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## **SHEEP LATERALITY**

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Turning preferences among 309 white-faced ewes were individually evaluated in an enclosed, artificially lighted, T-maze, followed by each ewe choosing either a right or left return alley to return to peers. Data recorded included time in the start box, time in the T-maze, exit arm chosen to leave the T-maze, and return alley. Right and left arms of the T-maze were chosen 65.7% and 34.3% of the time, respectively. In contrast, the right and left return alleys were chosen 32.4% and 67.6%, respectively. T-maze exit arm and return alley were not independently chosen ( $p < .0001$ ), with observed counts being higher than expected under independence when ewes made the same choice for exit and alley (RR or LL turn patterns) and being lower than expected for alternating choices (RL or LR). Specifically, out of the 309 ewes, 28.2% and 30.1% chose RR and LL turn patterns, respectively, while 37.5% chose the RL turn pattern, but only 13 out of 309 (4.2%) ewes chose the LR turning pattern. A slightly different interpretation of these data is to consider the choice of return alley conditioned on the initial exit choice. Thus, of the 203 ewes choosing the right arm, 116 (57.1%) demonstrated an alternating RL turning preference in choosing the left alley, while 87 (42.9%) of those ewes returned to peers through the right alley (RR). Of the 106 ewes exiting through the left arm, 93 (87.7%) demonstrated a non-alternating (LL) turning laterality by returning to peers through the left alley. In contrast, only 12.3% of those 106 ewes demonstrated an alternating (LR) laterality. Therefore, overall, ewes that initially turned right when presented a second turning opportunity had a slight preference to alternate their turning direction, while ewes that initially turned left tended to continue turning left when given another chance to turn. Exit arm and return alley laterality was not related ( $\alpha = .05$ ) to time of day the test was administered, ewe's age or genetics, most recent liveweight, or

most recent shorn fleece weight. The mean time spent in the start box (21 s) was not related to exit arm ( $p = .9472$ ) or return alley ( $p = .7790$ ). Mean time (15 s) spent in the T-maze was not related to exit arm ( $p = .0861$ ) or return alley ( $p = .9516$ ). More research will be required to understand sheep turning laterality and how it can impact working facilities and research equipment.

## **ACKNOWLEDGEMENTS**

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Laterality can be found throughout living systems beginning with DNA's double helix having a consistent clockwise or right-handed spiral (Bradley, 1994). During the 1970's, biologists began to consider that behavioral lateralization or asymmetry was possibly not just a human characteristic (Jackson, 1905) but was ubiquitous throughout the animal kingdom (Babcock, 1993). For a more in-depth understanding of vertebrate lateralization the reader is directed to several excellent recent comprehensive reviews (Rogers, 1989; Rogers & Andrew 2002; Vallortigara and Rogers, 2005).

Stress appears to play a role in the expression of lateral tendencies. If reindeer (*Rangifer tarandus*), are driven or crowded they invariably rotate counterclockwise (leftwards); however, free-ranging reindeer apparently demonstrate no right- or left- turning preference (Espmark & Kinderås, 2002). The left-turning bias when reindeer are stressed (Rogers, 2010) suggests that it is the right hemisphere of the brain that responds to escape and other emergency responses among these animals. Recent research by Versace, Morgante, Pulina, & Vallortigara (2007) suggest individual- and population-level lateralization can be observed in different tasks, and behavioral lateralization may provide insight into understanding stress responses (Morgante et al., 2007). Horses appear to be more reactive to a frightening stimulus when it is presented on the left (Austin & Rogers, 2007) suggesting that the left eye may be preferred by horses for assessment and evaluation of unfamiliar objects (Farmer, Krueger, & Byrne, 2010).

