Pharmacology Biochemistry and Behavior, Vol. 60, No. 2, pp. 423–430, 1998

0091-3057/98 $19.00 + .00

ELSEVIER

PII S0091-3057(97)00597-2

A New Look at an Old Task: Advantages and Uses of Sickness-Conditioned Learning in Day-Old Chicks

TERESA A. BARBER, AMANDA M. KLUNK, PAMELA D. HOWORTH, MELANIE F. PEARLMAN AND KRISTA E. PATRICK

Department of Psychology, Dickinson College, Carlisle, PA 17013-2896

Received 15 August 1997; Revised 22 October 1997; Accepted 12 November 1997

BARBER, T. A., A. M. KLUNK, P. D. HOWORTH, M. F. PEARLMAN AND K. E. PATRICK. *A new look at an old task: Advantages and uses of sickness-conditioned learning in day-old chicks.* PHARMACOL BIOCHEM BEHAV **60**(2) 423–430, 1998.—In sickness-conditioned learning, animals become ill after sampling a new substance and develop an aversion that is expressed as avoidance of that substance in subsequent presentations. We examined the parameters of a one-trial, nongustatory, sickness-conditioned learning task in day-old chicks. Chicks pecked a bead and were made ill by IP injection of lithium chloride (LiCl). Both 0.5 and 1.0 M LiCl (0.1 ml) produced reliable avoidance at test. Chicks injected with LiCl between 15 and 45 min after training avoided the bead at test, whereas those injected within 5 or 10 min or more than 45 min after training did not. Avoidance was present until 24 h posttraining and absent after 48 h. Therefore, robust learning of the sickness-conditioned learning task occurs in one trial without the need for gustatory cues, and memory for the task lasts at least 24 h. Uses of this task to study memory formation in the day-old chick are discussed. © 1998 Elsevier Science Inc.

Aversion Chicks Sickness-conditioned learning Lithium chloride Memory One-trial learning

A variety of behavioral tasks have been used to study learning and memory in day-old chicks, from autoshaping to Y-maze discrimination (22,38). Most commonly, chicks are trained and tested in one of two tasks, either filial imprinting or passive avoidance learning. In the chick filial imprinting task, the chick is exposed to a particular object, either the chick's natural mother or an artificial stimulus. Following exposure, the chick will approach or follow the object rather than novel objects and emit distress calls when separated from the object (8). In passive avoidance learning, chicks peck a bead coated with a bitter substance such as methylanthranilate. If the chick forms a memory of the bead and bad taste, it will not peck that color of bead again, but will peck other beads (12).

The passive avoidance task is especially appropriate for the study of learning and memory because of the duration of the training trial, which occurs as a brief and discrete event, taking a mere 10 s in many studies (25,29). Rose has argued that this feature of passive avoidance produces a separation of events surrounding the training experience from the processes that occur during memory formation (28). The timing of the training trial is precise enough to allow researchers to determine time-dependent processes involved in different phases of memory formation, even those occurring close to the training trial (2,17,25).

Considerable research using the passive avoidance task has examined the biochemical, electrophysiological, pharmacological, and anatomical correlates of memory formation. In the minutes following training on this task, there is upregulation of *N*-methyl-D-aspartate receptor activity, phosphorylation of the presynaptic membrane protein B50, and genomic activation of the immediate early genes c-*fos* and c-*jun* (1,4,35). During the next hours after training, increased incorporation of fucose into brain glycoproteins occurs (10). During this time, memory for the passive avoidance task can be impaired by inhibitors of glycoprotein synthesis injected around the time of training (31). Training in passive avoidance produces lasting changes in spontaneous neuronal activity recorded in a forebrain structure called the intermediate medial hyperstria-

Requests for reprints should be addressed to Teresa A. Barber, Department of Psychology, Dickinson College, Carlisle, PA 17013–2896.

tum ventrale [IMHV; (20)]. Changes in postsynaptic densities and increases in the number of dendritic spines have also been found in the IMHV after training (24,36).

Although the passive avoidance task has many advantages, there are some shortcomings inherent in its design. First, to measure changes in biochemistry, electrophysiology, and morphology following training, chicks that peck a bead coated with methylanthranilate are compared to chicks that do not experience the training, but still peck a bead. Normally the control procedure used is one in which the chicks peck at a bead coated with water. However, studies indicate that these control chicks may form a representation of the bead (5) and biochemical changes found in the brains of these control animals resemble to some degree the changes found in trained chicks (4). Researchers have recently turned to using a more complex multiple trial task in chicks to compensate for this problem (37); however, this multiple trial task has its own disadvantages because it does not use a brief and discrete training trial, which is the principal advantage of the passive avoidance task over other tasks. In addition, the passive avoidance task is ill-suited to the study of enhancement of memory, because the aversive substance used to coat the beads (methylanthranilate) produces high levels of avoidance for several days (11). Other versions of this task have been developed, using weaker training stimuli such as 10% methylanthranilate or quinine (9,32,33,34). However, in the weak training task, a second wave of glycoprotein synthesis (and presumably morphological change) does not occur, indicating, perhaps, some failure in long-term memory formation (9).

