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The influence of observing a maternal demonstrator on the ability of lambs to learn a virtual fence

Tellisa Kearton[A,B], Danila Marini[A,B], Caroline Lee[A,B,*] and Frances C. Cowley[A]

For full list of author affiliations and declarations see end of paper

***Correspondence to:**
Caroline Lee
Commonwealth Scientific and Industrial Research Organisation, Agriculture and Food, Locked Bag 1, Armidale NSW 2350, Australia
Email: caroline.lee@csiro.au

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ABSTRACT

Context. In virtual fencing, where an animal learns to remain within a set area by responding to an audio cue in order to avoid receiving an aversive electrical stimulus, maternal learning may play a role in facilitating successful learning. **Aims.** This study aimed to investigate the effect of early observation of virtual fence engagement using a maternal demonstrator on the ability of lambs to later learn to respond correctly to a virtual fence. **Method.** Merino lambs (n = 114) were assigned to one of three treatments prior to being trained to a virtual fence: (1) lambs from experienced demonstrators, in which the lambs observed their mothers interacting with a virtual fence having been trained prior to lambing; (2) lambs from naïve demonstrators, in which lambs observed their mothers learning the virtual fence system; and (3) unexposed lambs, in which lambs had not encountered a virtual fence prior to being trained. Following weaning, lambs were trained to a virtual fence and responses to stimuli were recorded. **Key results.** The number of audio cue and electrical pulse stimuli received by the lambs did not differ across the three treatments ($P > 0.05$). There were no significant differences between the proportions of correct behavioural responses to the audio cue stimulus across the three treatment groups ($P > 0.05$). Logistic regression analysis of learning curves showed that lambs from naïve demonstrators displayed a change in behaviour and learned the correct response to the audio cue, while the unexposed lambs and lambs from pre-trained demonstrators did not. **Conclusions.** These results suggest that maternal influences may be influencing the ability of lambs to learn a virtual fence, although the training protocol was limited due to time, space, equipment and environmental constraints. **Implications.** This work may help to inform producers on management decisions for the application of the virtual fencing, such as enabling lambs to observe their mothers interacting with a virtual fence prior to weaning to enhance learning the virtual fence when applied later in life.

Keywords: animal learning, animal welfare, livestock management, livestock technologies, maternal learning, sheep, social learning, virtual fencing.

Introduction

Social learning already plays a role in sheep production and management, through both naturally occurring learning such as grazing and feeding behaviour (Provenza and Burritt 1991) and self-medicating behaviour (Sanga *et al.* 2011). Artificial means, such as the use of hand-raised sheep, aid in reducing fear behaviour in a flock when introducing new facilities or procedures. Social buffering is a process by which physiological and behavioural stress responses can be minimised by the presence of conspecifics (Colditz *et al.* 2012), resulting in improved recovery from distressing experiences (Kiyokawa *et al.* 2007). Social buffering plays a role in reducing fear of novel situations, though this may be linked to isolation stress rather than the influence of behaviour of conspecifics (González *et al.* 2013). The introduction of new technologies such as virtual fencing presents new opportunities to utilise social learning to reduce labour requirements and training time. Social learning has the potential to improve welfare outcomes through the facilitation of successful learning and increased controllability for the animal's situation (Kearton *et al.* 2020). For virtual fencing, the sheep learns through associative learning that by responding to the audio cue via a change in behaviour, either stopping or turning around, it can avoid receiving the electrical pulse stimulus.

The influence of social learning has been investigated in sheep, and it has been shown lambs are more susceptible to social influences than ewes (Thorhallsdottir *et al.* 1990*b*). At 6 weeks of age, lambs were found to be more influenced by their mothers than lambs at 12 weeks of age (Mirza and Provenza 1990). However, much of the previous work in sheep has focused on dietary and grazing behaviour and novelty using social models (Villalba *et al.* 2012). It is not currently known whether social influences may play a role in the training of sheep. There is little research currently available on the social transmission of a training method. There is evidence in cetaceans (Pryor 2001) and in canines (Slabbert and Rasa 1997; Nicol 1995) that suggests training or reinforcing exposed conspecifics in the presence of their naïve counterparts may facilitate learning of the task. In sheep and cattle, there has been early indications that adult herd animals are influenced by their peers in learning a virtual fence even when not directly experiencing the cues or stimuli (Kearton *et al.* 2019*b*; Keshavarzi *et al.* 2020; Marini *et al.* 2020). This has led to consideration of whether a maternal social model could play a role in enhancing the ability of lambs to learn a virtual fence.

The aim of this study is to investigate whether early exposure of lambs to the virtual fence as 'observers', with their dams acting as 'demonstrators', influences the rate and capacity of lambs to learn to respond to a virtual fence audio cue following weaning. It investigated the influence of naïve or previously trained mothers as pre-weaning demonstrators on the lambs' ability to learn a virtual fence system post-weaning, compared to a control group of lambs which had not been exposed to the virtual fence stimuli prior to weaning. It was hypothesised that lambs pre-exposed to the virtual fence audio cue while at foot with pre-trained mothers would later learn to exhibit the correct responses to the audio cue more rapidly than lambs pre-exposed with naïve ewes and naïve lambs.

Materials and methods

Experimental design and treatments

The experiment was undertaken at CSIRO's FD McMaster Laboratory, Armidale, New South Wales (NSW), Australia. The protocol and conduct of the experiment were approved by the CSIRO Armidale Animal Ethics Committee under the NSW Animal Research Act, 1985 (Animal Research Authority numbers 19/08 and 19/23).

To test the effect of maternal experience and demonstration on the ability of lambs to learn the virtual fence, three training phases were implemented for ewes and lambs: (1) pre-training: a pre-lambing training phase for pregnant ewes; (2) demonstration training phase: a post-lambing training for ewes with lambs at foot, which served as a refresher training for ewes thathad participated in pre-training, and an initial training for naïve ewes; and (3) lamb training phase for all lambs post-weaning.

