Two Critical Periods of Protein and Glycoprotein Synthesis in Memory Consolidation for Visual Categorization Learning in Chicks

Anna A. Tiunova,^{1,3} Konstantin V. Anokhin,¹ and Steven P.R. Rose²

¹P.K. Anokhin Institute of Normal Physiology
103009 Moscow, Russia

²Brain and Behavior Research Group
Department of Biology
The Open University
Milton Keynes MK7 6AA, UK

Abstract

A protein synthesis inhibitor, anisomycin (ANI), and an inhibitor of glycoprotein synthesis, 2-deoxygalactose (2-D-gal), were used to investigate memory consolidation following visual categorization training in 2-day-old chicks. ANI (0.6 μ mole/chick) and 2-D-gal (40 μ moles/chick) were injected intracerebrally at different time intervals from 1 hr before to 23 hr after the training. Retention was tested 24 hr post-training. Both ANI and 2-D-gal injections revealed two periods of memory sensitivity to pharmacological intervention. ANI impaired retention when injected from 5 min before to 30 min after the training or from 4 hr to 5 hr post-training, thus demonstrating that consolidation of long-term memory in this task requires two periods of protein synthesis. 2-D-Gal first produced an amnesia when it was injected in the interval from 5 min before to 5 min after the training. Injections made between 5 min and 5 hr post-training were without effect on the retention. The second period of memory impairment by 2-D-gal started at 5 hr post-training and lasted until 21 hr after the training. Administration of 2-D-gal made 23 hr after the training did not influence retention in the test at either 24 hr or 26 hr. These results are consistent

with the hypothesis that two waves of protein and glycoprotein synthesis are necessary for the formation of long-term memory. The prolonged duration of performance impairment by 2-D-gal in the present task might reflect an extended memory consolidation period for a categorization form of learning.

Introduction

The cellular cascade underlying consolidation of memory is generally believed to involve successive steps starting from transmitter release and initiation of pre- and postsynaptic systems of second messengers and ultimately resulting in enhanced gene expression for a family of synaptic membrane proteins that modulate synaptic connectivity (Goelet et al. 1986; Rose 1991; DeZazzo and Tully 1995; Bailey et al. 1996). As early as 30 min after training day-old chicks on a one-trial passive avoidance task, there is a dramatic increase in brain synthesis of mRNA of the immediate-early genes (IEG) *c-fos* and *c-jun* (Anokhin et al. 1991). The expression of these transcription factors in the chick brain during learning has been shown to be associated with memory formation itself rather than with nonspecific neuronal or behavioral activation (Anokhin and Rose 1991). Administration of antisense oligonucleotides to *c-fos* mRNA 11 hr before training or the protein synthesis inhibitor anisomycin (ANI) around the time of training prevents synthesis of the relevant proteins and produces amnesia, which develops starting from 1 hr after training (Patterson

³Corresponding author.

et al. 1986; Crowe et al., 1994; Freeman et al. 1995; Mileusnic et al. 1996). Such a time course of amnesia reinforces the view that activation of gene expression is associated with the formation of long-term memory, whereas earlier stages of memory appear to be independent of protein synthesis (Ng and Gibbs 1991). Because ANI has been shown to suppress protein synthesis only for ~2 hr (Flood et al. 1973), the effect of ANI in these experiments must have been on an early stage of long-term memory formation, presumably involving the expression of proteins encoded by learning-inducible IEGs (Bailey et al. 1996).

However, our group has reported an additional, later time window of sensitivity to ANI in chicks trained on the passive avoidance task. In chicks tested 24 hr following training, amnesia was apparent if injections of ANI were made either between 30 min prior to and 1.5 hr after training or between 4 hr and 5 hr post-training. Injections made 2–3 hr or later than 5 hr post-training were without effect (Freeman et al. 1995). A similar second period of sensitivity of long-term memory to protein synthesis inhibitors around 4–6 hr after training has been reported previously for a brightness discrimination task in rats (Grecksch and Matties 1980) and more recently for habituation of neuronal responses to conspecific song in songbirds (Chew et al. 1995). Thus, there must be two successive “waves” of memory-relevant protein synthesis occurring during these ANI-sensitive time windows.

