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T-Maze Learning in Weanling Lambs

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Abstract

A major advantage of sheep models in experimental studies of neurodevelopmental disorders (e.g., with prenatal neurotoxicant exposure) is that the equivalent of all three trimesters of human brain development occurs in sheep entirely *in utero*. However, studies of learning and memory in sheep are limited. The goal of this study was to extend the analysis of spatial learning and memory in adolescent sheep using several traditional T-maze tasks. Both 9- and 14-week-old lambs acquired a delayed non-matching to place task, but the older lambs learned the task significantly faster. In contrast, acquisition of a matching to place task was significantly more difficult. Lambs, like rodents, appear to have a predisposition toward learning “win-shift” spatial problems in a T-maze under appetitive motivation. Lambs also rapidly acquired a position habit and showed typical reversal learning curves. These findings support the use of T-maze tasks to assess behavioral outcomes in various sheep models.

Keywords

sheep; ovine; matching-to-place; non-matching-to-place; position habit

INTRODUCTION

The simplicity of the T-maze apparatus belies its utility and versatility for examining learning and memory in animals. T-maze tasks have been used extensively to investigate various aspects of brain function, including spatial working memory (Zimmerberg, Sukel & Stekler, 1991; Wan, Pang & Olton, 1994; Nagahara & Handa, 1997), long-term reference memory (Olton & Papas, 1979), perseveration (Riley, Lochry, Shapiro & Baldwin, 1979), and foraging strategy such as the use of “win-shift” versus “win-stay” strategies in response to different reinforcement contingencies (Szelest & Cohen, 2006). Much of this work has been done in rodents, but when scaled appropriately, the T-maze can also be suitable for

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non-rodent species as well, including the ferret (Park & Baum, 1999), cat (Burgess, Villablanca, Levine, 1986), squirrel monkey (Peretti & Baird, 1976), horse (Heird, Lennon & Bell, 1981), goat and sheep (Hosoi, Swift, Rittenhouse & Richards, 1995).

The versatility of the T-maze derives from the task variants that can be used, separately or in combination, to address specific behavioral processes and neural systems. For example, spatial delayed alternation vs. position discrimination can be used, respectively, to assess spatial working memory and reference memory (Green & Stanton, 1989). In spatial delayed alternation, working memory capacity can be further characterized by increasing the delay interval, by increasing interference in working memory by varying the number of paired runs within a trial, or by including distracter activities within or between trials or trial components (Petrinovich & Bolles, 1957; Richman, Dember & Kim, 1986; Dudchenko, 2001). Reversal of T-maze position discrimination can also be used to assess “executive function” or “cognitive flexibility” (Erhard, Boissy, Rae & Rhind, 2004; Singer et al., 2009). Performance can also be tested under either appetitive (e.g., food or water reinforced) or aversive (e.g., swim escape or shock avoidance) conditions to assess motivational contributions to performance (Goodlett et al., 1988).

The T-maze preparation can be used to test hypotheses about alterations in brain function in systems that are known to contribute to performance of these various tasks, including the hippocampus (Ordy et al., 1988; Dudchenko, Wood & Eichenbaum, 2000; Clark et al., 2001), entorhinal cortex (Reeves & Smith, 1987; Goodlett et al., 1988), and frontal cortex (Bubser & Schmidt, 1990; de Brabander, de Bruin, & van Eden, 1991; Sánchez-Santed, de Bruin, Heinsbroek & Verwer, 1997). The hippocampal formation appears to encode spatial information regarding the location of a subject within the environment (O’Keefe & Dostrovsky, 1971; O’Keefe & Conway, 1978; O’Keefe, 1976; Wilson & McNaughton, 1993; Thompson & Best, 1990; Bohbot et al., 1998; Hollup et al., 2001a,b), and appears to be essential for the consolidation of long-term declarative memories (Zola-Morgan & Squire, 1990; Kim, Clark & Thompson, 1995; Thompson, Moyer & Disterhoft, 1996; Abel et al., 1997; Anagnostaras, Maren & Fanselow, 1999; Clark, Broadbent, Zola & Squire, 2002; Agnihotri, Hawkins, Kandel & Kentros, 2004), whereas frontal cortex has been implicated in such executive functions as reward evaluation, decision-making, error-correction, and reversal of previously acquired responses (Miller & Cohen, 2001; Fuster, 2008; Miller & Wallis, 2008). Additionally, both of these reciprocally-connected brain regions contribute to spatial working memory (Olton, 1983; Ordy et al., 1988; Wan, Pang & Olton, 1994; Clark et al., 2001).

Rodent models of various neurodevelopmental disorders in which hippocampal or frontal cortical dysfunction has been implicated often have used T-maze studies of spatial learning and memory (e.g., Chapman et al., 1999; Nagahara & Hand, 1997; Thomas et al., 1996; Volpe et al., 1992). The large experimental literature in rodents using T-maze tasks to investigate these brain systems mediating learning and memory and to assess their functional impairments in rodent models of neurodevelopmental disorders provides both a rationale and a comparative basis to pursue T-maze tasks to study spatial learning in sheep.

Sheep are particularly valuable for studies of neurodevelopmental disorders in which there must be a relatively good match between the duration and timing of various stages of brain developmental events (prior to birth) in the animal model compared to humans. For example, the advantages of a sheep model of human fetal alcohol spectrum disorder [FASD] are now well established (e.g., Cudd, Chen, Parnell & West, 2001; Cudd, 2005; Ramadoss, Hogan, Given, West & Cudd, 2006; Ramadoss, Tress, Chen & Cudd, 2008; Littner, Cudd, O’Riordan, Cwik & Bearer, 2008; Ramadoss, et al., 2007a,b). Many of the neurobehavioral deficits seen in clinical populations of FASD are consistent with functional impairment of the hippocampus and frontal cortex, including deficits in executive functioning and spatial working memory (Uecker & Nadel, 1996; Uecker & Nadel, 1998; Hamilton et al., 2003*****; Green et al., 2008; Kodituwakku, 2009). T-maze tasks have the potential to provide a relevant behavioral endpoint for the sheep model but, to date, T-maze performance has not been well-characterized in sheep (or other livestock species). Our main purpose in this study was to develop T-maze procedures to assess spatial learning in sheep that could provide a quantitative experimental method for use in future studies in our ovine model of FASD. Consistent with the goals of the study, five experiments were conducted using several T-maze task variants, separately or in conjunction, to assess their utility in normal peri-adolescent lambs. The task variants used were: non-matching-to-place (NMTP), matching-to-place (MTP), position habit (PH) and position habit reversal (PHR); greater detail is provided for each in the Methods section.