At the experiment commencement, 140 pregnant ewes (and subsequently, their respective lambs, $n = 114$) were allocated to three treatment groups described as follows (Fig. 1):

- Lambs from naïve demonstrator ewes: Ewes which were unexposed to the virtual fence system before lambing (no pre-training), and were initially trained to the virtual fence with their lambs at foot in the demonstration phase, approximately 6 weeks following lambing. The lambs from these ewes subsequently participated in the lamb training phase.
- Lambs from pre-trained demonstrator ewes: Ewes which had been pre-trained prior to lambing and which also received refresher training with their lambs at foot in the demonstration phase, approximately 6 weeks following lambing. The lambs from these ewes subsequently participated in the lamb training phase.
- Unexposed lambs: Ewes completely unexposed to the virtual fence system, and lambs unexposed until subsequent participation in the lamb training phase

Ewes and ewe management

One hundred and forty (140) pregnant Merino ewes (mean weight at pregnancy scanning 46.3 kg ± 4.3) aged 3 years were selected from a resource farm flock based on gestational age (around 70 days) and the detection of a single fetus on pregnancy scanning.

Ewes were housed in large paddocks and supplementary fed with hay (a variety of lucerne, oaten and canola hay) and Megamin® mineral blocks (Ag Solutions) with limited grazing available due to drought conditions, and were monitored daily. All management and treatments applied were in accordance with standard farm practice. Prior to lambing, the ewes were moved to lambing plots in groups of 15–20 and monitored twice daily. Rations consisting of chick peas, field peas, cottonseed meal pellets, corn and hay were fed at a rate sufficient for a rising plane of nutrition for 40 kg ewes carrying a single pregnancy in the final month (1.1 dry sheep equivalents, DSE), and 2.1 DSE following lambing throughout lactation, based on requirements of 10.0 and 15.0 MJ ME/hd/day for ewes in mid pregnancy and early lactation respectively (Australian Wool Innovation and Meat & Livestock Australia 2008). Following lambing, the ewes and lambs were returned to large paddocks where they remained until the completion of the ewe training phases. They were then returned to the farm flock for marking and weaning. Following weaning, 115 lambs were returned at approximately 5 months of age for the initial lamb training

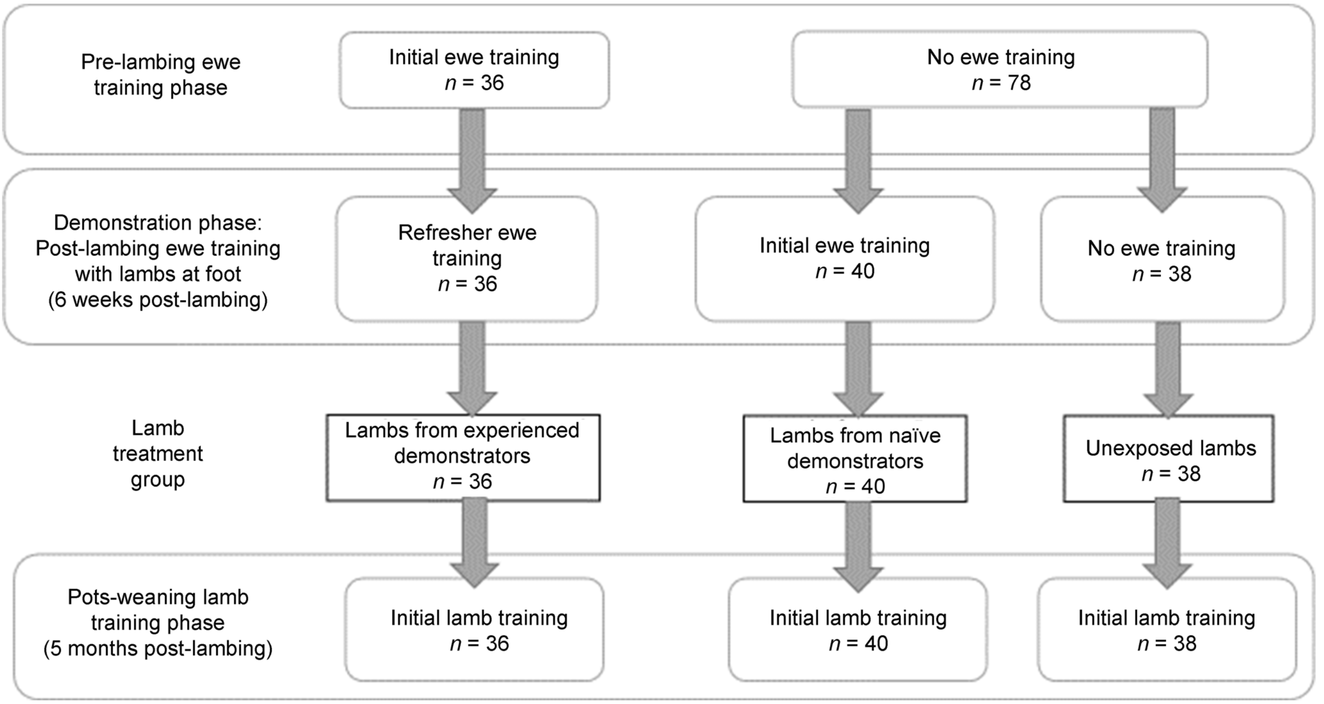

Fig. 1. Design of experiment, showing treatment groups and training phases.

phase, however one was excluded from the training due to being underweight, leaving 114 lambs to participate in the training. Lambs and ewes were identified throughout using visual ear tags and stock marking paint/spray.

Virtual fence training protocol

The following virtual fence training protocols were applied at each of the ewe pre-training, demonstration training and lamb training phases. Garmin dog control collars (Garmin TT15, Garmin Ltd., Kansas, KS, USA) were used to apply the virtual fence stimuli, consisting of an audio 'beep' cue (45–55 dB, 2.7 kHz) and an electrical pulse set to level 4 (320 V, 20 μs, 16 pulses/s for approximately 500–600 ms). This has been determined to be effective in training sheep to a virtual fence in previous work (Marini *et al.* 2018*b*) without adverse welfare experienced by the sheep from the stimuli (Kearton *et al.* 2019*a*). Two rectangular training arenas of approximately 15 m × 40 m were used (Fig. 2), with training sessions rotated between the arenas to reduce familiarisation and spatial associations developing which may influence the interactions with the virtual fence. The fences adjoining the yards and races were obscured with shade cloth to minimise distractions from handlers and other sheep. Encouraging movement of animals using attractants or humans was avoided where possible to reduce undue influences on the movement of the sheep around the arena. Natural movement was encouraged by scattering corn or Lucerne hay evenly around the arena to simulate grazing behaviour in the absence of pasture.