Though the exact molecules that are necessary for memory consolidation during the first and second waves of protein synthesis still remain to be identified, it is known that a number of them are further subject to post-translational modification, notably by glycosylation (Rose 1989; Bullock et al. 1990, 1992). Enhanced incorporation of fucose into rat hippocampal glycoproteins has been found following training on a brightness discrimination task (Popov et al. 1981). Increased incorporation of fucose has been also shown in the chick brain after passive avoidance training (Rose and Harding 1984; McCabe and Rose 1985, 1987). Intracerebral administration of 2-deoxygalactose (2-D-gal), a competitive inhibitor of glycoprotein fucosylation, produced amnesia in chicks both in passive avoidance and in a sickness-induced learning paradigm (Rose and Jork 1987; Barber et al. 1989; Barber and Rose 1991). However, as with ANI, we have recently found a second time window during which 2-D-gal administration affects memory consolida-

tion for the passive avoidance task. Intracerebral injections of 20 μ moles of 2-D-gal at 5.5 hr post-training resulted in amnesia in a test given 24 hr after training (Scholey et al. 1993). This time window is delayed by an hour by comparison with that for the ANI (Scholey et al. 1993; Freeman et al. 1995), which suggests that glycosylation might occur for new synthesized proteins. This second wave of glycoprotein synthesis has been found to involve a particular class of glycosylated cell adhesion molecules, notably the neural cell adhesion molecules NCAM and NgCAM (L1) (Scholey et al. 1993; Mileusnic et al. 1995).

The passive avoidance task that was used in most of our previous experiments is a particular form of one-trial learning that uses a strong negative reinforcement and requires formation of memory for an individual stimulus item (Andrew 1991). We have recently been comparing molecular processes underlying memory formation in this task with those involved in a different learning task in chicks (Tiunova et al. 1996). This new behavioral model is not based on such aversive stimulation as in passive avoidance and requires multiple-trial visual categorization learning (Anokhin and Tiunova 1996). In this task chicks are presented with a large number of different colored beads glued to the floor of the experimental chamber. Contrary to the passive avoidance task, the beads are not covered with any aversive substance, but they cannot be removed by chicks from the floor. Young chicks start pecking at beads vigorously but soon suppress this behavior after attempting to swallow only a small proportion of beads. Whereas avoidance behavior of chicks in the passive avoidance task is acquired toward a particular bead and is not transferred to beads of other color and size (Andrew 1991), in the “bead field” model chicks peck only at a small proportion of beads present on the floor (usually 20–25, which is about 10% of all the beads) and then generalize their reaction to the rest of the beads of different color, shape, and location on the floor of the chamber (Anokhin and Tiunova 1996; Tiunova et al. 1996). This task thus represents an animal model of category learning that is based on acquisition of a generalized reaction to a new class of objects after encountering only a few examples of the class (Smith and Medin 1981).

Our interest has been whether molecular processes involved in memory formation in this form of learning will be different from the passive avoidance model. The biochemical cascade underlying

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memory formation for this task has been studied by intracerebral injections of pharmacological agents before the training and analysis of their effect on retention 30 min, 3 hr, or 24 hr later. Although there were differences between the early stages of the biochemical cascade in the visual categorization and passive avoidance learning (notably the involvement of *N*-methyl-D-aspartate receptors, calcium channels, and nitric oxide synthesis), activation of protein kinases as well as protein synthesis was required for the long-term memory in both training models. In particular, bilateral administration of ANI into the intermediate medial hyperstriatum ventrale (IMHV) region of the chicken brain 5 min before visual categorization training resulted in significant amnesia on the test 3 and 24 hr later (Tiunova et al. 1996). Our conclusion was that while the initial steps in synaptic activation may be task specific, the later steps, including synaptic modulation, use more universal molecular mechanisms. The aim of the present study was to test this hypothesis by investigating the effects of pharmacological disruption of the later stages of memory consolidation; the prediction being that formation of long-term memory in the visual categorization task also requires two waves of protein synthesis and glycoprotein fucosylation.

Materials and Methods

ANIMALS AND TRAINING PROCEDURES

Ross 1 Chunky chicks of both sexes were hatched in a communal incubator maintained on a 12:12 hr light/dark cycle at 38–40°C. One-day-old chicks were placed in pairs into 20 × 25 × 20-cm aluminium pens, each illuminated with a 25-W red light, and left overnight with standard chick food and water freely available. The experiments started the following morning, when the chicks were 2 days old. The food was removed from the home pens 3 hr before training. Chicks were placed individually into an experimental cage, identical to their home holding cage except for a transparent anterior wall, small mirrors on the three other walls, and a perspex floor with colored beads glued onto it. The beads (3 mm in diam.) were of six colors and arranged in 12 rows, 15 beads in each, the distance between both beads and rows being 15 mm. Standard chick food (commercial starter crumbs, ~3 mm in diam.) was scattered on the floor between the beads. Each chick remained

in the pen until it had made a total of 80 pecks, which usually took between 5 min and 10 min. Each separate peck was manually scored as “correct” (a peck at food) or “error” (a peck at a bead instead of food). Immediately after the training session, the chick was returned to its home pen and left there until the test session that took place 24 hr after the training.