The majority of laterality studies involving ruminant livestock have been conducted using dairy cattle (Jackson, 1905; Beauchamp, Chapman, & Grebing, 1967; Uhrbrock, 1969; Yungblut, Albright, Hill, & Moeller, 1974; Albright, Yungblut, Arave, & Wilson, 1975; Arave & Walters, 1977; Albright, Yungblut, & Arave, 1978; Arave & Walters, 1980; Albright, 1981; Hopster, van der Werf, & Blokhuis, 1998; Arave, Lamb, Arambel, Purcell, & Walters, 1992;

Phillips, Llewellyn, & Claudia, 2003; Forsberg, Pettersson, Ljungberg, & Svennersten-Sjaunja, 2008; Tucker, Cox, Weary, & Špinka, 2009) especially with respect to lying. Although cows have been documented to switch sides between 50% and 60% of the time (Bao & Giller, 1991), Wagnon & Rollins (1972) found cows appear to lie predominantly on their left sides especially in the afternoon following a mid-morning feeding. Although dairy cattle show consistency in side of the milking parlor they prefer (Mateus, daCosta, & Broom, 2001), apparently no statistically significant level of discomfort or stress was recorded if milked on the non-preferred side of the parlor. These results suggest that the phenotypic expression of laterality is complex and probably cannot be attributable to only simple causes.

In contrast to cattle, many fewer studies exist on lateralization in sheep (Lane & Phillips, 2004). Using ten Suffolk ewes, Hansen, Esch, Hinds, Brown, Cabb, & Lewis (1978) reported that individual ewes varied ( $p < .001$ ) in their preference to use either the right or left arm when exiting a T-chute based on four trials. Overall, the left arm of the T - chute was chosen 62.5% of the time. However, two ewes turned right 100% of the time, five ewes turned left  $\geq 75\%$  of the time (of which 4 ewes turned left 100% of the time) while three sheep showed no laterality preference for turning (50% right and 50% left). These authors concluded that "because of the small sample size one can only speculate that sheep demonstrate laterality". Recently Erhard, Boissy, Rae, & Rhind, (2004) demonstrated that female sheep, if on a limited nutrition regime during early gestation, produced female offspring that had a left-bias ( $p < .05$ ) in a T-maze. Lane & Phillips (2004) found that neither gender nor number of siblings influenced laterality among 54 neonatal lambs. Simitzis et al. (2009) found male lambs between 2 and 5 months of age had a tendency of turning to the right-hand side of their test pen irrespective of nutritional treatments. *Mazes*. The single T-maze probably represents the simplest maze in use today (Silverman, 1978)

yet it is not free of introducing bias. Mazes can be considered complex environments (Liddell, 1925b; Silverman, 1978) requiring the animal to demonstrate neural processes controlled by multiple sensory stimuli, both internal and external to the maze itself. Walker, Dember, Earl, & Karoly (1955) indicate that when an animal was returned to a maze, it would likely use the same exit the second time, especially if it had been positively rewarded initially (Barnett, 1958). Furthermore, the route by which rats are taken to the test apparatus might have an effect on their subsequent behavior in the maze (Munn, 1933). Grandin, Odde, Schutz, & Behrns (1994) found this response held for cattle since cattle resisted change by looking back and forth at the decision point in a Y-maze once they associated a specific treatment effect with a specific side of a maze and the treatment was then switched.

Lambs exhibit a strong spatial memory (Nowak, 1994) and can learn simple tasks in as few as two trials (Bremner, Braggins, & Kilgour, 1980). Adult sheep have been shown to “learn” in a single trial, especially if the experience to which they are subjected is unpleasant (Hutson & Butler, 1978). Sheep exhibit good memory as they can remember where “home” is and will remain in specific locations on the landscape without fencing through a process called hefting (Hart, 2004). Under experimental conditions sheep have been shown to remember experimental protocols for up to 6 weeks (Morton & Avanzo, 2011). Therefore, each of the sheep evaluated in this study were only exposed once to the T-maze to eliminate a learning bias. Knowing how each of the sheep used in this study had been handled from birth until they were evaluated in the T-maze was not considered essential because Hosoi, Rittenhouse, Swift, & Richards (1995) suggests ungulates may find a maze environment completely atypical and as such the data may reflect behaviors quite unrelated to what may occur in its natural environment and daily habitat. Furthermore, Price & Thos (1980) suggest that effects of rearing are minimal during short-term



social isolation of sheep. In this study the protocol applied to each age class of sheep was consistent among years and followed routine extensive husbandry practices (Mathis & Ross, 2000). Furthermore, the ewes evaluated in this study had never been previously tested in a T-maze and were tested only once. Therefore, the objective of this research was to evaluate white-faced ewe laterality when exiting an enclosed T-maze followed by their choosing one of two return alley routes to return to peers.