Ewes in the pre-trained and naïve groups, and all lambs, were trained to the virtual fence using the protocol described by Lee *et al.* (2007). During each training session the animal grazed within an arena, with the virtual fence located at a random point determined by the experimenters between the sheep and the return gate (Fig. 2). The entry and return gates were rotated to different ends of the arena between training sessions to mitigate sheep associating the virtual fence location spatially as opposed to learning to associate the audio cue with the location of the fence. Upon approach to the virtual fence, an audio cue was applied by the collar from a manual controller operated by experimenters. If the sheep did not stop or turn around, an electrical pulse was applied. A maximum of five electrical pulses were received in any single training session. An animal was considered to have learned the system when it consistently showed correct responses to the audio cue by either stopping forward movement or turning around. Video camera footage was taken of all training sessions using a Sony Handycam (Sony Handycam HDR-XR550, Sony Electronics Inc., San Diego, CA, USA) set up outside the training arena.

The number of approaches to the fence (events), the number of audio cues received and the number of electrical pulses received were recorded for each animal each day during training. Additional behavioural data was collected which characterised responses to the stimuli as either 'desirable' (stopping or turning) or 'undesirable' (continuing forward), and 'aversive' (jumping or running) or 'non-aversive' (stopping or turning).

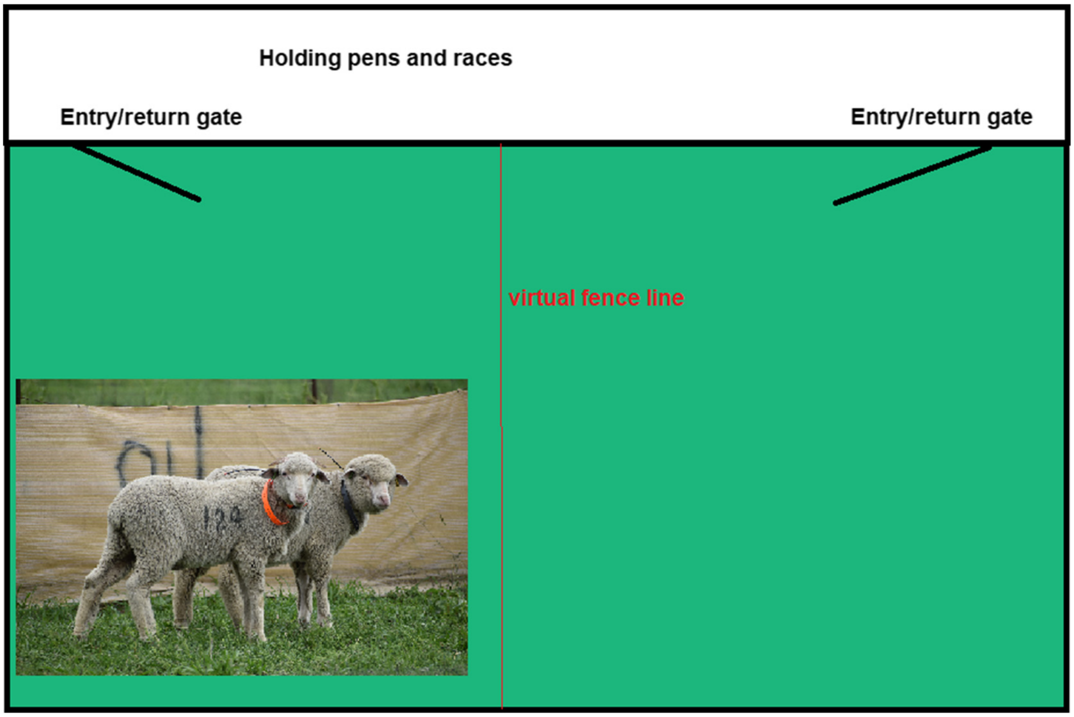

Fig. 2. Schematic of test arena where ewes and lambs underwent virtual fence training during all phases of the trial (initial ewe training, demonstration training and lamb training phase).

Pre-lambing ewe virtual fence interaction phase

All ewes in the pre-trained group were trained in pairs approximately 2 months prior to lambing in five sessions using the virtual fence training protocol outlined above, with each session lasting 3 min. The first four sessions were run on consecutive days, while the 5th was run approximately 2 weeks later to verify that the ewes had learned to respond consistently to the audio cue.

Demonstration of virtual fence interaction phase

Post lambing, the ewe demonstrators from the pre-trained and naïve demonstrator groups underwent a minimum of three (3) training sessions in pairs in the presence of the observers (their respective lambs), aiming to achieve at least one interaction with the fence (that is, where they stimulate at least the audio cue) per session. Each session was 3 min duration, with one session per day. As sheep learn the virtual fence (VF), they tend to stay within the boundaries of the fence, and so the number of interactions with the fence decreases (Marini *et al.* 2018*a*). In previous training with naïve adult sheep, 50% of animals achieve successful learning after an average of three interactions with the fence (Marini *et al.* 2018*a*), therefore it was deemed that a minimum of three interactions would be sufficient for the lambs to observe their mother's behavioural responses to the fence. While more interactions would be preferable to maximise the observations for the lambs, this was difficult to achieve given the limitations of time for training, equipment and space. Ewes were paired according to their training group so as to avoid confusion resulting from a trained and untrained animal being in the training arena at the same time. This is due to the likelihood that social interactions will influence responses to the virtual fence among peer groups (Kearton *et al.* 2019*b*). Throughout the demonstration phase, both pre-trained and naïve ewes interacted with the virtual fence in the presence of their lambs.

