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Exposure to humans after weaning does not reduce the behavioural reactivity of extensively reared Merino lambs

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ABSTRACT

Human-directed fear in extensively reared sheep is often high due to the aversive nature of common husbandry procedures and infrequent interactions with humans. This study investigated whether additional human exposure provided to weaned lambs reduced human-directed fear and behavioural reactivity. Ninety Merino lambs were either exposed to low or moderately stressful human exposure sessions, or had no additional human contact, and their fear responses and behavioural reactivity to humans, a startle stimulus and confinement were tested. Overall, the imposed interventions did not reduce behavioural reactivity during these tests, suggesting fear towards humans had not been altered.

1. Introduction

As a prey species, sheep are innately fearful of humans (Dwyer, 2004; Rushen, Taylor, and de Passillé, 1999). For extensively reared sheep in Australian commercial systems the first encounter with humans is generally at lamb marking at five to eight weeks of age, then at weaning at two to three months of age. Both events are highly aversive due to the experience of fear and/or pain (Grant, 2004; Mellor and Stafford, 2000; Freitas-de-Melo and Ungerfeld, 2016). Given that experiences early in life are known to influence future behaviours (Daskalakis et al., 2013; Dietz et al., 2018; Lyons et al., 2010), both events are likely to contribute to high human-directed fear. The ongoing absence of regular, close human contact in extensive systems sustains that fear (Dwyer, 2009; Turner and Dwyer, 2007). Producing a poor welfare state on its own, fear can also cause animals to become reactive and difficult to handle (Grandin and Shivley, 2015), can alter the experience of pain (Guesgen et al., 2013; Steagall et al., 2021), and chronic fear-induced stress has also been implicated in reduced production outcomes (Hemsworth and Coleman, 2010; Rushen et al., 1999).

Interactions with humans that occur during early, sensitive developmental periods may reduce human-directed fear and/or behavioural reactivity, providing both welfare and production benefits. For example, lambs that are handled within the first four weeks of life show a greater affinity towards humans than non-handled lambs (Markowitz et al., 1998; Coulon et al., 2015). Bateson (1979) has suggested that sensitive periods may represent any period of rapid behavioural reorganisation where the developing animal is particularly sensitive to external stimuli as a consequence of experiencing stress. In extensive sheep systems, lambs are permanently removed from their dams by abrupt weaning at around two to three months of age. As weaning is known to be highly stressful (Weary et al., 2008; Freitas-de-Melo and Ungerfeld, 2016), it may be a period where lambs are easily influenced by human exposure and could provide a more practical exposure window in commercial systems than the first few weeks of life. Indeed, increased neutral and positive human exposure during this period has been shown to reduce cortisol and neutrophil/lymphocyte ratios which are physiological indicators of acute and chronic stress respectively (Pascual-Alonso et al., 2015).

An animal that repeatedly experiences a low intensity, stress-inducing stimulus may habituate to it (Grissom and Bhatnagar, 2009) and over time exhibit a reduced fear response towards it. For example, sheep are known to habituate to a familiar human, resulting in reduced avoidance behaviours (Mateo et al., 1991; Destrez et al., 2013). However, as habituation is stressor specific it does not reduce behavioural reactivity towards other stimuli (Hargreaves and Hutson, 1990; Mateo et al., 1991). A more generalised reduction in fear responses may be achieved through exposure to moderate but manageable stressful challenges during early developmental stages. A phenomenon known as stress inoculation involves repeated bouts of such challenges that prime the individual's stress response systems in preparation for future stress

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(Franklin et al., 2012; Parker and Maestripieri, 2011). Too little or too much stress however, may result in stress vulnerability, where insufficient experience does not adequately prepare the individual for challenges later in life, and repeated experience with failing to cope with adversity leaves the individual vulnerable to stress (Daskalakis et al., 2013; Broom, 2001). Experimentally, stress inoculated animals have been shown to demonstrate lower behavioural reactivity through improved arousal regulation and inhibitory control which persisted for at least several months (Parker and Maestripieri, 2011) and into adulthood (Santarelli et al., 2017).

We hypothesised that sheep exposed to a human as a low intensity stressor would habituate to them, resulting in reduced human-directed fear responses, but have behavioural responses to other fearful situations that were comparable with the control group. We further hypothesised that sheep exposed to a human as moderate intensity stressor would show reduced behavioural reactivity in fearful situations, both including and excluding humans, compared to a control group. The overall aim of this study was to identify if exposure to different levels of stress during a potential sensitive period, immediately post weaning, would reduce the behavioural reactivity of sheep. Sexual dimorphism in fear responses has been reported in two- to six-month old lambs that indicate females are more fearful and more active in the presence of humans (Boissy et al., 2005) and show higher behavioural reactivity than males when startled and isolated from conspecifics (Viérin and Bouissou, 2003). The current study also measured the effect of sex to identify any confounding or interaction (with treatment) effects.

2. Method

2.1. Ethics approval

This study was approved by the University of Melbourne's Faculty of Veterinary and Agricultural Sciences Animal Ethics Committee, ethical review number 1814583.5.

2.2. Animals and housing

The lambs in the current study were of Merino breed, born on an Australian commercial farm and raised extensively since birth. At weaning 90 lambs were drafted from all lambs born in that season (approximately 1000) by alternately selecting lambs from the central, front and rear areas of a holding pen. As lambs were born up to eight weeks apart, any lamb weighing less than 18 kg or more than 35 kg was excluded from selection on the assumption they were among the youngest and oldest in the group, and the estimated range of the selected lambs at weaning (average weight 25 kg, 45 ewe-lambs, 45 castrated male lambs) was 2–3 months of age. After weighing, lambs were allocated into nine groups (n = 10 per group), balanced for sex using a random number generator, and with an even distribution of weight across groups (Table 3). Each group was assigned to one of three treatments (n = 30 per treatment). Groups were transported to nine outdoor treatment pens where the lambs were housed for eight weeks. Each pen measured approximately 100 m^2 and were a minimum distance of 45 m from each other meaning there was some visual and auditory overflow between neighbouring pens. Lambs from all pens were monitored twice daily from a distance and had brief human exposure during hay delivery on three occasions during the treatment period. Water and lucerne hay were available ad libitum.

2.3. Project timeline

Lambs were left undisturbed for one week to acclimate to the treatment pens (experimental week 1 – see Fig. 1). The treatment phase ran for six weeks (experimental weeks 2–7) and testing was conducted the following week across four days (experimental week 8). Experimental week 1 began in late October and experimental week 8 ended in mid-December, during late spring/early summer in central Victoria, Australia.

2.4. Treatments

The three treatment categories were controls (CON) and two human exposure treatments; LOW and MOD (Fig. 1).

(I) CON lambs had no additional human contact for experimental weeks 1–7.
(II) LOW lambs experienced low intensity, passive, predictable human behaviour which was intended to develop habituation towards a human presence. For the first two weeks of treatment (experimental weeks 2 and 3) the experimenter entered each pen, walked slowly to the centre and sat on the ground. For the remainder of the treatment (experimental weeks 4–7), the experimenter remained standing. After 5 mins the experimenter slowly and quietly exited the pen.
(III) MOD lambs experienced moderate intensity, active, unpredictable human behaviour which was intended to be a stressful yet manageable challenge without causing stress vulnerability. The experimenter entered the pen and performed a combination of upper and lower body movements and a noise for the duration of the session (Table 1). Combinations were

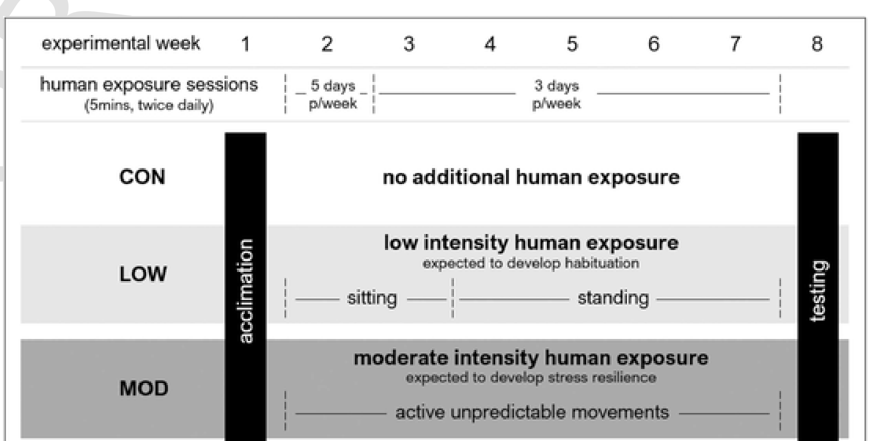

Fig. 1. Timeline for experimental weeks 1–8 indicating the acclimation, treatment and testing periods and a description of each treatment type. The frequency of human exposure sessions for the LOW and MOD groups was five days in the first week of exposure and three days per week for the remainder of the treatment phase. Each exposure day consisted of an AM and PM session lasting 5 min each.

