ASSESSING THE RELATIVE AVERSIVENESS OF TWO STIMULI: SINGLE SHEEP IN THE ARENA TEST

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In the test described here, sheep are exposed to a situation of conflict between the motivation to approach other sheep and the motivation to avoid a human handler. The distance that the test sheep keep from the handler is a reflection of the relative aversiveness of this handler to the sheep. The test itself requires only a minimum amount of handling and gives the test animals the opportunity to choose their distance from the aversive stimulus, thereby reducing stress during the test itself. The two aversive stimuli chosen for comparison were a human handler facing toward the test arena (more aversive) or the same handler turning his back to the arena (less aversive). Ten Scottish Blackface sheep were tested individually a total of ten times, five times with each of the two stimuli in alternate tests. During the first two tests, nine of the sheep stayed further away when the human was facing toward the arena, compared to when he was facing away; this shows that the test is able to discriminate differences in aversiveness between two stimuli as perceived by individual sheep. This difference was not apparent in the following eight tests, probably because of the fact that the stimuli were not reinforced during the tests. Because the test is concerned with sheep's reaction to a stimulus (eg handler), the procedure associated with the stimulus itself (eg shearing, castration) does not have to be repeated in the test, which means that this method is ideal for studying procedures which cause distress to the animals or which are difficult to repeat.

Keywords: *animal welfare, approach–avoidance conflict, aversion, motivation, sheep*

Introduction

A frequently asked question in animal welfare research is: "Which of these stimuli or procedures is perceived by the animals to be the least aversive?" (Rushen 1996). There are two main approaches to answering this question. The most direct way is to study the effect of a specific stimulus (eg an aspect of the environment, such as floor type) or of a procedure (eg type of handling) on an individual when it is in the presence of this stimulus or when it is undergoing the procedure. Bouissou and co-workers have developed a series of tests which assess the level of fear-related behaviour that sheep and cattle perform in the presence of various stimuli (eg Romeyer & Bouissou 1992; Vandenheede & Bouissou 1993; Boissy & Bouissou 1995). These tests were mainly used to find out whether fearfulness is a personality trait in sheep and cattle, and to discover which test situations and behaviours were the most appropriate for assessing the trait. However, the same methods can be used to compare the effects of different stimuli on the animals (eg human versus human-like model, Bouissou & Vandenheede 1995). Taylor and Weary (2000) used the type of vocalisation given by piglets during castration to identify the aspects of the procedure which caused the highest level of pain. A problem with using this approach to compare relative aversiveness is that the response of the animals may depend on the specific procedure, and there may not be a

common behavioural or physiological response to the procedures being compared (Rushen 1996). It also leaves the results open to interpretation. For example, is a short period of high stress or pain more aversive than a longer period of lower stress or pain? To answer that question, it is necessary to find out how these situations are perceived by the animals. Measuring an individual's response to these two situations may be a way of getting closer to finding out how it perceives them.

One approach that is concerned with the evaluation of procedures by animals uses associative learning and looks at an animal's reaction to stimuli that it has learned to associate with aversive experiences. The association can be formed with a location, for instance where a handling procedure is carried out. The aversiveness of the procedure can then be assessed by giving the animal the choice between different locations, often using mazes (eg Grandin *et al* 1986; Rushen 1986a), or by measuring the reluctance of the animal to return to the location (eg Rushen 1986b; Grigor *et al* 1998). The advantage of this method is that the behaviour that is measured is the same whatever the stimulus, which enables the comparison of different stimuli to be made on one scale. A disadvantage is that it requires the test animals to be repeatedly exposed to the procedure to be evaluated, which is not always possible (eg de-horning, clipping, castration). Animals can, however, learn to associate a procedure with the handler carrying out the procedure, or can learn that it was a human (any human) who carried it out, after only one exposure (Fell & Shutt 1989). The animal then shows a level of avoidance which is related to its recollection of the aversiveness of the procedure.

This latter method was used by Fell and Shutt (1989), who developed an arena test which exposes sheep to an approach–avoidance situation. In this test, which relies on the sheep's flocking instinct, a group of three or four test sheep is introduced into an oblong arena. At one end of the arena, a small flock of sheep (approach stimulus) is kept in a pen behind a wire mesh, with a human (avoidance stimulus) standing in front of it. Because the test sheep cannot simultaneously be far away from the human and close to their companions, they have to find a distance which reflects the trade-off between their conflicting motivations. If they moved any closer to their companions, they would be too close to the human; if they moved further away from the human, they would be too far away from their companions.

