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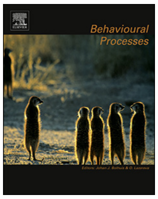

Time course of memory formation for an appetitive, one-trial, water-reward task in day-old chicks

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ABSTRACT

Researchers studying learning and memory in the day-old chick have developed a one-trial taste avoidance learning task, in which a chick pecks a bead coated with an aversive-tasting liquid such as methylanthranilate. The current study examined, in two experiments, whether chicks could demonstrate learning of an appetitive version of the one-trial task, and how long this memory might last. The results demonstrate that chicks show significantly decreased latencies to peck a test bead similar to that pecked at training coated with water, and that this memory lasted for at least 6 h after training. These results demonstrate that this appetitive task produces significant learning.

1. Introduction

Researchers in learning and memory have capitalized on the ability of newly hatched chicks to quickly learn the avoidance of aversive stimuli (Morgan, 1896) to produce the one-trial taste avoidance learning task, in which a chick pecks a bead coated with an aversive-tasting liquid such as methylanthranilate (MeA). The chick associates the bead with the bad taste and will, at test, avoid pecking a bead similar to that presented at training (Cherkin and Lee-Teng, 1965). This one-trial task is especially appropriate for the study of learning and memory because the training trial, a brief and discrete event, occurs quickly, taking on average 30 s in most studies (Rose, 2000, 2004; Gibbs et al., 2008).

Significant alterations in biochemistry, morphology and physiology are found in the chick forebrain following training on taste avoidance. For example, training results in increased glutamate release, an upregulation of NMDA-sensitive glutamate receptors, expression of immediate early genes c-fos and c-jun and increased synthesis of glycoproteins (Anokhin et al., 1991; Burchuladze et al., 1990; see Rose, 2004). These biochemical changes presumably lead to the increases in spontaneous multi-unit bursting activity and increased spine growth found in the hours after training (Mason and Rose, 1988; Patel et al., 1988). These many learning-induced changes have been localized to the IMM, in the anterior part of the forebrain roof in the avian brain (see Rose, 2004). The IMM has been described as a visual association area since it receives input from several sensory areas of the chick forebrain and visual connections are indirect (Bradley et al., 1985). In addition, several lesion studies have shown that the IMM is critical to learning and memory in this task (Davies et al., 1988; Patterson et al., 1990).

These many changes in the chick forebrain have been found by comparing changes in biochemistry, physiology, and morphology in trained animals to those that have not learned, and the control for these studies are most always animals that peck a bead that has water on it during training. Typically, learning is not measured in these control situations; the chick merely pecks the bead again during test as an indication of “no change” in its behavior. The changes that might occur in these control animals are considered changes found in motor behavior involved in pecking the training bead and general responses to the environmental stimulation of the training experience.

While this control procedure has been considered one in which the chick does not learn, it might also be considered an appetitive version of the one-trial taste avoidance learning task. Chicks are capable of learning many appetitive tasks. For example, newly hatched precocial chicks form a social attachment (imprint) to an object following exposure (see Bateson, 2015). Chicks can also learn a simultaneous learning (place and object) paradigm with a food reward (Vallortigara and Zanforlin, 1988; Vallortigara et al., 1990) and a color discrimination water reward task (Ogura et al., 2015). Chicks can learn color categorization using food reward (Jones et al., 2001). In addition, chicks displayed appetitive behavior in a behavioral spatial study (Seehuus et al., 2012).

In all of these paradigms, multiple trials were employed. Can chicks learn a single-trial appetitive task? The present experiments examined learning of a one-trial water-reward learning task in day-old chicks, using a fully counterbalanced design. It was hypothesized that if no learning occurs when chicks peck the water bead at training, then no

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change in behavior should occur during testing. To sufficiently measure change in behavior, the latency to peck the bead (during both training and testing) was measured.

2. Methods

2.1. Animals

Male leghorn-derived chicks were purchased from a local supplier (Hy-Line International, Elizabethtown, PA) and arrived at 8 a.m. the day after hatching. The chicks were placed in pairs in white opaque Plexiglas pens (22.8 cm x 22.8 cm x 22.8 cm) in the behavioral testing room, which was maintained on a 12 h light/dark cycle (lights on at 8 a.m.) at 38.5–40.5 °C and 45–51% humidity. The Plexiglas pens, which were open at the top and 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. Chick starter meal was scattered on the floor of each pen. The chicks were allowed to acclimate to the behavioral testing room for 1.5 h before pretraining.