GENERAL METHODS

Subjects

All lambs used in these experiments were offspring of Suffolk ewes bred at Texas A&M University, and all procedures were conducted with approval of the Institutional Animal Care and Use Committee (IACUC) at Texas A&M University. Lambs were weighed, ear-tagged, and given a subcutaneous 5-mL B-vitamin injection within a day of birth (Super B Complex, Vedco, Inc., St. Joseph, MO, USA); they were weighed weekly thereafter and remained with lactating maternal ewes until weaning at 8 weeks of age. All lambs were vaccinated against *Clostridium perfringens* (types C and D) and *Clostridium tetani* (Bar Vac CD/T, Boehringer Ingelheim Vetmedica, Inc., St. Joseph, MO, USA) at 4-weeks age and again at weaning. Weaned lambs were group-housed 2–3/pen inside a climate-controlled animal housing facility, with a 12-hr/12-hr light-dark cycle, and ad libitum access to water. Lambs were maintained on a daily ration of sheep feed (5% of body weight) formulated to National Research Council guidelines for growing lambs (Producers Cooperative Association, Bryan, TX, USA), supplemented by alfalfa (1% of body weight). The lambs used in Exps. 1, 4, and 5 had previously undergone two weeks of daily handling and eyeblink classical conditioning as part of another experiment that ended at least 2 weeks before the start of the T-maze procedures. The lambs in Exps. 2 and 3 were experimentally naive at the beginning of the T-maze procedures.

To increase the incentive salience of the food used as reinforcement in these experiments, the normal daily feeding schedule was modified on T-maze training days. Instead of receiving their daily ration as two equal portions in the morning and evening, lambs received

only a single evening feeding containing their total daily food ration, minus the amount of food eaten during T-maze trials. Thus, T-maze training trials provided the sole opportunities for lambs to obtain food during the day in advance of the evening feeding.

T-Maze Construction

A T-maze was constructed of plywood sealed against moisture with light-blue non-reflective “exterior” paint, with walls 3-ft (.91 m) high and dimensions suitable for use with post-weanling lambs. The modular construction included a 3-ft (.91 m) x 3-ft (.91 m) start box, a 3-ft-wide (.91 m) x 6-ft-long (1.82 m) stem (start-arm), and a 3-ft (.91 m) x 3-ft (.91 m) central intersection “choice-point”. Swinging doors on the start box remained closed and prevented lambs from entering the stem of the maze until the experimenter initiated a trial run. A weight attached to each door by cable caused the doors to simultaneously spring open when unlatched by the experimenter, thus initiating a trial. Extending from either side of the choice-point, at right angles to the stem, were two 3-ft wide (.91 m) x 4-ft-long (1.22 m) arms, leading to 3-ft (.91 m) x 4-ft (1.22 m) goal boxes. Food reinforcement was placed in 12-in (30 cm) x 16-in (41 cm) extensions of each goal box that protruded toward the front of the maze such that food bowls were not visible from the choice point. This part of each goal box was clad in wire mesh instead of plywood, which permitted the experimenter to readily establish when (or if) lambs found the food reward at either goal box. In an effort to minimize the contribution of food odor cues as determinants of side choice, two open buckets remained directly outside each goal box during all phases and trials of T-maze training (for all experiments), each bucket containing several kgs of sheep feed.

Habituation Procedures

Lambs were given a total of 15 T-maze habituation trials (3 trials/day, Mon–Fri) during the week prior to actual training. For the first six habituation trials (Mon–Tues), each lamb was led to and placed directly inside of one of the closed-off goal boxes, which was reinforced with 2 oz (~57 g) of sheep feed; lambs were permitted 2 min to explore the box and consume the food. Lambs were removed from the maze for 30 sec and then given a similar experience in the opposite goal box. The last nine habituation trials (Wed–Fri) were each comprised of pairs of reinforced forced-choice trials (i.e., one goal box available, the other closed off) to alternating sides. During these latter habituation trials, lambs again spent 2 min in each goal box, and trials were separated by a 30-sec inter-trial interval, but on each trial they entered the stem of the maze, instead of being led directly into a goal box. The order of sides visited by each lamb during habituation was pseudo-randomly determined, with an approximately equal number of left-right and right-left pairings across all 15 trials, and a limit of no more than three consecutive left-right or right-left pairings. At the conclusion of each habituation trial lambs were returned to the area where they were housed.

Training Procedures: Non-Matching to Place (NMTP)

A discrete-trials procedure was used for NMTP training trials (Figure 1); each trial consisted of a pair of runs, a “forced-run” followed by a “choice-run” (Olton, 1983; Green & Stanton, 1989). On forced-runs, one of the goal boxes was closed off and the other was open. At the outset of each run, lambs were led into the start box, where they remained for 5 sec. When the start-box doors were opened by the experimenter, lambs entered the stem of the maze

and proceeded to the available goal box, which always contained reinforcement, 2 oz (~57 g) of sheep feed. Once a lamb entered the available goal box on a forced-run, that goal box was closed off and the lamb was permitted 2 min in the goal box to consume the food. Lambs were then removed from the maze and the maze was prepared for the choice-run by insuring that both goal boxes were open and that food was available (only) in the goal box opposite the one that was open and reinforced on the forced-run. The sides blocked/reinforced for each forced-run were pseudo-randomly selected across trials, with a limit of no more than three consecutive forced-runs to either side, and with an equal number of forced-runs to the left and right goal boxes across blocks of 10 trials.