Each chick was trained and tested only once. The testing was conducted using blind protocol. In those experiments where drug injections had to be made after training, the chicks were post hoc divided into groups with equal mean number of mistakes during the training session. In the test session, chicks were allowed a total of 60 pecks. To estimate the level of retention during the test, the number of pecks at beads (errors) in the first 20 pecks was calculated. As we have demonstrated previously (Tiunova et al. 1996), the number of errors in untreated chicks during the first 20 pecks of the test was similar to the number of errors during the last 20 pecks of the training session. An increase in bead pecking during the test was regarded as demonstrating memory impairment (amnesia). Statistical comparisons of the retention level between drug-injected and control groups at each time point were made using a Mann-Whitney two-tailed *U* test.

DRUGS AND INJECTION PROCEDURE

Injections of ANI and 2-D-gal were made at different times from 1 hr before to 23 hr after the training session. The drugs were injected intracerebrally using a 50- μ l Hamilton microsyringe fitted with a plastic sleeve as a stop for an injection depth of 4 mm. Bilateral injections (10 μ l/hemisphere) were made using a Plexiglas head holder designed to direct the injections into the region of left and right IMHV (Davis et al. 1979). Young chicks have an unossified skull, which allows intracranial injections to be performed rapidly; no anesthesia or surgery is required. ANI (Sigma) was dissolved in 0.9% saline at a concentration 30 mM (8 mg/ml) by adding an equimolar amount of 3 M HCl and adjusting the pH of the solution to 7.0 with 3 M NaOH. The dose injected per chick was thus 0.6 μ mole. This dose is the one that has been shown to impair retention for the passive avoidance task in chicks tested at 24 hr when injected at appropriate times before or after training (Freeman et al. 1995). 2-D-Gal (Sigma) was dissolved in 0.9% saline at a con-

centration of 2 M (328 mg/ml). The dose per chick was 40 μ moles. Control groups of chicks received bilateral injections of 10 μ l of vehicle solution. Drugs were coded and injected blind.

Results

EFFECTS OF ANI AND 2-D-GAL INJECTIONS BEFORE TRAINING

As Figure 1A shows, bilateral injection of ANI (0.6 μ mole) or 2-D-gal (40 μ moles) 1 hr before training session did not affect either acquisition or retrieval of the task at the 24-hr test. Injection of ANI 5 min before training resulted in a small decrease in errors in the task acquisition, whereas a 2-D-gal injection 5 min before training produced a small increase in the number of errors in the last 20 pecks as compared with the control group (Fig. 1B). However, during the retention test given 24 hr after the training there was a substantial increase in the number of errors in the first 20 pecks in both ANI- and 2-D-gal-injected groups. Neither agent had any notable effect on the general behavior or pecking activity of the chicks during training and testing (data not shown), and there was no difference between the two drugs in respect to the degree of their effect in retention.

EFFECTS OF ANI INJECTIONS AT VARIOUS TIMES POST-TRAINING

ANI was injected at various times up to 23 hr

post-training, and chicks were tested 24 hr after training. Impairment of retention occurred in groups of chicks that received ANI injections at around the time of training (from 5 min before to 30 min after the training session) or 3–4 hr post-training. Injections of ANI made from 1 hr to 2 hr or after 5 hr post-training were without effect on the level of retention tested at 24 hr (Fig. 2).

EFFECTS OF 2-D-GAL INJECTIONS AT VARIOUS TIMES POST-TRAINING

Previous reports from our laboratory had described a dose of 20 μ moles, injected bilaterally around the time of training, as sufficient to produce amnesia for passive avoidance in chicks (Scholey et al. 1993). Our pilot experiments, however, did not demonstrate a reliable amnestic effect of this dose in the present learning model. As higher doses (40 μ moles and 80 μ moles) were both without overt behavioral effects, a dose of 40 μ moles per chick was chosen. This dose of 2-D-gal produced amnesia when the injections were made just before or just after the training session (5 min before or 5 min after the training) (Figs. 1 and 3). If injections were delayed to 15 min post-training, no amnesia was apparent at 24 hr (Fig. 3). However, amnesia did occur if injections were made at 4 hr post-training, and a high error rate on the test at 24 hr persisted if injections of 2-D-gal were made at any tested time up to 21 hr post-training. Injections made at 23 hr post-training were without effect on chicks tested at 24 hr (i.e., 1 hr post-injection).

