Physiology & Behavior 123 (2014) 67–75

Contents lists available at ScienceDirect

Physiology & Behavior

journal homepage: www.elsevier.com/locate/phb

Are hungry sheep more pessimistic? The effects of food restriction on cognitive bias and the involvement of ghrelin in its regulation

Else Verbeek, Drewe Ferguson, Caroline Lee *

CSIRO, Animal, Food and Health Sciences, Locked Bag 1, Armidale NSW 2350, Australia

HIGHLIGHTS

- Ghrelin administration tended to induce a pessimistic judgement bias
- Ghrelin administration tended to increase plasma cortisol concentrations
- Chronic food restriction tended to induce an optimistic judgement bias
- Chronic food restriction resulted in an attention bias towards a food-related cue
- Food restriction may alter the affective state of sheep

ARTICLE INFO

Article history:
Received 1 April 2013
Accepted 26 September 2013

Keywords:
Affective states
Attention bias
Cognitive bias
Food restriction
Ghrelin
Sheep
Welfare

ABSTRACT

Food restriction is considered to be a welfare issue in extensively reared animals. However, the effects of food restriction on the affective state, and its physiological regulation, are unknown. In Experiment 1, we aimed to assess the effects of increased plasma concentrations of acyl-ghrelin on judgement bias (an indicator of affective states) by fasting sheep for 24 h or by ghrelin administration. In Experiment 2, we aimed to assess the effects of chronic food restriction on judgement bias and attention bias towards a food-related cue. For the judgement bias test, sheep were trained in an arena to approach a positive location cue associated with conspecifics and not approach a negative location cue associated with a dog. Three non-trained, non-reinforced ambiguous location cues were situated between the positive and negative locations. Attention bias towards a food-related cue was assessed by placing an empty food bucket against the wall of the arena halfway between the entry point and the positive location. In Experiment 1, sheep were divided into three treatments; 24 h fast, ghrelin administration or control. Judgement bias, locomotor activity and plasma cortisol concentrations were assessed. The ghrelin treated group tended to express a more pessimistic bias compared to the control group ($P<0.1$), and plasma cortisol concentrations tended to be increased ($P<0.1$). In Experiment 2, sheep were subjected to a high feeding level (HF) or low feeding level (LF) for 7 days. The LF group tended to show a more optimistic judgement bias ($P<0.1$). When the food-related cue was presented, LF ewes took longer to reach the positive location ($P<0.001$), spent longer with their head inside the bucket ($P<0.001$) and more time interacting with the bucket ($P<0.01$). This study provides preliminary evidence that food restriction alters judgement bias and attention bias towards a food-related cue which may indicate altered affective states of sheep.

1. Introduction

Food restriction and the resulting negative energy balance impose a major threat to the health and reproductive success of animals. Many grazing ruminants, both wild and extensively farmed, are likely to be exposed to periods of undernutrition at some stage of their reproductive cycle. However, it is currently unknown whether food restriction also negatively impacts the affective state of the animal. Animal affective states are major determinants of welfare [1], and it is therefore important to investigate whether undernutrition leads to a negative affective state. Food restriction is associated with a number of physiological changes, such as decreases in plasma leptin and insulin concentrations and increases in plasma ghrelin concentrations [2–4]. Such homeostatic adaptations stimulate food intake and facilitate the mobilisation of energy substrates [5]. Physiological changes in food restricted animals are also accompanied by behavioural adaptation that aid in restoring energy homeostasis. For example, sheep fasted for 24 h [6] and chronically undernourished sheep in negative energy balance have an increased motivation for food [4].

* Corresponding author. Tel.: +61 267761459; fax: +61 267761333.
E-mail address: caroline.lee@csiro.au (C. Lee).

0031-9384/$ – see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.physbeh.2013.09.017

Furthermore, food restriction results in increased plasma concentrations of corticosterone and decreased brain serotonin turnover rates, as well as increased depression-like and anxiety-like behaviours in mice [7]. However, investigations into the effects of food restriction on the affective state are relatively limited, in particular in ruminants.

Assessing the affective state of food restricted animals is a challenge due to the subjective nature of such states. However, progress has been made in developing a methodology to measure affective states, called a judgement bias methodology [8]. This methodology measures whether animals have an increased expectation of positive events (optimistic) or negative events (pessimistic) when exposed to ambiguous stimuli [9]. Animals exposed to stressful unpredictable environments judged ambiguous cues as more pessimistic [8,10], while animals given access to environmental enrichment judged ambiguous cues as more optimistic [11]. Therefore, the judgement bias methodology is a promising indicator of affective states and may provide insight into the affective states of food restricted animals.

Alternative cognitive measures may also provide insight into the affective state of food restricted animals. For example, it has been shown that hungry people of normal weight are more likely to shift their gaze towards images of food (compared to images of neutral objects) and gaze at the food images for longer than satiated people [12]. In a different study, one food image and one neutral image were displayed simultaneously on a computer screen, and people were asked to respond to a probe that replaced one of the images (visual probe task); it was found that hungry individuals were slower to disengage their attention from the food-cues [13]. These results suggest that changes in the physiological state due to food restriction led to a biased attention toward food-cues. Similar changes in attention bias towards food-cues may occur in food restricted animals. For example, attention toward a food-related cue (e.g., an empty bucket, or other object, that is normally associated with food) may alter depending on the hunger state. The latency to detect and approach a food-related cue may be faster and the latency to disengage attention from the cue may be slower in food restricted compared to satiated animals. In addition, the duration of the time spend interacting with the food-related cue could be indicative of food searching/motivation behaviours.