For NMTP choice-runs, lambs were returned to the start-box for 5 sec, and then permitted to traverse the maze and choose a goal box to enter. Goal boxes were not closed on choice-runs until after a lamb had found the food, permitting an opportunity for “self-correction” after an incorrect choice. Lambs were free to enter (and re-enter) the left and right arms of the maze, as well as the stem, as often as necessary until they found and began to consume the food, at which time the latency to find the food was recorded and the arm to the reinforced goal box was closed off. Only the first choice (arm entry) was scored as correct or incorrect. Data recorded for each choice-run included the goal box side first chosen, whether that choice was rewarded (“correct”) or not (“incorrect”), and the number of arm (and stem) entries made by each lamb prior to finding the food reinforcement for each choice-run. A stopwatch was used to measure latencies to reach the goal box after the start-box doors were opened at the beginning of each run; latencies were recorded for forced-runs and choice-runs for each training trial. At the conclusion of each choice-run, lambs were returned to the area where they were housed.

Training Procedures: Matching to Place (MTP)

The procedures used for MTP training were similar to those used for NMTP training, i.e., a forced-run followed by a choice-run, except that instead of alternating the goal box location of the reinforcement between the forced-run and the choice-run of each trial, the choice-run food reward was consistently located in the *same* goal box visited on the forced-run (Figure 1).

Training Procedures: Position Habit (PH) and Position Habit Reversal (PHR)

Each PH training trial consisted of a single choice-run with both goal boxes open and available (Figure 1). For each lamb one of the goal boxes consistently contained a food reward during PH training, whereas the opposite-side goal box never did. Lambs were randomly assigned to either left-side or right-side reinforcement during PH training, with approximately equal numbers of lambs assigned to each side. Lambs underwent three training trials each day (Mon–Fri) for a total of 15 PH training trials. Following the week of PH training, the reinforcement contingency was switched once more for lambs in Experiment 1 (PHR, Figure 1). For each lamb, the location of the reinforced (CS+) and non-reinforced (CS–) goal boxes was reversed and lambs were then given three single-run training trials/day for five days, for a total of 15 PHR training trials.

Data analysis

For all experiments, mean daily percent correct choices served as the primary dependent measure of learning. For Experiments 1, 4, and 5, analysis of mean daily percent correct responses was performed using a mixed ANOVA with Sex as a grouping factor Day as the repeated measure. In Experiments 2 and 3, which had limited numbers of lambs of either sex, the daily percent correct were analyzed with repeated measures ANOVAs with Day as the within-subject factor. In the experiments involving different training phases, a one-way ANOVA was used for analyses of total mean percent correct during a given training phase. For comparisons of performance across experiments, a mixed ANOVA was used with Experiment as a grouping factor and Day as a repeated measure. Student-Newman-Keuls post-hoc analysis was used to identify difference in levels within significant factors. *A priori*, significance was established as $p < 0.05$. Measures of acquisition rate were obtained either by determining the number of days to reach a criterion (e.g., two consecutive errorless days, determined empirically) or by determining the slope of the line created by plotting the mean daily percent correct responses across days. These measures of acquisition rates were analyzed by performing either a repeated measures ANOVA followed by paired-samples t-test (comparing across tasks within subjects) or by comparing across experiments using one-way ANOVAs on the slopes, with Experiment as a grouping factor. Values are presented as means \pm SEM.

Experiment 1

Rationale

Experiment 1 addressed the question as to how readily 14-week-old lambs would learn to alternate during three weeks of NMTP training, and to what extent they would adapt their behavior when the reinforcement contingency was subsequently changed to position habit discrimination (and reversal). Because the primary goal of this work was to assess spatial working memory ability in lambs while retaining a within subjects approach to adaptation to changes in reinforcement contingencies, the task order of NMTP followed by PH and PHR was chosen to avoid any transfer effects on the primary task.

Specific Methods

The lambs trained in Experiment 1 (6 females, 8 males) were weaned at eight weeks of age and given eyeblink training procedures (as part of another experiment) at 9–11 weeks of age. Beginning at 13 weeks of age; they were habituated to the T-maze for one week and began training the following week (at 14 weeks age) using the same NMTP training procedures described in the Methods, with two exceptions: the duration of each trial run was one min, and the duration of the interval between forced- and choice-run was 30 sec. All 14 lambs were given three weeks of training (Mon–Fri) using the NMTP reinforcement contingency, for a total of 45 NMTP trials distributed over 15 training days. For 12 of the lambs, the NMTP was followed by a change to PH training for one week, and then a final week of PHR training. These tasks were studied successively in the same lambs in order to obtain maximum information from a limited number of lambs. There were insufficient lambs to counterbalance task order and so we chose to run the PH and PHR training phases following the NMTP phase for two reasons. First, NMTP learning was of primary interest and, second,

learning this task is less likely to interfere with subsequent PH-PHR learning than the other way around (see Discussion for further details and explanation).

Results

The mean daily percentage of correct choices increased significantly across days during the first (NMTP) phase of training (see Fig. 1, Panel A), from ~48% on the first training day to between 90 – 95% correct during the last several days of training. A mixed ANOVA with sex as a grouping factor and training day as a repeated measure indicated there were no main or interactive effects of sex, and there was a significant main effect of day [$F(14, 168)=5.326, p<0.001$], reflecting the significant improvement in performance by the lambs as training progressed.

On the first day of training following the switch to the PH reinforcement contingency (see Fig. 2, Panel A), mean percent correct responses fell to chance levels (~44% correct), but rapidly and significantly increased across the five days of PH training [$F(4, 40) = 10.359, p < 0.001$], with 100% correct responding by all lambs during the last two days of PH training. Again, there were no main or interactive effects of sex on the PH acquisition. Predictably, the percentage of correct responses also declined sharply when the training contingency was reversed with the change from PH to PHR. The mean daily percent correct responses went from 100% correct to <6% correct on the first day, but rapidly and significantly increased each day to an average of ~78% correct on each of the last two training days for RPH [main effect of day [$F(4, 40) = 21.046, p < 0.001$; again no main or interactive effects of sex].