Figure 1: The effect of ANI (0.6 μ mole) and 2-D-gal (40 μ moles), injected either 1 hr (A) or 5 min (B) prior to training, on acquisition of the visual categorization task and its retention tested 24 hr later. Each point represents the mean number of errors ($\pm$ S.E.M.) made by drug-injected and control groups of chicks in each block of 20 pecks during training and testing. The number of animals was 13–16 per group. (*) $P < 0.05$; (***) $P < 0.001$ between drug-injected [(Δ) ANI; ($\bullet$) 2-D-gal] and control group ($\circ$) within a single block.

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Figure 2: The effect of ANI, injected at different times before and after training, on the retention of the visual categorization task tested 24 hr after the training. The time of training is marked as zero. For each training to injection interval, a separate group of chicks was used. Each point represents the mean number of errors ($\pm$ S.E.M.) made by ANI-injected (●) and control (○) groups of chicks in the first 20 pecks during the test. The number of animals was 10–16 per group. (*) $P < 0.05$; (**) $P < 0.01$; (***) $P < 0.001$ between ANI-injected and control group in each point.

EFFECT OF TIME INTERVAL BETWEEN 2-D-GAL INJECTION AND TEST

To check the possibility that 2-D-gal injected 23 hr post-training did influence memory processing but that the effect was not yet apparent in the test 1 hr later, two additional control experiments were performed. In the first experiment, 2-D-gal was injected 23 hr after training, but the test was given 3 hr later, that is, 26 hr after the training. Thus, the delay between injection and testing was exactly the same as in the case in which a 2-D-gal injection was given at 21 hr post-training that produced a significant amnesia on the test at 24 hr (Fig. 3). However, no difference in the number of mistakes made by the 2-D-gal-injected and control chicks was detected in the case of the injection at 23 hr and the testing at 26 hr (Fig. 4).

In the second experiment, 2-D-gal was injected 21 hr after training, and the test was given 1 hr later, that is, 22 hr after the training. This 1-hr injection-to-test interval imitated the experiment with 23-hr post-training 2-D-gal injection, which produced no effect on task retention. However, there was a significant increase in the number of mistakes if chicks were injected with 2-D-gal 21 hr post-training and tested 1 hr later (Fig. 4). Administration of 2-D-gal 21 and 23 hr after training did

not affect the general pecking rate of chicks at any of the testing times.

EFFECTS OF POST-TRAINING GALACTOSE INJECTION

To control for the chemical specificity of the delayed 2-D-gal effects on memory formation, chicks were injected with the equimolar amount (40 μ moles) of D-(+)-galactose that does not interfere with protein fucosylation processes. No signs of retrieval disturbances were detected if chicks were injected with D-(+)-galactose 21 hr post-training and tested 24 hr after the training (mean number of mistakes in the D-(+)-galactose group, 1.7 ± 0.4 , $n = 13$; and in the saline group, 2.1 ± 0.5 , $n = 12$, $P > 0.05$).

Discussion

The conventional approach in studies of the role of protein synthesis in long-term memory formation uses the administration of protein synthesis inhibitors close to the time of training (Davis and Squire 1984). If the time of the administration of

Figure 3: The effect of 2-D-gal, injected at different times before and after training, on the retention of the visual categorization task tested 24 hr after the training. The time of training is marked as zero. For each training to injection interval a separate group of chicks was used. Each point represents the mean number of errors ($\pm$ S.E.M.) made by 2-D-gal-injected (●) and control (○) groups of chicks in the first 20 pecks during the test. The number of animals was 10–20 per group. (*) $P < 0.05$; (**) $P < 0.01$; (***) $P < 0.001$ between 2-D-gal-injected and control group in each point.

Figure 4: The effect of 2-D-gal injections 21 or 23 hr post-training on task retention in the test given either 1 or 3 hr after the injection. Each bar represents the mean number of errors ($\pm$ S.E.M.) made by 2-D-gal-injected (hatched bars) and control (open bars) groups of chicks in the first 20 pecks during the test. Groups of chicks: 21/22 hr—injection made 21 hr post-training, test given 22 hr post-training ($n_1 = 14$ for saline group, $n_2 = 17$ for 2-D-gal group); 21/24 hr—injection made 21 hr post-training, test given 24 hr post-training ($n_1 = 11$, $n_2 = 13$); 23/24 hr—injection made 23 hr post-training, test given 24 hr post-training ($n_1 = 18$, $n_2 = 19$); 23/26 hr—injection made 23 hr post-training, test given 26 hr post-training ($n_1 = 12$, $n_2 = 14$). (**) $P < 0.01$; (***) $P < 0.001$ between 2-D-gal-injected and control group.

the inhibitor is delayed for more than a short period, usually not exceeding 2 hr after the training, the injections are ineffective in producing amnesia, and no further extension of training-treatment interval is examined (e.g., Patterson et al. 1986; Rose and Jork 1987; Crowe et al. 1994). The general assumption behind such an experimental design is that long-term memory consolidation occurs within a short time window after the end of training (Goelet et al. 1986; Bailey et al. 1996) and that once long-term memory is established its maintenance can not be disrupted by inhibitors of macromolecular synthesis (Davis and Squire 1984).