Before the demonstration phase training sessions, the ewes and lambs were penned separately for a short period so that ewes could have training collars fitted. The ewes and lambs were reunited prior to their entry into the test arena, and were released into the training arena in pairs of two ewes and their respective lambs. Lambs were not fitted with collars at this stage and were simply observing the behaviour of the two dams. Due to the necessity of handling lambs during the demonstration trainings, similar handling was also conducted with the unexposed lambs, so that all lambs experienced a similar amount of handling before the post-weaning lamb training phase.

Logistic regression analysis was performed to investigate the pattern of learning for ewes during in the pre-lambing and demonstration phases, as described for lambs, below.

Ewes that were pre-trained to the virtual fence when pregnant and then participated in a refresher training with lambs at foot in the demonstration phase displayed similar responses to the virtual fence during the demonstration phase as seen in previously-trained sheep in previous studies (Marini *et al.* 2018*a*; Table 1). When these ewes received refresher training with lambs at foot in the demonstration phase, they had retained much of their previous experience, as seen by 28 out of the 37 ewes responding to the audio cue alone (and therefore not receiving the electrical pulse) on the first interaction with the VF. During the demonstration phase the maximum number of interactions with the VF by an individual ewe in the pre-trained group was 11. During the 9th, 10th and 11th VF interactions, of those ewes still approaching the VF, more than 50% were receiving an electrical pulse. We were not able to fit a logistic regression for naïve ewes receiving initial training in the demonstration phase, with lambs at foot, however their interactions with the VF were similar to initial training observations in previous experiments (Marini *et al.* 2018*a*) with an average proportion of 0.43 electrical stimuli received for each interaction with the VF throughout training in the demonstration phase. Meanwhile, pre-trained ewes averaged 0.45 electrical stimuli received for each interaction with the VF during the demonstration phase.

Ewe behavioural data was analysed in RStudio (RStudio Team 2015; R Core Team 2018), did not meet assumptions of normality using a Shapiro–Wilk test for normality, and did not improve with transformation. This data was then analysed with a two-sample test for equality of proportions for correct behavioural responses to stimuli, a Fisher's exact test for aversive behavioural responses, and a generalised linear model with Poisson function (Bates *et al.* 2015) for number of stimuli received. There were no differences in the number of audio cues received per ewe during the demonstration phase for Days 1 ($P = 0.555$, $z = -0.590$), 2 ($P = 0.142$, $z = 1.467$), 3 ($P = 0.994$, $z = -0.007$) or across all days ($P = 0.529$, $z = -0.630$). There were no differences between the number of electrical pulses received per ewe for each day of training ($P = 0.139$, $z = 1.481$; $P = 0.085$, $z = 1.724$; $P = 0.676$, $z = -0.417$ for Days 1, 2 and 3 respectively) nor for the total number received for all days ($P = 0.084$, $z = 1.727$) (Table 2).

The proportion of correct behavioural responses from ewes with lambs at foot on each day of the demonstration phase was analysed with a two-proportions z-test due to the low numbers of animals and observations. There was a significant treatment difference within day for the proportion of correct responses to the audio cue (where the ewe changed behaviour and so avoided the electrical pulse) observed on Day 1 ($P = 0.016$, $z = 2.397$), but not Days 2 or 3 or overall (Table 3).

During the demonstration phase, the percentage of aversive responses exhibited by the pre-trained ewes analysed by group using a two-sample test for equality of proportions was lower than that exhibited by naïve ewes for Days 1 ($P = 0.000$, $z = 18.029$), and 3 ($P = 0.033$, $z = 4.525$), and for the total proportion of aversive vs non-aversive responses to the electrical stimulus ($P = 0.009$, $z = 6.803$; Table 3). The number of aversive responses to receiving the pulse stimulus, when analysed on a per ewe basis using Fisher's exact test, was not significantly different between naïve and trained ewes for Days 1, 2 and

Table 1. Logistic regression analysis for pre-trained ewes undergoing refresher training during the demonstration phase.

Upper asymptote	Lower asymptote	P, difference in asymptotes	Point of inflection	Slope	P, slope ≠ 0
0.21	0.48	0.37	5.81	−0.67	0.59

Logistic regression training parameters are presented for all training days for each group.
The upper asymptote indicates the proportion of ewes that received a stimulus at the beginning of training. The lower asymptote is the proportion of animals that continued to receive a stimulus after the point of inflection. The difference between the asymptotes is tested for significance. The slope indicates the speed of transition between the asymptotes. The point of inflection is the mean number of attempts it takes for half of the animals to change their behaviour.

Table 2. Ewe interactions with the virtual fence during the demonstration phase with lambs at foot.

Treatment	Day 1	Day 2	Day 3	Overall
Mean number of interactions with the virtual fence per ewe				
Naïve ewes	2.0 ± 1.2a	2.8 ± 1.1a	1.7 ± 0.9a	2.2 ± 1.3a
Pre-trained ewes	2.2 ± 1.1a	2.3 ± 1.2a	1.7 ± 1.1a	2.04 ± 1.2a
P (*z*)	0.555 (−0.590)	0.142 (1.467)	0.994 (−0.007)	0.529 (0.630)
Mean number of electrical pulses received per ewe				
Naïve ewes	0.7 ± 0.5a	1.1 ± 0.9a	0.6 ± 0.7a	0.80 ± 0.8a
Pre-trained ewes	0.5 ± 0.6a	0.7 ± 0.7a	0.6 ± 0.8a	0.6 ± 0.7a
P (*z*)	0.139 (1.481)	0.085 (1.724)	0.676 (−0.417)	0.084 (1.727)

Values sharing a common letter within a column within section were not significantly different and data were analysed by group ($P < 0.05$).

Table 3. Behavioural responses to virtual fence stimulus for pre-trained and naïve ewes during the demonstration phase training, with lambs at foot.

