THE ROYAL SOCIETY

Received 14 April 2004
Accepted 9 June 2004
Published online 7 September 2004

Face pictures reduce behavioural, autonomic, endocrine and neural indices of stress and fear in sheep

Ana P. da Costa, Andrea E. Leigh, Mei-See Man and Keith M. Kendrick*

Laboratory of Cognitive and Behavioural Neuroscience, Babraham Institute, Cambridge CB2 4AT, UK

Faces are highly emotive stimuli and we find smiling or familiar faces both attractive and comforting, even as young babies. Do other species with sophisticated face recognition skills, such as sheep, also respond to the emotional significance of familiar faces? We report that when sheep experience social isolation, the sight of familiar sheep face pictures compared with those of goats or inverted triangles significantly reduces behavioural (activity and protest vocalizations), autonomic (heart rate) and endocrine (cortisol and adrenaline) indices of stress. They also increase mRNA expression of activity-dependent genes (c-*fos* and *zif*/268) in brain regions specialized for processing faces (temporal and medial frontal cortices and basolateral amygdala) and for emotional control (orbitofrontal and cingulate cortex), and reduce their expression in regions associated with stress responses (hypothalamic paraventricular nucleus) and fear (central and lateral amygdala). Effects on face recognition, emotional control and fear centres are restricted to the right brain hemisphere. Results provide evidence that face pictures may be useful for relieving stress caused by unavoidable social isolation in sheep, and possibly other animal species, including humans. The finding that sheep, like humans, appear to have a right brain hemisphere involvement in the control of negative emotional experiences also suggests that functional lateralization of brain emotion systems may be a general feature in mammals.

Keywords: face attraction; stress; fear; temporal cortex, amygdala; emotion

1. INTRODUCTION

Sheep, like humans and non-human primates, have sophisticated social recognition skills using visual cues from faces. They also have specialized neural systems in the temporal and frontal brain cortices for performing this task and, like humans, there appears to be some degree of hemispheric specialization with the right being more important than the left for face identification (Broad *et al.* 2000; Peirce *et al.* 2000; Peirce & Kendrick 2002). In sheep, face-sensitive cells in these regions tend to categorize faces in terms of their emotional and behavioural significance rather than on the basis of a common perceptual appearance. The most striking example of this is that human and dog faces, which differ markedly in appearance but share a common negative emotional significance for sheep are usually encoded by the same cells (Kendrick & Baldwin 1987). The importance of this emotional characterization is further illustrated by our finding that when a specific human becomes very familiar to the sheep, and is always associated with positive interactions, their face starts to be encoded more by cells that are normally only responsive to the faces of socially familiar sheep (Kendrick *et al.* 2001). This implies close interactive links between brain regions controlling face recognition and those controlling emotional responses. Such links often appear to become dysfunctional in human developmental (autism) and psychiatric (schizophrenia and depression) disorders where face recognition abilities seem to function relatively well but responses to emotional cues from facial expressions are impaired (George *et al.* 1998; MacDonald *et al.* 1989; Ross *et al.* 2001; Edwards *et al.* 2002).

This study has attempted to establish the strength of this link between face recognition systems and those mediating emotional responses by assessing the behavioural, autonomic and endocrine effects of exposing sheep to familiar face pictures when they are stressed through social isolation. The measures taken were those typically sensitive to stressors in sheep and in many other species, and included changes in vocalizations and locomotion, heart rate and concentrations of the adrenal hormones cortisol and adrenaline (Parrott *et al.* 1994, 1998). Altered patterns of neural activation have then been visualized in face and emotion/stress-associated brain regions using quantification of mRNA changes in two immediate early genes, c-*fos* and *zif*/268. We have already used mRNA changes in these two genes to show patterns of brain activation during face and olfactory recognition and physiological stress in sheep (Vellucci *et al.* 1995; Da Costa *et al.* 1997; Broad *et al.* 2000). The human brain not only exhibits a right-hemisphere dominance for face recognition but also for the processing of intense negative emotional cues (Davidson 1995). We have therefore also investigated whether any alleviation of negative emotional states caused by viewing familiar faces in sheep is associated with neural activation changes lateralized to the right hemisphere.

2. MATERIAL AND METHODS

(a) *Subjects*

Forty Clun Forest sheep (*Ovis aries*) were used for the study (19 were used for behavioural analysis—11 of these were also used

* Author for correspondence (keith.kendrick@bbsrc.ac.uk).