However, a second sensitive period from 4 hr to 6 hr after training has been discovered for ANI-induced amnesia in a brightness discrimination task in rats (Grecksch and Matties 1980) and more recently in one-trial passive avoidance task in chicks (Freeman et al. 1995). Similarly, a second time window for the amnestic effect of 2-D-GAL between 5 hr and 6 hr after training has been demonstrated in passive avoidance learning in chicks (Scholey et al. 1993). Unlike the first 2-D-gal-sensitive period near to the time of training, the second time window has been proved to be concerned with synthesis of NCAMs, which are assumed to play a crucial role in stabilizing synaptic changes

following training (Scholey et al. 1993; Mileusnic et al. 1995; Rose 1995). This period, 6–8 hr downstream of training, has also been shown to be vulnerable to the effect of antibodies to NCAMs in experiments involving passive avoidance and radial arm maze learning in rats (Doyle et al. 1992; Alexinsky et al. 1997).

Our aim in the present study was to investigate the role of protein and glycoprotein synthesis in the late phases of memory processing in a different form of learning that involves categorization of novel objects (Anokhin and Tiunova 1996). Categorization learning usually requires an encounter with a series of objects and leads to the establishment of memory that has been shown to depend on different neuroanatomical substrates than memory of individual object items (Knowlton and Squire 1993; Squire and Knowlton 1995; Rees et al. 1997). In the current work we wanted to know whether a molecular dynamics of protein synthesis for memory consolidation in an animal model of a categorization learning task would be similar or different from a dynamics in a related noncategorization single-object memory task.

Our previous data demonstrated that intracerebral bilateral injections of the protein synthesis inhibitor ANI just before training in the visual categorization task prevented formation of long-term memory for the categorized objects in 2-day-old chicks (Tiunova et al. 1996). In the present experiments injections of ANI and the specific inhibitor of glycoprotein synthesis 2-D-gal were given at different time points both before and after the training, the effect of post-training injections being of particular interest.

The experiments showed that administration of both ANI and 2-D-GAL around the time of visual categorization training produced amnesia in chicks tested 24 hr after the training. Injections made 1-hr before training were without effect, which is in accord with the facts that (1) protein synthesis is inhibited by ANI for 2 hr after which it resumes; (2) 3 hr after injection of 2-D-gal, fucosylation of proteins reverts to its original level; and (3) for at least 30 min after training, memory is supported by protein synthesis-independent mechanisms (Flood et al. 1973; Gibbs and Ng 1977; Patterson et al. 1986; Rose and Jork 1987; Tiunova et al. 1996). The first time window for the influence of ANI in a bead field task was from 5 min before training to 30 min after training, which basically coincides with data obtained for passive avoidance training in chicks (Patterson et al. 1986; Freeman et al.

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1995). The sensitive period for 2-D-gal started simultaneously with that for ANI and was shorter, thus confirming the fact that the first wave of synthesis of glycoproteins occurs independently from de novo protein synthesis (McCabe and Rose 1985) and presumably involves mainly fucosylation of already synthesized proteins. Similar amnesic effects of pre- and post-training injections of ANI and 2-D-gal excludes the interpretation that effects in this time period were owing to their influence on task acquisition itself. In general, the time course of the effects of ANI and 2-D-gal injected close to the time of training in the studied categorization task is in agreement with the data obtained in other training models (Patterson et al. 1986; Rose and Jork 1987; Crowe et al. 1994; Freeman et al. 1995).

Both ANI and 2-D-gal injected 5 min before the training influenced the number of errors made during training (Fig. 1). As we have found previously, the changed task acquisition curve in ANI-injected chicks did not impair performance tested 30 min later (Tiunova et al. 1996). In 2-D-gal-injected chicks there was a small but significant increase in the number of errors at the end of training in comparison with that in the control group (Fig. 1). However, 2-D-gal injected 5 min post-training also produced a comparable amnesic effect on the test at 24 hr, which suggests that the effect of 2-D-gal injected around the time of training on the retention was owing to its influence on memory formation rather than on task acquisition.