Treatment	Day 1	Day 2	Day 3	Overall
Mean number of aversive responses per ewe receiving pulse				
Pre-trained ewes	0.4 ± 0.5a	0.2 ± 0.4a	0.2 ± 0.4a	0.3 ± 0.4a
Naïve ewes	0.7 ± 0.4b	0.3 ± 0.5a	0.3 ± 0.5b	0.5 ± 0.5b
P (z)	0.000 (18.029)	0.237 (1.397)	0.033 (4.525)	0.009 (6.803)
Mean number of correct behavioural responses per ewe receiving audio				
Pre-trained ewes	0.7 ± 0.3a	0.6 ± 0.4a	0.6 ± 0.4a	0.6 ± 0.4a
Naïve ewes	0.4 ± 0.4b	0.6 ± 0.3a	0.6 ± 0.4a	0.5 ± 0.4a
P (z)	0.042 (4.133)	0.784 (0.076)	0.846 (0.038)	0.197 (1.668)

Correct = stop or turn; aversive = jump or run forward; non-aversive = stop or turn. Values sharing a common letter within a column within a section were not significantly different and data were analysed by group as a proportion ($P < 0.05$).

3 (P = 0.061, 0.604 and 0.495 respectively), and the total number of aversive responses per sheep across all days of training was higher for naïve ewes than pre-trained ewes during the demonstration phase (P = 0.032) (Table 3, analysed by group).

Post-weaning lamb virtual fence training phase

Following weaning, a total of 115 lambs, approximately 5-month-old, were retained for training (unexposed lambs $n = 36$, lambs from pre-trained demonstrators $n = 38$, lambs from naïve demonstrators $n = 40$). Lambs were divided into two cohorts according to date of birth, with the first cohort being trained during the first week and the second cohort being trained during the following week.

Lambs were trained to the virtual fence using the protocol described above (section *Virtual fence training protocol*).

Statistical analysis of lamb training

The statistical analysis software R (R Core Team, ver. 3.3.3, R Foundation for Statistical Computing, Vienna, Austria), and packages ggplot2 (Wickham 2016), dplyr (Wickham *et al.* 2018), tidyverse (Wickham 2017) and multcomp (Hothorn *et al.* 2008) were used for analysis of the lamb training data.

Total number of audio cues and electrical stimuli received per animal over the lamb training period were analysed for each day of training, with data not meeting normality assumptions using a Shapiro–Wilk test but not able to be improved with transformation. As a result, data of audio cues and electrical stimuli received per sheep were subsequently analysed using a generalised linear model with a Poisson distribution (Bates *et al.* 2015).

To analyse the proportion of interactions resulting in a correct response to the audio cue, i.e. where the animal avoided the electrical stimulus after receiving the audio cue, data were calculated for each animal for each day as:

$$\frac{(n \text{ audio cues received} - n \text{ electrical stimuli received})}{n \text{ audio cues received}}$$

Due to the low numbers of animals and interactions recorded, the proportion of correct behavioural responses by lambs for each day of training was analysed using a three-sample test for equality of proportions. Values of $P < 0.05$ were considered significant for the purposes of interpretation of this data.

Logistic regression analysis was performed to investigate the pattern of learning across the treatment groups. The logistic regressions were fitted to the data using the non-linear least squares function, as previously described Lee *et al.* (2009). The application of the audio cue and electrical stimulus from each test day was paired to create a binary variable, starting at the first audio cue received. The audio event number X_{ij} and a binary variable Z_{ij} is zero if the sheep received an audio cue not followed by an electrical stimulus during an interaction with the VF, and one if the audio cue was followed by an electrical stimulus. A general logistic curve of the form

$$\pi = a + \frac{c}{1 + \exp(-b(x - m))}$$

was fitted, where π is the probability that $Z_i = 1$. The upper asymptote is the sum of a and c, and is the proportion of animals receiving an electrical stimulus on the first interaction. The lower asymptote, a, is the proportion of animals that still receive an electrical stimulus after behavioural change. The slope parameter, b, is related to the rate of behavioural change, with a negative slope indicating that the proportion of the animals receiving an electrical stimulus during a VF interaction decreases with repeated interactions, indicating that the animals are increasingly responding correctly to the audio cue. The point of inflection, m, is the number of interactions it takes for half of the animals to change their behaviour. This is the midpoint on the curve where behavioural change is observed. No constraints were applied in fitting the curve, allowing for the slope parameter to be greater than zero and the asymptote to be outside the meaningful range of zero to one.

Results

Application of stimuli

The number of audio cues received by lambs showed no significant effect of treatment when lambs from naïve demonstrators were compared to lambs from pre-trained demonstrators, for training Days 1, 2, 3 and overall ($P = 0.427$, $z = -0.795$; $P = 0.890$, $z = -0.139$; $P = 0.090$, $z = -1.695$; and $P = 0.103$, $z = -1.631$ respectively; Table 4). Similarly, lambs from pre-trained demonstrators did not differ significantly in the number of audio cues received when compared to the unexposed lambs for Days 1, 2 and 3 of training and overall ($P = 0.097$, $z = 11.130$); $P = 0.196$, $z = 1.294$; $P = 0.002$, $z = -3.082$ and $P = 0.838$, $z = 0.204$ respectively; Table 4).

The number of electrical pulse stimuli received did not differ between lambs from naïve demonstrators and those of unexposed lambs for Days 1, 2 and 3 of training and overall ($P = 0.794$, $z = -0.262$; $P = 0.570$, $z = -0.568$; $P = 0.499$, $z = -0.676$; $P = 0.397$, $z = -0.848$ respectively; Table 4). When lambs from pre-trained demonstrators were compared with unexposed lambs there were no significant differences recorded for the number of electrical pulses received on Days 1, 2, 3 and overall ($P = 0.708$, $z = 0.375$; $P = 0.191$, $z = 1.307$; $P = 0.066$, $z = -1.840$; $P = 0.901$, $z = 0.124$ respectively; Table 4).

Individual behavioural analysis was not possible due to low numbers of sheep and stimuli received, therefore behaviours were analysed in terms of the proportion of correct responses shown out of all audio cues received in a training session. The proportion of correct responses recorded for the lambs for each day of training showed no significant differences across all treatment groups for Days 1, 2 and 3 ($P = 0.659$, $z = 0.833$; $P = 0.561$, $z = 1.155$; $P = 0.324$, $z = 2.251$; $P = 0.325$, $z = 2.248$ respectively; Table 4).