The advantages of this test are that it requires no handling during the test itself, that it requires no expensive equipment and that it presents the animals with a situation which is very close to their natural behaviour (ie they do not require training for the test, as they do for mazes and runways). This is of particular importance when working with animals such as hill sheep, which are kept under extensive conditions and have little contact with people. Testing them in groups (Fell & Shutt 1989) avoids putting the test animals under the stress of social isolation and reduces the influence of individual differences on behaviour during the test. However, group testing requires a larger sample size and may reduce the individual's motivation to approach the stimulus sheep in the adjacent pen, particularly in breeds of sheep with low sociability such as the Scottish Blackface (Lynch *et al* 1992).

In the experiment reported here, I wanted to find out whether the approach–avoidance test can be used on single sheep. Before the test can be used to answer general questions of aversiveness, it first has to be shown that it can detect known differences in aversiveness. I chose the orientation of the handler as the stimulus, since a predator facing toward an animal has been shown to be more aversive than one facing away (eg human facing toward sparrows [Hampton 1994]; hawk facing toward chickens [Gallup *et al* 1971]). This difference is

reinforced every time that sheep are handled during routine husbandry procedures, as a handler facing them is associated with handling (eg for clipping or administration of medication), whereas a handler facing away is associated with no handling. The difference between these two orientations can, therefore, be expected to be meaningful to sheep without any additional training. The test sheep were not handled in the arena, so that the orientation of the handler would lose its meaning with repeated exposure (extinction). The hypotheses to be tested were that single sheep in the arena would stay further away from their companions when a human handler stood between them and the companions; that this distance would be greater when the handler was facing toward the test sheep than when the same handler was facing away; and that this latter effect would decrease with repeated exposure (as a result of extinction).

Materials and methods

Animals and housing

The experimental subjects were 10 one-year-old female Scottish Blackface sheep. For the duration of the experiment (five days), they were housed in a group pen (4.5 m × 6 m) indoors and bedded on sawdust, with hay and water provided *ad libitum*. Testing began two days after the sheep had been moved from pasture to the group pen. Prior to the start of the test sessions, the sheep were introduced into the arena in groups of three (each sheep six times) to habituate them to the test situation. After the experiment, the animals were returned to the flock.

The experiment was carried out in a building which people entered from time to time. To mask any resulting disturbances, a radio was played from 0700h to 2000h on test days, tuned to a station which broadcasts people talking.

The test arena

The layout of the test arena (Figure 1) was similar to that used by Adams and Fell (1997), Chapman *et al* (1994), Fell *et al* (1991), and Kilgour and Szantar-Coddington (1997), being 3 m × 13 m, with lines painted 1 m apart on the concrete floor. At one end of the arena, separated by wire mesh, was a 3 m × 3 m group pen with sawdust, in which the remaining nine sheep were kept while the test sheep was in the arena. A wooden board (approximately 0.8 m × 2 m) was placed in front of part of the wire mesh, to prevent the sheep in the group pen from seeing the handler while still allowing the test sheep to see them. There were two gates to the arena, one 7 m from the group pen (gate 1) where the test sheep entered, and one beside the group pen (gate 2) where the handler entered and left and where the test sheep left the arena. All handling during the test was carried out by the same person, who acted as the avoidance stimulus.

The test procedure

Before testing, the entire group of 10 sheep was moved into the group pen. A test sheep was then separated from the rest of the group and moved into the arena via gate 1. Two minutes later, the handler entered through gate 2 and stood in front of the wooden board, where he could not be seen by the sheep in the group pen. After two minutes, the handler left the arena through gate 2. Two minutes later, he opened gate 2 again, to let the test sheep return to the handling area, where it was reunited with the group. If a sheep did not leave the arena voluntarily, then the handler entered the arena via gate 1 to encourage the test sheep to leave.