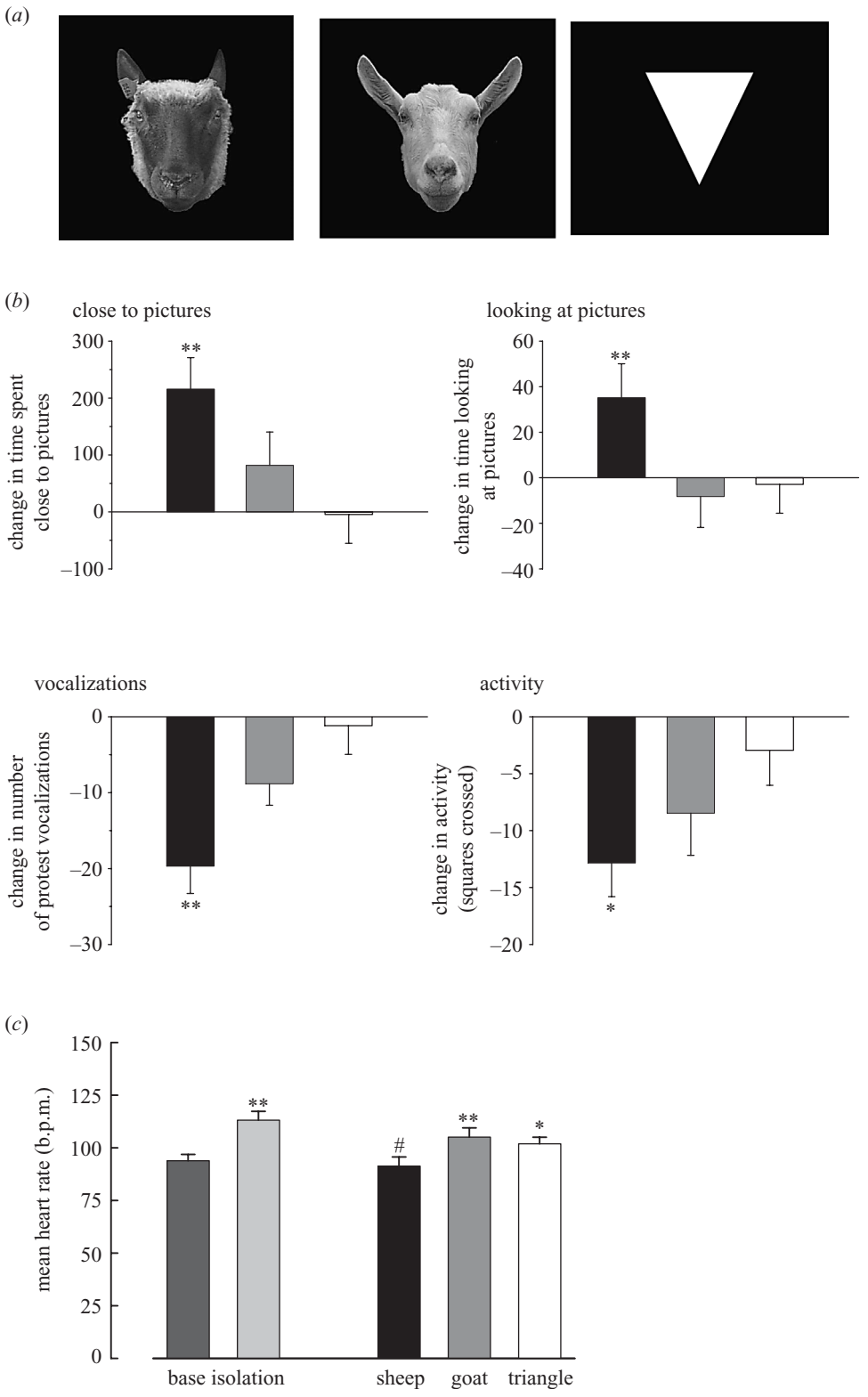

Figure 1. (*a*) Sheep, goat and inverted triangle pictures used as stimuli. (*b*) Histograms show mean + s.e.m. differences in time spent near to each of the stimulus pictures or looking directly at them and frequencies of high-pitched protest vocalizations and activity (number of gridline crossings) between the first 15 min period of social isolation and a second 15 min period when either the face pictures (sheep, black bars or goat, grey bars) were shown or the inverted triangle (open bars) continued to be shown (control). $^{**}p < 0.01$ or $^{*}p < 0.05$ compared with inverted triangle or goat ($n = 19$ animals). (*c*) Mean + s.e.m. heart rate (beats per minute, b.p.m.) before and during the first 15 min of isolation and then during the remaining 15 min period of isolation where either the triangle, sheep or goat face pictures were viewed. $^{**}p < 0.01$, $^{*}p < 0.05$ versus baseline and $^{\#}p < 0.05$ versus inverted triangle or goat ($n = 11$ animals).

for taking endocrine and autonomic measures—and 21 for analysis of altered patterns of brain activation using quantification of c-*fos* and *zif*/268 mRNA expression). These animals were kept together in flocks and had had some previous exposure to tasks requiring discrimination between sheep face pictures.

