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Brain Metabolic Activity Associated with Long-Term Memory Consolidation

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Contents

Abstract

The use of day-old chickens trained on a single-trial passive avoidance task provides a useful paradigm for investigations into cellular mechanisms underlying memory formation. Pharmacological intervention studies indicate that there are three temporally identifiable stages of memory processing leading to the consolidation of information for this task. These stages, designated as short-term (STM; up to 15 min), intermediate-term (ITM; 15–55 min), and long-term (LTM; more than 55 min) memory, have been found to be sequentially dependent (Ng and Gibbs, 1989). In addition, ITM appears to consist of two physiologically distinguishable phases, A and B. Evidence in this laboratory suggests that the crossover between these ITM phases (at 30 min after training) represents a critical time-point for the triggering of LTM.

Introduction

In this task, chicks are pretrained to peck at water-coated red and blue beads, but learn to avoid the red bead after it is coated with the chemical aversant methylanthranilate (MeA). Typically, memory for this task lasts for weeks and is considered to be relatively permanent. However, whether LTM forms appears to depend upon the intensity of the aversive stimulus. Our studies show that diluted MeA (weak training) fails to initiate ITM (B) and, subsequently, LTM (Crowe et al., 1989). Specifically, memory diminishes after 30 min posttraining (the crossover between Phase A and B of ITM). However, it has been found that if additional arousal is induced (for example, via hormonal treatment or further reinforcement), all three stages of memory develop (Crowe et al., 1990).

Assuming that the temporal parameters manifest in this model have distinct cellular correlates and that these can be localized to specific regions

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of the chick brain, it should be possible to detect distinct differences in brain metabolic activity with regard to the stages of memory formed. The study presented here attempted to determine:

1. Whether memory for the one-trial passive avoidance task involves specific brain structures;
2. Whether there is a corresponding change in the pat-tern of regional metabolic activity with the formation of LTM; and
3. Whether hormonal treatments of subthreshold stimuli yield a pattern of regional metabolic activity similar to that observed with normal threshold learning.

The [^{14}C]-labeled 2-deoxyglucose (2DG) technique was used to compare regional metabolic activity in weakly and strongly reinforced birds at: (a) 30 min after learning (the crossover time-point between Phase A and B of ITM), and (b) 60 min after learning in the absence of hormones (when LTM should only be present in strongly reinforced birds), or with the hormonal treatment, adrenocorticotropic hormone, fragment 1–24 (ACTH 1–24), applied immediately after learning (which should initiate LTM in weakly reinforced birds).

Methods

Two separate experiments were performed. In both of these, 1-d-old black-Australorp, white-Leghorn cross cockerels were trained on a one-trial passive avoidance task as previously described (Ng and Gibbs, 1989).

Five min before training, 36 birds from a total of 180 birds were randomly selected for the autoradiographic study, given intracardiac injections of 2DG (10 μCI; SA 31.0 mCi/m*M*), and included in one of the following conditions (*see* Table 1).

All birds were tested for retention at 30 or 60 min postlearning. The 2DG injected birds were killed by decapitation immediately after testing for retention. Coronal sections (25 μm) of the forebrain were exposed to autoradiographic film prior to histological staining. Selected brain regions on the autoradiographs were sampled for activity using a computerized image processing system.

Results and Discussion

Figure 1 shows the retention for birds from the various conditions. These results were consistent with previous behavioral findings, with retention being low for all water-trained birds (no discrimination between colored beads) and high for all strongly reinforced birds, at both test times. Weakly trained birds showed high retention at the 30-min time-point (at the end of Phase A of ITM), but low retention at 60 min. Hormonal treatment increased the level of retention in weakly trained birds to that of their strongly trained counterparts.

Figure 2 shows the metabolic activity for the various brain regions for weak and strong training, at 30 min postlearning, and at 60 min (with and without ACTH) after learning, expressed as the percentage difference in 2DG accumulation relative to H_2O trained birds. At 30 min posttraining, no substantial differences were observed in the level of metabolic activity between weakly and strongly trained animals, nor were there any differences in these training regimes relative to water-trained controls (Fig. 2A). This suggests that, up to this point in the memory trace (STM and Phase A of ITM), the mechanisms underlying memory formation do not involve metabolic processes that are assayable by 2DG uptake in the structures measured.

At the 60-min time-point (Fig. 2B)—which also incorporated any metabolic activity occurring between 30 and 60 min posttraining—a markedly different picture emerged. In particular, the medial neostriatum (NM) of strongly reinforced birds showed a substantial increase in metabolic activity relative to water-trained controls (19.7%). For all other brain structures measured, any differences in weakly and strongly trained birds from water controls remained negligible. However, it is instructive to note that, in contrast to

Table 1

	Treatment		
	Saline†		ACTH†
Train—sacrifice interval (min):	30†	60†	60*
Water training	$n = 4$	$n = 4$	$n = 4$
Weak training	$n = 4$	$n = 4$	$n = 4$
Strong training	$n = 4$	$n = 4$	$n = 4$

*50 µg injections of ACTH 1–24. † Ogger Saline injection.