There is another chick one-trial task in which the temporal separation between training and memory formation is even greater than that found in passive avoidance. It has been known for some time that chicks can learn to avoid visual stimuli associated with sickness, a task similar to that in which a rat learns to avoid food associated with injection of a substance that produces gastric distress (15). In early studies of sickness-conditioned learning, the chick drank from a colored sucrose solution or ate food of a distinct color, then was injected with lithium chloride (LiCl), a substance that produces reliable symptoms of sickness such as diarrhea and lethargy. Chicks injected with LiCl did not drink the colored solution or eat the colored food at test, and showed a preference for control substances not paired with the LiCl injection (16,19).

In these studies of sickness-conditioned learning in the chick, the training trial lasted up to an hour, and the chicks were injected with LiCl at the end of training. Given such a long training trial duration, it was difficult to determine when an association was formed. Barber, Gilbert, and Rose modified the sickness-conditioned learning task to better define this training time and to remove the gustatory component from the task (5). In this newer paradigm, the chick was offered a dry bead, allowed to peck, and then injected 30 min later with LiCl. At test, the chicks avoided the bead presented before the LiCl injection, but pecked novel beads not associated with the LiCl injection. Removing the gustatory component of the task changed it from a specialized form of learning to a more traditional visual association task. The change also made the training trial a discrete 30-s event, in which the chick pecked a bead and presumably learned about the visual characteristics of the training stimulus that allowed it to distinguish the training bead from other beads at test. Learning of this task was impaired by an inhibitor of glycoprotein fucosylation injected near the time of presentation of the bead, whereas injection of the inhibitor near the time of LiCl injection was not amnestic (5). This indicates that a memory for the visual characteristics of the bead, dependent upon glycoprotein fucosylation, is made at the time of pecking, even if there are no immediate consequences to that behavior.

Despite the long separation between presentation of the conditioned and unconditioned stimuli, the sickness-conditioned learning task shares many features with the passive avoidance task. The chicks distinguish and identify the characteristics of the training stimulus to respond correctly at test and associations must be made between pecking the bead and its consequences. This suggests that memory formation following training in both passive avoidance and sickness-conditioned learning may be similar in biochemistry, electrophysiology, and morphology, with differences in anatomical location and time of changes related to differences between the two tasks. We have recently used the sickness-conditioned learning task to examine the effects of lesions of the IMHV, a forebrain structure known to be involved in memory formation for passive avoidance learning in the chick (26). Bilateral pretraining lesions of the IMHV impair sickness-conditioned learning (6), which is consistent with results found in passive avoidance, indicating that the location, and possibly the mechanisms of memory formation might be similar in both types of learning.

These studies and other experiments in our laboratory (7,13) suggest that the sickness-conditioned learning task can be used as an important addition to the study of memory and learning in young chicks. Before this can be concluded, however, we need to know the optimal parameters of training, such as doses of LiCl, times of LiCl injection, and the time course of memory retention. The present study examined the different training parameters in this task. First, we determined the dose response curve for LiCl to identify the most effective dose that produced robust and consistent avoidance of the bead. Then we examined the effects of varying the time of LiCl injection on retention, to determine the most appropriate time of injection and to determine the longest possible time window between pecking the bead and injection of LiCl. Finally, we examined the time course of memory for the sickness-conditioned learning task to determine how long the memory endures for this task.

GENERAL METHOD

Animals

Male leghorn-derived chicks were purchased from a local supplier (Hy-Line Hatchery, Elizabethtown, PA) and arrived at 0800 h the day after hatching. The chicks were placed in pairs in white opaque Plexiglas pens (9 × 9 inches) in the behavioral testing room, which is maintained on a 12 L:12 D cycle (lights on at 0800 h) at 38.5–40.5°C and 45–51% humidity. The Plexiglas pens, which were open at the bottom, sat on white paper towels that were replaced before each experiment. A chick in each pen was marked on the back to distinguish one chick from the other.

Drug Injection

The saline (0.9%) and LiCl solutions were made up fresh each morning. LiCl was purchased from Sigma Chemical Company (St. Louis, MO) and was made up in saline solution (0.9%). Intraperitoneal (IP) injections of either saline or specific doses of LiCl, depending upon the experiment, were given in a volume of 0.1 ml using a 27-gauge needle at prespecified times after training.

Training and Testing Procedure

Upon arrival from the hatchery, the chicks were housed in pairs in the training pens for at least 1 h before the experiment began to allow them to acclimate to the behavior testing room. The chicks were then pretrained by a 30-s presentation of a 2-mm diameter pearl bead to initiate pecking behavior. A high number (98%) of chicks pecked at this small bead. Ten minutes after pretraining, the chicks were trained by a 30-s presentation of a dry 3-mm diameter chrome bead. Again, high numbers of chicks (91%) pecked the training bead. The chicks were then injected with saline or LiCl according to the specific experiment. Two hours after injection, all birds were provided with a small dish of tap water, to aid the recovery of the LiCl-injected chicks.

The chicks were tested by two sequential 30-s presentations of beads. The chick was offered a 3-mm chrome bead similar to that used in training, then a novel 3-mm gold bead. During the retention tests, the behavior of each chick was recorded as either pecking or avoiding the test bead. Chicks that receive saline injections following training normally peck the beads again at test, indicating that they do not form any aversions to the training bead because of the injection, nor have they habituated to presentation of the bead. Chicks that receive LiCl after training and avoid the chrome bead but peck the gold bead at test are considered to have learned an aversion between pecking the chrome bead and the aversive consequences of the LiCl injection. Chicks that peck both beads after receiving LiCl are considered amnesic for the association.