The rate at which the lambs adapted to the change in reinforcement contingency from NMTP to PH was remarkably fast. For the PH discrimination, the lambs reached a criterion of 2 consecutive days of errorless performance on average in only 3.5 (± 1.3) days, compared to 8.2 (± 4.2) days to achieve the same criterion for the prior NMTP training [$t(11df)=3.30, p=0.007$]. For PH reversal, 7 of the 12 lambs met the same criterion by the fifth (last) day of training. Slopes of the acquisition curves for the 12 lambs given all three phases were determined for each phase, using the first 8 days for the NMTP (slope (m) = 4.9 ± 1.0), the first 4 days for the PH ($m = 17.9 \pm 4.3$), and all five days for PHR ($m = 19.2 \pm 2.7$). A one-way repeated measures ANOVA on these slopes across the three training phases yielded a significant main effect of Phase [$F(2, 22) = 9.8, p = 0.0009$], and follow up t-tests indicated that the acquisition slope during the NMTP phase was significantly lower than either the PH phase [$t(11df)=2.93, p=0.0137$] or the PHR phase [$t(11df)=6.533, p<0.0001$]; the PH and PHR slopes did not differ significantly from each other.

Conclusions

The results of Experiment 1 confirmed that 14-week-old lambs were capable of learning the alternation rule and were clearly able to retain the side information provided by the forced-run throughout the inter-run interval to guide subsequent side-choice on the choice-run during NMTP training phase. Lambs were also clearly able to respond adaptively to changes in reinforcement contingency, as indicated by the rapid improvement across days during the PH and PHR training phases. The rapid acquisition of the PH and PHR contingencies

following the switch from NMTP suggests that these training variants are demonstrably easier for lambs to acquire than NMTP (see Experiment 5 and Discussion below).

Experiment 2

Rationale

Experiment 2 was conducted to determine whether acquisition of NMTP would be seen in lambs that were 6 weeks younger than those tested in Experiment 1, and, if so, how they would respond to a switch from NMTP to MTP training. This switch represents a form of reversal learning in which the discriminative cue is represented in working memory, rather than perceptually present as in reversal of position habit (switch from PH to PHR). Because reversal of a working memory task, represented by the switch from NMTP to MTP, is more challenging than reversal of a reference memory task (PH to PHR), we sought to determine whether young lambs could learn the NMTP-to-MTP reversal task.

Specific Methods

Lambs (2 males, 5 females) were habituated to the T-maze for one week beginning at eight weeks age, and began four consecutive weeks (Mon–Fri) of NMTP training the following week, for a total of 60 NMTP trials distributed over 20 training days. The extra week of training was scheduled in case the younger lambs needed more training to reach asymptotic performance. Lambs were 13 weeks old when the reinforcement contingency was changed from NMTP to MTP; they were trained for an additional 25 days over the course of five weeks, with three training trials each day (Mon–Fri), for a total of 75 MTP training trials.

Results

As shown in Fig 2 Panel B, the mean percentage of correct choices increased significantly across days during NMTP training, from ~53% during the first week of training to ~85% during the fourth and final week [$F(19, 114) = 3.092, p < 0.05$]. However, even with the additional week of NMTP training, younger lambs as a group did not attain the same consistently high level of terminal performance seen in the older lambs at the end of their third week of NMTP training (Fig. 2, compare Panel B NMTP phase with Panel A NMTP phase). To permit cross-experiment statistical comparisons of NMTP performance between nine-week-old lambs (Experiment 2) and 13-week-old lambs (Experiment 1), NMTP performance measures for Experiment 2 were truncated at 15 days, matching the total number of NMTP training days for Experiment 1. Experiment 1 lambs performed the NMTP task significantly better than Experiment 2 lambs, an observation confirmed by a significant main effect of Experiment [$F(1, 19) = 7.036, p = 0.016$] on mean daily percent correct choices across days. However, this difference appeared to reflect differences in performance at the outset of training rather than differences in rate of learning the NMTP task. Lambs in both experiments improved over the course of training, as reflected in a significant main effect of Day [$F(14, 266) = 6.277, p < 0.001$]; the Day x Experiment term was not significant. A separate analysis of the total mean percent correct over the 15 days of NMTP acquisition for lambs in the two experiments confirmed that overall means were significantly greater for Experiment 1 [$F(1, 19) = 8.108, p = 0.010$]. However, the analysis

of the slopes of the acquisition curves revealed only non-significant differences between the lambs of Experiment 2 and those of Experiment 1.

Following the switch from NMTP to MTP, mean percent correct responding fell to significantly below chance levels. Within-group analysis of mean daily percent correct choices across the first 20 days of each training condition confirmed that performance was significantly better during the NMTP training phase (main effect of Phase, $[F(1, 12) = 54.676, p < .001]$), and this was confirmed by significantly greater overall means during the NMTP training phase (69.76 ± 5.07) than during the first 20 days of MTP training (23.93 ± 3.94) [paired-samples t-test, $t(6) = 5.160, p = .002$].

There was a general tendency for improvement across days during both training phases (main effect of Day $[F(19, 228) = 2.829, p < .001]$). Closer inspection of data from individual lambs indicated that five of the seven lambs used the formerly adaptive alternation strategy during the first week of MTP training, and thus performed at levels significantly below chance, whereas performance by the other two lambs appeared to be random. The improvement by the group as a whole was therefore attributable to the gradual abandonment of the formerly adaptive alternation response by individual lambs as MTP training progressed, but even by the fifth and final week of training, none of the lambs in Experiment 2 ever showed evidence of having acquired the new MTP contingency. In fact, during the final week of MTP training, four of the lambs had clearly adopted a side-bias, and the remaining three lambs showed no discernible strategy at all; their performance during that week with respect to left-right choice appeared to be random, and their correct choice performance was at chance levels.

In contrast to the results with the percent-correct-choice measure, no significant differences were seen between NMTP and MTP training phases for overall mean daily number of arm entries (NMTP = 1.35 ± 0.07 , MPT = 1.90 ± 0.07), mean daily choice-run latencies (NMTP = 8.19 ± 0.31 , MPT = 11.28 ± 0.49), or for the rates of reduction in number of arm entries (NMTP slope = -2.42 ± 0.77 , MPT slope = -4.31 ± 1.39) or choice-run latencies to find food (NMTP slope = -0.49 ± 0.09 , MPT slope = -0.86 ± 0.22) when individual slopes were calculated and analyzed for these measures.