## **METHODS**

*The T-maze.* The data used in this study were collected prior to the USDA-ARS Jornada Experimental Range livestock research program participating under the guidelines of the New Mexico State Universities Institutional Animal Care and Use Committee's protocol. However, the senior author, who is currently on this committee at NMSU, can attest to the fact that this study was conducted with the highest regard for the animal's health and welfare and that none of the 309 ewes were mishandled or harmed in any way prior to, during or following the study.

The T-maze (Figure 1A) was constructed in a location remote from human traffic and other animals in an attempt to reduce external cues that could have influenced the sheep's behavior once it was put inside the T-maze. In addition, the pens constructed to hold pre and post-tested sheep were located behind the door opening into the start box. Pen D, held the sheep immediately before they were tested. Its solid sides prevented sheep returning from the T-maze from being seen by those sheep yet to be tested. The wooden T-maze consisted of two sides and a roof constructed of unpainted commercially available plywood sheets and pine lumber. It did not have a floor but sat directly on the soil. The joints between adjacent plywood sheets were

sealed to prevent sunlight from entering the maze. To exclude sunlight from entering through the start-box door frame, a foam seal was maintained around the opening to exclude light once it was closed (Figure 1B). A viewing port located above and to the right of the entrance door into the start box (Figure 1B) provided an observer, standing outside of the T-maze, the ability to watch the sheep while it was inside the T-maze. The viewing port too was surrounded by foam rubber to exclude external light from entering the T-maze. The inside of the T-maze was illuminated from twelve 40-watt fluorescent tube-lights located in three ceiling fixtures positioned at the intersection of the arms of the T-maze (Figure 1B). This placement kept the start box dimly illuminated compared to the intersection. Grandin (1990) found sheep prefer to move from a dimly illuminated area to a more brightly illuminated area. Electricity was provided by a portable gasoline generator located north and east of Pen D approximately 24 m.

The longest runway in the T-maze (Figure 1C) was 4.9 m (16.1'). The left and right exit arms or branches as they have been called (Heird, Lennon, & Bell, 1981) were each 2.4 m (7.8') long and extended at 90° angles on either side of the 3.7 m (12.1') long runway. The 1.2 m (3.9') x 0.5 m (1.5') start box was located in the center of the of the 1.2 m (3.9') wide runway just inside the door through which animals entered the T-maze.

The T-maze was oriented so sheep entered through the south end and exited through either a left (west) or right (east) arm (Figure 1B). To insure the right and left exit arms provided similar sensory stimulation among sheep, especially illumination, and wind movement, the exit arms were narrowed to 0.9 m (3.0') and were covered with plywood (Figures 1B and 1C). Franklin & Hutson (1982) demonstrated sheep used both arms of a Y-maze equally when one had been traversed 25 times by a group of 20 sheep leaving visual cues including footprints, wool and feces while the other arm was "clean." This is in contrast to Liddell (1925a) who reported that

even a few small tufts of grass and seeds in his labyrinth tended to interrupt the movement of sheep through his test apparatus. Therefore, the day before each test, all plant material in or on the soil was pulled and raked out of the T-maze and the soil inside the maze was wetted to eliminate dust during testing. Immediately preceding testing, the soil floor inside the T-maze was again raked to eliminate all foreign objects including animal tracks. Raking was repeated after every 20<sup>th</sup> animal tested. This was less frequent than that used by Kilgour (1981) who raked his test apparatus following the testing of every 3<sup>rd</sup> cow to obliterate animal tracks. However, if it was necessary for a technician to enter the T-maze for any reason prior to the 20<sup>th</sup> animal being tested, the T-maze was raked to eliminate both human and animal tracks.

Weather parameters recorded included ambient wet and dry air temperatures outside and inside the T-maze, along with outside atmospheric conditions throughout the two-day study. Temperatures inside the maze were recorded at the intersection of the long alley and exit arms.