In all experiments, chicks not pecking at training were not included in the final analyses. To eliminate sources of confound due to generalized avoidance of both beads at test or any lingering sickness produced by the LiCl during the test phase, only chicks that pecked the gold bead were included in the analyses. All training and testing procedures were carried out blind as to the treatment the chick received. The performance of saline-injected and LiCl-injected animals was compared using chi-square tests of independence.

Experiment 1: Dose Response Function of Lithium Chloride

This experiment was designed to determine the optimum doses of LiCl. Chicks were pretrained and trained as described above. One-half hour after training the chicks were given 0.1-ml IP injections of either saline solution or varying doses of LiCl (0.1, 0.5, or 1.0 M). In pilot studies it was determined that higher doses than 1.0 M of LiCl produced behavioral side effects that limited the behavior of the animals at test, such as swaying back and forth, inability to peck the bead when attempting to, and excessive diarrhea. Four hours after training the chicks were tested by sequential presentations of the dry chrome and gold beads.

As can be seen in the results presented in Fig. 1, control saline solution injected 30 min after training did not produce avoidance of the test bead; high numbers of animals pecked the bead again at test (100%). The low dose of LiCl (0.1 M LiCl) did not produce avoidance at test (75% pecked), presumably because it was not strong enough to make the animals ill enough to produce a conditioned aversion. The two higher doses of LiCl (0.5 M and 1.0 M LiCl) both produced significant avoidance of the test bead. Sixty-five percent of the birds injected with 0.5 M LiCl and 20% of the birds injected with 1.0 M LiCl pecked the test bead ($\chi^2 = 5.73$, saline compared to 0.5 M LiCl, $p < 0.05$, $\chi^2 = 15.94$, saline compared to 1.0 M LiCl, $p < 0.01$). The 1.0 M dose of LiCl produced significantly greater avoidance than did the 0.5 M dose ($\chi^2 = 5.04$,

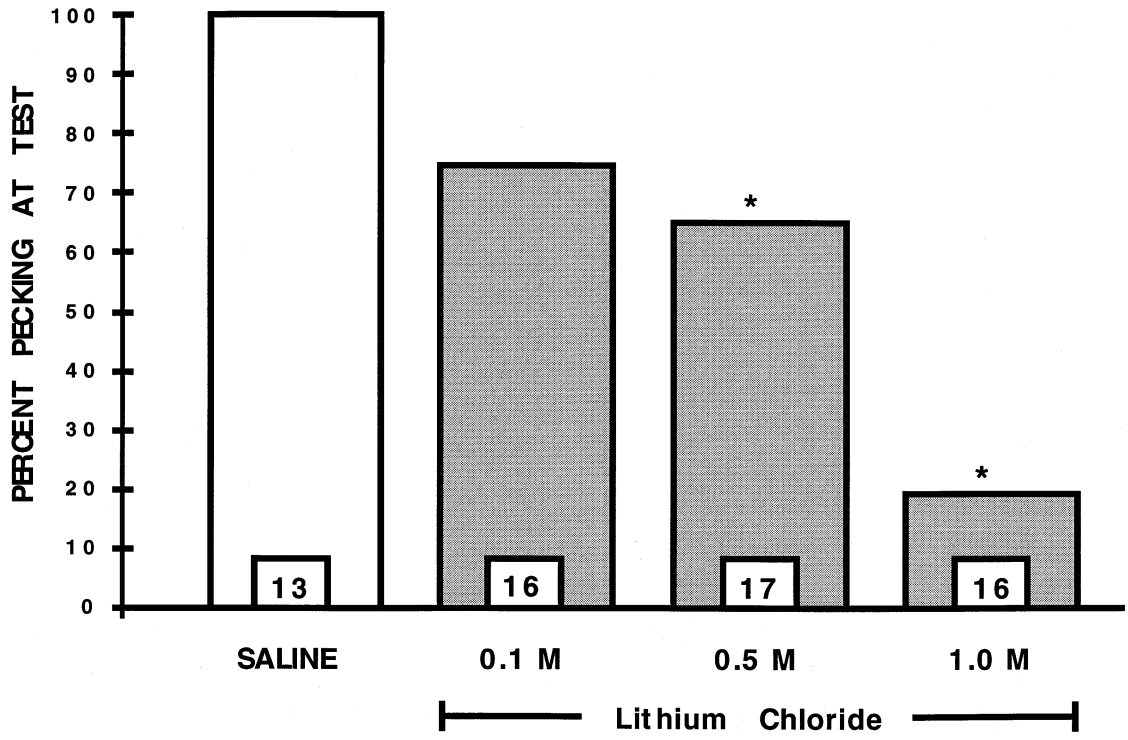

FIG. 1. Effects of different doses of lithium chloride (LiCl) on percent pecking of beads at test. *$p < 0.05$, saline-injected group compared to LiCl-injected group. *n* for each group is shown at the bottom of each histogram.

$p < 0.05$), without any of the behavioral side effects found with higher doses. The 1.0 M dose of LiCl produced diarrhea within 15 min of injection and made the chicks noticeably "sick" (lethargy with eyes closed) for about 1.5 h. This dose of LiCl is the same dose used by Barber et al. (5). Although 0.5 M LiCl produced some avoidance at test, the current results indicate that 1.0 M LiCl should be used to produce consistent avoidance of the training bead.