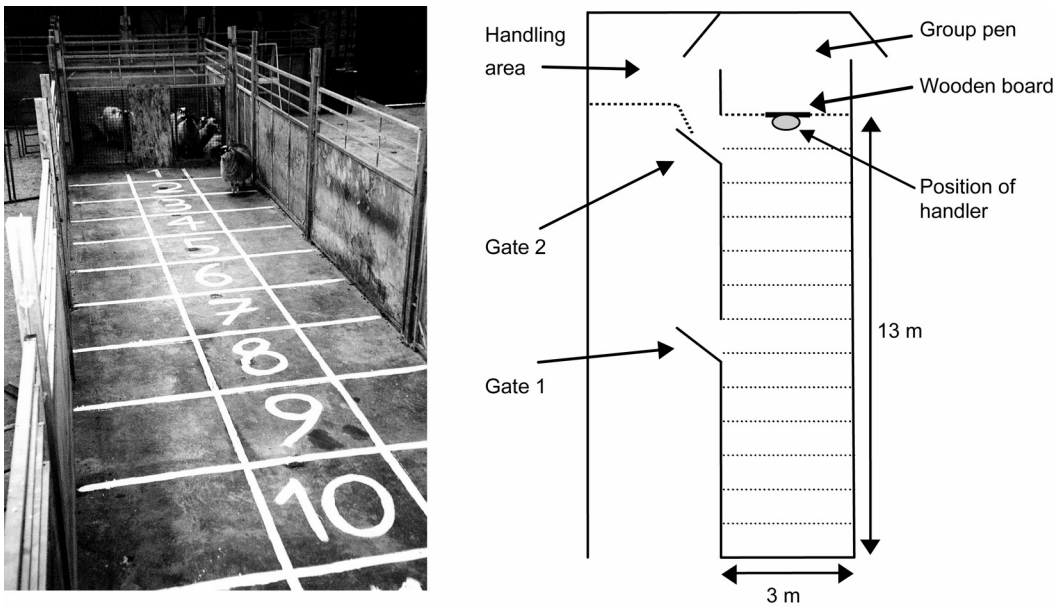

Figure 1 **The test arena and handling area.**

Each sheep was tested once per session for 10 sessions, so each sheep was tested 10 times. The three 2 min phases will be referred to as BEFORE (starting when the test sheep's front legs first entered the arena); HUMAN present (starting when the handler had assumed his position in the arena); and AFTER (starting when gate 2 was closed behind the handler). During the HUMAN phase, the handler was either facing toward the arena (FACE; eyes directed toward the centre of the arena), or facing away from the arena toward the wooden board (BACK). The BEFORE period allowed the test sheep to calm down after having been put into the test arena, and the AFTER period was used to dissociate the human handler in the arena from the handling required to return the sheep to the group.

The sheep were randomly assigned numbers 1 to 10. Sheep with odd numbers had the treatment FACE during odd-numbered sessions, sheep with even numbers during even-numbered sessions. Session 1 was carried out on day 1, sessions 2–5 on day 2, sessions 6–8 on day 3, and sessions 9 and 10 on day 4, between 0820h and 1830h. The sheep were tested in the same order each time, but after every two sessions the two sheep tested ninth and tenth were tested first and second in the next session.

The lines on the arena floor sectioned it into areas of 1 m × 3 m, with the distances (in m) painted in the centre of the areas (see Figure 1). The time spent by a test sheep in each of the areas was recorded using the Observer© software (http://www.noldus.com). Urination and/or defecation between introduction to the arena and the end of the test, and number of vocalisations during the HUMAN phase, were also recorded.

Measurements

Locomotion was estimated by calculating the number of areas entered (lines crossed). The mean distance of the test sheep from the group pen during a particular phase of the test was calculated by multiplying the distance from the group pen by the proportion of time spent

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there. The effect of the presence of the handler was calculated by subtracting the distance during the BEFORE phase from that during the HUMAN phase.

Statistical analysis

The experiment was designed to compare the effect of the human's orientation during the test in the two treatments FACE and BACK.

Short term effects (first exposure to stimuli): sessions 1 and 2 and the treatment FACE versus BACK were organised in a 2×2 factorial design. The hypothesis that sheep find the handler's face more aversive than his back (greater distance for FACE) was tested with a sign test (one-tailed) on the direction of the difference FACE minus BACK, as well as with an analysis of variance with session and orientation (FACE versus BACK) as the treatments, blocked within individual.

Medium term effects (repeated exposure): For each sheep, the average distances for FACE and BACK across sessions 3–10 were calculated. Because the means for both treatments were identical, a statistical analysis was considered unnecessary.