Neither ANI or 2-D-gal administered 1 or 2 hr after the training affected task performance on the test at 24 hr. However, ANI injected at 3 or 4 hr post-training produced an amnesia equal to the amnesic effect of ANI around the time of training. Thus, this result demonstrates that establishing long-term memory in visual categorization learning, just as in passive avoidance, requires two waves of protein synthesis. The fact that the second window in visual categorization learning was shifted to a somewhat earlier time compared with passive avoidance might be attributable to the task-specific differences, particularly the longer duration of the training procedure in the task used in this paper.

Unlike the action of ANI, injections of 2-D-gal made 3 hr after training were ineffective. However, starting from 4 hr post-training, we have found a second time window for the amnesic effect of 2-D-gal. Such a displacement in the time of effect of 2-D-gal resembles that between the two inhibitors found for passive avoidance (Freeman et al. 1995)

and suggests that in contrast to the processes blocked during the first time window, glycoproteins subjected to fucosylation during the second wave must be synthesized de novo. Because the second wave of glycoprotein synthesis has been shown to be related to the role of cell adhesion molecules, it is likely that translation of these molecules occurs on ribosomes during the second wave of protein synthesis, after which they are glycosylated and inserted into the synaptic membrane (Scholey et al. 1993). Our recent experiments have demonstrated that antibodies to the cell adhesion molecule L₁ produced amnesia when injected at 5.5 hr after bead field training, thus confirming the role of this glycoprotein in memory consolidation in this task (Tiunova et al. 1997).

Although the beginning of the second time window for 2-D-gal in visual categorization was similar to that obtained in passive avoidance training, the further time courses in the two training models appeared to be completely different. In passive avoidance, the second wave of glycosylation became insensitive to the inhibitor at 8 hr after the training (Scholey et al. 1993); in contrast, the amnesic action of 2-D-gal on retention in visual categorization learning continued as late as 21 hr post-training (Fig. 3). This result was quite unexpected, because it seemed hardly likely that consolidation of memory is fucosylation-dependent or could be influenced by the inhibitor at such delayed times following training. Of equal interest was the fact that 2-D-gal injected 23 hr after the training did not result in amnesia at 24 hr, thus suggesting that the second sensitive period for the drug finally terminated 17 hr after its onset.

The possibility that 2-D-gal administration at 23 hr post-training was ineffective because of too short an interval between the injection and testing was ruled out in the additional control experiments. In the first experiment the interval was extended to 3 hr, thus matching the case of the injection at 21 hr and the testing at 24 hr, which produced amnesia. In the case of injection at 23 hr and testing at 26 hr, however, there was no amnesic effect. By contrast, injection of 2-D-gal made at 21 hr post-training did produce amnesia even if the testing was conducted 22 hr after the training, that is, 1 hr after the injection. These data suggest that the memory processes influenced by 2-D-gal naturally decline between 21 hr and 23 hr after learning in the studied task.

Thus, although the existence of the two waves of sensitivity to the inhibitors of protein synthesis

and glycosylation and their times of onset suggest that similar biochemical mechanisms underlie consolidation of memory for these two rather different learning tasks in chicks, the extensive prolongation of the period of sensitivity to 2-D-gal is a distinctive feature of visual categorization learning.

One question that cannot be answered by the present study is the precise neuroanatomical location of the consolidation-related protein synthesis in the categorization task. Although the injections of drugs were made into the region of IMHV, which is critical for memory consolidation in the passive avoidance task (Rose 1991), the possibility of their influence on protein synthesis in other brain areas cannot be ruled out. Our control experiments have demonstrated that the dye injected into the IMHV in the volume of 5 μ l also penetrates in the lateral cerebral ventricles and stains periventricular brain structures. Additionally, injection of ANI into the IMHV in the dose twice lower than used in our study has been shown to produce inhibition of the protein synthesis in the whole chick brain for 35% during the first 30 min following the injection (Patel et al. 1988; Patterson et al. 1989), which suggests much wider anatomical action of the inhibitor than just the IMHV region. However, the suppression of the brain protein synthesis after such ANI injections is not complete, thus leaving an opportunity that certain brain areas might maintain learning-induced protein synthesis sufficient for concomitant long-term synaptic changes. This may account for the fact that amnesia produced by ANI in our experiments was only partial (see Fig. 1).

It is noteworthy that several critical periods of memory dependence on protein synthesis has been reported recently for long-term habituation of neuronal responses to conspecific song in songbirds (Chew et al. 1996). Whereas the first two time windows when injection of protein synthesis inhibitor cycloheximide disrupted habituation in songbirds (0.5–3 hr and 6.5–7 hr) might correspond to two critical time windows revealed by ANI injections in our study, the third and the fourth periods (14–15 hr and 17.5–18.5 hr) are covered by the prolonged amnesic effect of 2-D-gal in our experiments.