(b) *Procedure for behavioural analysis*

Animals were introduced individually into an enclosed funnel-shaped arena (1 m wide at the entrance, 4 m long and 4 m wide at the far end) and kept in isolation for a short period (30 min) to simulate typical conditions encountered during normal husbandry practices. When the animals first entered the arena they were presented with four static pictures of an inverted white triangle (to simulate a face shape) on a black background on the rear wall (spaced 1 m apart). Although, similar to some human studies, we have previously used inverted faces as a control, we decided against this in the present experiments because exposure to stimuli was long (15 min). In our experience some sheep can, given time, discriminate between inverted faces, whereas the inverted triangle stimuli we chose are totally unfamiliar even though they simulate a generic face shape. After an initial 15 min period, the control triangle pictures were then either maintained for a further 15 min (control condition to simulate a face shape) or changed to face pictures of an unfamiliar sheep of the same breed or an unfamiliar species: a goat (figure 1*a*). Three different individual sheep and goat faces were used during the study. The different picture conditions were presented in a random sequence to each sheep at 48 h intervals. Animals that were also used for autonomic and endocrine measures were fitted with remote heart-rate recorders (PolarS610) both before and during the isolation period. Blood samples were taken from them by jugular venipuncture before and immediately after the isolation period for measurement of the concentrations of cortisol, noradrenaline (NA) and adrenaline (AD).

To assess the differential effects of the picture stimuli on brain activity, 20 animals were killed (i.v. lethobarb—30 ml) 15 min following the end of the isolation period (i.e. 30 min after they were exposed to the picture stimuli). Their brains were removed and frozen at −80 °C for subsequent measurement of c-*fos* and *zif*/268 mRNA by *in situ* hybridization histochemistry.

(c) *Behaviour and heart-rate measures*

Several different behavioural measures were taken, including those considered to be sensitive indices of stress (number of protest high-pitched bleat vocalizations, and locomotor activity—the isolation chamber was divided up into 8 × 1 m squares and activity was measured as the number of squares crossed during each of the phases of the experiment), and interest in the different images projected onto the four panels at the rear of the chamber (time spent within 1 m of the pictures and time spent looking directly at one of the pictures).

(d) *Assays*

Blood concentrations of cortisol were measured in a single radioimmunoassay as previously described (Vellucci *et al.* 1995). Concentrations of AD and NA were measured, after a perchloric acid extraction, by high-performance liquid chromatography with electrochemical detection (as previously described by Vellucci *et al.* (1995)). Detection limits were 1.7 nM for cortisol and 0.1 pM for AD and NA.

(e) In situ *hybridization*

Anterior–posterior coronal sections (12 μm) of the sheep brains were cut on a cryostat (Bright) and thaw-mounted onto slides coated with poly-L-lysine. Sections were fixed for 5 min in phosphate-buffered paraformaldehyde, washed twice in phosphate-buffered saline (PBS—pH 7.4) for 2 min, dehydrated in an ascending ethanol series, stored in 95% ethanol and kept at 4 °C until required.

Oligonucleotide probes were 3′ end-labelled using terminal deoxynucleotidyl transferase (Pharmacia) with an α-35S-thiophosphate dATP (NEN) at 30 °C for 1.5 h. Labelled probes for c-*fos* and *zif*/268 (Broad *et al.* 2002; da Costa *et al.* 1997) were purified using Sephadex G-50 gel exclusion columns (Pharmacia) and diluted to a concentration of 3000 dpm μl^{-1} in hybridization buffer (50 mM sodium phosphate, 1 mM sodium pyrophosphate, Denhardt's solution (5×), heparin (120 $\mu g\,ml^{-1}$), salmon sperm DNA (200 $\mu g\,ml^{-1}$), polyadenylic acid (100 $\mu g\,ml^{-1}$), dithiothreitol (40 mM), formamide (50%) and dextran sulphate (10%). Hybridization was performed overnight in humidified chambers at 42 °C for c-*fos* and *zif*/268. Sections were then washed in 1× SSC, once at room temperature and a second time at 55 °C. They were dipped in 0.1× saline sodium citrate, then in an ascending ethanol series, air-dried and exposed to Amersham hyperfilm for 15 days at room temperature to visualize labelled c-*fos* and *zif*/268 mRNA expression on the brain sections. Sections required for cellular resolution were dipped in liquid photographic emulsion (Ilford K-5) for 10 weeks, developed in phenisol (Ilford) and fixed and counterstained in methylene blue.