The results demonstrate that chicks can form and maintain a representation of the training stimulus for at least 30 min before an association with sickness is made. Barber et al. (unpublished experiments) reported that the injection of LiCl could be delayed up to 60 min posttraining, but the aversion produced was less than that found with the 30-min posttraining injection (5). Experiment 2 examined the optimum period of delay between training and LiCl injection in greater detail.

Experiment 2: Time Windows of Effectiveness for Lithium Chloride Injection

In sickness-conditioned learning there is a significantly longer conditioned stimulus-unconditioned stimulus interval compared to more traditional forms of learning such as passive or active avoidance (15). It was hypothesized that the strength of the association between pecking the bead and sickness produced by LiCl would vary based on the time between the two events. This experiment examined learning following different times between presentation of the training bead and LiCl injection. Groups of chicks were given 0.1-ml IP injections of either 0.9% saline or 1.0 M LiCl 5, 10, 15, 20, 30, 45, 60, or 120 min after pecking the training bead. Chicks were tested 4 h after training as described above.

Chicks injected with saline at any time point after training pecked the bead again at test (see Fig. 2). Chicks injected with LiCl 5 or 10 min after training also pecked the bead at test and there were no significant differences between saline-injected and LiCl-injected chicks at these time points. In contrast, chicks injected with LiCl 15, 20, 30, or 45 min after training avoided the bead at test (15 min, 44% pecked, $\chi^2 = 5.04$; 20 min, 31% pecked, $\chi^2 = 6.75$; 30 min, 21% pecked, $\chi^2 = 13.27$; 45 min, 18% pecked, $\chi^2 = 12.44$; all significant at $p < 0.05$). By 60 min posttraining, the LiCl was no longer effective in producing an aversion, and there were no significant differences between chicks injected with saline or LiCl at the 60 or 120 min time points.

The results of Experiment 2 demonstrate that the aversion to pecking the bead can be produced even with a relatively long interval between pecking and injection of LiCl. Chicks can acquire an aversion to green sucrose solution if they are injected immediately following a 1-h exposure to the stimulus (16) and can form aversions to colored food if injected with LiCl immediately following a 15-min feeding session (19). However, in these studies, chicks were exposed to the stimulus for a considerable time, and it cannot be precisely deter-

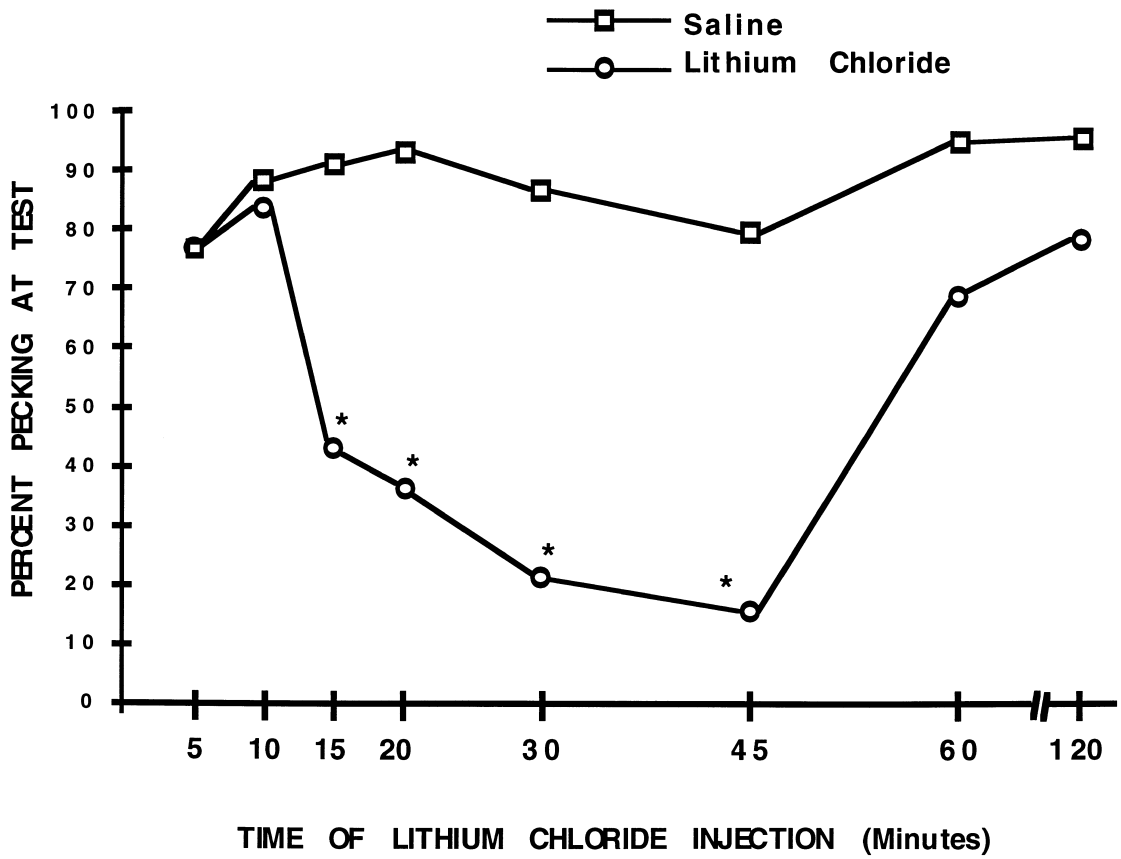

FIG. 2. Effects of different times of injection of lithium chloride (LiCl) on percent pecking of beads presented 4 h after training. *$p < 0.05$; saline group compared to LiCl group at each time point. *n* for each group ranged from 9 to 18 animals per group.