Although we have no obvious explanation for the extended period of memory disruption by 2-D-gal, we are presently investigating the kinetics of this process and the nature of glycoproteins involved. It would also seem important to check the duration of the second glycoprotein synthesis-sen-

sitive window in other species and tasks, such as the rat passive avoidance and maze-learning tasks, where the time of onset matches that found in the chick, but the offset time has not been established.

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References

- Alexinsky, T., J. Przybyslawski, R. Mileusnic, S.P.R. Rose, and S.J. Sara. 1997. Antibody to day-old chick brain glycoprotein produces amnesia in adult rats. *Neurobiol. Learn. Mem.* **67**: 14–20.
- Andrew, R.J., ed.. 1991. *Neural and behavioral plasticity: The use of the domestic chick as a model*. Oxford University Press, Oxford, UK.
- Anokhin, K.V. and S.P.R. Rose. 1991. Learning-induced increase of immediate early gene messenger RNA in the chick forebrain. *Eur. J. Neurosci.* **3**: 162–167.
- Anokhin, K.V. and A.A. Tiunova. 1996. Interocular memory transfer following visual categorization learning in chicks. *Proc. Russian Acad. Sci.* **348**: 564–566.
- Anokhin, K.V., R. Mileusnic, I.Y. Shamakina, and S.P.R. Rose. 1991. Effects of early experience on *c-fos* gene expression in the chick forebrain. *Brain Res.* **554**: 101–107.
- Bailey, C.H., D. Bartsch, and E.R. Kandel. 1996. Toward a molecular definition of long-term memory storage. *Proc. Natl. Acad. Sci.* **93**: 13445–13452.
- Barber, A.J. and S.P.R. Rose. 1991. Amnesia induced by 2-deoxygalactose in the day-old chick: Lateralization of effects in two different one-trial learning tasks. *Behav. Neural Biol.* **56**: 77–88.
- Barber, A.J., D.B. Gilbert, and S.P.R. Rose. 1989. Glycoprotein synthesis is necessary for memory of sickness-induced learning in chicks. *Eur. J. Neurosci.* **1**: 673–677.
- Bullock, S., J. Potter, and S.P.R. Rose. 1990. Effects of the amnesic agent 2-deoxygalactose on incorporation of fucose into chick brain glycoproteins. *J. Neurochem.* **54**: 135–142.