Consistency across sessions: Because the distances had several outliers, which could have resulted in artificially high correlations, Spearman's rank order correlation coefficients were calculated. The average correlation coefficient was calculated by converting the correlation coefficients into z-values, calculating the mean of these, and then converting them back into a correlation coefficient (Snedecor & Cochran 1989). The median correlation coefficient is also given.

Differences between the periods BEFORE, HUMAN and AFTER were analysed within animal and session, using analysis of variance with 'period' as the treatment and individual by session interaction as blocks.

Ethical considerations

Assessing aversiveness of procedures or stimuli is an essential part of the study of animal welfare. It is, however, important that care is taken to avoid imposing unnecessary stress on the experimental subjects. The method described in this paper minimises stress by providing the test animal with both visual and auditory contact with familiar conspecifics, and by minimising handling (movement of the test sheep from the group into the test arena only). Giving the animals the opportunity to stay away from the aversive stimulus further reduces the potential stress imposed on them. The aversive stimuli chosen required no special aversion learning prior to the test.

Results

The test sheep walked less and stayed at a greater distance from the companion sheep in the presence of the handler (locomotion: 11.7 ± 0.85, 2.4 ± 0.49 and 5.9 ± 0.83 lines crossed; distance: 2.98 ± 0.49, 7.18 ± 0.67 and 3.73 ± 0.86 metres during the BEFORE, HUMAN and AFTER phases, respectively [mean ± SEM; ANOVA, $P < 0.001$ in all cases]). Three of the 10 sheep vocalised while the handler was present, during three, four, and nine of the 10 tests, respectively. When vocalising, the sheep bleated 3.5 ± 0.6 times (frequency per 2 min: mean ± SEM for those 16 tests in which sheep vocalised). The number of bleats, of those sheep which vocalised, decreased with exposure to the test. From session 5 onwards, only one bleat per sheep which vocalised was recorded. Six of the test sheep urinated during at least one test (3.3 tests on average); eight defecated during at least one test (4.1 tests on

average). There was no apparent effect of session on number of sheep that vocalised or urinated/defecated.

The presence of the handler increased the distance from the companion sheep in 93 out of the total of 100 tests. Four of the remaining seven tests were with one particular individual. The overall effect of the presence of the human handler was to increase this distance by 4.2 m (see Figure 2).

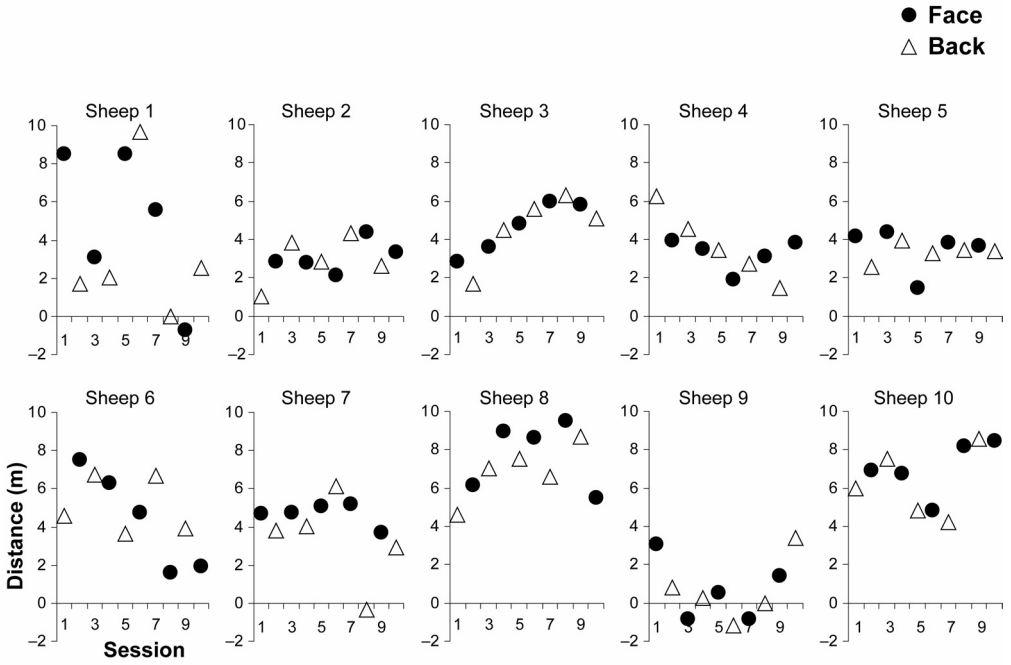

Figure 2 **The change in distance from before the handler entered the arena to when he was present for FACE and BACK across the ten sessions for each sheep.**

