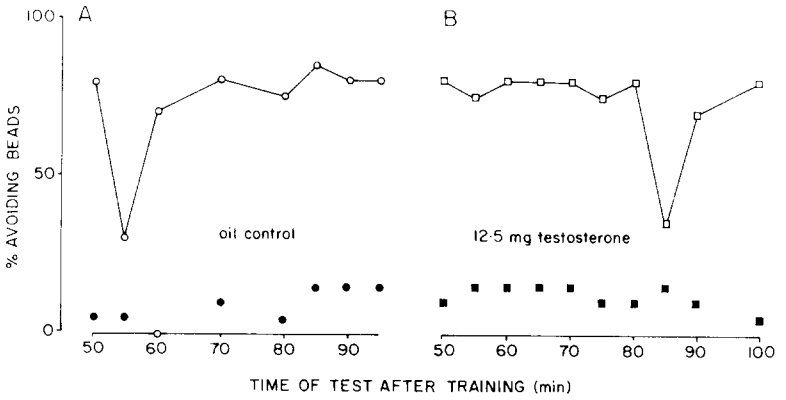


FIG. 1. Retention time course for control chicks administered arachis oil (A) and experimental chicks given 12.5 mg testosterone (B) 30 min before learning. Unfilled symbols represent avoidance of the red bead. Filled symbols represent avoidance of the blue bead.

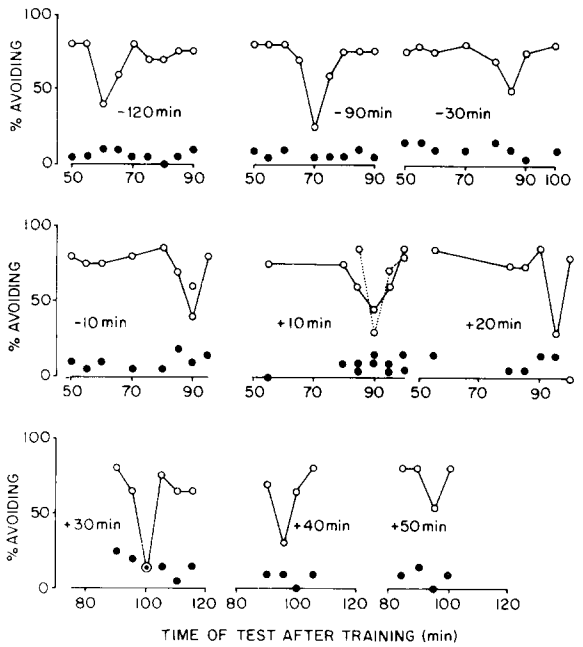


FIG. 2. Retention time course for chicks given 2.0 mg testosterone at various times before and after learning. Note that chicks injected between 120 and 30 min before learning were not exposed to pretraining trials. Part of the retention function was replicated for testosterone administered 10 min after learning, and the retention level at 90 min for testosterone administered 10 min before learning was also replicated. Unfilled symbols represent avoidance of the red bead. Filled symbols represent avoidance of the blue bead.

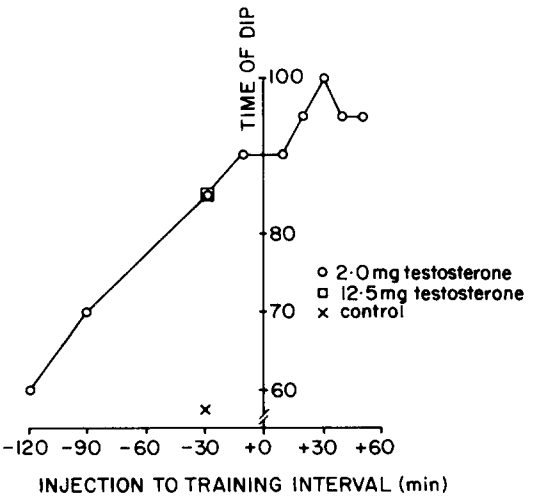


FIG. 3. Relationship between time of administration of testosterone relative to the learning trial and the time after learning when the second temporary reduction in retention level occurs. Note that control chicks show this retention dip at 55 min after learning. Only avoidance of the red bead is shown.

subcutaneously between 120 minutes before and 50 minutes after training, and retention tested at various times between 50 and 120 min after training. For comparison purposes, a control group of chicks was administered the vehicle (arachis oil) 30 minutes before training, and another group of chicks was administered 12.5 mg of testosterone, also 30 minutes before training. These doses of testosterone were selected on the basis of previous studies [3,5] showing that they have significant effects on acquisition of aversive learning in young chicks.