*Animals and testing procedure.* A total of 309 white-faced range ewes between 8 months and 10 years-of-age were each evaluated in a single trial for turning laterality when exiting the enclosed T-maze January 30 and 31, 1997. The genetics of these sheep represented straight Polypay and 3/4 Polypay x 1/4 Rambouillet breeding. The night before testing the sheep had been provided *ab libitum* drinking water and a restricted amount of feed. Just prior to testing, ewes were moved to Pens A through C (Figure 1A). Once the ewes were in Pen D they were sequentially (non-randomly) selected based on who was closest to the door leading into the start box. Two technicians then gently moved the sheep through the plywood door of the T-maze and into the start box. A stopwatch was immediately activated as the entrance door was shut behind the sheep and one of the technicians standing in Pen D behind the closed plywood door began

watching the animal through the viewing port with his right eye. An open-mesh counter-balanced guillotine gate at the front of the start box was left in the closed position for a minimum of 10 s to allow the animal time to orient itself to its surroundings. Only after 10 s, or longer if necessary to ensure that the animal's head was observed oriented towards the exit arms, was the guillotine gate raised by pulling on a rope activated by the technician watching the animal through the viewing port. The open position of the guillotine gate was maintained until the ewe was no longer visible in the T-maze. At that time the guillotine gate was allowed to drop shut with a bang. The sound generated by this procedure may have hastened the ewe's return to peers through the return alley; however, its exact effect on the ewes was not evaluated.

If the animal did not immediately leave the start box, at the end of 60 s the plywood door leading into the start box was opened and the animal was given a gentle prod. When a prod had to be administered, the animal's ID along with notes on the animal's reaction to the prod was recorded. Once the animal stepped into the long runway of the T-maze, the first stopwatch was stopped and a second stopwatch was activated. The second stop watch was stopped when no fleece on the ewe was observed through the viewing port as the animal moved out of the T-maze through either the right or left exit arms. At the point where the ewe was no longer visible through the viewing port the second stopwatch was stopped and the guillotine gate was released. The second stopwatch recorded the time the animal spent in the T-maze, which has been referred to by Kratzer, Netherland, Pulse, & Baker (1977) as the animal's "latency of escape." In addition to time in the start box and latency time, the right or left T-maze exit arm chosen and the right or left return alley chosen to return to Pen B (Figure 1A) were recorded for each animal.

*Statistics.* Percents, standard errors and approximate 95% confidence intervals (*CI*) (Snedecor &

Cochran, 1967, p 210) were calculated for the categorical responses: maze exit (right or left), return alley (right or left) and the four combinations of maze exit and return alley. Mean responses between right versus left choice (for maze exit and alley return) were compared using two-sample *t*-tests (with homogeneity of variance being checked by *F*-test) for the numeric responses: time-in-start box, time-in-maze, fleece weight, sheep liveweight, and individual sheep's percent Polypay. Chi-square tests of homogeneity were performed for right/left maze exit and right/left return alley versus age of sheep (in years) and hour tested. Lastly, a Chi-square test of independence was performed for right/left maze exit versus right/left return alley. All analyses were performed using SAS procedures (SAS Institute, 1989; 1990).

## RESULTS

Wet and dry bulb ambient air temperatures ranged between  $< -1.11$  °C ( $< 30$  °F) at 0710 hr to a 20 °C (68 °F) at 1415 hr over the two-day study. Overall, temperatures inside the maze were about 0.5 °C (1 °F) warmer than outside the maze throughout the study. Both days were clear with no wind to intermittent wind out of the west north-west and south, south-west.

A summary of percents (with 95% confidence intervals) for choice of right/left T-maze exit and right/left return alley for the 309 ewes in this study is given in Table 1 “(Table 1 about here)”. Neither direction of turning when exiting the T-maze nor the alley chosen to return to peers occurred with equal probability (50/50) among the 309 ewes. Right and left arms of the T-maze were chosen 65.7% and 34.3% of the time, respectively. In contrast, the right and left return alleys were chosen 32.4% and 67.6%, respectively. T-maze exit arm and return alley were not independently chosen ( $p < .0001$ ), with observed counts being higher than expected under