mined when learning about the visual characteristics of the colored and flavored solution occurred. The use of the term "immediately after training" is misleading, because training (that is, learning about the visual characteristics of the training stimulus) could have occurred much earlier and the delay between training and injection much longer than that indicated in these earlier studies. The present experiment, through the use of a well-defined, discrete training event lasting 30 s, allows us to determine, with greater precision, that injections of LiCl given earlier than 15 min after training are not effective in producing a conditioned aversion to the training bead.

Experiment 2 also demonstrates that memory for the conditioned aversion is not produced when LiCl is injected more than 45 min after training. Chicks can form aversions to the color of food when injected up to 4 h posttraining and aversions to the texture of food when injected up to 7 h posttraining (19). These are both relatively long time periods compared to the present experiment. However, given the differences in the training trial duration (15 min vs. 30 s) and the fact that in earlier studies the chicks actually ingested the training stimulus, it is not surprising that conditioned aversions could be produced with such long delays.

Experiment 3: Time Course of Memory for Sickness-Conditioned Learning

Training for passive avoidance is brief, yet the memory has a long duration, lasting up to 9 days (11). This allows researchers to examine memory formation processes that occur minutes or days after training. Previous studies of sickness-conditioned learning in chicks showed that memory for this task is present 24–48 h after training; however, the duration of the memory has not been measured (16,19). Experiment 3 determined the duration of memory for the current sickness-conditioned learning task. Chicks pecked the chrome bead and 30 min later were injected with either 0.9% saline or 1.0 M LiCl (0.1 ml). Groups of chicks were then tested at either 4, 6, 8, 24, or 48 h after training.

As can be seen in Fig. 3, the saline-injected chicks pecked the bead again at test, regardless of the time tested. The chicks injected with LiCl, however, showed a distinct time course in avoidance of the bead. The LiCl-injected chicks showed strong avoidance at 4 h (23% pecked, $\chi^2 = 28.67$, $p < 0.01$) and 6 h (35% pecked, $\chi^2 = 18.53$, $p < 0.01$). The avoidance was still present in LiCl-injected chicks 8 and 24 h after training, although it was considerably smaller (8 h, 72% pecked, $\chi^2 = 9.25$; 24 h, 73% pecked, $\chi^2 = 7.05$; both $p < 0.01$). When tested 48 h after training, the LiCl-injected chicks pecked the bead again and were not significantly different from the saline-injected control animals.

The results of Experiment 3 indicate that memory for the conditioned aversion remains strong for 6 h, weakens considerably between 6 to 24 h, and then is absent 2 days after training. It is not surprising that chicks fail to retain the task as long as they do in other types of sickness-conditioned learning tasks. This is most likely due to the fact that in the present task, chicks peck a dry bead at training rather than eating a novel colored or flavored substance. An aversion can be maintained over a 5-day period if chicks are given a highly novel food (19). The memory for pecking the dry bead followed by sickness might be highly susceptible to interference

