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A forced lateralisation test for dairy cows and its relation to their behaviour

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ABSTRACT

Dairy cows' emotional state can sometimes be inferred from their behaviour, for example previous studies have suggested that those passing a novel person to the right are more likely to be anxious than those passing to the left. We undertook two studies of cow behaviour as they passed a novel person, to validate these behaviours as emotional indicators, in addition to determining correlations to other indices of emotional state. Cows passing to the right were more likely to have a raised or tucked tail, sniff the ground, walk slowly and a faster exit when put in a crush, compared with those passing to the left, which had their ears held forwards. From a principal component analysis, it was determined that cows passing on the right side were also most likely to pass without turning their head towards the person, pass singly and defecate whilst passing. However, those passing to the left side were most likely to turn to look at the person and pass in pairs. Cows with high milk yields were more likely to pass on the right side. Measurements of side of passage were repeatable between experiments but those of ear position were not. It is concluded that side of passage past a person correlates with other behavioural indicators of the cow's emotional state, with those passing to the right (i.e. left eye/right brain *hemisphere)* apparently more anxious. Evidence was also provided that high yielding cows are more anxious, as assessed by right side passage. With further validation, side of passage past a person could be developed as a simple measure of emotional state in dairy cows that can be conducted under field conditions.

1. Introduction

In cattle, levels of arousal are related to the lateralisation of their behaviour (Phillips et al., 2003; Robins and Phillips, 2010; Phillips et al., 2015). Lateralisation is defined for the purposes of this paper as functioning on one side of the body, at either an individual or population level (Vallortigara and Rogers, 2005). Lateralised behaviour may derive from functional cerebral asymmetries (FCAs), which are specialized processes to enhance adaptive fitness (Vallortigara et al., 1999; Vallortigara and Rogers, 2005; Vallortigara, 2006; Ocklenburg et al., 2013; Rogers et al., 2013; Ströckens et al., 2013; Green and Jutfelt, 2014; Okamoto, 2014). The left hemisphere is specialised for temporal memory, and the right for spatial memory activity, sympathetic nervous system activation and vigilance (Bianki, 1988; Chernisheva, 2006). Vigilance is evidenced by alertness and scanning in threatening circumstances (Lazarus and Symonds, 1992), and is related to predation risk (Elgar, 1989; Quenette, 1990). It may be a useful indicator of anxiety, and an elevated head and pronounced opening of the eyes have been observed in cattle exposed to threatening situations (Sandem et al., 2002; Welp et al., 2004).

The neural processes in the right brain hemisphere associated with enhanced vigilance involve the amygdala, which reduces the neuronal thresholds in the animal's sensory systems and orientes attention their attention to affective stimuli (Holland and Gallagher, 1999; Davis and Whalen, 2001). Although generalisation about function across an entire hemisphere may be unwise because of variation between regions of the right hemisphere (Da Costa et al., 2004), it is still clear that aspects of the processing of emotions is hemispherically lateralized (Cabanac, 2002). The right-hemisphere processes fear/anxiety and aggression, i.e. negative emotions, and the left hemisphere is involved in perceptions of food rewards, i.e. positive emotions (Tucker, 1981). This is in line with left hemisphere responding to familiar stimuli and the right hemisphere to novel and unexpected stimuli. Furthermore, the left hemisphere consolidates and retrieves long-term memories for visual stimuli (Robins, 1997; Robins et al., 2005; Robins and Rogers, 2006). Thus, the right hemisphere of the vertebrate brain responds to definitive (i.e. identified), immediate cues, and the left for recalled cues (Rogers and Andrew, 2002; Vallortigara and Rogers, 2005).

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Recent approaches to animal emotions have categorized them by valence (ranging from negative to positive) and arousal (from low to high) dimensions (Mendl and Paul, 2004; Paul et al., 2005; Mendl et al., 2010; Zebunke et al., 2011; Puppe et al., 2012). Lateralisation in cows may assist in understanding their emotional valence and what stimuli they perceive to be threatening and stressful. This can be ssessed by a forced lateralization test, in which cows are forced to decide which side of a centralised person to pass when they walk down a lane (Phillips et al., 2015). Cattle passing on the right side, viewing the person with their left eye, connected to their right brain hemisphere, appear to be more stress susceptible, as evidenced by an increased stress score in a crush (Phillips et al., 2015). Most cattle, and especially subordinate ones, preferentially use their left eye to observe a threat (Robins and Phillips, 2010). The relations with cow performance are unclear, but it has been observed that a feed wagon approaching from the left side increased cow milk yield, enhanced reproduction and increased longevity, compared with one approaching from the right (Rizhova and Kokorina, 2005).

The forced lateralisation test is relatively new and comparison with other measures is prudent. There is evidence that emotional valence is also related to facial characteristics (Stiedl et al., 2004; Forkman et al., 2007). Cattle can move their ears asymmetrically (Nickel et al., 1968), and more pendulous ears have been observed when cattle are groomed, suggesting that their emotions are aroused and/or positively valenced (Schmied et al., 2008). In sheep and dogs, respectively, ear postures relate to cognitive appraisal of a stimulus (Quaranta et al., 2007) and probably contralateral hemispheric brain activity (Davidson et al., 1992; Wager et al., 2003). Sheep respond to food deprivation with lateralised ears, left in front of right (Reefmann et al., 2009). As well as facial expressions, tail posture and frequency of movement can indicate pain following castration (Petherick et al., 2014) and other stressors applied to cattle (Grant, 2004).