independence when ewes made the same choice for exit and alley (RR or LL turn patterns) and being lower than expected for alternating choices (RL or LR). Specifically, out of the 309 ewes, 28.2% and 30.1% chose RR and LL turn patterns, respectively, while 37.5% chose the RL turn pattern, but only 13 out of 309 (4.2%) ewes chose the LR turning pattern. A slightly different interpretation of these data is to consider the choice of return alley conditioned on the initial exit choice. Thus, of the 203 ewes choosing the right arm, 116 (57.1%) demonstrated an alternating RL turning preference in choosing the left alley, while 87 (42.9%) of those ewes returned to peers through the right alley (RR). Of the 106 ewes exiting through the left arm, 93 (87.7%) demonstrated a non-alternating (LL) turning laterality by returning to peers through the left alley. In contrast, only 12.3% of those 106 ewes demonstrated an alternating (LR) laterality. Those ewes that exited the T-maze through the left arm but returned to peers through the right alley had a statistically smaller confidence interval compared to the confidence intervals for the other three turning combinations. Montgomery (1952) has attributed spontaneous alternation of rat lateralities when evaluated in a maze to occur due to exploratory behavior. Walker et al. (1955) found laterality alternation behavior was least likely among male albino rats when intra- and extra-maze stimuli were as similar as possible. This test facility did not present the ewes with identical conditions at the two locations (arm and alley) where turning decisions had to be made. Our intra-maze and extra-maze environments were definitely different. Light, shadow, and wind, cues were kept constant or eliminated in our intra-maze environment while our extra-maze environment was highly influenced by biotic as well as abiotic cues. One obvious cue that may have differed between inside and outside of our T-maze was the resonant sound coming from the gasoline generator through the earth and various wood and metal structures surrounding and comprising the T-maze. Environmental cues, although external to the T-maze itself, may have

been recognized by the animal but missed by experimenters and may have been important in influencing the exit alley chosen by the sheep.

Overall, the ewes spent between 11 s and 185 s in the start box and between 4 s and 136 s for latency times. Mean time (21 s) in the start box was not related to exit arm ( $p = .947$ ) or return alley ( $p = .779$ ) chosen. On only three occasions was it required to prod the ewes to get them to leave the start box. Mean latency time (15 s) may have had some effect on exit arm chosen ( $p = .086$ ) while return alley and mean latency time appeared unrelated ( $p = .952$ ). Age of our ewes (between 8 months and 10 years-of-age) was not related to exit arm chosen ( $p = .356$ ).

Of the 309 ewes, only three were slow or deliberate in exiting the maze. These three sheep appeared to be observing the plywood sides and ceiling, and returned into the start box, smelling it and the soil, but did not appear to be under stress. This apparent lack of stress when working with individual animals was also found by Morton & Avanzo (2011) who found they could individually test 5 month old female Welsh Mountain sheep for periods lasting up to 30 minutes without the animals showing signs of isolation distress. Although our remaining 306 sheep moved with determination down the center of the long runway, they did not run. At the intersection they did not exhibit thigmotaxis but simply turned to exit through the arm they had chosen. According to Sluckin (1972), the lack of thigmotaxis behavior indicates lack of fear. Liddell (1925b), on the other hand, attributed the failure of his adult ewes to leave the starting compartment to nervousness or timidity that was exhibited by all sheep and lambs he tested when they were isolated from the flock. Bleating of our sheep while in the T-maze was minimal while Liddell (1925b) found that most of his animals bleated almost continuously during their first trial in his maze. The explanations for the difference between our sheep and those of Liddell's with respect to vocalizations remain unanswered.

We believe the motivating force which moved our sheep through the maze was their desire to return to peers. This same stimulus was successfully used by Lee, Colegate, & Fisher (2006) to move sheep through a maze without solid sides. The majority of our sheep moved quickly, unidirectionally and quietly from the start box through the T-maze and through either the right or left alley to return back to peers. However, a few sheep, which were not individually identified on their return to peers, hesitated or turned around at various points outside the T-maze before passing through the single bayonet gate (Anderson & Smith, 1980) located at the entrance to each of the two return alleys.

