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RAPID COMMUNICATION

Forebrain Noradrenaline Concentration Following Weakly Reinforced Training

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CROWE, S. F., K. T. NG AND M. E. GIBBS. *Forebrain noradrenaline concentration following weakly reinforced training.* PHARMACOL BIOCHEM BEHAV **40**(1) 173–176, 1991.—Day-old chicks trained on a single-trial discriminated passive avoidance task using a concentrated taste aversant, methyl anthranilate, have been shown to exhibit three stages of memory processing; short-, intermediate-, and long-term memory. If the aversant is diluted to 20% v/v methyl anthranilate in absolute ethanol, only the short-term and some of the intermediate stage are observed. In this study we investigated the whole forebrain levels of noradrenaline in response to differing intensities of the training experience. The results show a profound difference in the level of whole forebrain NA at all training-sacrifice intervals for the trained as compared to the untrained controls, except at 15- and 20-minute posttraining, when a substantial reduction in the level of NA was achieved under all training conditions. Furthermore, subjects which received treatments which resulted in the emergence of behavioural evidence of long-term memory tended to have higher levels of whole-forebrain NA at 30 minutes after initial training. This is the time when we have postulated that triggering of protein synthesis associated with long-term memory formation takes place.

Day-old chicks Long-term memory Triggering Noradrenaline Training stimulus intensity

THE dependence of learning on reinforcement and attentional mechanisms is well established at the behavioural level and there is an obvious adaptive advantage to an organism which retains in long memory only those experiences which are necessary for survival (15,18). These suggestions have prompted a number of investigations in our laboratory into the processing of weakly reinforced training experiences. Our results indicate that day-old chicks trained on a single-trial passive discrimination avoidance task using a concentrated aversant substance exhibit three stages of memory processing; short-, intermediate- and long-term memory (10). Futhermore, the intermediate stage is shown to have two phases, a phase A [ITM(A)] sensitive to inhibition by dinitrophenol (DNP) and a non-DNP sensitive phase B [ITM(B)].

In a variant of the task where the aversant has been diluted to a 20% v/v concentration in absolute ethanol the training leads to short- and intermediate-term memory, but not long-term memory (5). The intermediate stage appears to only contain phase A. It was possible to induce long-term memory formation in chicks which had been trained initially with the weakly reinforced training experience by two means: giving the chicks a second training trial with the same aversant stimulus (6) or applying the neurohormonal effects of a strong training experience closely contiguous to the weakly reinforced learning trial (7).

In each instance when long-term memory was produced in the initially weakly trained subject the emergence of long-term memory was invariably associated with the alteration of the intermediate-memory stage by the emergence of the non-DNP sensitive ITM(B) phase (6–7). We have previously argued that the initiation of a protein synthesis associated with long-term memory formation may occur at the transition from the ITM(A) to the ITM(B) phase of the intermediate memory stage (8,11).

The facilitatory effect of noradrenaline on weak training regimes has been numerously reported in the literature over the last twenty years (15, 18–19). We have shown that the β-noradrenergic blocker propranolol inhibits ITM(B) and subsequent LTM formation, when LTM is induced by either strong training or weak training coupled with the neurohormonal peptide ACTH 1–24 (8). Similar results have been observed with another β-blocker, sotalol, following strong learning (21).

A number of laboratories have reported a correlation between posttraining release of whole brain noradrenaline (NA) and later retention performance (9, 12–13, 16–17). Gold and his colleagues have observed that in the rat, there is a transient decrease in NA levels maximal at 10 minutes after initial training. In weakly trained subjects, brain NA levels did not seem to show such a decrease and later retention performance was weak. In subjects which were initially weakly trained and given the neurohormonal consequences of strong reinforcement, a similar decrement in NA levels 10 minutes after training was observed (12,14). More recent experiments indicate that the observed decrement in NA levels is widespread throughout the brain and does not appear to be delimited to particular brain areas (17).

The aim in this study was to determine the levels of NA in the whole forebrain of day-old chicks at various times following learning, under five training regimes: no training, a single 20% aversant training experience, a single 100% aversant training experience, a single 20% aversant training trial followed by a 50 μg/chick dose of ACTH 1–24, and an initial 20% aversant