Conclusions

Results from Experiment 2 showed that lambs are capable of learning the NMTP contingency at an age even earlier than those tested in Experiment 1, although as a group, the older lambs performed at a higher level because their initial (baseline) performance was higher than the lambs in Experiment 2. Lambs in Experiment 2 did not readily adapt to the change in reinforcement contingency (to MTP training) after the NMTP training phase. Even after five weeks of training with the MTP contingency, not only did group means never consistently exceed 50%, not one of the lambs appeared to have ever adopted consistent use of the appropriate return-response strategy. Although, lambs in Experiment 2 acquired the NMTP contingency more readily (at nine weeks age) than they acquired the MTP contingency (at 13 weeks age), further inferences about MTP performance in general would clearly be confounded by the negative transfer from prior NMTP training. A further study was therefore conducted to determine whether the training contingency itself (NMTP

or MTP) affected acquisition, or whether lambs initially trained with the MTP contingency acquire the task at the same rate as same-age NMTP-trained lambs.

Experiment 3

Rationale

Experiment 3 was conducted specifically to assess naïve MTP acquisition beginning at nine-weeks-age, for comparison with naïve NMTP acquisition of the nine-week-old lambs in Experiment 2.

Specific Methods

Just as in Experiment 2, lambs in Experiment 3 (Experiment 3, 2 males, 3 females) were weaned and habituated to the T-maze at 8 weeks age, and began T-maze training at 9 weeks. (A fourth female lamb was removed from the study because it showed little interest in the food reward, and frequently attempted to jump out of the maze, rendering the behavioral data uninterpretable.) The lambs were given five consecutive weeks of MTP training (three trials/day, Mon–Fri), for a total of 75 MTP trials over 25 training days.

Results

An Experiment X Day mixed ANOVA on mean daily percent correct responses comparing naïve acquisition between lambs of Experiment 2 and Experiment 3 confirmed that performance by the Experiment 2 lambs (during the initial 20 days of NMTP training) was significantly better than that of Experiment 3 lambs during a comparable period of MTP training [main effect of Group, $F(1, 10) = 11.735$, $p < .001$]. A main effect of Day [$F(19, 190) = 3.145$, $p < .001$] and positive acquisition curve slopes suggested that both groups improved over the course of training (Experiment 2 = 2.07 ± 0.57 , Experiment 3 = 1.17 ± 0.56), but a within-subjects comparison of daily means from the first and last training weeks for the lambs in Experiment 3 revealed no statistically significant differences. Lambs in the MTP training of Experiment 3 chose the correct side on ~43% of the choice-runs during the first week of training, and on ~51% of the choice-runs during the final week of MTP training. There was no indication that any of the lambs in Experiment 3 had learned to consistently return (on the choice-run) to the goal box that had been reinforced on the previous forced-run. For Experiment 3 the main effect of day appeared to be due to the erratic performance of the group as a whole, and not due to a tendency toward progressive improvement across training, as was seen in the performance by lambs trained with NMTP in Experiments 1 and 2. A separate independent-samples t-test further confirmed that the better acquisition across the first 20 training days by lambs of Experiment 2 was due to significantly greater overall mean percent correct for Experiment 2 (69.76 ± 5.07) than for Experiment 3 (50.00 ± 3.12) during the comparable period [$t(10) = 3.201$, $p = .009$].

Conclusions

The MTP results from the lambs in Experiment 3, coupled with the performance data from both training phases for Experiment 2, suggest that the NMTP response contingency is acquired much more readily by young peri-adolescent lambs than the MTP contingency. The nine-week-old lambs in Experiment 2 acquired the NMTP contingency more readily than

the nine-week-old lambs in Experiment 3 acquired the MTP contingency. In fact, under the training conditions of Experiment 3, the lambs did not acquire the appropriate response during MTP training. The small number of lambs ($n=5$) in this study raises concerns about the reliability of the learning curve, and it precludes a definitive conclusion about whether or at what rate lambs can learn the MTP task. Nevertheless, given that Experiment 3 was designed to explicitly compare acquisition of MTP with that of NMTP of Experiment 2, this small sample size still had sufficient power to detect significant group differences (observed power of 0.824), mainly due to the very large effect size attained (Cohen's $d=1.88$ for the sample estimate). The Experiment factor accounted for 50.7% of the variance (partial eta squared = 0.507). Thus, even with the small n for Experiment 3, the study did have sufficient power to show that performance on MTP acquisition was significantly worse than on NMTP, over the course of the first fifteen training days than for the NMTP groups (post-hoc, $p < 0.05$). The poorer performance on MPT relative to NMTP tasks suggest that Suffolk lambs may be predisposed to perform better when the task requires an alternation strategy (win-shift) than when it requires a return to the previously reinforced location (win-stay).

Experiment 4

Rationale

Experiment 4 was conducted to determine whether NMTP acquisition in nine-week-old lambs is robust across procedural variables such as the physical size of the maze, the number of training trials administered each day, and the use of a correction procedure. NMTP performance of Experiment 4, which differed along these dimensions, was compared with NMTP performance in previous experiments.

Specific Methods

The lambs used for training in Experiment 4 (10 males, 15 females) had been previously tested in a behavioral study of the classically conditioned eyeblink response; they also were only available for participation in this study for a total of 13 training days. Lambs in Experiment 4 were given only two training trials on each training day, for a total of 26 NMTP training trials distributed across 13 training days (Mon–Fri for two weeks, Mon–Wed of final week). Experiment 4 began habituation at eight weeks of age, and began NMTP training starting when the lambs were nine weeks old. For Experiment 4, goal boxes were located directly proximal to the choice point (4' arm-extension on each side was removed) and instead of the 3' x 3' start box used in Experiments 1–3, lambs were entered directly into the gated stem of the maze, thus substantially reducing the overall length and width of the maze apparatus. In contrast to the training methods used for the other experiments, the goal box was closed immediately after the first goal box entry on each choice-run for lambs in Experiment 4. Lambs in this experiment were denied the opportunity to “self-correct” after an incorrect side choice and thus obtained no food reinforcement following an incorrect side choice. Regardless of whether or not the side-choice was correct or not, the lambs remained in the chosen goal box for two minutes before being removed from the testing area and returned to the housing area.