Lamb logistic regression

The logistic model did not fit the data well for the lambs from pre-trained demonstrators or the unexposed lambs, as can be seen by the observed proportions (numerals on the graph) not being close to the line (Fig. 3*a*–*c*). Very few lambs achieved large numbers of interactions with the VF (*x*-axis, Fig. 3), and this made data of the proportion of lambs receiving the electrical pulse after the audio cue at these interactions highly variable. Related, the slope of the regression did not differ from zero for any group, which would normally suggest that there was no change in behaviour. However, a point of inflection, indicating a change in behaviour once sheep began to learn the VF, was able to be fitted for all groups. The upper asymptotes were low for all groups, indicating that few lambs received the electrical pulse, from the beginning of training. A low value for the lower

Table 4. Lamb interactions with and responses to the virtual fence during post-weaning training.

Treatment	Day 1	Day 2	Day 3	Overall
Mean number of interactions with the virtual fence per lamb				
Unexposed lambs	3.6 ± 2.3	2.5 ± 2.1	3.4 ± 2.2	3.2 ± 2.2
Lambs from naïve demonstrators	3.2 ± 2.1	2.5 ± 2.0	2.6 ± 2.1	2.9 ± 2.2
P (*z*)	0.427 (–0.795)	0.890 (–0.139)	0.090 (–1.695)	0.103 (–1.631)
Lambs from pre-trained demonstrators	4.7 ± 3.1	3.1 ± 1.6	2.0 ± 1.4	3.3 ± 2.4
P (*z*)	0.097 (11.130)	0.196 (1.294)	0.002 (–3.082)	0.838 (0.204)
Mean number of electrical pulse stimuli received per lamb				
Unexposed lambs	1.4 ± 0.9	1.0 ± 1.1	1.1 ± 0.8	1.2 ± 0.9
Lambs from naïve demonstrators	1.3 ± 0.7	0.9 ± 1.0	1.0 ± 0. 8	1.1 ± 0.9
P (*z*)	0.794 (–0.262)	0.570 (–0.568)	0.499 (–0.676)	0.397 (–0.848)
Lambs from pre-trained demonstrators	1.5 ± 0.9	1.4 ± 1.3	0.7 ± 0.7	1.2 ± 1.0
P (*z*)	0.708 (0.375)	0.191 (1.307)	0.066 (–1.840)	0.901 (0.124)
Mean number of correct behavioural responses per lamb receiving audio				
Unexposed lambs	0.4 ± 0.4	0.4 ± 0.4	0.3 ± 0.3	0.4 ± 0.4
Lambs from naïve demonstrators	0.3 ± 0.3	0.5 ± 0.4	0.3 ± 0.4	0.3 ± 0.4
Lambs from pre-trained demonstrators	0.3 ± 0.3	0.5 ± 0.4	0.4 ± 0.4	0.4 ± 0.4
P (*z*)	0.659 (0.833)	0.561 (1.155)	0.324 (2.251)	0.325 (2.248)

Number of interactions and pulses received per animal, proportion of correct responses analysed by group.