Table 1
The list of activities used to randomly assign unpredictable behavioural combinations for the MOD treatment group. Each day included one activity from each of the three activity groups. Both sessions within the same day used the same behavioural combination.

Activity group	Lower body	Upper body	Noise
Activity	Stationary	Stationary	No Noise
	Moving Quickly	Waving arms above head	Shouting
	Big Steps	Waving a scarf	Music
	Dribbling a Ball	Clapping (also the noise)	Bell

randomly assigned prior to commencement of the trial and varied each day but were consistent for both sessions within the same day.

Human exposure sessions for LOW and MOD lambs were conducted twice a day for 5 min per session. There were five exposure days (ten sessions) in week 1 and three exposure days per week (six sessions) in weeks 3–7 (Fig. 1).

2.5. Testing

Testing was conducted eight weeks after weaning, when lambs were 4–5 months of age. Four behavioural tests that measure fear and reactivity that have been previously validated in sheep were conducted in the following fixed order: attention bias (AB), moving human (MH), startle (S) and an isolation box/temperament (IB) test (see full descriptions below). The first three tests were conducted in an enclosed arena measuring 5 × 5 × 2 m with separate entry and exit doors (Fig. 2). Walls were constructed with plywood and a grid of twenty-five 1 × 1 m squares was spray painted on the ground. A small pile of hay was placed in the centre square for the AB test. The isolation box was a modified weigh crate measuring 1.25 × 0.5 × 1 m that was enclosed on all 4 sides and partially enclosed on top. Two GoPro HERO3 Silver edition cameras (GoPro, Inc., San Mateo, California, U.S.) were mounted at floor level at each end of the box and two on the top of opposing walls of the arena for continuous recording. One pen of lambs (n = 10) at a time was moved quietly and calmly on foot approximately 200 m from their treatment pen to a holding yard adjacent to the arena. Each lamb was weighed and spray marked (Dy-Mark steadfast stock mark aerosol paint) for identification on camera, and the group was left to settle in the yard for 30 min. Individuals were randomly selected and ushered into the arena to commence testing. Vocalisations for all tests were recorded on the day and all other behaviours were logged from video playback. Once all ten lambs of a pen had been tested, they were walked back to their treatment pen and the process was repeated with the next pen. Pens 1–3 were tested on day one, pens 4 and 5 were tested on day two, pens 6–8 on day three and pen 9 on day four. Lambs that were to be tested in the morning and midday sessions (pens 1, 2, 4, 6, 7 and 9) were fasted the day prior to ensure the motivation to eat was present for the AB test. Lambs that were to be tested in the late afternoon sessions (3, 5 and 8) were fed a half ration the day prior to ensure they weren't restricted from food for too long, but that the motivation to eat was still present.

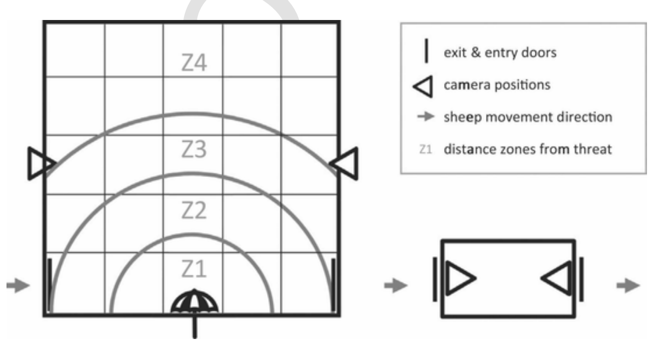

Fig. 2. Layout of the testing arena (left) and the isolation box (right) indicating the location of doors, positioning of cameras, direction of animal movement through the tests and distance zones from the umbrella as measured with SZones.

2.5.1. Attention bias (AB) test

An AB test has been validated in sheep to identify differences in anxiety states by measuring attentional-orienting behaviours (Lee et al., 2016). A modified version of this test, using an unfamiliar human as the threat, was used to establish if the human exposure treatments caused the lambs to become more anxious towards a human than the lambs from the CON treatment. Five seconds after a lamb entered the arena, an unfamiliar human opened the exit door, remained in the doorway until the lamb oriented their head towards them, then closed the door. Behaviours recorded are listed in Table 2. Time spent vigilant (ABVig) and attentive to the threat (ABAtt), and the latency to eat the hay in the centre square (ABEat) were measured as indicators of the valence (positive or negative) of each lamb's affective state. Squares crossed (ABSq) and escape attempts (ABEsc) were recorded as possible indicators of the arousal (degree) of the affective state. This test concluded after 1 min (Monk et al., 2018).

2.5.2. Moving human (MH) test

A variant of a forced approach or open field test (Forkman et al., 2007) was used to test the fear responses of lambs towards a human stimulus. A moving human, as opposed to a stationary one, was chosen to identify differences in human-directed fear responses between the three treatment groups. The current version was modified from a test used by Hemsworth et al. (2018). After 1 min the same human from the AB test re-entered the arena and timing commenced once the door was closed. The human walked around the inside perimeter for 1 min at approximately 0.33 m/s. with their head facing forward and did not make eye contact with the lamb. Where the lamb was blocking their path, they paused until the lamb moved then continued to walk. Behaviours recorded are listed in Table 2. Closest approach zones for this test (MHZones) were measured in relation to the position of the human as they moved around the arena (Fig. 3).

2.5.3. Startle (S) test

The startle response is a reflex reaction to a sudden or threatening stimulus that is modulated by affective state (Lang et al., 1990). It is enhanced during a fear state, attenuated in a pleasant state and is often followed by a defensive or flight reaction (Broom and Johnson, 1993). Therefore, the degree of behavioural reactivity exhibited can indicate how fearful an animal perceives their current situation to be (Broom and Johnson, 1993; Salvin et al., 2020). A modified S test used previously by González et al. (2013) was used to measure both the startle response and the subsequent fear response towards the novel startle stimulus. Once the human had exited the arena a purple and white patterned umbrella was inserted through a previously covered hole in a side wall. The umbrella was popped open quickly once the lamb's head was oriented towards it and the open umbrella was rested against the wall where it remained for 5 min (Fig. 4). Behaviours recorded are listed in Table 2. Closest approach zones for this test (SZones) were measured in relation to the static umbrella (Fig. 2). After 5 min the lamb was ushered out of the arena and into the nearby isolation box.

2.5.4. Isolation box (IB) test

The IB test has been validated in sheep as a measure of temperament due to its high repeatability (Murphy et al., 1994). The test measures agitation or behavioural reactivity in response to isolation and confinement, the latter intended as the third fear stimulus for the current study. A variation of the original test, as used previously by Rice et al. (2016)

Table 2
An ethogram of the final behaviours assessed for the attention bias (AB), moving human (MH), startle (S) and isolation box (IB) tests and corresponding identification codes. For binary measures 0 = lamb did not perform the behaviour and 1 = lamb did perform the behaviour.