Movement of the ewes through the T-maze appeared free and unconstrained; however, some ewes did stop or momentarily hesitate at the intersection of the exit arms as if they were deciding which way to turn. Small (1901) observed this same phenomenon with rats. This hesitation may have been an attempt by the ewes in our study to pick up on a cue or cues they could use in their attempt to exit. Both olfactory and spatial cues, especially those involving the walls surrounding the maze, apparently provide salient distal cues to mice and must be carefully controlled when evaluating this species (Roullet, Lassalle, & Jegat, 1993).

Our data appear to be the first reported test in which sheep were evaluated with only a single trial in an enclosed T-maze followed by their returning to peers through either a right or left return alley to determine laterality without the bias of learning or memory entering into the interpretation of the data. The challenge in a complete and accurate interpretation of these data lies in the fact that a standardized test for determining laterality in livestock does not exist and as such hinders in developing the science of applied ethology as stated by Forkman, Boissy, Meunier-Salaün, Canali, & Jones (2007).

Subtle cues can be important in affecting animal behavior (Pfungst, 1965). Overall, the



interpretation of data from T-maze tests involves both anatomic and environmental factors, thus making accurate explanations complicated (Warren, 1958). However, our findings agree with Vallortigara, Rogers, & Bisazza (1999) that vertebrate animal's exhibit functional lateralization.

Although the sheep ranged between 8 months and 10 years-of-age, there was no statistical relationship between age and T-maze arm chosen ( $p = .356$ ) or return alley chosen ( $p = .504$ ). Furthermore, neither amount of wool that had been shorn in 1996, or the individual's most recent liveweight taken in 1996 or the sheep's genetic make-up appeared to have had any significant relationship on arm chosen by any of the sheep when exiting the T-maze ( $p = .314, .649$  and  $.905$ , respectively) or the return alley they used to return to peers once they were outside the T-maze ( $p = .748, .403$  and  $.154$ , respectively). Furthermore, the ambient weather conditions (as indicated by hour tested) did not appear to influence exit arm ( $p = .179$ ) chosen or return alley ( $p = .818$ ) chosen.

The T-maze was entered only once by technicians to remove an animal that would not exit on its own. Based on *a priori* protocol, the soil was raked after this event.

## **DISCUSSION**

Animal management today should foster low-stress animal handling (Smith, 1998). There is evidence to suggest that applying knowledge of laterality to husbandry practices can positively impact such things as reproductive performance in cows (Rizhova & Kokorina, 2005). Therefore, if the majority of sheep are found to have an initial bias to turn right (clockwise) it may be preferable to build sheep working facilities that curve counterclockwise to foster low-stress animal handling. A working facility that initially causes sheep to turn clockwise might

tend to cause sheep to crowd together into a tighter mob as a result of “centripetal behavior” thus making it more challenging to move individual animals through an alley or a race. In contrast, a facility that moves sheep initially in a counterclockwise direction may tend to produce “centrifugal behavior” that may separate individuals from the perimeter of a group and subsequently allow them to move more rapidly through an alley or race. Obviously such conjecture will require testing to determine its practicality.

Although Ganskopp (1995) did not find right and left turning tendencies among foraging angora goats ( $p \geq .01$ ), most likely turning direction during foraging is not random since more than 68 factors have been associated with free-ranging animal distribution (Anderson, 2010). Therefore, knowledge of an animal’s innate laterality may be useful in understanding and controlling animal movement. Although sheep exhibit parting and whorls in facial wool, their patterns are difficult to classify (Craft & Warner, 1933). In contrast, hair whorls (trichoglyphs) in the face of horses are readily classifiable and have been found to correlate positively with the animal’s laterality (Murphy & Arkins, 2008). Farmer et al. (2010) suggest further research is needed to determine if there is correlation between the strength of a horse’s laterality and its rank in herd hierarchy. If such relationships occur within all livestock species it might be possible to use laterality in developing a “leadership test” making it possible to justify instrumenting only those animals deemed to be leaders in a group. Furthermore, Basile, Boivin, Boutin, Blois Beulin, Hausberger, & Lemasson (2009) found horses exhibited lateralized responses to auditory signals although this too has not been evaluated in other livestock species to date.