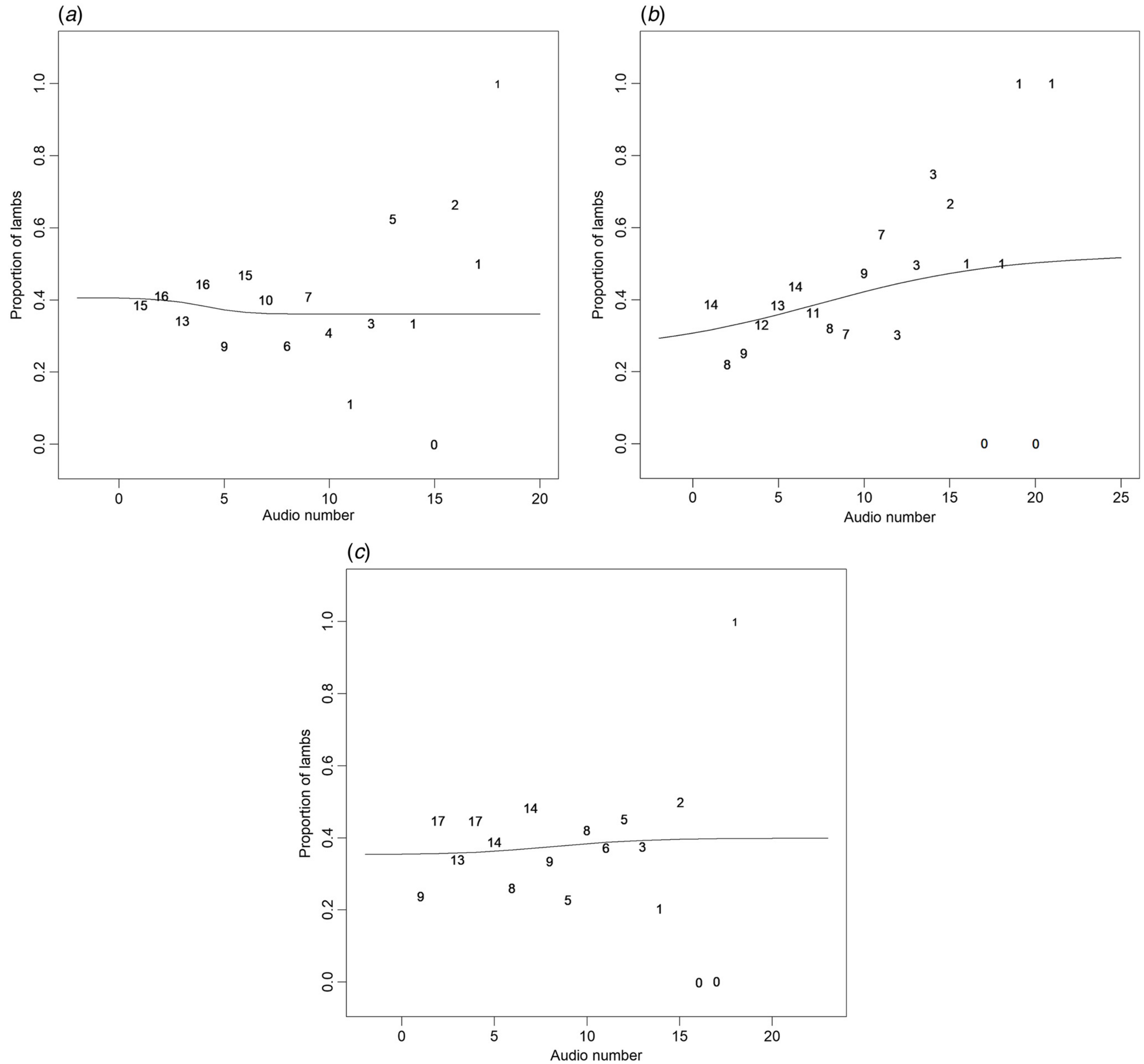

Fig. 3. Logistic regression of (*a*) lambs from naïve demonstrators, (*b*) lambs from pre-trained demonstrators and (*c*) unexposed lambs failing to respond appropriately to virtual fence audio cue during the demonstration phase. *y*-axis is the proportion of lambs that received an electrical pulse after receiving an audio cue. *x*-axis is number of interactions with the virtual fence throughout the entire training. The numerals are the number of animals that received an electrical stimulus for that event number, and their placement with respect to the *y*-axis indicates the proportion of animals receiving the electrical stimulus. The line indicates the fit of the logistic regression.

asymptote indicates that animals are responding correctly to the audio cue. However, because the difference in asymptotes was not significant, and the slopes of the regressions did not differ from zero, in the present study it is not possible conclude whether this was indicative of lambs learning and thus changing their behaviour. Lambs from naïve demonstrators were the only group to have a decline in value from the upper to the lower asymptote. The fitted slope for the lambs from naïve demonstrators was very high (0.89), even though it did not differ from zero, and the point of inflection for this group occurred much sooner than for lambs from pre-trained demonstrators and unexposed lambs. These observations suggest that lambs from naïve demonstrators learned the VF more successfully than the other groups. The fitted regressions for lambs from pre-trained demonstrators and unexposed lambs showed an upward trend in the logistic model, indicating that the proportion of lambs receiving an electrical stimulus increased, although the change in behaviour was not significant (Table 5, significance of slope).

Table 5. Lamb training logistic regression parameters for all training days for each group.

Group	Upper asymptote	Lower asymptote	*P*, difference in asymptotes	Point of inflection	Slope	*P*, slope ≠ 0
Lambs from pre-trained demonstrators	0.25	0.53	0.82	7.36	−0.18	0.85
Lambs from naïve demonstrators	0.41	0.36	0.76	3.98	−0.95	0.89
Unexposed lambs	0.35	0.39	0.87	8.19	−0.39	0.92

The upper asymptote indicates the proportion of lambs that received a stimulus at the beginning of training. The lower asymptote is the proportion of animals that continued to receive a stimulus after the point of inflection. The difference between the asymptotes is tested for significance. The slope indicates the speed of transition between the asymptotes. The point of inflection is the mean number of attempts it takes for half of the animals to change their behaviour.

Discussion

Adult cattle have utilised social facilitation when learning a virtual fence (Keshavarzi *et al.* 2020) and there is some evidence that this may also occur in sheep (Kearton *et al.* 2019*b*), therefore it is reasonable to surmise that some maternal facilitation of learning may occur in the context of virtual fence training. The results of the present study suggest that, contrary to the initial hypothesis, lambs which observed pre-trained dams using the virtual fencing system did not have improved rates of learning the virtual fence compared to unexposed lambs which had no prior experience of the virtual fence. Lambs which had observed naïve demonstrators learning the virtual fence appeared to benefit from their experience.

The logistic regression analysis showed that unexposed lambs followed a similar learning pattern to adult sheep in similar studies, which showed no clear point of inflection at which there is a change of behaviour evident (Marini *et al.* 2018*a*). The exposed lambs differed from this pattern in opposite ways. The learning pattern analysis showed that the lambs which had observed naïve demonstrators reached a clear inflection point (the point at which a change in behaviour occurs) when compared with lambs which had no prior experience with the virtual fence and did not show a clear turning point. However, lambs from pre-trained demonstrators became *less* likely to change their behaviour, resulting in an increased probability of receiving an electrical stimulus. It is important to note that these treatment group differences were not statistically significant. These trends may be attributable to a number of factors, such as the novelty of the audio cue, the number of demonstrator ewe interactions with the virtual fence, the number of incorrect responses to the audio cue (which resulted in the demonstrator ewe receiving the electrical pulse), and the aversiveness of the demonstrator ewe's response to the electrical pulse.

The clear turning point of the logistic regression curve for the lambs from naïve demonstrators may correspond with a reduction in the novelty aspect of the audio cue. In previous studies with merino sheep it has been found that sheep are alert to the audio cue upon first hearing it (Kearton *et al.* 2019*a*) and this increased vigilance to the novel sound may influence initial responses as they appraise it (Désiré *et al.* 2004). Once the novel sound is perceived to be non-threatening in itself, then sheep more confidently move forward and encounter the electrical pulse, and the result of fewer audio cues experienced by the naïve group on the first day of training may be reflective of this. It is possible that the early exposure to this benign sound during training of the mothers may reduce the novelty factor so that the naïve lambs more rapidly progress to the associative learning phase of the virtual fence. Of importance, however, is that this early exposure to the audio cue did not appear to benefit the lambs from pre-trained demonstrators.

Because the electrical pulse, which the demonstrators received only after failing to correctly respond to the audio cue, was silent, maternal facilitation of learning relied on the lamb's perception of their maternal demonstrator's reaction to receiving the audio cue (which the lamb could hear). For demonstrators which had learned the VF successfully, the maternal response to the audio cue demonstrated to the lamb was stopping or turning (a correct response). For demonstrators which had not yet learned the VF successfully, the response demonstrated to the lamb was a reaction to the electrical pulse. On the first day of the demonstration phase, pre-trained ewes were more likely to demonstrate a correct response to the audio cue than naïve ewes, and less likely to demonstrate an aversive response when they did receive the electrical pulse. However, naïve ewes increased their frequency of demonstration of correct responses and reduced their frequency of aversive responses on Days 2 and 3.