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- Bullock, S., S.P.R. Rose, and R. Zamani. 1992. Characterization and regional localization of pre- and post-synaptic glycoproteins in the chick forebrain showing changed fucose incorporation following passive avoidance training. *J. Neurochem.* **58**: 2145–2154.
- Chew, S.J., C. Mello, F. Nottebohm, E. Jarvis, and D.S. Vicario. 1995. Decrements in auditory responses to a repeated conspecific song are long-lasting and require two periods of protein synthesis in the songbird forebrain. *Proc. Natl. Acad. Sci.* **92**: 3406–3410.
- Chew, S.J., D.S. Vicario, and F. Nottebohm. 1996. Quantal duration of auditory memories. *Science* **274**: 1909–1914.
- Crowe, S.F., W.-Q. Zhao, G.L. Sedman, and K.T. Ng. 1994. 2-Deoxygalactose interferes with an intermediate processing stage of memory. *Behav. Neural Biol.* **61**: 206–213.
- Davis, H.P. and L.R. Squire. 1984. Protein synthesis and memory: A review. *Psychol. Bull.* **96**: 518–559.
- Davis, J.L., D.T. Masouka, D.T. Gerbrandt, and A. Cherkin. 1979. Autoradiographic distribution of L-proline in chick after intracerebral injection. *Physiol. Behav.* **22**: 177–184.
- DeZazzo, J. and T. Tully. 1995. Dissection of memory formation: From behavioral pharmacology to molecular genetics. *Trends Neurosci.* **18**: 212–217.
- Doyle, E., P. Nolan, R. Bell, and C.M. Regan. 1992. Intraventricular infusions of anti-neural cell adhesion molecules in a discrete posttraining period impair consolidation of a passive avoidance response in the rat. *J. Neurochem.* **59**: 1570–1573.
- Flood, J.F., M.R. Rosenzweig, E.L. Bennett, and A.E. Orme. 1973. The influence of duration of protein synthesis inhibition of memory. *Physiol. Behav.* **10**: 555–562.
- Freeman, F.M., S.P.R. Rose, and A.B. Scholey. 1995. Two time windows of anisomycin-induced amnesia for passive avoidance training in the day-old chick. *Neurobiol. Learn. Mem.* **63**: 291–295.
- Gibbs, M.E. and K.T. Ng. 1977. Psychobiology of memory: Towards a model of memory formation. *Biobehav. Rev.* **1**: 113–136.
- Goelet, P., V.F. Castelluci, S. Schachner, and E.R. Kandel. 1986. The long and short of long-term memory—a molecular framework. *Nature* **322**: 419–423.
- Grecksch, G. and H. Matties. 1980. Two sensitive periods for the amnesic effect of anisomycin. *Pharmacol. Biochem. Behav.* **12**: 663–665.
- Knowlton, B.J. and L.R. Squire. 1993. The learning of categories: Parallel brain systems for item memory and category knowledge. *Science* **262**: 1747–1749.
- McCabe, N.R. and S.P.R. Rose. 1985. Passive avoidance training increases fucose incorporation into glycoproteins in chick forebrain slices in vitro. *Neurochem. Res.* **10**: 1083–1095.
- . 1987. Increased fucosylation of chick brain proteins following training: Effects of cycloheximide. *J. Neurochem.* **48**: 538–542.
- Mileusnic, R., S.P.R. Rose, C. Lancashire, and S. Bullock. 1995. Characterization of antibodies specific for chick brain N-CAM which cause amnesia for a passive avoidance task. *J. Neurochem.* **64**: 2598–2606.
- Mileusnic, R., K. Anokhin, and S.P.R. Rose. 1996. Antisense oligodeoxynucleotides to c-fos are amnesic for passive avoidance in the chick. *NeuroReport* **7**: 1269–1272.
- Ng, K.T. and M.E. Gibbs. 1991. Stages in memory formation: A review. In *Neural and behavioral plasticity: The use of the domestic chick as a model*. (ed. R.J. Andrew), pp. 305–328. Oxford University Press, Oxford, UK.
- Patterson, T.A., M.G. Alvarado, I.T. Warner, E.L. Bennett, and M.R. Rosenzweig. 1986. Memory stages and brain asymmetry in chick learning. *Behav. Neurosci.* **100**: 856–865.
- Patterson, T.A., S.P.R. Rose, and P.M. Bradley. 1989. Anisomycin and amnesia in the chick: State dependent effects are not present with intracranial injections. *Develop. Brain Res.* **49**: 173–178.
- Popov, N., S. Schulzeck, W. Pohle, and H.-J. Matties. 1981. Changes in the incorporation of ^3H fucose into rat hippocampus after acquisition of a brightness discrimination reaction: An electrophoretic study. *Neuroscience* **5**: 161–165.
- Rees, G., R. Frackowiak, and C. Frith. 1997. Two modulatory effects of attention that mediate object categorization in human cortex. *Science* **275**: 835–838.
- Rose, S.P.R. 1989. Glycoprotein synthesis and postsynaptic remodelling in long-term memory. *Neurochem. Int.* **14**: 299–314.
- . 1991. How chicks make memory: The cellular cascade from c-fos to synaptic remodelling. *Trends Neurosci.* **14**: 390–397.
- Rose, S.P.R. 1995. Cell adhesion molecules, glucocorticoids and long term memory formation. *Trends Neurosci.* **18**: 502–506.
- Rose, S.P.R. and S. Harding. 1984. Training increases [^3H] fucose incorporation into chick brain only if followed by memory storage. *Neuroscience* **12**: 663–667.
- Rose, S.P.R. and R. Jork. 1987. Long-term memory formation in chicks is blocked by 2-deoxygalactose, a fucose analogue. *Behav. Neural Biol.* **48**: 246–258.
- Scholey, A.B., S.P.R. Rose, M.R. Zamani, E. Bock, and M. Schachner. 1993. A role for the neural cell adhesion molecule in a late, consolidating phase of glycoprotein

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synthesis six hours following passive avoidance training of the young chicks. *Neuroscience* **55**: 499–509.

Smith, E.E. and D.L. Medin. 1981. *Categories and concepts*. Harvard University Press, London, UK.

Squire, L.R. and B.J. Knowlton. 1995. Learning about categories in the absence of memory. *Proc. Natl. Acad. Sci.* **92**: 12470–12474.

Tiunova, A., K. Anokhin, S.P.R. Rose, and R. Mileusnic. 1996. Involvement of glutamate receptors, protein kinases and protein synthesis in memory for visual discrimination in the young chick. *Neurobiol. Learn. Mem.* **65**: 233–243.

Tiunova, A., K. Anokhin, M. Schachner, and S.P.R. Rose. 1997. Several time windows for amnestic effect of antibodies to cell adhesion molecules L1 in chicks. *NeuroReport* (in press).

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A A Tiunova, K V Anokhin and S P Rose

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