Both chicks in each pen were assigned to the same experimental conditions and were trained and tested together in the pen. There may be some social facilitation in pecking behavior between the two birds, however, studies in our laboratory show that these minor influences far outweigh the isolation stress produced when chicks are housed singly (DeVaus et al., 1980). Examination of the results indicates that there were no significant effects due to social facilitation in any of the experiments reported here. A total of 294 chicks were used in these experiments. Chicks that did not peck at least once during pre-training or at training were eliminated from the study, and elimination rates were between 5% and 8% for each group (total N = 22). The Dickinson College Animal Care and Use Committee approved these experiments.

2.2. Training and testing procedure

2.2.1. Experiment 1

Chicks were trained in one of eight training paradigms. In all conditions, the chicks were pretrained with a dry 2-mm chrome bead to initiate pecking behavior. Ten minutes later, groups of chicks were given a 30 s presentation of either a 3 mm white bead coated with water (N = 49) or left dry (N = 46), or a 3 mm red bead coated with water (N = 46) or left dry (N = 46). The latency to peck was recorded for each chick. At test, four hours after training, the chicks were given two 30 s trials in which they were either presented first with a 3 mm red or 3 mm white bead followed by a 3 mm red or 3 mm white bead. This procedure divided the four groups into eight groups, one group trained on a wet white bead, then tested first on a white bead, then a red bead (WWR Wet, N = 25), one group trained on a dry white bead, then tested first on a white bead, then a red bead (WWR Dry, N = 24), one group trained on a wet white bead, then tested first on a red bead, then a white bead (WRW Wet, N = 24), one group trained on a dry white bead, then tested first on a red bead, then a white bead (WRW Dry, N = 22), one group trained on a wet red bead, then tested first on a red bead, then a white bead (RRW Wet, N = 24), one group trained on a dry red bead, then tested first on a red bead, then a white bead (RRW Dry, N = 23), one group trained on a wet red bead, then tested first on a white bead, then a red bead (RWR Wet, N = 22), and one group trained on a dry red bead, then tested first on a white bead, then a red bead (RWR Dry, N = 23). Again, the latency for each chick to peck the beads was recorded. The test results of the pecking behavior towards the test bead were compared using a Wilcoxin Signed Rank Test.

2.2.2. Experiment 2

The goal of Experiment 2 was to determine the time course of memory in the water reward task, using the training paradigm that produced the best discrimination at four hours after training. Here, the chicks were again pretrained with a 2-mm chrome bead to initiate pecking behavior. Ten minutes later, the chicks were given a 30 s presentation of a 3 mm white bead either coated with water or left dry. The latency to peck was recorded for each chick. Groups of chicks were tested at six, eight, and 24 h after training. At test, chicks were given two 30 s trials in which they were presented first with a novel 3 mm red bead followed by a 3 mm white bead similar to that used at training. Both beads were dry at test. Again, the latency for each chick to peck the beads was recorded. The test results of the pecking behavior towards the white bead were compared using a Wilcoxin Signed Rank Test.

Ten minutes later, the chicks were given a 30 s presentation of a 3-mm white bead coated with water. The latency to peck was recorded for each chick. At test, chicks were given two 30 s trials in which they were presented first with a novel 3-mm red bead followed by a 3-mm white bead similar to that used at training. Both beads were dry at test. Again, the latency for each chick to peck the beads was recorded. Groups of chicks were tested at six hours (wet bead, N = 26; dry bead, N = 12), eight hours (wet bead, N = 22; dry bead, N = 14), or 24 h (wet bead, N = 19; dry bead, N = 14) after training. The test results of the pecking behavior towards the white bead were compared using a Wilcoxin Signed Rank Test.

3. Results

3.1. Experiment 1

As Fig. 1 shows, chicks responded to the beads differently depending on which bead was presented first at test. It is important to note that all chicks pecked the beads at test. Chicks tested first with the same color bead as pecked in training (either on wet or dry white or red beads), did not show any significant changes in behavior towards the test bead. However, chicks tested first with the opposite color seen at training showed different responses depending on whether the bead was wet or dry. Chicks trained on the wet white bead, then tested first with the dry red bead, then the dry white bead, showed a significant decrease in the latency to peck the white bead (W, 23df = 66.0, p = 0.017). Chicks trained with the wet red bead, then tested with the white bead first (and red bead second) also showed a significant reduction in latency to peck the red bead (W, 21df = 45.0, p = 0.008).