Very little is known about the underlying physiological processes that are involved in the regulation of behavioural adaptations to food restriction. Ghrelin, secreted by the oxyntic cells in the gut, is a particularly interesting peptide, as it is the only gut peptide that stimulates food intake by acting on several nuclei in the hypothalamus [14]. In sheep, plasma ghrelin concentrations are higher in fat than in lean animals [3] and ghrelin plays an important role in the short-term regulation of feeding and meal initiation [15,16]. Furthermore, intravenous injections of ghrelin stimulates feed intake in sheep [17]. There is also evidence to suggest that ghrelin provides a link between homeostatic processes and cognitive and affective behaviours [18,19]. Intracerebroventricular administration of ghrelin increased locomotor activity and may be implicated in reward and motivated behaviours, most likely by increasing brain dopamine concentrations [20]. Moreover, intracerebroventricular administration of ghrelin increased anxiety-like behaviour in an open field test and elevated plus maze and depression-like behaviour in a forced swim test in rats [21,22]. Peripheral administration of ghrelin also increased anxiety-like behaviours in the elevated plus maze and led to an increase in corticosterone in mice, suggesting that ghrelin may play a role in mediating behavioural and neuroendocrine responses to stress [23]. Therefore, increased concentrations of ghrelin may lead to changes in the affective state, and ghrelin may be a physiological signal involved in altering the affective state of food restricted animals.

The aim of this study was to conduct two different experiments in order to investigate the effects of acute (no change in body weight) and chronic food restriction (leading to a reduction in body weight) on the affective state of sheep. In Experiment 1, we aimed to assess the effects of increased plasma concentrations of acyl-ghrelin on judgement bias by fasting sheep for 24 h or by ghrelin administration. We hypothesise that a 24 h fast leads to a pessimistic judgement bias in sheep, and that similar changes in judgement bias occur after ghrelin administration in satiated animals. In addition, we aim to measure locomotor activity and vocalizations during the judgement bias test as additional behavioural indicators of the affective state, and plasma cortisol concentrations as a physiological indicator of distress. Because the effects of the 24-h fast on judgement bias in Experiment 1 were relatively minor, we also investigated the effects of a chronic 7-day food restriction period on judgement bias as well as an additional cognitive measure; attention bias towards a food-related cue. We hypothesise that chronic food restriction leads to a pessimistic judgement bias and an increased attention bias towards a food-related cue. This study will therefore contribute to a better understanding of changes in the affective state depending on the physiological hunger state and its regulation. A better understanding of the affective state of food restricted animals is an important step toward improving animal welfare.

2. Methods

2.1. Ethical note

This study was approved by the CSIRO Chiswick Animal Ethics Committee (#11–18). All animals were monitored closely for health and wellbeing during and after the experiment. No long-term negative effects on health and wellbeing as a consequence of the study were observed.

2.2. Animals, housing and management

Forty-one Merino ewes (14–16 months old) with an average body weight (BW) of 42.6 ± 0.6 kg and a body condition score [BCS, scored on a scale of 1–5; 24] of 2.7 ± 0.04 were used for the experiment. All ewes had been accustomed to indoor housing conditions in group pens (12–14 ewes per pen) during a previous experiment. Sheep were fed a mixture of concentrate pellets (900 g per sheep, 11.9 MJ/kg DM containing wheat, lucerne, pollard, bran, salt and ammonium chloride, with 22% crude protein, 2.5% fat, 11% ash, 14% acid detergent fibre, 30% neutral detergent fibre) and oaten chaff (300 g per sheep, 8.9 MJ/kg DM with 11% crude protein, 9% ash, 33% acid detergent fibre, 57% neutral detergent fibre). The diet corresponded to 110% of theoretical maintenance requirements [25].

2.3. Judgement bias facility and training

Judgement bias was assessed in a 3 × 3 m arena of which the back wall was divided into five different locations (60 cm wide, 80 cm long and 40 cm high) made of fixed wooden panels that could be closed/opened by inserting/removing a wooden panel (Fig. 1). Behind the two most outer locations was a sliding door that could be lifted to reveal either a dog or two companion sheep. Identical looking dummy doors were positioned behind the three middle locations (ambiguous locations, see below), although these could not be physically opened. Each location was also associated with a coloured cue printed on A3 size laminated paper attached to the sliding and dummy doors. The cues were all of the same colour green, but differed in brightness (0, 25, 50, 75 and 95%). The degree of brightness was adjusted in Microsoft PowerPoint. Previous research has shown that sheep have a good ability to discriminate between different degrees of brightness [26]. Therefore, five different location/colour cue combinations were used for judgement bias testing: positive (P, closest to the companion sheep), near positive (NP), middle (M), near negative (NN) and negative (N,