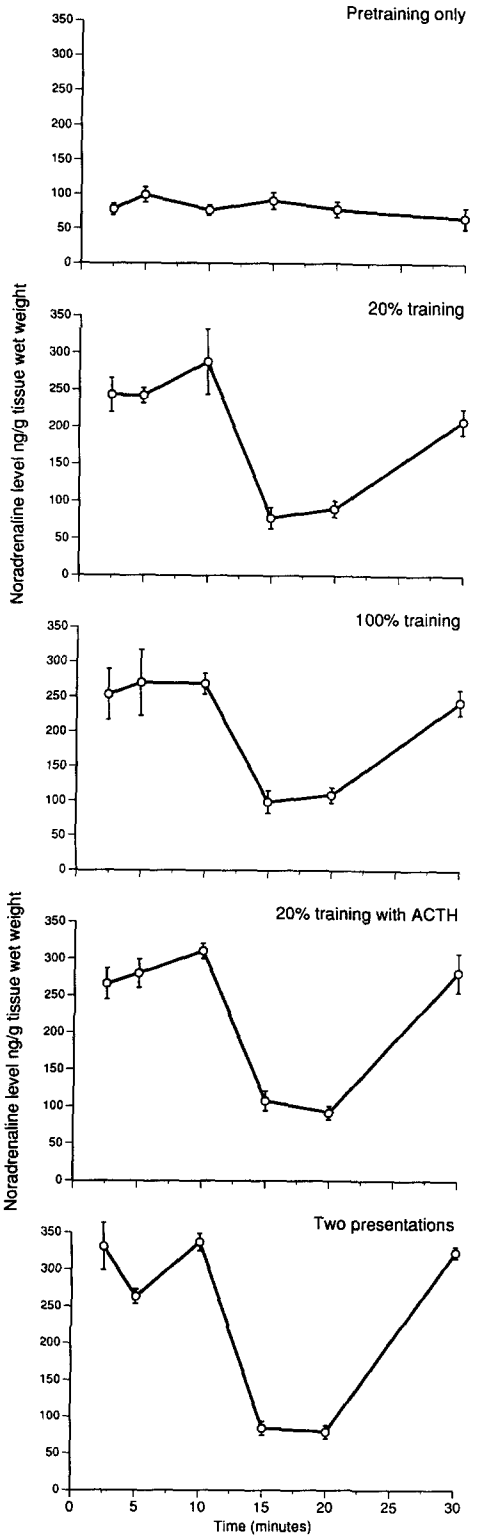

FIG. 1. Levels of whole forebrain NA (ng/g wet weight of tissue) following five different training regimes: no training, 20% anthranilate, 100% anthranilate, 20% anthranilate plus 50 μg ACTH and two presentations of 20% anthranilate training at 15-minute intervals.

training experience followed by a second such training experience 15 minutes later. The first treatment condition should yield no evidence of memory, while the second should yield only STM and ITM(A) (5).

METHOD

Animals

Day-old black Australorp, white Leghorn chicks were obtained from the local hatchery on the morning of each experiment. Six chicks were used for each data point.

Procedure

The experimental paradigm is described elsewhere (10). Chicks were housed in pairs and given two exposures with a chrome lure coated with water at approximately 30-minute intervals. This procedure encourages the chicks to peck on the subsequent pretraining and training trials. Thirty minutes after the second exposure chicks were pretrained 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 aversant solution and presented to each of the chicks in the pair. Two training stimulus intensities were employed; concentrated methyl anthranilate (Sigma) and 20% v/v methyl anthranilate dissolved in absolute ethanol. The chicks were not retention tested as it was considered that this may interfere with NA levels possibly as a consequence of retrieval cues.

Six training-sacrifice intervals (TSI) were employed: 2.5, 5, 10, 15, 20 and 30 minutes after initial training. In the case of the subjects receiving two training trials, these measures were taken 17.5, 20, 25, 30, 35 and 45 minutes after initial training. The second training trial was administered 15 minutes after the first. In the untrained controls, the sacrifices were at the prescribed intervals beginning 30 minutes after the second exposure to the chrome lure.

Forebrain Sample Removal and NA Assay

At each TSI the chicks were removed from their enclosure in pairs and one bird was randomly chosen for sacrifice. The other bird was discarded. The chick was decapitated, its scalp was removed and the forebrain was exposed by dissection away of the skull cap. The forebrain was separated from the remainder of the tissue and weighed, then placed in a nitrogen-safe tube and frozen in liquid nitrogen. The samples were stored at −73°C until they were assayed. The noradrenaline concentration in each sample was determined by high-performance liquid chromatography using a variant of the method described by Anderson, Batter, Young, Shaywitz and Cohen (1) for rat brain. The final concentration of NA, corrected for percentage recovery, was divided by the wet weight of the forebrain and the level of NA expressed as ng/g NA per gram of tissue wet weight.