One practical application in knowing if there is a predictable relationship between laterality and leadership as well as a lateralized responses to auditory signals may be in choosing which individuals to instrument and how intense the cues need to be when using directional virtual

fencing (DVF<sup>TM</sup>; Anderson, 2007). This method of managing animals relies on producing directional changes in a free-ranging animal's direction of movement by applying audio as well as electrical stimulation cues to either the animal's right or left side to elicit movement in the direction opposite the side to which the cues are applied. Possibly, stimulating movement towards the side reflecting an animal's innate lateral tendency may require less intense cues compared to those required to move animals in the opposite direction. Employing pro-active husbandry that focuses on understanding an animal's behavioral lateralization is necessary for minimizing stress and promoting positive animal welfare (Morgante & Vallortigara, 2009).

## CONCLUSIONS

Among 309 white-faced ewes 8 months to 10 years of age, naïve to a T-maze, different turning lateralities were demonstrated. Those ewes that initially turned right to exit the T-maze when presented a second turning opportunity (alley to return to peers) showed a slight preference to alternate their turning direction. In contrast, ewes that initially turned left when leaving the T-maze tended to continue turning left when given another chance to turn and returned to peers in a left return alley. An accurate explanation of these turning lateralities will require further research. Furthermore, age of the sheep ( $p = .356$ ), time of day between 0800 and 1500 hr when testing was done ( $p = .179$ ), seconds spent in the start box ( $p = .947$ ) and latency within the T-maze ( $p = .086$ ) were not shown to be statistically related to laterality. T-mazes may serve not only as a method of presenting animals pairs of choices within a research context but the use of mazes may provide insight into developing more efficient and humane animal handling procedures.

TABLE 1

Chi-square test<sup>a</sup> with 95% confidence intervals for independence among 309 ewes each tested once when exiting an enclosed T-maze and their returning to peers located behind the T-maze through one of two open return alleys