Behaviour	Description	Unit	AB test	MH test	S test	IB test
Vocalisations	All low- & high-pitched bleats	count	ABVoc	MHVoc	SVoc	IBVoc
Escape Attempts	Lamb jumps against the arena wall and all feet leave the ground, or both front feet leave the ground in the box	0/1	ABEsc	MHEsc (count)	SEsc	IBJumps
Level of Activity	*Squares crossed* where both front feet cross the line or one foot crosses and the other is on the line (but not both feet on the line).	count	ABSq	MHSq	SSq	
	Single steps where either front foot is lifted and put back down	count				IBSteps
	Lamb turns body 180 degrees to face opposite door of the box	count				IB180T
Attention to Threat	Head oriented towards the threat (exit door/human/umbrella)	seconds	ABAtt	MHAtt	SAtt	
Vigilance	Head at or above shoulders	seconds	ABVig			
Proximity to Threat	The closest zone relevant to the threat (human/umbrella) the lamb entered: Z1 = approached to within < 1 m Z2 = approached to within 1–2 m Z3 = approached to within 2–3 m Z4 = stayed 3 m + away	1–4		MHZones	SZones	
	Interacted with the umbrella	0/1			SIntUmb	
Willingness to Feed	Ate hay from centre of arena	0/1	ABEat			
Startle Magnitude	1 = jumps/startles but takes no steps 2 = takes steps but moves < 1 square 3 = runs or moves > 1 square 4 = flees and may attempt escape	1–4			SPop	

was conducted. Once the lamb entered the box and the rear door was closed the timer began for 2 min. Behaviours recorded are listed in Table 2. After 2 min the lamb was released from the front of the box and could make its way back to the holding area.

2.6. Statistical analyses

Three months after the current study the same group of lambs completed a second round of testing in the MH, S and IB tests for a related, but separate study (Atkinson et al., 2022). For ease of modelling, both rounds of data were modelled together, and this process has been described. However, as only the round 1 data is relevant to the current study, point estimates (means of the posterior distributions) were predicted for round 1 only. Conditional and marginal coefficients of determination (R^2) are more informative for understanding the influence of treatment and sex on each behavioural response and these have been calculated from both rounds of data.

All data were analysed using R (R Core Team, 2020) in RStudio version 1.3.1093. A series of univariate generalised mixed effects models were constructed using a frequentist approach to assess the response distributions for each variable. Univariate models were used to fit two Bayesian generalised non-linear multivariate multilevel models which were used for the principal analysis.

The combination of repeated measures, non-continuous responses including ordinal and count data, and interacting terms motivated a careful regression modelling approach in this study. Though multilevel generalized linear models are widely supported in the frequentist paradigm, as in the popular 'lme4′ package (Bates et al., 2015), a Bayesian approach offers increased flexibility, clearer expression of uncertainty, and regularization (Dunson, 2001), and also evades interpretational difficulties associated with frequentist methods, especially under multiplicity (Vasishth et al., 2018). Computational and programming demands tend to make Bayesian methods less accessible, but the high performance of Hamiltonian Monte Carlo (HMC) in Stan (Carpenter et al., 2017), combined with accessibility of the high-level 'brms' interface

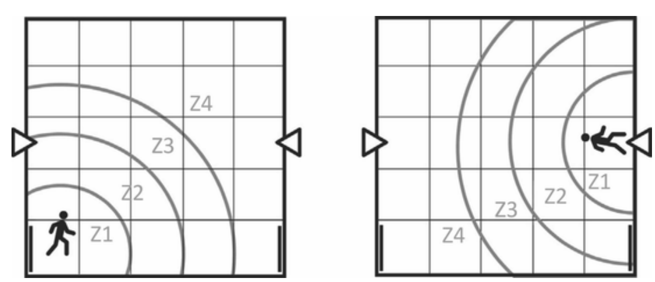

Fig. 3. Distance zones from the human in the MH test (MHZones) remained relative to the human as they walked around the arena.

Fig. 4. After the umbrella was popped open to startle the lamb, it was rested against the inside wall of the arena where it remained for the five-minute duration of the startle (S) test.

(Bürkner, 2017), facilitated efficient analysis in this study. Practical uptake of Bayesian methods is apparently increasing, potentially driven by recognition of abundant misuse of frequentist statistics, with introductory materials now widely available for applied scientists (Etz and Vandekerckhove, 2018; Korner-Nievergelt et al., 2015). In particular, recent examples in animal behaviour (Kühl et al., 2019; Williamson et al., 2019), ecology (Barneche et al., 2018), cognition (Bürkner et al., 2017), and psychology (Arango et al., 2019) emphasize the potential for Bayesian methods as general tools.

With weakly informative priors used throughout, the selection of a Bayesian approach was largely pragmatic, rather than motivated by expressing existing knowledge. A key advantage of the approach was the capability to simultaneously express the results, regarding diverse outcome variables, in a common format using posterior predictions from the model. Though this may require complicated bootstrapping approaches in frequentist models, it is a natural result of Bayesian analysis (Bürkner and Vuorre, 2019). The joint model structure allowed in the 'brms' package (Bürkner, 2017) also allowed the specification of correlated subject parameters between responses, which is explored elsewhere (Atkinson et al., 2022).

The attention to threat variables for the MH, S and AB behavioural tests were log transformed. These and vigilance in the AB test were reasonably continuous, so were fit with a normal distribution. The ordinal ranked variables of closest approach zone entered for the MH and S tests and the startle response to the umbrella 'pop' were fit with a cumulative link model. Due to a low number of occurrences, latency to eat in the AB test, time spent interacting with the umbrella in the S test and number of escape attempts in the AB, S and IB tests were all dichotomised and fit using a binomial response distribution with logit link. The count variable 180 degree turns in the IB test was fit using a Poisson distribution, and all other count variables were fit using a negative binomial distribution. Table 2 lists all final behaviours, final units and their identification codes.

Univariate models were reconstructed using the 'brms' package (see Equation 1 for the general model expression) to incorporate priors and HMC algorithm settings were checked to ensure each model would converge. Residual analysis was conducted where appropriate, depending on the response type. Priors for the fixed effects were selected based on the scale of all response variables (N(0,2) or N(0,3)), were normal with mean zero and were intended to be weakly informative. Priors for random effects were left as the package defaults. The variable weight-at-testing was mean-centred and used as a linear predictor variable and fit as a fixed effect along with treatment, sex and a treatment*sex interaction. The effect of pen was poorly identifiable and so was removed from both final multivariate models. Individual animal ID was also fit as a random intercept to account for repeated measures across both testing rounds for these three tests. Intermediate multivariate models were constructed according to behavioural test (AB, MH, S and IB) and the Gelman-Rubin convergence statistic (R-hat) and effective sample sizes (ESS) were checked as indicators of convergence. The AB intermediate multivariate model remained separate as there were no round 2 data for this test. A larger multivariate model was constructed in sequence by progressively adding the intermediate models for each of the other tests and was retested for convergence and ESS. Point estimates (mean of the posterior distribution) split by sex, were then extracted for each behaviour.

$$f(y) = \beta_0 + \beta_1 Treatment * \beta_2 Sex * \beta_3 Round + \beta_4 Weight + \gamma$$

Equation 1: A generalised model expression for all behavioural variables where β_0 = the global intercept term, β_n = intercept terms (or priors) for fixed effects, * denotes an interaction between terms and γ = the random intercept term.

Conditional and marginal coefficients of determination (R^2) were extracted (Nakagawa et al., 2017) for each behaviour in the MH, S and IB tests to better understand how much treatment and sex influenced each behavioural response. Conditional R^2 (R^2_{Cond}) indicates the proportion of variance explained by the full model while marginal R^2 (R^2_{Marg}) indicates the proportion explained by only the fixed effects (treatment, sex and testing round).

3. Results

Complete records for 75 lambs were included in the final analysis. Four lambs were not tested due to injury (LOW n = 1) or escape from the holding pen (CON n = 1, MOD n = 2). The results of two lambs were excluded from the AB test due to the human exiting the arena before the lamb had seen them (CON n = 1, MOD n = 1). Technical issues with video recordings resulted in the exclusion of a further two lambs from the AB test (CON n = 1, LOW n = 1), one lamb from the S test (LOW) and 11 from the IB test (CON n = 8, LOW n = 1, MOD n = 2). Table 3 summarises total numbers of lambs for each treatment group along with median and range (min, max) values for each behaviour. Results from both multivariate analyses are presented as posterior means [95% credible intervals]. The posterior mean represents the predicted value of the posterior distribution, that is, the distribution of each response variable that is conditional on the data collected.

3.1. Attention bias test

The multivariate analysis of the AB behaviours indicated that overall, the effects of treatment were small in magnitude with a large degree of uncertainty around the direction of the effects on both measures of valence and arousal (Fig. 5, see also Table S1). In general, posterior means for CON females were higher than their male counterparts, although the magnitude of this sex effect was small (Fig. 5). The opposite was seen for the LOW group indicating a consistent, albeit small, interaction effect between treatment and sex within this group. Time spent attentive to the threat (ABAtt) and number of vocalisations (ABVoc) were relatively consistent across treatment groups and sex. There was no apparent pattern in the effect of the MOD treatment.