*Procedure*

Chicks given 2.0 mg testosterone between 10 min before and 50 min after learning were pretrained to peck at a red and a blue glass bead (3.0 mm diameter) dipped in water and presented in succession for 10 seconds each. Chicks given the testosterone between 120 and 30 min before training were not given a pretraining trial because of the potential for interference with the training trial through enhancement of the pretraining experience [3,5]. Pretrained chicks were given the training trial 120 min after pretraining. The training phase consisted of a 10 second presentation of the red glass bead dipped in the chemical aversant, methyl anthranilate. Chicks were considered to have acquired the aversive association if they pecked at the bead during the training trial and showed the typical disgust reactions of head-shaking and beak-wiping after tasting the aversant. On retention tests, chicks were presented with a dry red and a dry blue bead in succession for 10 seconds each. Retention was indexed by the pro-

portion of chicks avoiding the red bead during retention tests. The proportion of chicks avoiding the blue bead during retention tests provided a check on possible performance effects produced by the steroid. All chicks were trained and tested in pairs.

## RESULTS

Control chicks, administered the arachis oil 30 minutes before learning and tested for retention between 50 and 95 minutes after learning, showed normal retention levels at all times of retention tests except at 55 minutes after learning. These results are comparable to those reported previously for untreated chicks [9] and the dip in retention level at 55 minutes after learning represents the postulated normal cross-over in recall from the intermediate to the long-term stage of memory (Fig. 1A). In contrast, chicks given either 12.5 mg (Fig. 1B) or 2.0 mg of testosterone (Fig. 2) 30 minutes before learning yielded a temporary retention deficit at 85 minutes after learning, suggesting the possibility that testosterone at these concentrations and at this time of administration may have extended the duration of availability of intermediate memory.

Varying the time of administration of 2.0 mg testosterone between 30 min before and 50 min after learning yielded a relatively small variation in the time of occurrence of the retention dip. The dip was delayed by between 30 and 45 min (Fig. 2). However, with testosterone given 120 minutes before learning, the dip was delayed by only 5 minutes, while testosterone given 90 minutes before learning produced a retention deficit at 70 minutes after learning, a delay of 15 min. The reliability of the findings is emphasized by the results of the replication study with testosterone give 10 min after learning. Figure 3 summarizes the functional relationship between the time of injection of 2.0 mg of testosterone relative to learning and the time after learning at which the temporary retention deficit occurs. While the function appears virtually linear in the $-120$ to $-10$ min range, the absence of a $-60$ min time of administration cautions against drawing this conclusion too readily.

## DISCUSSION

The present findings suggest the possibility that testosterone, like the pituitary and adrenal hormones, is capable of extending the duration of intermediate memory. In doing so it is also possible that formation of long-term memory is also delayed [10]. Preliminary evidence from our laboratories indicate that testosterone does not alter the time of occurrence of the first dip found in our normal retention function at 15 min post-learning [9]. Thus short-term memory duration appears unaffected. This requires further confirmation. If the effect of testosterone is in fact on the duration of the intermediate stage, the size of this effect appears to be a function of the time at which the hormone is administered. The effect seems to increase with proximity of injection to the time of learning. However, injections later than 30 minutes after learning do not produce delays substantially different from that observed with steroid administration 30 minutes before learning. This suggests that there may be a biological limit to the extent to which the duration of intermediate memory can be modulated under the present behavioural paradigm. It may be noted that the pituitary and adrenal hormones yielded about the same extent of delay when given immediately after learning [11]. In this context, it is interesting to note also that in several mammalian species, ACTH stimulates secretion of testosterone by the gonads [7,13]. In the rat, the retardation of extinction of conditioned aversion by ACTH depends on the presence of functional testes [4].

The present findings also provide a possible mechanism for the reported action of testosterone in interfering with normal learning of the aversive association when it is given at a time close to the pretraining trials [3,5]. Testosterone may strengthen and extend the duration of memory for the pretraining experience sufficiently to prevent the change in information about the red bead occurring at the learning trial from registering or from being consolidated into long-term memory.

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