Although side of passing (or lateralised eye gaze) may indicate emotional valence in dairy cows, there is only limited information from previous studies. The objective of the present study was to examine emotional correlates of side of passing (i.e. lateralised eye gaze), in order to validate them as emotional indicators, and also to determine what contribution a forced lateralisation test could make as a test for emotions in cows. Our hypothesis was that cows walking to the right-hand side of a person, who view them mainly wth the left eye, would demonstrate behaviour indicative of anxiety, and in particular that ear position would be more likely to be forward and upright.

2. Materials and methods

Approval for the studies was obtained from the Animal Ethics Committee of the University of Queensland. Data were collected utilising the commercial dairy herd of 202 milking cows at the University of Queensland, 79% of which were Holstein-Friesians and the remainder Jersey, Brown Swiss, Red Swiss and mixed breeds. The milking herd were split into early (n = 110) and late (n = 92) lactation cow groups, both of which were milked twice daily, beginning at 05:00 and 15:00 h, in a Westfalia Surge rapid exit herringbone parlour. Cows were at pasture between the morning and afternoon milking and on a feed pad overnight, where they had access to a Total Mixed Ration.

2.1. Experiment 1

2.1.1. Forced Lateralisation Test (FLT)

Cows in the herd were tested for 9 consecutive days for side of passing past a person who had no previous contact with the cattle (a novel person) in a laneway down which the cows exited the milking parlour after afternoon milking. The laneway was of concrete construction (4.3 m wide by 23.5 m long from the parlour to the novel person, with an identical 3 barred metal fence on either side). This laneway had previously been used to demonstrate lateralised responses

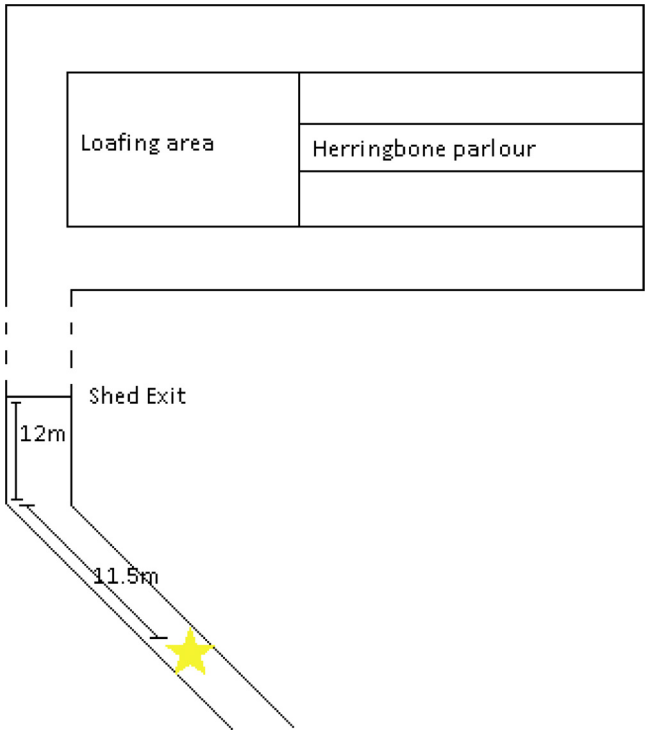

Fig. 1. Position of the person (star) in the lane exiting the parlour shed.

to a person in some of the same cows (two years previously), with the person standing in the lane on alternate days (Phillips et al., 2015). In the current 9-day test, the novel person stood in the centre of the lane, at a point equidistant to the left and right side fences (Fig. 1). She wore khaki overalls, which were assumed to be differentiated from farm and veterinary staff, who wore high visibility yellow overalls, and students, who wore blue overalls. The novel person stood facing the cows, which were identified by ear tags, and filmed their passage with an iPhone 5 mobile camera held in both hands. Video recordings were subsequently analysed to determine mean ratios of cows passing on the left and right sides of the passage (L and R, respectively) from the cow's perspective. Ratios were calculated as L + 1/R + 1, to avoid 0 being a dominator or numerator. An individual cow was assumed to be left lateralized when the value of ratio was > 1 or right when < 1. The distribution of cows passing to one side showed evidence of bimodality, with 41 cows going only to the right and 22 cows only to the left (Fig. 2).

The ear positions (EP) of each cow were classified, following the scoring system of Proctor and Carder (2014): EP1: ear held upright above the neck with the pinna facing forwards or to the side, EP 2: ear pinna directed forwards in front of cow and ear held horizontally, EP 3: ear held backwards on cow's head and EP 4: ear hung loosely downwards, falling perpendicular to head. They suggested that ear positions 3 and 4 reflect a low arousal, positive emotional state, since the duration of time spent in these postures increased during stroking, and positions 1 and 2 reflect high arousal and neutral or negative state, since they were increased before and after stroking. Ears were scored from video recordings by two observers working independently. Ear position was recorded at the moment that the front legs of the animal passed a line 1.5 m in front of the novel person. To ensure concordance for scoring the four unique postures described by Proctor and Carder (2014), the two observers separately scored the position of 16 cows in two repeated tests, which were statistically analysed by analysis of variance and the scoring system was established at the point when there was no difference ($P < 0.05$) between the two. The % of days each cow passed the observer in either EP1 or EP2 (EP1 + 2), or EP3 or EP4 (EP3 + 4) and the ratio of these was then calculated, representing the