3.2. Human approach, startle and isolation box tests

Overall, the data for 15 lambs were excluded from the analysis of the remaining three tests due to incomplete records. Fig. 6 presents the R^2_{Cond} and R^2_{Marg} for behaviours from these tests. R^2_{Cond} ranged from 0.39 (SEsc) to 0.8 (HAVoc) with all but three behaviours above 0.5. The R^2_{Marg} were considerably lower than the R^2_{Cond} for all behaviours. The R^2_{Marg} for SPop, SAtt, SIntUmb and IB180T were 0.26, 0.27, 0.28 and 0.31 respectively while the R^2_{Marg} for the remaining behaviours were all below 0.15.

The multivariate analysis of the three fear-based tests again indicated that overall there was a small effect of treatment on some behaviours, also with a large degree of uncertainty around the direction of this effect (Fig. 7, see also Table S2). A consistent pattern indicating a treatment by sex interaction is again suggested for the LOW group in behaviours in the MH test. LOW females were 2 times more likely to attempt escape (MHEsc) than LOW males ((F: 0.4 [0.09, 1.06], M: 0.19 [0.04, 0.52]), crossed 5 more squares (MHSq, F: 20 [14, 27], M: 14 [10, 19]) and spent 9.93 fewer seconds (out of a possible 60) attentive to the human (MHAtt) than their male counterparts (F: 15.77 [12.15, 20.46], M: 25.7 [20.26, 32.57]). Lambs in all groups were more likely to approach to within < 1 m of the umbrella than any of the other approach zones (SZones, Fig. 8, see also Table S3). An effect of sex was seen in the CON and LOW groups with males 1.5 and 2 times more likely to approach to within < 1 m than their female counterparts respectively. The largest difference between the sexes was again seen in the LOW group (Z1, F: 0.43 [0.08, 0.87], M: 0.81 [0.45, 0.98]) and LOW females had the largest degree of uncertainty across all zones (Z2, F: 0.14 [0.05,

Table 3
Descriptive statistics identifying the number of lambs included in the analyses for each test, the median and the range (min, max) values for each behaviour. The behaviours MHZones, SZones and SPop were measured on an ordinal scale and are not included.

		n lambs			Median			Range (min, max)		
Measure	Unit	CON	LOW	MOD	CON	LOW	MOD	CON	LOW	MOD
ABVoc	c	29	29	28	13	12	11	(0, 32)	(0, 36)	(0, 29)
ABEsc	0/1[a]	27	28	27	0	0	0	(0, 1)	(0, 1)	(0, 1)
ABSq	count	27	28	27	24	27.5	29	(12, 54)	(13, 48)	(10, 46)
ABAtt	seconds	27	28	27	5.7	5.5	5.8	(2.7, 13.5)	(2.3, 11.1)	(2.7, 12.4)
ABVig	seconds	27	28	27	51.9	53.2	57.3	(34.1, 60)	(24.1, 60)	(43.9, 60)
ABEat	0/1[a]	27	28	27	0	0	0	(0, 1)	(0, 1)	(0, 1)
MHVoc	count	29	29	28	1	1	1	(0, 16)	(0, 21)	(0, 20)
MHEsc	count	29	29	28	0	0	0	(0, 8)	(0, 6)	(0, 3)
MHSq	count	29	29	28	14	11	12	(1, 87)	(0, 58)	(1, 72)
MHAtt	seconds	29	29	28	23.6	22.7	28.9	(5.7, 53.9)	(5.2, 46.7)	(4.3, 46.6)
SVoc	count	29	29	28	26	27	20	(0, 83)	(0, 119)	(0, 108)
SEsc	0/1[a]	29	28	28	0	0	0	(0, 1)	(0, 1)	(0, 1)
SSq	count	29	28	28	35	31	26.5	(1, 98)	(0, 121)	(0, 100)
SAtt	seconds	29	28	28	46.8	56.3	66.2	(15.8, 158.8)	(21.2, 139.3)	(18.8, 219.5)
SIntUmb	0/1[a]	29	28	28	1	1	1	(0, 1)	(0, 1)	(0, 1)
IBVoc	count	29	29	28	0	0	0	(0, 30)	(0, 21)	(0, 17)
IBJumps	0/1[a]	21	28	26	0	0	0	(0, 1)	(0, 1)	(0, 1)
IBSteps	count	21	28	26	42	35	35	(2, 134)	(3, 111)	(9, 135)
IB180T	count	21	28	26	0	1	0	(0, 11)	(0, 8)	(0, 5)
Weight[b]	Kg				25.7	24.6	24.9	(19, 35)	(18.5, 33)	(18.5, 31.5)

[*] Recorded at weaning
[d] Recorded at weaning

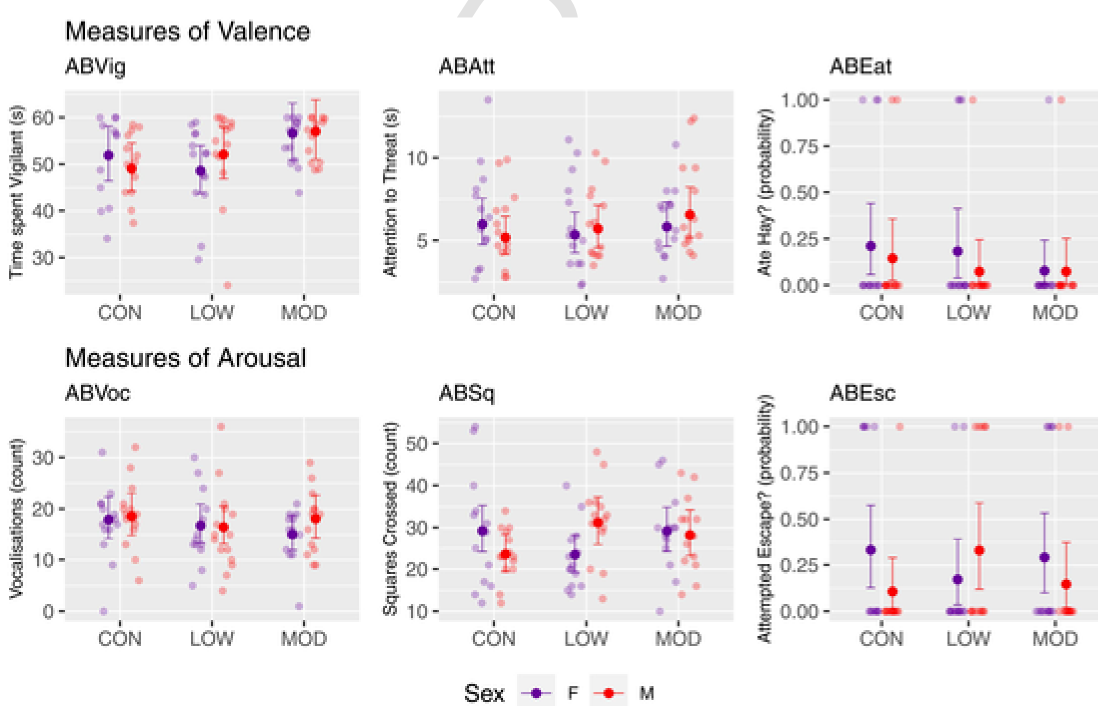

Fig. 5. Interval plots for all behaviours from the attention bias (AB) test with point estimates of the population mean (posterior mean) and 95% credible intervals. For the binary measures ABEat and ABEsc, 0 = lamb did not perform the behaviour and 1 = lamb did perform the behaviour.

0.32], M: 0.09 [0.01, 0.24], Z3, F: 0.3 [0.05, 0.57], M: 0.09 [0.01, 0.28], Z4, F: 0.1 [0.01, 0.37], M: 0.02 [0.001, 0.07]). A small treatment by sex interaction effect is also evident for the MOD group in level of activity in the IB test (IBSteps, Fig. 7). Within the two-minute timeframe, MOD females took 23 fewer steps than their male counterparts (F: 28 [19, 40], M: 51 [35, 74]). Results for the remaining behaviours across all three tests were largely inconsistent with no further patterns within or across treatment groups, sex or tests.