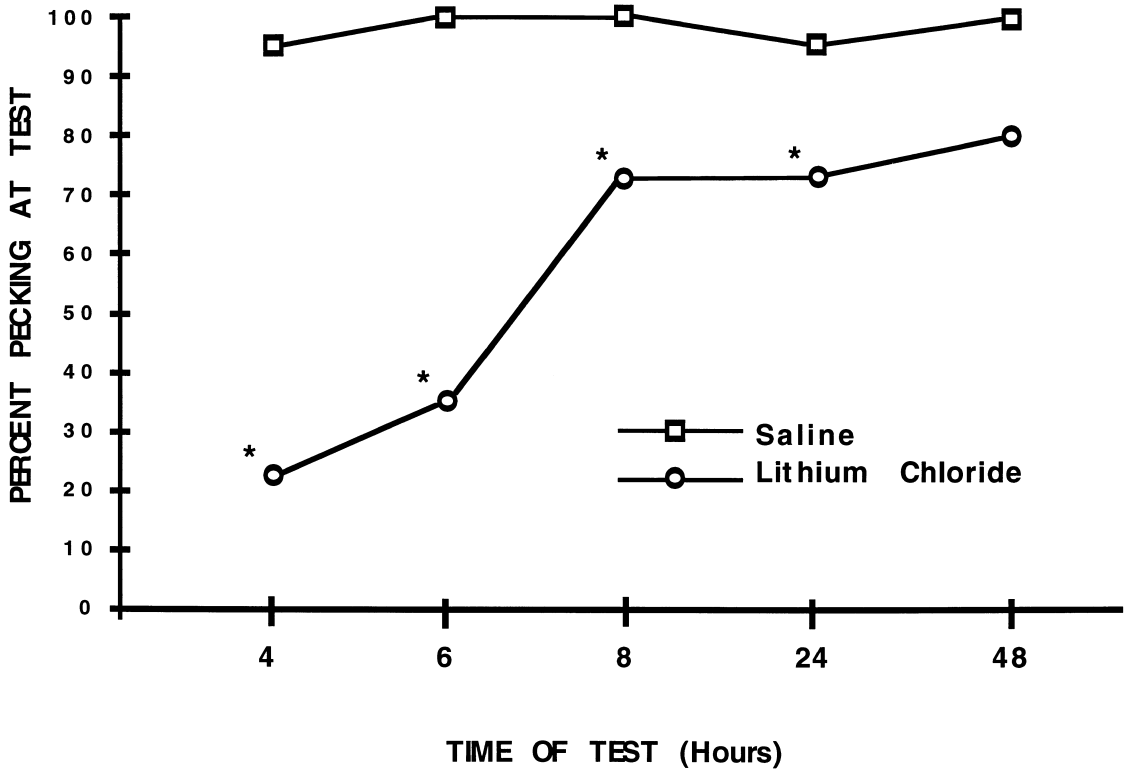

FIG. 3. Time course of memory formation for sickness-conditioned learning. *$p < 0.05$; saline group compared to LiCl group at each time point. *n* for each group ranged from 14 to 36 animals per group.

or retrieval cues less salient after 24 h. It is also probable that higher doses of LiCl would lead to a more lasting memory in the current task; however, higher doses of LiCl produce long-lasting behavioral side effects that make testing unreliable.

We can conclude that the memory for sickness-conditioned learning in the present study produces a truly long-term memory of the training bead. In passive avoidance, 4 to 8 h after training is considered long-term memory and many of the biochemical, electrophysiological, and anatomical studies of training-induced changes have been conducted using these time points.

GENERAL DISCUSSION

These data demonstrate that significant avoidance of a particular bead can be produced by pairing pecking of that bead with sickness produced by LiCl injection. The sickness produced by LiCl injection can be given between 15 and 45 min after the chicks peck the training bead, and memory for the task is strong for several hours after training, gradually diminishing into amnesia by 48 h after training.

This sickness-conditioned learning task has several intriguing qualities that make it an appealing addition to the body of learning tasks used to study learning and memory in day-old chicks. First, it is clear that the association between sickness and the bead is based only on the visual characteristics of the training bead because the chicks do not ingest anything during training. It is possible that chicks receive some information from tactile senses when pecking the bead at training, but the clear avoidance of the bead at test (rather than pecking once or twice to confirm the tactile characteristics) indicates that this is primarily a visual association task. Thus, this task shares some of the features of other visual discrimination tasks (3,14,37).

It has been suggested that the passive avoidance paradigm is advantageous over the imprinting task because the brief training trial separates the biochemical processes of training from those of learning, allowing researchers to examine memory formation that occurs minutes after training (28). The sickness-conditioned learning task produces an even greater separation than that found for passive avoidance; this should provide a good opportunity for researchers to distinguish memory formation for the training stimulus (how and where in the brain the visual aspects of the bead are stored) from the association with LiCl.

Sickness-conditioned learning separates out the training experience from the stressful effects of the UCS (in this case the LiCl injection) because they can be separated from each other by 15 min or more. The training stimulus is no longer present when memory formation for the association of pecking the bead and sickness occurs. This is an important difference between passive avoidance and sickness-conditioned learning. Researchers using the passive avoidance task have had to rule out the possibility that concomitant CNS changes found after learning are due to stress encountered during training. One procedure used to accomplish this is to train the chicks on passive avoidance and then give brief subconvulsive electroshock 5 min after training, which produces amnesia in some of the chicks. Animals that are amnesic do not show training-induced changes in glycoprotein synthesis, electrophysiology, or morphology (21,23,30). However, this procedure must produce some changes in the brain, and care must be taken to show that subconvulsive electroshock itself does not produce detectable differences between shocked and unshocked animals. The sickness-conditioned learning tasks offers a much simpler paradigm. Chicks can be injected with LiCl 5 min after pecking the bead or they can be injected 20 min after pecking the bead. Both groups undergo exactly the same procedures; however, one group will form an aversion (the 20-min group), and the other group will not (the 5-min group). It is predicted that training-induced changes found in the brains of animals trained with the 20-min delay between training and injection would not be present in those animals trained with the 5-min delay.

It is clear that the chicks are able to distinguish the chrome bead from the gold bead because they consistently avoid one and peck the other if they are given LiCl after pecking the chrome bead. However, at the time the chick pecked the chrome bead, there were no immediate consequences to pecking. Therefore, the chick must make some type of representation of the training bead, at the time of training, to allow discrimination at the time of test. Whether the chicks learn more about the bead beyond its color is not yet known, and we are currently examining whether chicks can distinguish shape and size characteristics of beads using the sickness-conditioned learning task. However, the finding that chicks learn a great deal about beads that they peck, regardless of the consequences of pecking, raises some questions concerning the interpretation of data in studies using chicks that peck water-coated beads as a control group. The results of the current experiments would suggest that this control group also forms a representation of a control bead for at least 45 min. This finding would account for the changes in c-*fos* expression found following control procedures in passive avoidance (4). The sickness-conditioned learning task does not utilize these types of control procedures. Using the sickness-conditioned learning task should provide all the necessary controls for accurate determination of training-induced biochemical, electrophysiological, and anatomical correlates of memory formation.