The lack of aversive responses to the paired VF stimuli by the pre-trained ewes may have hindered the ability of their lambs to understand the significance of the behavioural response in relation to the audio cue and subsequent application of the electrical pulse. This behaviour by the demonstrator ewes may have resulted in a lack of clarity for the lambs as to the reasons for the ewe's reaction to the audio cue, and a weaker association by the lambs of the audio cue with the subsequent change in behaviour (stopping or turning around). The pairing of the audio cue with the electrical pulse is a key aspect in learning the virtual fence (Marini *et al.* 2019). Previous work in cattle has investigated the role of social facilitation of virtual fence learning, and shows that observations of behavioural responses of group members interacting with the fence helps the observers to learn (Keshavarzi *et al.* 2020), showing that observation of strong behavioural responses can assist group members to learn more quickly. By responding correctly to the audio cue, and therefore not demonstrating the paired experience of the audio cue and electrical pulse, the pre-trained ewes were less likely to demonstrate this strong association to their lambs. As a result, it appears the lambs from pre-trained demonstrators may have experienced difficulty in interpreting the significance of the audio cue.

Conversely, the naïve ewes were able to demonstrate the active learning experience of associating the audio cue with receiving an electrical pulse encouraging them to change their behaviour. Therefore, they were able to act as more effective demonstrators to their lambs. By observing the ewe's reactions to the negative stimuli, the lambs were more likely to understand the association between audio cue and the negative behavioural response of the ewe. These results were borne out in the lamb training during which the lambs from naïve demonstrators learned to respond correctly to the VF audio cue after fewer interactions with the VF than both the unexposed lambs and the lambs from pre-trained demonstrators. So while the reduced novelty of the audio cue may have played a role in the reduced time taken to learn for the lambs from naïve demonstrators, it appears that the observation of the learning process may be more influential on the turning point at which lambs begin to show correct responses to the audio cue.

The observational interaction criteria were slightly modified during the demonstration phase in response to the behaviours exhibited by the ewes. More interactions with the virtual fence were preferred, *ex ante*, so as to maximise the exposure the lambs received to these interactions, however, the behavioural responses of the ewes changed over the course of the training and they were less willing to move around the training arena after having had several interactions with the virtual fence. Therefore, the number of interactions was revised down to a minimum of three to more closely reflect the conditions under which adult naïve sheep learn to avoid a virtual fence. This was an unexpected limitation occurring due to the need for a controlled experimental environment, it is anticipated that this would be less likely to occur under normal paddock grazing conditions due to larger area and greater flock influences.

The consequence of the differences in ewe interactions with the VF between the pre-trained and naïve ewes was fewer opportunities for the lambs to learn as the mother's inclination to interact with the fence diminished, and this was particularly noticeable in the pre-trained demonstrators receiving fewer electrical stimuli than the naïve demonstrators. This is likely to have impacted on the ability of lambs to benefit from observing the mother's behaviour. In previous feed aversion studies, lambs have been exposed to social influences for 5 min per day for 5 days (Thorhallsdottir *et al.* 1990*a*), which has been sufficient to impact on previously-trained aversions, however exposure to unconditioned peers can quickly ameliorate these aversions (Provenza and Burritt 1991). Another maternal learning study using trained drug detection dogs utilised as many as 14 interactions over a period of 6 weeks in which to observe their trained mother demonstrating a learned behaviour (Slabbert and Rasa 1997), and it is likely that motivation and reward played a role in the successful transfer of behaviours in that research compared with the pre-trained demonstrators in the present study. While Slabbert and Rasa (1997) did not record specific behaviours, the pups saw the mother being praised by the handler, and it is possible that the mother exhibited behaviours which facilitated the pup's understanding. In contrast, the lambs from pre-trained demonstrators in this study did not observe as great a proportion of aversive reactions to the VF stimuli as did the lambs from naïve demonstrators, suggesting that it may not be enough to have the demonstrator already know the correct response to allow the transmission of information, particularly in the context of the artificial training which may suppress the expression of normal behaviours.

There were limitations in the interpretation of the findings due to the training protocol itself, such as the artificial arena training area and the need for manually controlled collars. This may have been the cause of poor evidence of a learning progression comparable with that seen in previous studies performed on groups of sheep naturally grazing in a paddock (Marini *et al.* 2018*a*), except in lambs from naïve demonstrators.

This work has implications for the understanding of the role that maternal learning may play in the demonstration of associative learning within the context of virtual fencing. While previous work in social learning in sheep has focused on feeding behaviour and the passive transfer of information (Provenza and Burritt 1991), the importance of understanding more about the role of social learning becomes more evident as technology that requires more active animal participation plays a greater role in livestock management. The results suggest that observing maternal demonstrators actively learning the virtual fence may have a positive effect on the future learning of the lambs, and it may be of benefit to retain lambs at foot when first implementing a virtual fence system for ewes. Further work on the influence of maternal learning in the context of training and associative learning is recommended. In the context of virtual fencing, further investigations of maternal and peer learning with larger groups in a grazing setting, and over a longer period of time would be beneficial to overcome the limitations of this study.

Conclusions

The results of this study suggest that early exposure to a virtual fence may influence the way in which lambs go on to learn a virtual fencing system. Further, the experience of observing the ewes undergoing initial training has the potential to be beneficial to lambs compared to observing previously trained ewes interacting with a virtual fence. This work may assist researchers and producers when making decisions about timing of implementing virtual fencing into their livestock management systems and whether to keep trained ewes together with naïve lambs for example. However, further investigation is needed to examine whether these findings are replicable in a flock situation.

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Data availability. The data produced from this work will be made available on the CSIRO data access portal. Available at: https://data.csiro.au/.

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Author affiliations
[A]School of Environmental and Rural Science, University of New England, Armidale, NSW 2350, Australia.
[B]Commonwealth Scientific and Industrial Research Organisation, Agriculture and Food, Locked Bag 1, Armidale NSW 2350, Australia.