3.3. Weight

There was no effect of weight on the outcome of any of the AB behaviours. There was a small negative effect on four behaviours across the other three tests (HAVoc: −0.09 [−0.16, −0.03], SIntUmb: −0.29 [−0.57, −0.05], IBSteps: −0.04 [−0.07, −0.01], IB180T: −0.17 [−0.23, −0.11]) and a small positive effect on two behaviours from the S test (SZones: 0.19 [0.01, 0.38], SPop: 0.15 [0.04, 0.27]).

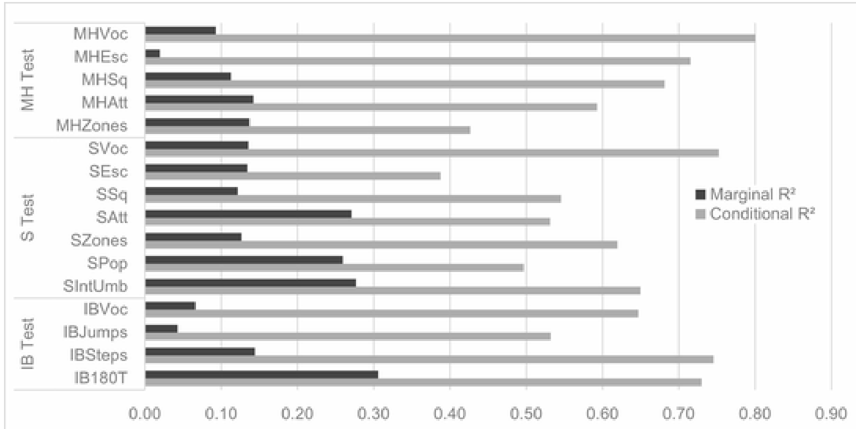

Fig. 6. Conditional and marginal R^2 for behaviours from the moving human (MH), startle (S) and isolation box (IB) tests.

4. Discussion

Extensively reared sheep have high human-directed fear due to infrequent and often aversive interactions with humans. The aim of this study was to reduce human-directed fear and behavioural reactivity in weaned lambs by either habituating them to a human presence or challenging them with a manageably stressful experience that did not overwhelm or sensitise them. The current study analysed a total of 22 behavioural responses to four fear-based stimulus tests and found evidence that the additional human exposure treatments did influence fear-based responses, however, overall, the treatment effect was weak.

The purpose of the AB test was to identify if the LOW or MOD treatments sensitised the lambs to a human presence, i.e., if they were more anxious compared with CON lambs. As suggested by Monk et al. (2018) the consistency in the way the test is applied across all animals allows for the reasonable assumption that differences in behavioural responses are more strongly influenced by the affective state of the animal coming in to the test, as opposed to the influence of the test procedure itself. The similarities across treatment groups for the three valence measures suggests the perception of the human as a threat was similar, and that the human exposure treatments did not induce a heightened state of anxiety, but also did not reduce it.

The current study saw a small but consistent treatment by sex interaction effect for the LOW group across four out of the six AB behaviours suggesting LOW females may have been slightly less anxious than their CON female and LOW male counterparts, and LOW males slightly more anxious than their CON male counterparts. However, while a small treatment by sex interaction was also present for the LOW group for the MH test, it suggests the opposite. LOW females showed more behavioural reactivity towards the moving human than both their CON female and LOW male counterparts, the latter of which agrees with our hypothesis and previous studies in both juvenile and adult sheep (Vandenheede and Bouissou, 1993; Boissy et al., 2005). A possible explanation for the difference in female behaviour is that they habituate to a stationary and predictable threat better than their male counterparts but find an active and unpredictable threat in a novel environment too overwhelming for these effects to persist. Indeed, in the current study, a small number of LOW lambs willingly approached the human during the treatment phase from week 3 onwards and by week 6 one lamb had made brief contact on two occasions. This indicates that some habituation to the human had occurred in the treatment pens, however the sex of these lambs was not recorded. Previously, gentled lambs showed increased approach behaviours towards a stationary human compared with non-gentled lambs, but this effect did not generalise to humans in situations where they had a more active role (Mateo et al., 1991). However, that study did not report on the effect of sex and further studies are needed to investigate this possible treatment by sex interaction, particularly as to date there have been no attentional bias studies with sheep of both sexes for comparison.

The lack of a consistent pattern within and across tests for the MOD lambs and the similarities between behavioural responses for the S and IB tests compared with CON lambs indicates the MOD treatment did not reduce behavioural reactivity. This is further supported by assessment of the R^2 (Fig. 6). While the R^2_{Cond} indicates the components of the multivariate model were quite explanatory of most behaviours, the low R^2_{Marg} indicates most of the variation measured was attributable to individual animal differences, suggesting the effect of either treatment was minimal. It is possible that the MOD treatment was not sufficiently challenging to induce stress inoculation. It is also possible that the timing of the intervention in the current study was unsuitable for reducing human-directed fear in extensively reared lambs. In stress inoculation studies on juvenile squirrel monkeys (Lyons et al., 2010), the maternal separation sessions were the infants' first experience with moderate to high levels of stress. As the current study occurred after weaning, lambs had already undergone lamb marking as is consistent with commercially reared lambs in Australian extensive systems. This mismatch between remaining largely undisturbed by humans for the first five to eight weeks of life followed by the sudden and highly aversive human interactions involved in lamb marking may have overloaded existing coping thresholds. It is therefore possible the lambs had already developed strong human-directed fear well before the commencement of the trial, compromising the effectiveness of any intervention to come afterwards. This has implications for all behavioural studies with extensively reared sheep. Therefore, the delivery of interventions prior to the highly aversive event of lamb marking should be further investigated. It should be noted however, that maternal separation may not be a suitable stress inoculation paradigm for sheep. When separated from their dams over several sessions, pre-weaned lambs demonstrated a progressive increase in behavioural reactivity in response to being separated, indicating greater separation distress (Mora et al., 2017), but no change in behavioural reactivity towards isolation and novelty post-weaning when compared with non-separated lambs (Simitzis et al., 2012). Further investigation into the benefits of human exposure prior to lamb marking however, is warranted.

Investigation of the effects of positive reinforcement should therefore also be considered to reduce human-directed fear and behavioural reactivity. Previously, young lambs have been shown to develop an affinity for human caregivers without food association (Boivin et al., 2000; Tallet et al., 2008). When additional exposure to humans, including tactile contact (known as gentling), occurred within two months of age, lambs exhibited increased ease of handling (Uetake et al., 2000). Additionally, there is some evidence that gentled lambs showed reduced pain sensitivity to tail docking (Guesgen et al., 2013). While gentling may not be practical in extensive systems, these studies provide