Short term effects (sessions 1 and 2)

The effect of the presence of the handler was calculated as the distance during the HUMAN phase minus that during the BEFORE phase. There was a significant effect of treatment on this difference (FACE and BACK [mean ± SEM]: 5.1 ± 0.66 m and 3.3 ± 0.63 m; ANOVA: $F_{1,19} = 6.3$, $P = 0.02$, one-tailed). Individual sheep was not a significant source of variation, although there was a weak tendency (ANOVA: $F_{9,19} = 2.33$, $P = 0.13$). The effect of FACE was greater than that of BACK in nine of the 10 sheep (sign test: $P = 0.011$; Figure 2). The order in which the stimuli were presented did not affect this difference (Mann-Whitney test: $W_{5,5} = 29.5$, not significant).

Medium term effects (sessions 3 to 10)

Across sessions 3 to 10, there was no difference between the FACE and the BACK treatments on the change in distance between the BEFORE and the HUMAN phase (FACE and BACK [mean ± SEM]: 4.2 ± 0.71 m versus 4.2 ± 0.62 m; Figure 2).

The overall tendency ($P < 0.07$) of sheep to move further away from the human when he was facing them compared to when he turned his back to them, averaged across all ten sessions, was due to the effect during the first two sessions (Figure 3).

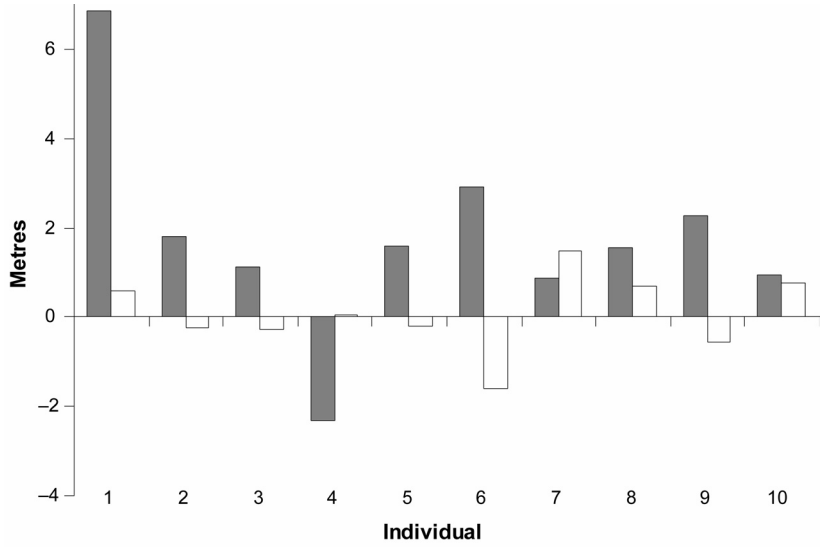

Figure 3 **The difference between the effect of human for FACE and BACK (FACE minus BACK [m]) for the ten test sheep. Dark bars represent sessions 1 and 2, open bars represent sessions 3 to 10.**

Consistency across time

Some sheep showed considerable change in the effect of HUMAN across the ten sessions, and there were large differences between individuals in the nature of this change (Figure 2). The correlation of the effect of HUMAN between consecutive sessions was low between sessions 1 and 2 (Spearman's rank order correlation $r_S = 0.27$), but high from sessions 3 to 10 ($r_S = 0.71$).

Discussion

By staying, on average, more than 4 m further away from their companions after the handler entered the arena, the sheep showed that they found being in close proximity to the handler aversive. For nine of the ten test sheep, this distance was greater when the handler was facing toward the arena than when he was turned away, which suggests that the arena test can indeed pick up not only that a stimulus is aversive, but also differences in aversiveness between stimuli. The order in which the two treatments FACE and BACK were first presented did not affect the sheep's response to them.