3.2. Experiment 2

The results of Experiment 2 are presented in Fig. 2. Again, it is important to note that all chicks pecked the testing beads. When chicks were tested six hours after training, the chicks responded similarly to that seen in Experiment 1. That is, chicks trained with the wet white bead at training, and tested with red bead first, then the white bead, showed significant decreases in latency to peck the white bead (W, 25df = 90.0, p = 0.044). The chicks also showed a significant increase in latency to peck the red bead at test (W, 25df = 87.0, p = 0.024). Chicks tested eight hours or 24 h after training did not show these differences.

4. Discussion

The results of Experiment 1 indicate that chicks significantly change their behavior towards a bead dipped in water during training, such that they peck that same colored bead faster during test four hours after training. The results of Experiment 2 suggest that these changes occur for at least six hours after training but are no longer present by eight hours after training. These results clearly demonstrate that learning occurs during the one-trial, water-reward task. The learning is strong for several hours after training but diminishes rapidly. This appetitive learning task complements the learning found in the one-trial taste avoidance task.

Memory for the water reward task closely resembles the weak

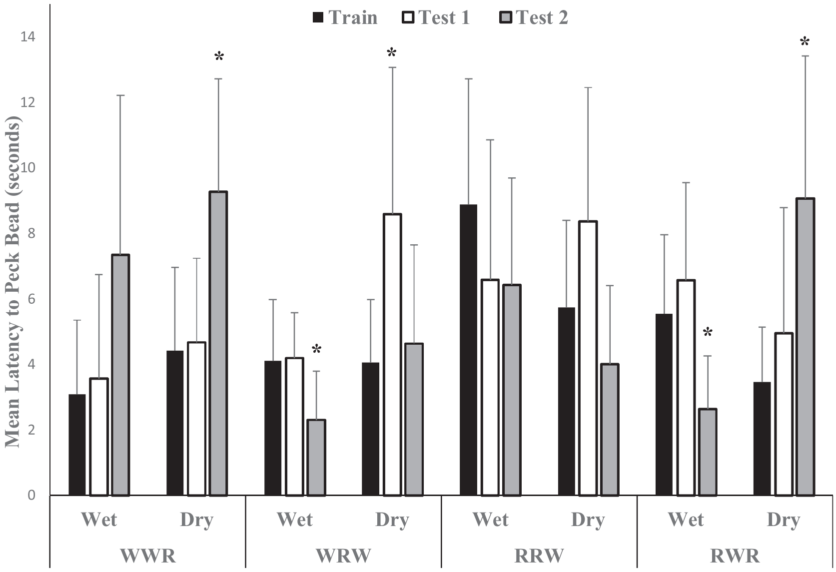

Fig. 1. Percent avoidance at test (4 h after training) of chicks trained to peck a 3 mm red or white bead coated with water and tested with 3 mm red and white beads in a fully counter-balanced order of presentation.

Median latency to peck the training and test beads in chicks trained with either wet or dry red or white beads and tested 4 h after training with eight different presentations of white or red beads (Wet White, White, Red, (Wet WWR), N = 25, Dry White, White, Red, (Dry WWR), N = 24, Wet White, Red, White, (Wet WRW), N = 24, Dry White, Red, White, (Dry WRW), N = 22, Wet Red, Red, White, (Wet RRW), N = 24, Dry Red, Red, White, (Dry RRW), N = 23, Wet Red, White, Red, (Wet RWR), N = 22, or Dry Red, White, Red, (Dry RWR, N = 23). *, $p < 0.05$, latency to peck the test bead compared to latency to peck the training bead.

version of the one-trial taste avoidance task, in which chicks trained with a weaker aversive stimulus (less MeA diluted in ethanol do not form long-term memory (Crowe et al., 1989; Sandi and Rose, 1994). Differences in retention appear to arise primarily from the differences in pre-training and training methods (Crowe and Hamalainen, 2001), but memory found using diluted MeA longer than 9 h has not been observed.

It is apparent that the chick is capable of learning both aversive and appetitive one-trial tasks. The ability to learn these tasks involves discrimination of the training bead along a variety of stimulus dimensions, such as size, shape, color and odor. Color is an important stimulus cue for the taste avoidance learning task (Patterson and Rose, 1992). Possible classificatory cues besides color include the size and shape of the stimulus object. Chicks can discriminate beads of different sizes, but not different shapes, when trained in the taste avoidance task (Barber, 2016). Chicks are able to categorize objects according to color, shape and position, and show a preference for pecking round objects over angular objects (Fantz, 1957; Vallortigara et al., 1990). Salzen and Meyer (1968) demonstrated that chicks have an innate preference for round compared to square objects and this preference leads to more easily and strongly learned imprinting with the round objects. It would be interesting to determine the role of these other stimulus dimensions in the water-reward task.