Although the results of Experiment 3 suggest that the memory for sickness-conditioned learning is brief, substantial evidence exists to support the hypothesis that long-term memory is formed in this task. The chicks are capable of showing clear aversion to the training bead for 8 h after training. In both passive avoidance and imprinting tasks, processes occurring in the brain at this time period would be those considered to underlie long-term memory formation (1,6,25,28). Therefore, biochemical and electrophysiological processes of long-term memory formation could be accurately measured in the sickness-conditioned learning task.

Given the robust avoidance 4 to 6 h after training, the sickness-conditioned learning task seems well suited for pharmacological investigation of memory formation. The separation of CS (conditioned stimulus, in this case pecking the bead) and US (unconditioned stimulus, in this case the sickness produced by LiCl injection) in this task allows researchers to determine the effect of agents on the processes involved with the initial representation of the bead. For example, learning is impaired by 2-deoxygalactose, an inhibitor of glycoprotein synthesis, when injected near the time of training (5). Furthermore, unpublished experiments in our laboratory show that scopolamine [a muscarinic receptor antagonist known for its amnestic effects in a variety of tasks (18,27)] produces amnesia when injected before, but not after, presentation of the training bead, indicating that the cholinergic system is required for this short-term nonassociative memory.

Last, the comparatively brief duration of long-term memory found in the sickness-conditioned learning task makes this an ideal model to study memory enhancement, in addition to memory impairment. In the sickness-conditioned learning

task, memory can be tested 48 h after training for enhancement, a paradigm with a brief enough test point to rapidly gather data. This means that there needs to be no special modification to the training paradigm to adequately measure any enhancement of memory. We are currently examining agents that improve memory on retention of sickness-conditioned learning.

In conclusion, the sickness-conditioned learning paradigm produces robust learning with a brief training trial. The characteristics of the training protocol suggest that memory for this task is similar to other forms of visual discrimination tasks. The parameters of this task suggest the interesting possibility that associative memory formation can be delayed, and further that a nonassociative visual memory is suppressed (or at least uncoupled from potential associations) for a short (5–10 min) period of time. This memory is then recovered or made available to delayed association for a 30-min period, followed by either erasure or decay. We suggest that the biochemistry of memory formation be investigated using the sickness-conditioned learning task. We have used this task to examine the effects of forebrain lesions on learning and retention and have begun to examine the pharmacology of memory impairment and enhancement. The results obtained thus far suggest that using this task will provide a promising avenue of research.

ACKNOWLEDGEMENTS

Teresa A. Barber has previously published as Teresa A. Patterson. These experiments were carried out with the support of student/faculty research funds from Dickinson College, the Whittaker Foundation, and the Charles A. Dana Foundation.