Results

Daily mean percent correct responses for Experiment 4 were analyzed with a mixed ANOVA with sex as a grouping factor and training day as a repeated measure. There were no main or interactive effects of sex, and there was a significant main effect of day [$F(12, 276)=4.138, p<0.001$], reflecting the significant improvement in performance by the lambs as training progressed (see Fig. 2, Panel B NMTP phase). Lambs in Experiment 4 chose the correct side on 48% of the choice-runs on the first day of training, reaching a high of 80% correct responses by the final day of training.

Mean daily percent correct responses from the 9-week-old lambs in Experiment 4 (Fig. 2, Panel B) were compared to the data from the first 13 NMTP training days for the lambs trained in Experiment 1 (Fig. 2, Panel A, NMTP phase) and in Experiment 2 (Fig. 2, Panel B NMTP phase). Overall, performance was significantly better in the lambs of Experiment 1 that began training at 14 weeks age than for either of the 9-week-old groups (Experiment 2 or 4). Perhaps the most striking cross-experiment comparison was how similar the performance over days of the Experiment 4 lambs was to that of the lambs tested in Experiment 2 (see Fig. 2 Panel B, NMTP phase), in which there were no significant differences in percent correct responding over the first 13 training days for those two groups. The NMTP performance in the two groups of 9-week-old lambs was virtually indistinguishable, despite the differences in prior experimental history (prior eyeblink conditioning in Experiment 4) and the differences in the t-maze procedures used (number of daily trials, correction procedures, maze configuration and spatial dimensions). The lack of performance differences between these two groups indicates that acquisition of the adaptive alternation response is a robust learning phenomenon in young post-weaning lambs.

Analysis of Forced-Run Latency

To examine the potential contribution of differences in motor function or motivation across the different experiments in these experiments, we measured approach response latencies on forced runs. Forced runs were chosen as a measure because they are not confounded by cognitive or motivational factors, such as decision time or inconsistency of reinforcement outcome that can influence response latencies on choice trials.

A mixed ANOVA with Experiment as a grouping factor used to analyze forced-run latencies as a repeated measure across the first 13 days of T-maze training (matching the total duration of training for Experiment 4). There was a main effect of Experiment [$F(3, 47) = 81.123, p < .001$]; post-hoc follow-up confirmed significantly shorter latencies for Experiment 4 (3.1 ± 0.1 sec); latency differences between Experiments 1 (5.6 ± 0.2 sec), 2 (6.1 ± 0.2 sec) and 3 (6.2 ± 0.4 sec) were not statistically significant. The significantly shorter latencies for the young lambs in Experiment 4 presumably reflected the fact that they were trained with a physically smaller maze configuration. This pattern of data indicates that differences in T-maze performance across experiments are not attributable to differences in motor capabilities or motivation to obtain the food reward of the lambs, including age differences between Experiments 1 & 2.

Experiment 5

Rationale

In Experiment 1, lambs that had been given 15 days of acquisition training on NMTP then switched to a position habit contingency were remarkably fast to learn the PH (reaching 100% correct on the 4th and 5th days), as well as the reversal of the position habit (from <10% correct choices on the first day of PHR to 75% correct on the 4th and 5th days of PHR training). However, it is not clear the extent to which the initial NMTP training may have influenced the position habit and reversal performance. The goal of experiment was to assess acquisition and reversal of position habit learning in the absence of prior NMTP training.

Specific Methods

The lambs used in Experiment 5 (4 females, 7 males) followed the same experimental protocol (up until the first T-maze acquisition training day) as those used in Experiment 1, being weaned at eight weeks of age, given eyeblink training (as part of another experiment) at 9–11 weeks of age, then habituated to the T-maze for one week at 13 weeks of age. The lambs were then first trained on PH for 5 days (beginning at 14 weeks of age), followed by 5 days of PHR training in the following week.

Results

The position habit acquisition and reversal performance of the naïve lambs of this experiment (Figure 3) was very similar to the PH and PHR performance of the lambs in Experiment 1 that had first undergone NMTP training (see Fig. 1, Panel A). The mean percent correct responses on PH Day 1 was slightly above chance levels (60% correct), but rapidly and significantly increased across the five days of PH training [main effect of Day, $F(4, 36) = 3.699$, $p < 0.05$] and reached 97% and 100% correct responding on the last two days of PH training, respectively. Again, there were no main or interactive effects of sex on the PH acquisition. On reversal, the percentage of correct responses dropped to 0% on the first day, and rapidly and significantly increased each day to reach 82% and 88% correct on Days 4 and 5 of PHR [main effect of Day, $F(4, 36) = 22.209$, $p < 0.001$; again no main or interactive effects of sex].

Conclusions

Given the similarity of acquisition and reversal curves for position habit learning in the lambs of Experiment 4 and Experiment 1, it appears that position habit contingencies are highly salient for lambs, given the relatively rapid PH and PHR learning evident in both experiments. Position habit and reversal tasks appear to be well suited for studies of procedural learning in sheep.

GENERAL DISCUSSION

Though this is not the first study to use a maze-type apparatus to examine learning and behavior in sheep (e.g. Peirce, Leigh & Kendrick, 2000; Kendrick, da Costa, Leigh, Hinton & Pierce, 2001; Camm, Gibbs, Cock, Rees & Harding, 2000; Hosoi, Swift, Rittenhouse &

Richards, 1995; Erhard, Boissy, Rae & Rhind, 2004), it is, to our knowledge, the first to use the T-maze specifically to investigate appetitively-motivated spatial learning and working memory in weanling/peri-adolescent lambs. Several key findings emerged from this study:

1. Consistent with the developmental learning literature for other, more extensively studied species, including humans (Diamond, 1990; Fox & Bell, 1990; Overman, 1990), non-human primates (Bachevalier, 1990; Alvarado & Bachevalier, 2000), and rodents (Campbell & Spear, 1972; Green & Stanton, 1989), age-dependent effects on performance were evident, with older lambs making more correct choices on the NMTP task than younger lambs. However, there were no age differences in learning the NMTP task, as reflected in the rate of improvement in performance across training trials, at least over the range of ages employed in this study. This pattern of results is similar to that seen with this task in developing rats, which show ontogenetic differences in spontaneous alternation but not reinforced NMTP learning at specific stages of development (Green & Stanton, 1989). As previously mentioned, latency data on forced runs suggest that motor and motivational factors do not account for these age differences.
2. NMTP acquisition in the post-weanling lamb is robust across a range of task parameters. A comparison of NMTP performance by the younger lambs in Experiments 2 and 4 revealed that lambs learned at a similar rate regardless of whether they were trained with two or three trials per day, whether or not they had the opportunity to self-correct after an incorrect side choice, or whether a one- or two-minute inter-run interval was imposed between forced and choice runs of each NMTP training trial. The remarkably similar learning by lambs in these two experiments shows that these differences in training procedures had minimal effect on learning and performance of the NMTP task.
3. Rapid acquisition of the PH reinforcement contingency in Experiment 1 (starting when the lambs were 17 weeks old, after 3 weeks of NMTP training) and in Experiment 5 (starting when lambs were 14 weeks old, with no prior T-maze training) showed that lambs are able to quickly learn the reinforcement contingency of a simple position habit, even if it involves a change from the previous NMTP contingency. The large number of errors on the first day of PHR training further confirmed that the lambs in Experiment 1 and Experiment 5 had fully acquired the original position habit, but they rapidly learned the new (reversal) contingency. The similarity of performance of lambs in Experiment 1 and Experiment 5 on the PH and PHR training phases is consistent with the view that prior NMTP had limited effects on subsequent position habit learning (either positive or negative transfer). This is because NMTP learning does not produce side-biases that can interfere with PH learning, and omission of the forced run following the transition from NMTP trials to PH trials should limit generalization across tasks. The similarity in position habit performance regardless of the prior NMTP experience suggests that PH and PHR tasks could be used in within-groups designs to study task dissociations in lambs.

4. Different tasks yielded different rates of acquisition, even by same-age lambs, revealing potentially important constraints on T-maze learning in peri-adolescent sheep. The three groups trained with the NMTP task learned rapidly. In contrast, lambs in Experiment 2 failed to perform above chance levels following the switch in reinforcement contingency from NMTP to MTP, even after five weeks of MTP training, and experimentally naïve lambs in Experiment 3 also failed to acquire the MTP task to above-chance levels. Thus, Experiments 2 and 3, together, showed that performance by younger lambs was clearly better with the NMTP task than with MTP task.

The difference in NMTP vs. MTP performance suggests that, like rodents (Montgomery, 1952; O'Connell, 1965; Still, 1966; Henderson, 1970), lambs have a propensity to alternate between different maze arms when seeking food reward. This finding contrasts with the results of another T-maze study investigating lateral preference and foraging strategy in sheep (Hosoi et al., 1995). However, in that study, adult sheep were given trials consisting of a pair of free-choice-runs. Notably, of the five sheep purportedly using a win-stay strategy in the Hosoi et al., (1995) study, four clearly demonstrated lateral biases, consistently choosing the same side on the first run of each trial at levels significantly greater than would be predicted by chance. Thus, the apparent "win-stay strategy" in that study likely reflected a side-preference. Side-biases could not be confused with win-shift or win-stay strategies in our study because sheep chose on the basis of the preceding forced run and forced runs occurred equally to both sides of the T-maze in a pseudorandom sequence. The Hosoi et al (1995) study also differed from the present one in other potentially important ways. The lambs used in the current study were younger and their developmental history in our lab, including a lack of foraging experience, was known. Sheep in the Hosoi study were acquired at auction when they were about one year old; they presumably had experience with pasture foraging and it is unknown how such experience (or lack thereof) might affect subsequent food-seeking behavior under experimental conditions.

In addition to these contributions, there are also some important limitations of the current study that warrant discussion. First, it was beyond the scope of this study to systematically evaluate a number of variables that may well be important determinants of maze performance by sheep in general and lambs in particular. For instance, it may be the case that lambs can begin to learn these tasks or other T-maze variants at an even earlier age than reported here; we chose to wait until post-weaning to implement these appetitively-motivated training procedures to provide better experimental control over the incentive-salience of the food reward. Performance by the older lambs in this study was markedly superior to that of the younger lambs, yet it remains to be determined at what age performance is optimized, beyond which no further age-related improvement in performance is seen. Second, the present study also left unanswered the question as to how well, or even if, older lambs can acquire the MTP task. Third, no lambs younger than 14 weeks old were trained with the PH (or PHR) contingencies, so it is also unresolved as to whether or not age-related performance differences would be evident for this apparently easier T-maze task. Finally, it is also not known how parametric manipulations that affect T-maze performance in other rodent or primate species, such as substantially increasing the duration of the delay

between the forced- and choice-runs, would affect performance in developing lambs (e.g., Stanton, Thomas & Brito, 1984).

The primary objective of this work was to develop T-maze training procedures appropriate for use with adolescent lambs. The success described in this report provides a behavioral platform to pursue neural systems analyses of spatial learning and working memory functions in sheep. For example, the larger brain (especially cortex) compared to laboratory rodents may facilitate more precise analysis of regions involved in these functions, using reversible inactivation, neurophysiological recording, or local neurochemical analysis. More importantly for our purposes, these findings will enable us to apply these methods to lambs given prenatal alcohol exposure to assess its consequences on behavioral measures of learning that are in many respects analogous to the types of alcohol-induced neurobehavioral deficits commonly reported in children with FASD. If spatial learning is affected by prenatal alcohol, similar T-maze tasks could then be used to gauge the efficacy of interventions attempting to mitigate those alcohol-induced neurobehavioral deficits.