The difference between FACE and BACK was apparent only during the first two exposures to these stimuli. This could be attributable to the fact that they were tested in extinction — in other words, the stimuli had no consequences during the test, as the presence of the handler was separated from any handling of the test sheep by the 2 min periods before and after the human presence. This is a major difference from other tests of aversion, in which animals are moved toward a location where they then undergo a procedure. In the latter case, every exposure reinforces the association between the location and the procedure, and the number of exposures needed to create a difference can be a measure of the relative aversiveness of the treatments (Rushen 1986b; Grigor *et al* 1998). Fell and Shutt (1989) found the effect of one aversive treatment to be persistent across nine tests and 37 days. If the

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duration of aversion is related to the level of aversiveness in a similar way as the time taken to form an association, then it seems that the difference between the two stimuli used in the present experiment was not very large. Hampton (1994) found that sparrows (*Passer domesticus*) responded to the direction of a human face rather than to where the eyes were pointing. It is therefore possible that after only two exposures, the sheep interpreted both FACE and BACK as 'looking away'.

Testing of single sheep as opposed to small groups has potential implications for the entire test procedure and its interpretation. Kilgour and Szantar-Coddington (1997) tested sheep singly as well as in small groups, but in visual isolation from the companion sheep. They found that, when tested singly, sheep vocalised and walked a lot, whereas when tested in small groups they vocalised and walked hardly at all. Their results led them to conclude that testing of single sheep in the arena measured temperament rather than avoidance of the human. The levels of locomotor behaviour and vocalisation in the experiment described here are similar to those shown by sheep in small groups in visual isolation (Kilgour & Szantar-Coddington 1997), or in visual contact with other sheep (Fell & Shutt 1989). This suggests that the visual contact with the companion sheep in the pen adjacent to the arena provided enough of a social environment for the sheep not to feel isolated. It is, therefore, reasonable to assume that the test sheep were showing an avoidance of the handler as opposed to a reaction to social isolation.

Testing sheep in small groups provides the additional information of the spread of the group. Most breeds of sheep have the tendency to flock together in a situation of perceived danger, so the degree of flocking can be used as an indicator of the fear experienced at the moment of testing (Fell & Shutt 1989). This information is not available when sheep are tested singly. Testing of small groups also reduces the effect of individual differences, which may remove noise from the data. Individual differences, however, are not only shown during the test, but may also occur during the administration of the aversive treatment. In such cases, the aversiveness of a treatment may depend on the individual animal undergoing it. Under these circumstances, testing the animals singly is an advantage.

Testing sheep singly, as opposed to in small groups, reduces the number of animals required for a given sample size. This may be desirable when it is difficult to obtain a large number of test animals or when the procedure to be investigated causes considerable distress.

A potential use for the arena test is to compare different handling methods or procedures, provided that the sheep can associate these procedures with the person carrying them out. An example of this is provided by Chapman *et al* (1994), who studied the reaction of sheep after either surgical or non-surgical mulesing. In the surgical Mules operation, plasma cortisol levels rose immediately, while in the non-surgical procedure they rose more slowly, reaching their peak 24 h after the procedure, suggesting immediate pain in one case, and delayed pain in the other. When the authors tested the sheep in the arena test, the animals that had undergone surgical mulesing showed a lasting aversion to the person who had handled them during the procedure, while non-surgical mulesing did not lead to such an aversion. This is probably because the sheep linked the handler to the immediate pain, while failing to link the handler to pain which occurred 24 h after handling. The sheep in the present experiment distinguished between the two stimuli only during the first exposure, suggesting that they learned that even when the handler faced them, he did not move, and his orientation was therefore of no consequence to them. They may have seen the face as a stimulus associated with being handled, and the back as a stimulus associated with not being handled. It is

therefore conceivable that the arena test can be used to assess the relative aversiveness of two procedures by measuring the sheep's reaction to the person who carried out the procedure.

Animal welfare implications

The arena test can be used on individual sheep to study the relative aversiveness of stimuli. It puts the test animals in an approach–avoidance situation; this is a very basic conflict with which most animals will be familiar and which they should, therefore, be able to understand without training. The potential stress caused to the test animals is likely to be reduced by giving them visual contact with other sheep, by the requirement of the test itself for only a minimum of handling, and by the test animals being given the opportunity to choose their distance from the aversive stimuli, which reflects a trade-off between their conflicting motivations.

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