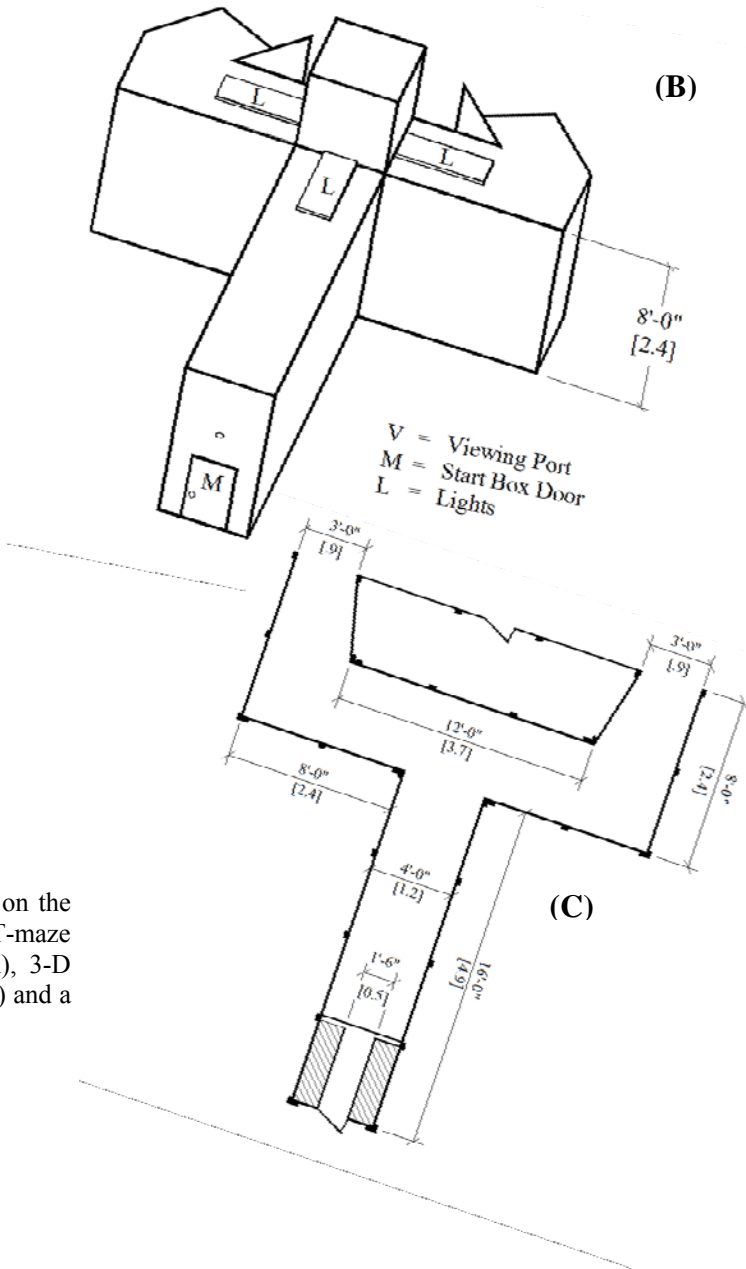
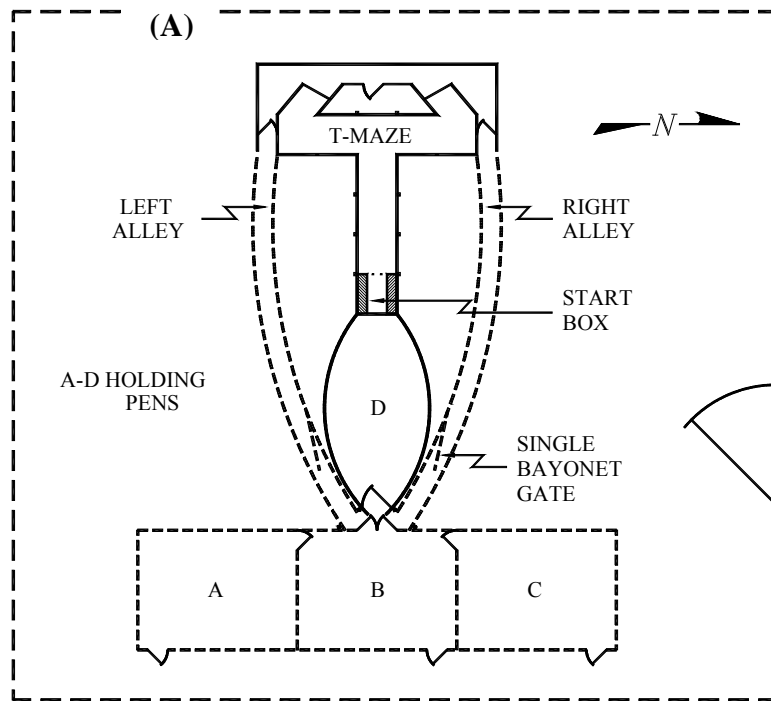
<i>Arm</i>	<i>Alley</i>		<i>Row Statistics</i>
	<i>Left</i>	<i>Right</i>	
<b><i>Left</i></b>			
Observed frequency	93	13	106
Expected frequency <sup>b</sup>	71.7	34.3	
Percent out of 309	30.1	4.2	34.3
Confidence interval <sup>c,d</sup>	(L = 25.0, U = 35.2)	(L = 2.0, U = 6.4)	(L = 29.0, U = 39.6)
<b><i>Right</i></b>			
Observed frequency	116	87	203
Expected frequency <sup>b</sup>	137.3	65.7	
Percent out of 309	37.5	28.2	65.7
Confidence interval <sup>c,d</sup>	(L = 32.1, U = 42.9)	(L = 23.2, U = 33.2)	(L = 60.4, U = 71.0)
<b><i>Column Statistics</i></b>			
Observed frequency	209	100	
Percent out of 309	67.6	32.4	
Confidence interval <sup>c,d</sup>	(L = 62.4, U = 72.8)	(L = 27.2, U = 37.6)	

<sup>a</sup>Overall Chi-square ( $\chi^2$ ) = 29.78.  $p < .0001$  with 1df

<sup>b</sup>Expected frequency under independence between T-maze choice and return alley choice

<sup>c</sup>L = lower confidence limit

<sup>d</sup>U = upper confidence limit



**Figure 1.** Three views of an enclosed plywood T-maze that sits directly on the soil for evaluating sheep laterality behavior. Corral and associated T-maze structures constructed of solid wood {—} and wire {--} not to scale (A), 3-D representation of the T-maze with height dimensions in feet and [meters] (B) and a plan view of the T-maze with length dimensions in feet and [meters] (C).

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