The ovine model offers several potential advantages over rodent models of developmental disorders caused by in utero neurotoxicant exposure, including a closer correspondence to the human condition on several key factors, such as maternal and fetal body mass, number of offspring per pregnancy, and length of gestation. The relatively large size of the sheep facilitates instrumentation of the fetus prior to parturition, providing a means by which to manipulate and monitor the intrauterine environment. A longer gestation (~5 months in sheep, versus ~3 weeks in rats and mice) permits better temporal resolution and precision for modeling neurotoxic insults at specific developmental time-points or during particular periods of brain growth. Lambs are also much more precocial at birth than rodent pups (or human infants), offering the potential for various types of behavioral testing beginning relatively early in life. The primary limitation of the sheep model has been the sparse behavioral literature and lack of information about cognitive performance in developing lambs. The findings from the present study show that lambs perform well on T-maze tasks that are widely used to study the impact of developmental neurotoxicant exposure on the neural mechanisms of spatial working memory in developing and adult rodents. Future work involving these tasks in a sheep model of *in utero* alcohol exposure has the potential to importantly advance the effort to use animal models to devise intervention strategies to mitigate or prevent human FASD (Cudd, 2005; Goodlett, Horn & Zhou, 2005).

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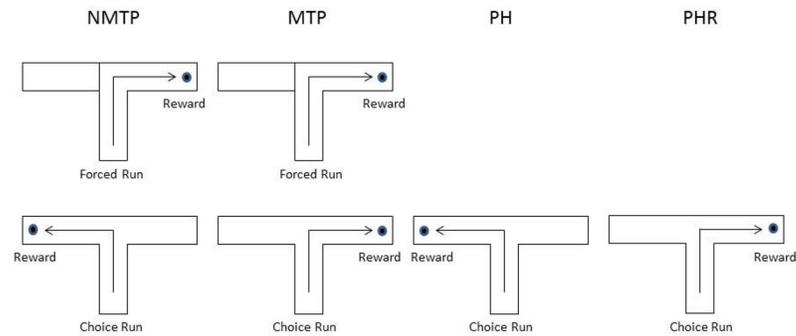


Figure 1. Schematic summary of the four tasks employed in this study. NMTP, Non-matching-to-place: trials consisted of a forced-run (top) followed by a choice run (bottom) with reward (dark circle) contingent on choosing the arm of the T-maze that was *not* rewarded on the preceding forced-run. The direction of forced runs was balanced across right and left arms in a quasi-random sequence (not shown). MTP, Matching-to-place: same as NMTP except that reward was contingent on choosing the T-maze arm that *was* rewarded on the preceding forced run. PH, Position Habit: trials consisted only of choice runs with the same arm being rewarded on every trial (left for half the animals and right for the other half, randomly determined). PHR, Position Habit Reversal: Same as for PH except that reward was present in the opposite arm from the one that was rewarded during PH training.

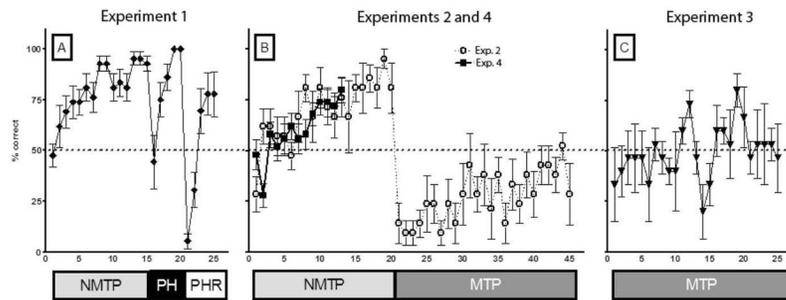


Figure 2.

Mean daily percent correct side choices (\pm SEM). [A] Lambs in Experiment 1 started T-maze training at 14 weeks age (3 trials/day), beginning with 15 days non-matching-to-place (NMTP) training, followed by 5 days of position habit (PH), and then a final 5 days of position habit reversal (PHR). [B] Lambs in Experiments 2 and 4 began NMTP training at nine weeks age. Experiment 2 received three daily training trials, starting with 20 days of NMTP training followed by 25 days of matching-to-place (MTP) training, whereas Experiment 4 was trained for only 13 days, with a smaller T-maze configuration and only two daily NMTP training trials. [C] Experiment 3 was given 25 days of matching-to-place (MTP) training (3 trials/day) beginning at nine weeks age. Dashed horizontal line indicates percent correct predicted by chance performance.

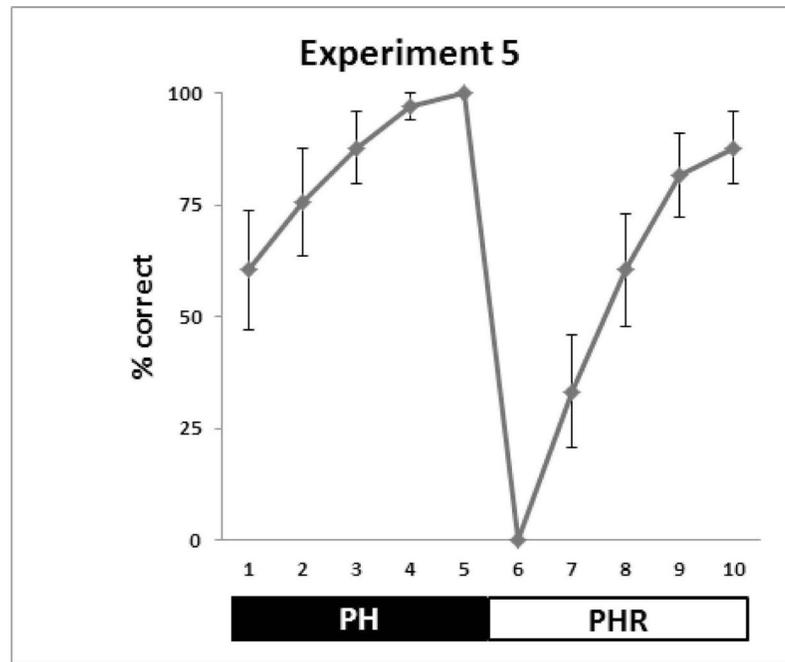


Figure 3.

Mean daily percent correct side choices (\pm SEM) for Experiment 5. Lambs were given five days of training on the position habit (PH) in the T-maze at 14 weeks of age followed by 5 days of training on position habit reversal (PHR) at 15 weeks of age.