Significant discrimination occurred in the present study when the

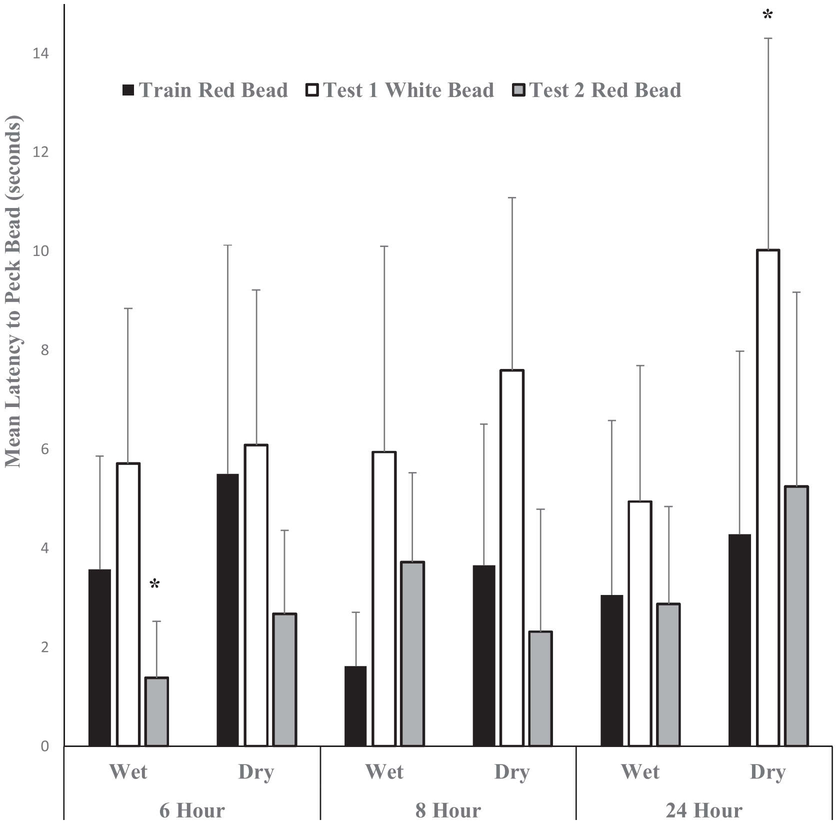

Fig. 2. Percent avoidance at test (6, 8, or 24 h after training) of chicks trained to peck a 3 mm red bead coated with water and tested with 3 mm white bead followed by a red bead.

Median latency to peck the training and test beads in chicks tested 6, 8, or 24 h after training with red training bead, presented with white, then red bead at test, (6 h, N = 26, 8 h, N = 16, 24 h, N = 19). *, $p < 0.05$, latency to peck the test bead compared to latency to peck the training bead.

novel bead was presented first at test. These same effects are found with color discriminations in both the taste avoidance and sickness-conditioned learning tasks (Patterson and Rose, 1992; Barber et al., 1998). Presentation of the aversive bead first at test seems to potentiate neophobia in the chicks. Poison-enhanced neophobia has been demonstrated with sickness-conditioned learning in rodents (see Domjan, 1977 for review). Ionescu and Bures (1976) also found that young chicks display neophobia towards novel objects. Presentation of the novel bead after the aversive bead may accentuate this process. It may be possible to reduce neophobia (and increase discrimination) at test by increasing the interval between presentation of the two test beads. Research with rodents indicates that neophobia is reduced if animals are exposed to the conditioned stimulus without the aversive consequences (Nachman and Jones, 1974).

These results also provide some caution to the interpretation of the results found following one-trial taste avoidance training, especially those found with the first few hours after training (see Rose, 2004). Pecking a bead coated with water, often the control procedure in many of these studies, does lead to learning of some duration, and changes found during these early hours after training could reflect memory primarily associated with the aversive stimulus, rather than general memory processes. Chicks respond strongly to the odor of the MeA used in one-trial taste avoidance training (Marples and Roper, 1997; Richard and Davies, 2000; Roper and Marples, 1997) and it has been argued that many of the learning-dependent changes in brain function might be due to the strong smell of the substance used to coat the aversive bead (Bourne et al., 1991). Learning induced by the water reward task might be located in other areas of the avian brain rather than the IMM. Neuroimaging studies have pointed out that primary (i.e., snacks or drinks) and secondary (i.e., money) reinforcers activate some common brain regions (e.g., striatum) but also entail distinct patterns of activation (Delgado et al., 2011; Levy and Glimcher, 2011). Future studies should determine the areas associated with memory for the water reward task.

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