REFERENCES

1. Ali, S. M.; Bullock, S.; Rose, S. P. R.: Phosphorylation of synaptic proteins in chick forebrain: Changes with development and passive avoidance training. J. Neurochem. 50:1579–1587; 1988.
2. Andrew, R. J.; Brennan, A.: Sharply timed and lateralised events at time of establishment of long-term memory. Physiol. Behav. 34:547–556; 1985.
3. Andrew, R. J.; Rogers, L. J.: Testosterone, search behavior and persistence. Nature 237:343–346; 1972.
4. Anokhin, K.; Mileusnic, R.; Shamakina, I.; Rose, S. P. R.: Effects of early experience on c-*fos* gene expression in the chick forebrain. Brain Res. 544:101–107; 1991.
5. Barber, A. J.; Gilbert, D. B.; Rose, S. P. R.: Glycoprotein synthesis is necessary for memory in sickness-induced learning in chicks. Eur. J. Neurosci. 1:673–677; 1989.
6. Barber, T. A.; Howorth, P. D.; Klunk, A. M.; Cho, C. C.: Localization of memory for sickness-conditioned learning in day-old chicks. (submitted).
7. Barber, T. A.; Pearlman, M. F.; Patrick, K. E.: Sickness-conditioned learning in day-old chicks: Memory formation depends on time of lithium chloride injection. Soc. Neurosci. Abstr. 21:1451; 1995.
8. Bateson, P. P. G.: The characteristics and context of imprinting. Biol. Rev. 41:177–220; 1966.
9. Bourne, R. C.; Davies, D. C.; Stewart, M. G.; Csillag, A.; Cooper, C.: Cerebral glycoprotein synthesis and long-term memory formation in the chick (*Gallus domesticus*) following passive avoidance training depends on the nature of the aversive stimulus. Eur. J. Neurosci. 3:243–248; 1991.
10. Bullock, S.; Potter, J.; Rose, S. P. R.: Identification of chick brain glycoproteins showing changed fucosylation rates after passive avoidance training. J. Neurochem. 54:135–142; 1990.
11. Cherkin, A.: Retrograde amnesia: Impaired memory consolidation or impaired retrieval? Commun. Behav. Biol. 5:183–190; 1970.
12. Cherkin, A.; Lee-Teng, E.: Interruption by halothane of memory consolidation in chicks. Fed. Proc. 24:328; 1965.
13. Chromiak, W.; Barber, T. A.; Kyler, K.: When memories are too new: Selective associations in day-old chicks. (submitted).
14. Deyo, R. A.; Nix, D. A.; Parker, T. W.: Nifedipine blocks retention of a visual discrimination task in chicks. Behav. Neural Biol. 57:260–262; 1992.
15. Garcia, J.; Ervin, F. R.; Koelling, R. A.: Learning with prolonged delay of reinforcement. Psychol. Sci. 5:121–122; 1966.
16. Gaston, K. E.: An illness-induced conditioned aversion in domestic chicks: One-trial learning with a long-delay of reinforcement. Behav. Biol. 20:441–453; 1977.
17. Gibbs, M. E.; Ng, K. T.: Psychobiology of memory: Towards a model of memory formation. Biobehav. Rev. 1:113–136; 1977.
18. Levin, E. D.; McGurk, S. R.; Rose, J. E.; Butcher, L. L.: Cholinergic–dopaminergic interactions in cognitive performance. Behav. Neural Biol. 54:271–299; 1990.
19. Martin, G. M.; Bellingham, W. P.: Learning of visual food aversions by chickens (*Gallus gallus*) over long delays. Behav. Neural Biol. 25:58–68; 1979.
20. Mason, R. J.; Rose, S. P. R.: Lasting changes in spontaneous multi-unit activity in the chick brain following passive avoidance training. Neuroscience 21:931–941; 1987.
21. Mason, R. J.; Rose, S. P. R.: Passive avoidance learning produces focal elevation of bursting activity in the chick brain: Amnesia abolishes the increase. Behav. Neural Biol. 49:280–292; 1988.
22. Mattingly, B. A.; Zolman, J. F.: Resistance to extinction in the developing chick: Effects of punishment and preextinction training. Bull. Psychol. Soc. 20:317–320; 1982.
23. Patel, S. N.; Rose, S. P. R.; Stewart, M. G.: Training induced dendritic spine density changes are specifically related to memory formation processes in the chick, *Gallus domesticus.* Brain Res. 463:168–173; 1988.
24. Patel, S. N.; Stewart, M. G.: Changes in the number and structure of dendritic spines, 25 h after passive avoidance training in the domestic chick, *Gallus domesticus.* Brain Res. 449:34–46; 1988.
25. Patterson, T. A.; Alvarado, M. C.; Warner, I. T.; Bennett, E. L.; Rosenzweig, M. R.: Memory stages and brain asymmetry in chick learning. Behav. Neurosci. 100:856–865; 1986.
26. Patterson, T. A.; Gilbert, D. B.; Rose, S. P. R.: Pre- and posttraining lesions of the intermediate medial hyperstriatum ventrale and passive avoidance learning in the chick. Exp. Brain Res. 80:189–195; 1990.
27. Patterson, T. A.; Lipton, J.; Bennett, E. L.; Rosenzweig, M. R.: Disruption of muscarinic cholinergic receptor activity impairs formation of intermediate-term memory in the chick. Behav. Neural Biol. 54:63–74; 1990.
28. Rose, S. P. R.: Biochemical mechanisms involved in memory formation in the chick. In: Andrew, R. J., ed. Neural and behavioral plasticity: The use of the domestic chick as a model. Oxford: Oxford University Press; 1991:277–304.
29. Rose, S. P. R.: How chicks make memories: The cellular cascade from c-*fos* to dendritic remodeling. Trends Neurosci. 14:390–397; 1991.
30. Rose, S. P. R.; Harding, S.: Training increases (^{3}H) fucose incorporation in chick brain only if followed by memory storage. Neuroscience 12:663–667; 1984.
31. Rose, S. P. R.; Jork, R.: Long-term memory formation in chicks is blocked by 2-deoxygalactose, a fucose analogue. Behav. Neural Biol. 48:246–258; 1987.
32. Rosenzweig, M. R.; Lee, D. W.; Mean, M. K.; Bennett, E. L.; Martinez, J. L., Jr.: Effects of varying training strength on short-, intermediate-, and long-term formation of memory for a one-trial passive avoidance task in chicks. Soc. Neurosci. Abstr. 15:1171; 1989.
33. Sandi, C.; Rose, S. P. R.: Corticosterone enhances long-term retention in one-day old chicks trained in a weak passive avoidance learning paradigm. Brain Res. 647:106–112; 1994.
34. Steele, R. J.; Dermon, C. R.; Stewart, M. G.: D-Cycloserine causes

transient enhancement of memory for a weak aversive stimulus in day-old chicks (*Gallus domesticus*). Neurobiol. Learn. Mem. 66: 236–240; 1996.
35. Stewart, M. G.; Bourne, R. C.; Steele, R. J.: Quantitative autoradiographic demonstration of changes in binding to NMDA-sensitive [^{3}H]Glutamate and [^{3}H]MK801, but not [^{3}H] AMPA receptors in chick forebrain 30 minutes after passive avoidance training. Eur. J. Neurosci. 4:936–943; 1992.
36. Stewart, M. G.; Rose, S. P. R.; King, T. S.; Gabbott, P. L. A.; Bourne, R.: Hemispheric asymmetry of synapses in chick medial hyperstriatum ventrale following passive avoidance training: A stereological investigation. Dev. Brain Res. 12:261–269; 1984.
37. Tiunova, A.; Anokhin, K.; Rose, S. P. R.; Mileusnic, R.: Involvement of glutamate receptors, protein kinases, and protein synthesis in memory for visual discrimination in the young chick. Neurobiol. Learn. Mem. 65:233–243; 1996.
38. Vallortigaro, G.; Zanforlin, M.: Position learning in chicks. Behav. Proc. 12:23–32; 1986.