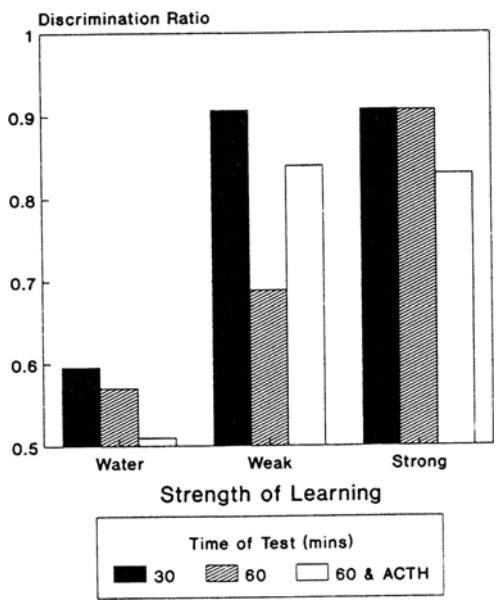

Fig. 1. Discrimination ratio at 30 min and 60 min for water, weakly and strongly trained chicks injected with either saline or ACTH 1–24 immediately after learning.

the strongly trained chicks tested at 30 min, the level of regional 2DG uptake for similarly reinforced chicks tested at 60 min was in every case higher than water-trained controls. It would appear, therefore, that increases in the level of arousal brought about by adequate learning alone produce substantial increases in metabolic activity in the NM during Phase B of ITM and LTM.

The introduction of ACTH had two major effects on regional metabolic activity up to 60 min (Fig. 2C). First, hormonal treatment removed the

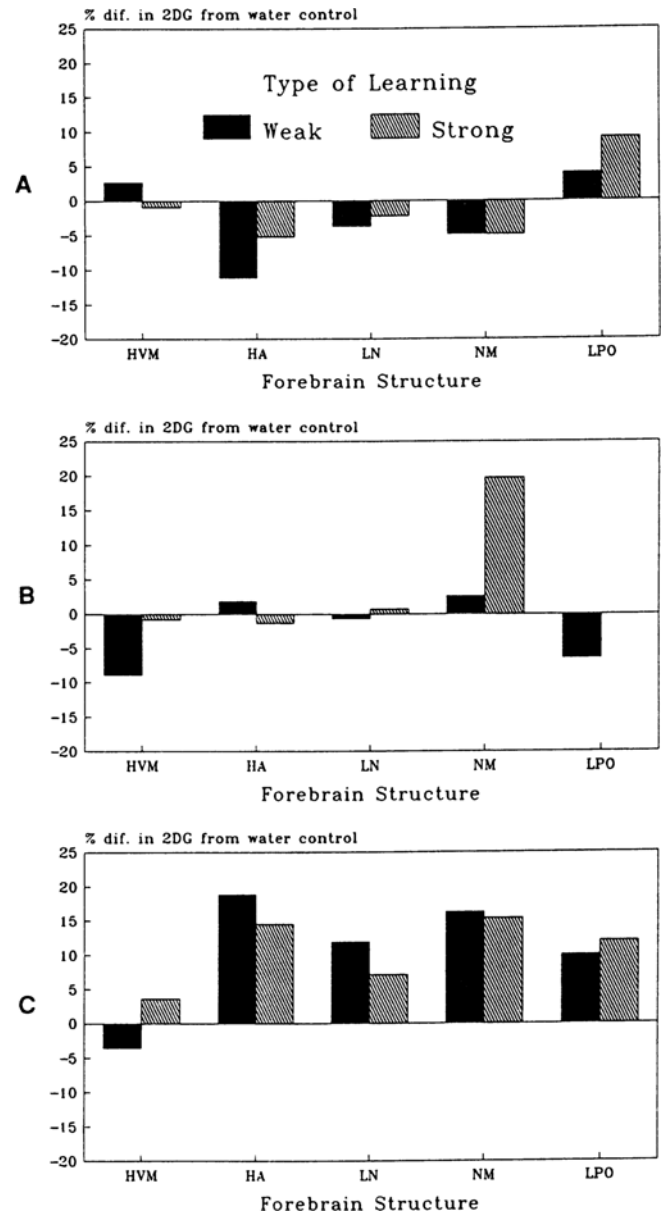

Fig. 2. Percentage differences in 2DG uptake in five regions of the chick brain; medial hyperstriatum ventrale (HVM), hyperstriatum accessorium (HA), lateral neostriatum (LN), medial neostriatum (NM), and lobus paraolfactorius (LPO) after (a) 30 min of training in weakly and strongly reinforced chicks, (b) 60 min of training in weakly and strongly reinforced chicks, and (c) 60 min of training following 50 µg subcutaneous doses of ACTH 1–24. All percentage differences are expressed relative to water-trained birds. To control for the effects of injection *per se*, birds not injected with ACTH 1–24 were given injections of saline.

difference between weakly and strongly reinforced birds in the NM, and secondly, regional metabolic activity in both weakly and strongly reinforced birds was increased relative to controls in all areas except in the medial hyperstriatum ventrale (HVM). Thus, the increased levels of arousal in ACTH-treated birds yielded more intense 2DG labeling in all areas of the weakly and strongly trained birds relative to water-trained birds. Further, the difference between weakly and strongly trained animals observed in the NM in the absence of hormones was removed by the ACTH treatment.

The results indicate that arousal-dependent metabolic activity occurring during Phase B of ITM is critical to LTM consolidation. Weakly reinforced learning does not normally produce a sufficient level of arousal to elicit ITM (B) and, hence, long-term consolidation. Contingently applied exogenous hormones in weak learning mimic the effects of strongly reinforced learning.

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