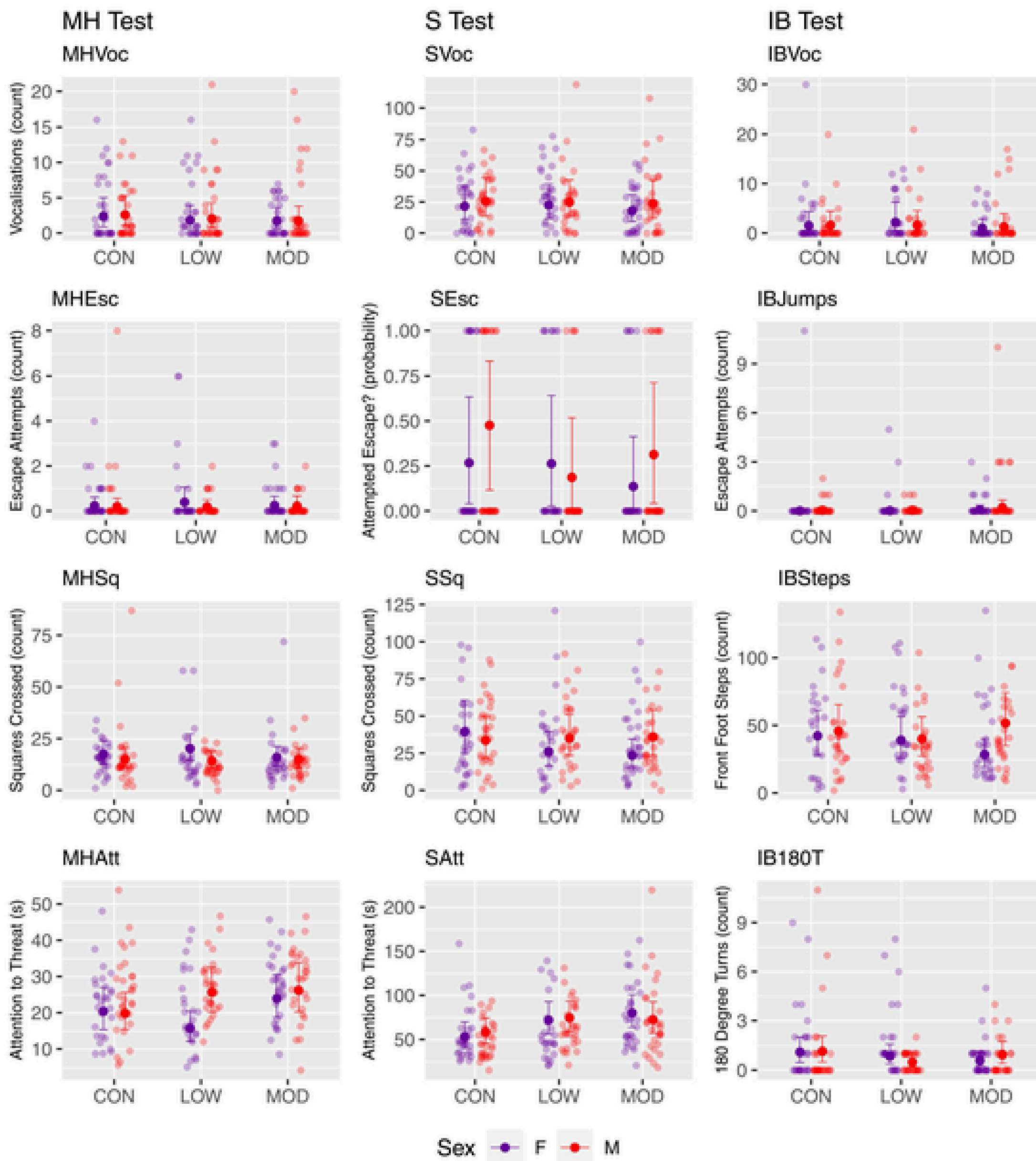

Fig. 7. Interval plots for most behaviours from the moving human (MH), startle (S) and isolation box (IB) tests with point estimates of the population mean (posterior mean) for each behaviour. Intervals are 95% credible intervals for the population mean. For the binary measures SEsc and IBJumps, 0 = lamb did not perform the behaviour and 1 = lamb did perform the behaviour.

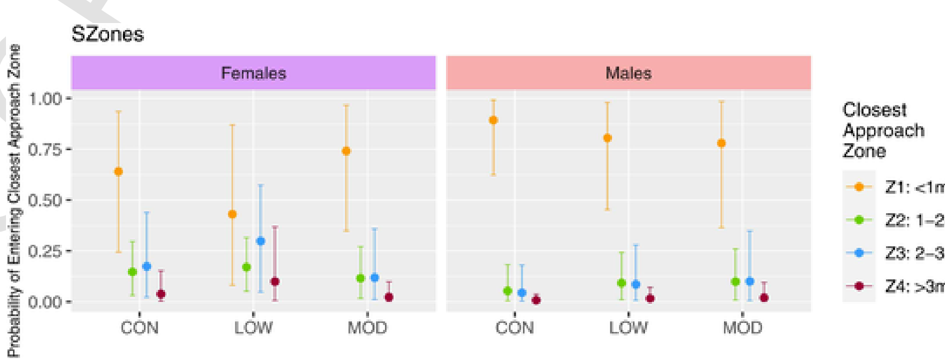

Fig. 8. Interval plots comparing female and male responses for the ordinal SZones behaviour. Point estimates and 95% credible intervals indicate the probability of lambs approaching to within < 1 m, 1–2 m, 2–3 m, or > 4 m of the umbrella in the startle (S) test.

some evidence that the development of positive associations towards humans prior to lamb marking may also be beneficial in reducing the negative impacts of marking by reducing human-directed fear, pain perception, and improving the lambs' overall ability to cope.

As already mentioned, the high degree of uncertainty around behavioural responses in the current study and the big difference between R^2_{Cond} and R^2_{Marg} are indicative of high inter-animal variation. While often considered noise in intervention studies applied at a group level, individual variation is often attributable to temperament, which has been shown to affect the behavioural reactivity of sheep towards humans (Murphy et al., 1998; Beausoleil et al., 2008). Temperament also influences social structure, which has been shown to affect fear responses in goats (Miranda-de la Lama et al., 2013). As a fundamental driver of behaviour (Réale et al., 2007; Finkemeier et al., 2018), temperament may also impact the effectiveness of an intervention across different individuals. It should also be considered that commercially bred sheep, such as those used in the current study, are much more genetically diverse than laboratory species which allows for greater variability in temperament within the same cohort, and therefore greater variability in responses, and is likely to have contributed to the high degree of uncertainty around the measured responses. The influence of temperament on behavioural responses during standardised tests of the lambs in the current study has been investigated further and will be discussed in a subsequent publication.

It is possible the one-month age range of lambs in the current study has also contributed to the uncertainty around the behavioural responses. Age related differences have been seen previously in lambs of a similar age to those in the current study in response to behavioural reactivity to fear inducing stimuli (Viérin and Bouissou, 2003) although a large variation in responses was also seen. Although each experimental group was balanced for lamb weight, the exact age of each lamb, ewe parity and litter size were unknown and could act as confounding factors and are therefore limitations of the current study.

Overall, the imposed interventions did not reduce the behavioural reactivity of lambs in the tests used, suggesting neither fear towards humans nor fear in general had been altered. This may have been due to the treatments being unsuitable to produce the mechanisms of habituation or stress inoculation, the timing of the interventions, which occurred several weeks after the highly aversive experience of lamb-marking or a combination of the two. Further research into interventions imposed before lamb-marking are warranted.

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CRediT authorship contribution statement

Leigh Atkinson : Project administration, Funding acquisition, Conceptualisation, Methodology, Formal analysis, Investigation, Data curation, Writing – original draft, Visualisation, Project administration, Funding acquisition. **Rebecca E. Doyle :** Conceptualization, Methodology, Supervision, Writing - review & editing. **Andrew Woodward :** Data curation, Formal analysis, Visualization, Writing - original draft, Writing - review & editing. **Ellen C. Jongman :** Conceptualization, Methodology, Supervision, Writing - review & editing.

Declaration of Competing Interest

The authors declare they have no conflicts of interest.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.beproc.2022.104709.

References

Arango, A., Cole-Lewis, Y., Lindsay, R., Yeguez, C.E., Clark, M., King, C., 2019. The protective role of connectedness on depression and suicidal ideation among bully victimized youth. J. Clin. Child Adolesc. Psychol. 48, 728–739. https://doi.org/10.1080/15374416.2018.1443456.

Atkinson, L., Doyle, R.E., Woodward, A. and Jongman, E.C., 2022. Behavioural reactivity testing in sheep indicates the presence of multiple temperament traits. xxxx.

Barneche, D.R., Robertson, D.R., White, C.R., Marshall, D.J., 2018. Fish reproductive-energy output increases disproportionately with body size. Science 360, 642–645. https://doi.org/10.1126/science.aao6868.

Bates, D., Mächler, M., Bolker, B., Walker, S., 2015. Fitting linear mixed-effects models using lme4. J. Stat. Softw. 67, 1–48. https://doi.org/10.18637/jss.v067.i01.

Bateson, P., 1979. How do sensitive periods arise and what are they for. Anim. Behav. 27, 470–486. https://doi.org/10.1016/0003-3472(79)90184-2.

Beausoleil, N.J., Blache, D., Stafford, K.J., Mellor, D.J., Noble, A.D.L., 2008. Exploring the basis of divergent selection for 'temperament' in domestic sheep. Appl. Anim. Behav. Sci. 109, 261–274. https://doi.org/10.1016/j.applanim.2007.03.013.

Boissy, A., Bouix, J., Orgeur, P., Poindron, P., Bibé, B., Le Neindre, P., 2005. Genetic analysis of emotional reactivity in sheep: effects of the genotypes of the lambs and of their dams. Genet. Sel. Evol. 37, 381–401. https://doi.org/10.1051/gse:2005007.

Boivin, X., Tournadret, H., Le Neindre, P., 2000. Hand-feeding and gentling influence early-weaned lambs' attachment responses to their stockperson. J. Anim. Sci. 78, 879–884. https://doi.org/10.2527/2000.784879x.