RESULTS

The results of the assay are presented in Fig. 1. It can be seen from the graph that there is a marked difference between trained and untrained chicks. The untrained chick level of NA is quite consistent at about 75 ng/g throughout the times sampled. Trained chicks feature a quite marked rise in NA levels immediately after training, in the range of 250–350 ng/g. However, all trained animals show a marked decrement in NA levels at the 15- and 20-minute training-sacrifice interval (TSI). Moreover, at most TSIs, the weakly trained animals show the lowest level of NA concentration in whole forebrain, while the animals receiv-

ing two presentations of the aversant display consistently higher levels of NA than the other trained animals. At the 30-minute TSI, a spread of NA levels is observed, with the single weakly reinforced training condition yielding the lowest level of NA concentration, relative to the other training regimes. A two-way ANOVA [training regime (5) by time (6)] yielded significant main effects for training, $F(4,150)=56.11$, $p=0.00$, and time, $F(5,150)=70.56$, $p=0.00$, and a significant training by time interaction, $F(20,150)=5.94$, $p=0.00$. Simple main effects analysis ($\alpha=0.05$) indicated significant training within time effects at all times except at 15 and 20 minutes after training and significant time within training effects for all training regimes except the untrained controls. A comparison of all training regimes at 30 minutes after training yielded significant differences (Newman-Keuls procedure, $\alpha=0.05$) between trained versus untrained animals and between the animals trained with 20% aversant only and those receiving ACTH plus 20% aversant or two 20% training trials. Those subjects receiving the 100% aversant training proved to be significantly different from those receiving two presentations, but no significant difference between 100%-MeA- and 20%-MeA-trained subjects was observed. This is not surprising, given the small number of subjects used, and the almost linear trend in NA levels over the various training conditions. It is possible training with 100%-MeA may define some threshold value of NA necessary for long-term memory consolidation.

DISCUSSION

The results show a profound difference in the level of whole forebrain NA at all TSIs for the trained as compared to the untrained controls, except at 15 and 20 minutes posttraining, when a substantial reduction in the level of NA was achieved under all trained conditions. Furthermore, subjects which received treatments which resulted in the emergence of behavioural evidence of long-term memory tended to have higher levels of whole-forebrain NA at 30 minutes after initial training.

The baseline level of NA observed in this experiment is consistent with previously observed mean control levels (wulst 175 ng/g, medial forebrain 115 ng/g, basal forebrain 100 ng/g: (9) and with the levels of NA observed at 30 minutes after concentrated methyl anthranilate training [295 ng/g (4); 242 ng/g (3)]. The latter studies also suggest that levels of NA remain at a similar level of elevation at 60 (3), 90 and 210 minutes (4) after concentrated methyl anthranilate training.

It is interesting to compare these findings with those observed by Gold and van Buskirk (12) in the rat. These researchers found that levels of NA 10 minutes after passive avoidance training predicted later retention performance of rats trained with high and low footshock, and predicted the retroactive effects of post-training adrenaline injections. In the chicks in our study the decrement in NA levels observed in whole forebrain seems to occur between 15 and 20 minutes after training rather than at the 10-minute TSI. Nor did we obtain as close an association between level of training and NA levels as that observed by Gold and van Buskirk (12), although there was some support for the development of this difference at the 30-minute TSI. Interestingly, in the Gold and van Buskirk (12) study the untrained controls demonstrated a baseline level of NA (499 ng/g telencephalon/diencephalon, 480 ng/g brainstem, 861 ng/g adrenals; 10 minutes posttraining) from which the levels for trained animals declined (357 ng telencephalon/diencephalon, 274 ng/g brainstem, 625 ng/g adrenals; 10 minutes posttraining). In the chick case it would appear that the baseline level is low and then rises dramatically immediately after training. They dip at the 15–20 minutes TSI only to rise again by 30 minutes after training. While the rats used in the experiments by Gold and his associates (12, 16–17) showed only a 20% decrement in posttraining NA level, in the chick the decrement was as high as 75% of immediate posttraining levels.

It is difficult to determine the exact relationship between the whole forebrain NA levels observed in this study and the proposed modulatory role assigned to them (15). In our study no clear association between degree of depression of NA levels and subsequent retention performance could be established. Rather, it would appear that the training experience itself causes a marked initial surge in NA, which abates 15–20 minutes after training only to rise again at the 30-minute TSI. Previous behavioural studies suggest that the levels of NA at this time correlate well with the emergence of long-term memory (5–6, 8), with the weakly reinforced training experience showing the lowest level of NA at this time. This is also the time of emergence of phase B of intermediate memory in the Gibbs-Ng model (7), a phase thought to be crucially involved in the subsequent emergence of LTM (7–8, 11).

The precise role of NA in modulating memory processes is still unclear. Interpretation of changes in whole forebrain levels of NA is difficult, given the distinction between a high use/high turnover pool of NA and a low turnover pool (2). The fact that the changes are associated with the strength of reinforcement and occur at a time when pharmaco-behavioural studies suggest the involvement of NA is encouraging. Preliminary studies from our laboratories indicate that whole forebrain levels of cAMP increase shortly after this time. The possibility that NA may drive a cAMP-mediated mechanism involved in the triggering of long-term memory consolidation (20) is being explored, as is the issue of training-induced changes in NA levels in regions of the chick forebrain shown by autoradiographic studies to be selectively metabolically active during memory consolidation.

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