Broom, D., 2001. Coping, stress and welfare. In: Broom, D. (Ed.), Coping with Challenge: Welfare in Animals Including Humans. Dahlem University Press, Berlin.

Broom, D.M., Johnson, K.G., 1993. Stress and Animal Welfare. Kluwer Academic Publishers, The Netherlands. https://doi.org/10.1007/978-94-024-0980-2.

Bürkner, P.-C., 2017. Brms: an R package for Bayesian multilevel models using Stan. J. Stat. Softw. 80. https://doi.org/10.18637/jss.v080.i01.

Bürkner, P.-C., Vuorre, M., 2019. Ordinal regression models in psychology: a tutorial. Adv. Methods Pract. Psychol. Sci. 2, 77–101. https://doi.org/10.1177/2515245918823199.

Bürkner, P.-C., Williams, D.R., Simmons, T.C., Woolley, J.D., 2017. Intranasal oxytocin may improve high-level social cognition in schizophrenia, but not social cognition or neurocognition in general: a multilevel Bayesian meta-analysis. Schizophr. Bull. 43, 1291–1303. https://doi.org/10.1093/schbul/sbx053.

Carpenter, B., Gelman, A., Hoffman, M.D., Lee, D., Goodrich, B., Betancourt, M., Brubaker, M., Guo, J., Li, P., Riddell, A., 2017. Stan: a probabilistic programming language. J. Stat. Softw. 76. https://doi.org/10.18637/jss.v076.i01.

Coulon, M., Nowak, R., Peyrat, J., Chandèze, H., Boissy, A., Boivin, X., 2015. Do lambs perceive regular human stroking as pleasant? Behavior and heart rate variability analyses. PLOS One 10, e0118617. https://doi.org/10.1371/journal.pone.0118617.

Daskalakis, N.P., Bagot, R.C., Parker, K.J., Vinkers, C.H., de Kloet, E.R., 2013. The three-hit concept of vulnerability and resilience: Toward understanding adaptation to early-life adversity outcome. Psychoneuroendocrinology 38, 1858–1873. https://doi.org/10.1016/j.psyneuen.2013.06.008.

Destrez, A., Coulon, M., Deiss, V., Delval, E., Boissy, A., Boivin, X., 2013. The valence of the long-lasting emotional experiences with various handlers modulates discrimination and generalization of individual humans in sheep. J. Anim. Sci. 91, 5418–5426. https://doi.org/10.2527/jas.2012-5654.

Dietz, L., Arnold, A.-M.K., Goerlich-Jansson, V.C., Vinke, C.M., 2018. The importance of early life experiences for the development of behavioural disorders in domestic dogs. Behaviour 155, 83–114. https://doi.org/10.1163/1568539X-00003486.

Dunson, D.B., 2001. Commentary: practical advantages of bayesian analysis of epidemiologic data. Am. J. Epidemiol. 153, 1222–1226. https://doi.org/10.1093/aje/153.12.1222.

Dwyer, C.M., 2004. How has the risk of predation shaped the behavioural responses of sheep to fear and distress? Anim. Welf. 13, 269–281.

Dwyer, C.M., 2009. Welfare of sheep: providing for welfare in an extensive environment. Small Rumin. Res. 86, 14–21. https://doi.org/10.1016/j.smallrumres.2009.09.010.

Etz, A., Vandekerckhove, J., 2018. Introduction to Bayesian inference for psychology. Psychon. Bull. Rev. 25, 5–34. https://doi.org/10.3758/s13423-017-1262-3.

Finkemeier, M.-A., Langbein, J., Puppe, B., 2018. Personality research in mammalian farm animals: concepts, measures, and relationship to welfare. Front. Vet. Sci. 5. https://doi.org/10.3389/fvets.2018.00131.

Forkman, B., Boissy, A., Meunier-Salaün, M.C., Canali, E., Jones, R.B., 2007. A critical review of fear tests used on cattle, pigs, sheep, poultry and horses. Physiol. Behav. 92, 340–374. https://doi.org/10.1016/j.physbeh.2007.03.016.

Franklin, Tamara, Saab, B., Bechara, J., Mansuy, Isabelle, M., 2012. Neural mechanisms of stress resilience and vulnerability. Neuron 75, 747–761. https://doi.org/10.1016/j.neuron.2012.08.016.

Freitas-de-Melo, A., Ungerfeld, R., 2016. Artificial weaning in sheep: stress response and animal welfare. Review. Rev. Mex. De. Cienc. Pecu. 7, 361–375.

González, M., Averós, X., Heredia, I.Bd, Ruiz, R., Arranz, J., Estevez, I., 2013. The effect of social buffering on fear responses in sheep (Ovis aries). Appl. Anim. Behav. Sci. 149, 13–20. https://doi.org/10.1016/j.applanim.2013.09.011.

Grandin, T., Shivley, C., 2015. How farm animals react and perceive stressful situations such as handling, restraint, and transport. Animals 5, 1233–1251. https://doi.org/10.3390/ani5040409.

Grant, C., 2004. Behavioural responses of lambs to common painful husbandry procedures. Appl. Anim. Behav. Sci. 87, 255–273. https://doi.org/10.1016/j.applanim.2004.01.011.

Grissom, N., Bhatnagar, S., 2009. Habituation to repeated stress: get used to it. Neurobiol. Learn. Mem. 92, 215–224. https://doi.org/10.1016/j.nlm.2008.07.001.

Guesgen, M.J., Beausoleil, N.J., Stewart, M., 2013. Effects of early human handling on the pain sensitivity of young lambs. Vet. Anaesth. Analg. 40, 55–62. https://doi.org/10.1111/j.1467-2995.2012.00746.x.

Hargreaves, A.L., Hutson, G.D., 1990. Some effects of repeated handling on stress responses in sheep. Appl. Anim. Behav. Sci. 26, 253–265. https://doi.org/10.1016/0168-1591(90)90141-Y.

Hemsworth, P., Rice, M., Borg, S., Edwards, L., Ponnampalam, E., Coleman, G., 2018. Relationships between handling, behaviour and stress in lambs at abattoirs. Animal 1–10. https://doi.org/10.1017/s1751731118002744.

Hemsworth, P.H., Coleman, G.J., 2010. Human-Livestock Interactions: The Stockperson and the Productivity and Welfare of Intensively Farmed animals. CABI, Chippenham, UK. https://doi.org/10.1079/9781845936730.0000.

Korner-Nievergelt, F., Roth, T., von Felten, S., Guélat, J., Almasi, B., Korner-Nievergelt, P., 2015. Chapter 3 – The Bayesian and the frequentist ways of analyzing data. In: Korner-Nievergelt, F., Roth, T., von Felten, S., Guélat, J., Almasi, B., Korner-Nievergelt, P. (Eds.), Bayesian Data Analysis in Ecology Using Linear Models with R, BUGS, and STAN. Academic Press, Boston.

Kühl, H.S., Boesch, C., Kulik, L., Haas, F., Arandjelovic, M., Dieguez, P., Bocksberger, G., McElreath, M.B., Agbor, A., Angedakin, S., Ayimisin, E.A., Bailey, E., Barubiyo, D., Bessone, M., Brazzola, G., Chancellor, R., Cohen, H., Coupland, C., Danquah, E., Deschner, T., Dowd, D., Dunn, A., Egbe, V.E., Eshuis, H., Goedmakers, A., Granjon, A.-C., Head, J., Hedwig, D., Hermans, V., Imong, I., Jeffery, K.J., Jones, S., Junker, J., Kadam, P., Kambere, M., Kambi, M., Kienast, I., Kujirakwinja, D., Langergraber, K.E., Lapuente, J., Larson, B., Lee, K., Leinert, V., Llana, M., Maretti, G., Marrocoli, S., Martin, R., Mbi, T.J., Meier, A.C., Morgan, B., Morgan, D., Mulindahabi, F., Murai, M., Neil, E., Niyigaba, P., Ormsby, L.J., Orume, R., Pacheco, L., Piel, A., Preece, J., Regnaut, S., Rundus, A., Sanz, C., Schijndel, Jv, Sommer, V., Stewart, F., Tagg, N., Vendras, E., Vergnes, V., Welsh, A., Wessling, E.G., Willie, J., Wittig, R.M., Yuh, Y.G., Yurkiw, K., Zuberbühler, K., Kalan, A.K., 2019. Human impact erodes chimpanzee behavioral diversity. Science 363, 1453–1455. https://doi.org/10.1126/science.aau4532.

Lang, P.J., Bradley, M.M., Cuthbert, B.N., 1990. Emotion, attention, and the startle reflex. Psychol. Rev. 97, 377. https://doi.org/10.1037/0033-295X.97.3.377.

Lee, C., Verbeek, E., Doyle, R., Bateson, M., 2016. Attention bias to threat indicates anxiety differences in sheep. Biol. Lett. 12. https://doi.org/10.1098/rsbl.2015.0977.

Lyons, D.M., Parker, K.J., Schatzberg, A.F., 2010. Animal models of early life stress: Implications for understanding resilience. Dev. Psychobiol. 52, 616–624. https://doi.org/10.1002/dev.20429.

Markowitz, T.M., Dally, M.R., Gursky, K., Price, E.O., 1998. Early handling increases lamb affinity for humans. Anim. Behav. 55, 573–587. https://doi.org/10.1006/anbe.1997.0640.

Mateo, J.M., Estep, D.Q., McCann, J.S., 1991. Effects of differential handling on the behaviour of domestic ewes (Ovis aries. Appl. Anim. Behav. Sci. 32, 45–54. https://doi.org/10.1016/S0168-1591(05)80162-5.

Mellor, D., Stafford, K., 2000. Acute castration and/or tailing distress and its alleviation in lambs. N. Z. Vet. J. 48, 33–43. https://doi.org/10.1080/00480169.2000.36156.

Miranda-de la Lama, G.C., Pinal, R., Fuchs, K., Montaldo, H.H., Ducoing, A., Galindo, F., 2013. Environmental enrichment and social rank affects the fear and stress response to regular handling of dairy goats. J. Vet. Behav. 8, 342–348. https://doi.org/10.1016/j.jveb.2013.03.001.

Monk, J.E., Belson, S., Colditz, I.G., Lee, C., 2018. Attention bias test differentiates anxiety and depression in sheep. Front. Behav. Neurosci. 12. https://doi.org/10.3389/fnbeh.2018.00246.

Monk, J.E., Doyle, R.E., Colditz, I.G., Belson, S., Cronin, G.M., Lee, C., 2018. Towards a more practical attention bias test to assess affective state in sheep. PLOS One 13, e0190404. https://doi.org/10.1371/journal.pone.0190404.

Mora, P., Mota, D., Arch-Tirado, E., Roldán, P., Vázquez-Cruz, C., Terrazas, A.M., Rosas, M., Orihuela, A., 2017. Behavior of lambs at different ages during brief periods of increased sensorial isolation from their mothers. J. Vet. Behav. 22, 29–34. https://doi.org/10.1016/j.jveb.2017.09.004.

Murphy, P.M., Purvis, I.W., Lindsay, D.R., Leneindre, P., Orgeur, P., Poindron, P., 1994. Measures of temperament are highly repeatable in merino sheep and some are related to maternal behaviour. Anim. Prod. Aust.

Murphy, P., Lindsay, D. and Le Neindre, P. 1998. Temperament of Merino ewes influences maternal behaviour and survival of lambs. In: Proceedings of the 32nd Congress of the International Society for Applied Ethology.

Nakagawa, S., Johnson, P.C., Schielzeth, H., 2017. The coefficient of determination R 2 and intra-class correlation coefficient from generalized linear mixed-effects models revisited and expanded. J. R. Soc. Interface 14, 20170213. https://doi.org/10.1101/095851.

Parker, K.J., Maestripieri, D., 2011. Identifying key features of early stressful experiences that produce stress vulnerability and resilience in primates. Neurosci. Biobehav. Rev. 35, 1466–1483. https://doi.org/10.1016/j.neubiorev.2010.09.003.

Pascual-Alonso, M., Miranda-de la Lama, G.C., Aguayo-Ulloa, L., Ezquerro, L., Villarroel, M., Marín, R.H., Maria, G.A., 2015. Effect of postweaning handling strategies on welfare and productive traits in lambs. J. Appl. Anim. Welf. Sci. 18, 42–56. https://doi.org/10.1080/10888705.2014.941107.

R. Core Team. 2020. R: A Language and Environment for Statistical Computing, R Foundation for Statistical Computing, Vienna, Austria.

Réale, D., Reader, S.M., Sol, D., McDougall, P.T., Dingemanse, N.J., 2007. Integrating animal temperament within ecology and evolution. Biol. Rev. 82, 291–318. https://doi.org/10.1111/j.1469-185X.2007.00010.x.

Rice, M., Jongman, E.C., Borg, S., Butler, K.L., Hemsworth, P.H., 2016. Characterisation of shy-feeding and feeding lambs in the first week in a feedlot. Appl. Anim. Behav. Sci. 179, 39–45. https://doi.org/10.1016/j.applanim.2016.03.011.

Rushen, J., Taylor, A.A., de Passillé, A.M., 1999. Domestic animals' fear of humans and its effect on their welfare. Appl. Anim. Behav. Sci. 65, 285–303. https://doi.org/10.1016/S0168-1591(99)00089-1.

Salvin, H., Cafe, L., Lees, A., Morris, S., Lee, C., 2020. A novel protocol to measure startle magnitude in sheep. Appl. Anim. Behav. Sci. 104996. https://doi.org/10.1016/j.applanim.2020.104996.

Santarelli, S., Zimmermann, C., Kalideris, G., Lesuis, S.L., Arloth, J., Uribe, A., Dournes, C., Balsevich, G., Hartmann, J., Masana, M., Binder, E.B., Spengler, D., Schmidt, M.V., 2017. An adverse early life environment can enhance stress resilience in adulthood. Psychoneuroendocrinology 78, 213–221. https://doi.org/10.1016/j.psyneuen.2017.01.021.

Simitzis, P., Petrou, M., Demiris, N., Deligeorgis, S., 2012. Effect of pre-weaning temporary isolation within different age periods on the early post-weaning behaviour of juvenile lambs. Appl. Anim. Behav. Sci. 141, 43–48. https://doi.org/10.1016/j.applanim.2012.07.004.

Steagall, P.V., Bustamante, H., Johnson, C.B., Turner, P.V., 2021. Pain management in farm animals: focus on cattle, sheep and pigs. Animals 11, 1483. https://doi.org/10.3390/ani11061483.

Tallet, C., Veissier, I., Boivin, X., 2008. Temporal association between food distribution and human caregiver presence and the development of affinity to humans in lambs. Dev. Psychobiol. 50, 147–159. https://doi.org/10.1002/dev.20254.

Turner, S., Dwyer, C., 2007. Welfare assessment in extensive animal production systems: challenges and opportunities. Anim. Welf. 16, 189.

Uetake, K., Yamaguchi, S., Tanaka, T., 2000. Psychological effects of early gentling on the subsequent ease of handling in lambs. Nihon Chikusan Gakkaiho 71, 515–519. https://doi.org/10.2508/chikusan.71.515.

Vandenheede, M., Bouissou, M., 1993. Sex differences in fear reactions in sheep. Appl. Anim. Behav. Sci. 37, 39–55. https://doi.org/10.1016/0168-1591(93)90069-2.

Vasishth, S., Mertzen, D., Jäger, L.A., Gelman, A., 2018. The statistical significance filter leads to overoptimistic expectations of replicability. J. Mem. Lang. 103, 151–175. https://doi.org/10.1016/j.jml.2018.07.004.

Viérin, M., Bouissou, M.F., 2003. Responses of weaned lambs to fear-eliciting situations: origin of individual differences. Dev. Psychobiol.: J. Int. Soc. Dev. Psychobiol. 42, 131–147. https://doi.org/10.1002/dev.10090.

Weary, D.M., Jasper, J., Hötzel, M.J., 2008. Understanding weaning distress. Appl. Anim. Behav. Sci. 110, 24–41. https://doi.org/10.1016/j.applanim.2007.03.025.

Williamson, C.M., Lee, W., DeCasien, A.R., Lanham, A., Romeo, R.D., Curley, J.P., 2019. Social hierarchy position in female mice is associated with plasma corticosterone levels and hypothalamic gene expression. Sci. Rep. 9, 7324. https://doi.org/10.1038/s41598-019-43747-w.