Both optical density and grain counts were quantified using an image analysis system (AIS, Haverhill, UK). Optical density readings were analysed bilaterally in all animals using 2–8 sections per region and with reference to ^{14}C standards (ARC Inc., St Louis, USA) to produce readings in $nCi\,g^{-1} \times mm^2$. This allowed an overall estimate of gene expression within a region to be made. For cell autoradiography, grain counts were made for 50–100 cells in a particular region in 3–4 different sections. The brain regions analysed include those regulating stress (hypothalamic paraventricular nucleus: PVN; Vellucci *et al.* 1995), fear (central and lateral amygdala; Le Doux 2000) and responses to faces (frontal and temporal cortices and basal amygdala; Broad *et al.* 2000; Haxby *et al.* 2000) as well as some others associated with emotional responses (orbitofrontal and cingulate cortex; Le Doux 2000) and aggression (septum; Albert & Walsh 1984). To our knowledge, no detailed work has been carried out establishing the role of the amygdala, septum, orbitofrontal and cingulate cortices in emotional behaviours in sheep, although it has in rodents, monkeys and humans. Additional areas quantified included the entorhinal cortex and hippocampal sub-fields, the piriform and somatosensory cortices and the diagonal band.

(f) *Statistics*

For behavioural measures, data were expressed as differences between the initial 15 min of isolation compared with the following 15 min of isolation when either sheep or goat faces were shown. This was the same for hormonal measures, although the differences were between pre-isolation levels and those seen at the end of the 30 min period of isolation. These data were analysed by a repeated measures parametric or non-parametric (Friedman) ANOVA and, where this gave $p < 0.05$; the Tukey or Dunn's test was used for *post-hoc* comparisons. Non-parametric tests were used only where data were not normally distributed. A mean heart rate was calculated for each animal during the period prior to isolation and the first and second 15 min periods of the isolation. A repeated-measures ANOVA, followed by a *post-hoc* Tukey test, were then used to test for significant changes. For the gene expression analysis, mean optical density or grain counting measures obtained in the three independent treatment groups (triangle, sheep and goat) were subjected to ANOVA tests and, where $p < 0.05$, these were followed by a *post-hoc* Tukey test.

3. RESULTS

(a) *Behavioural effects*

There were significant changes in the numbers of high-pitched protest bleats ($F = 6.19$, d.f. 2, 18, $p = 0.005$), activity (Friedman ANOVA, $Fr = 9.562$, d.f. 2, 18, $p = 0.008$), time spent close to the pictures ($F = 4.96$, d.f. 2,18, $p = 0.013$) and time spent looking at the pictures ($F = 4.96$, d.f. 2, 18, $p = 0.013$) in the different experimental conditions. During the initial period of isolation the animals showed high frequencies of high-pitched protest bleats and locomotor activity, and paid little attention to the triangle pictures. Exposure to the sheep faces, but not the goat faces significantly decreased both vocalizations ($p < 0.01$) and locomotor activity ($p < 0.05$) and increased the amount of time spent close to the pictures ($p < 0.01$) and looking directly at them ($p < 0.01$; figure 1*b*).

(b) *Autonomic and endocrine effects*

The effects of initial isolation on increasing heart-rate levels did not vary significantly across the three experimental conditions ($F = 0.2643$, d.f. 2, 10, $p = 0.770$) so an overall mean was calculated. Heart-rate levels varied significantly across conditions ($F = 9.981$, d.f. 4, 10, $p < 0.001$). As expected, there was a significant increase in heart-rate levels during the initial period of isolation (mean ± s.e.m.: 93.8±3.01 beats per minute (b.p.m.) before isolation versus 113.1±4.23 b.p.m. during it; $p < 0.001$; figure 1*c*). When sheep were exposed to the sheep face pictures, heart-rate was reduced significantly to pre-isolation levels (91.3±4.38 b.p.m.; $p < 0.001$; figure 1*c*). With continued exposure to the triangles (101.98±3.1, $p > 0.05$) or pictures of goat faces (105.1±4.4, $p > 0.05$), heart rates were not significantly reduced.

There was a highly significant increase in blood cortisol concentrations at the end of the period of isolation compared with those found pre-isolation for the triangle and goat face conditions (baseline versus triangle: $t^{10} = 3.208$, $p = 0.0094$, baseline versus goat: $t^{10} = 5.481$, $p < 0.001$; figure 2*a*). When the animals were exposed to sheep faces there was only a slightly significant increase (baseline versus sheep: $t^{10} = 2.38$, $p = 0.039$). The proportionate changes between the three conditions were significant ($F = 5.83$, d.f. 2, 10, $p = 0.0101$). The magnitude of the increase was significantly higher in the triangle (454.2±117%, $p < 0.05$) and goat face (488.7±106%, $p < 0.05$) conditions than that seen with the sheep faces (204.6±48.1%; figure 2*a*).

Blood AD concentrations had also increased significantly following the period of isolation in the triangle but not the goat face conditions (baseline versus triangle: $t^{10} = 3.874$, $p = 0.003$, baseline versus goat: $t^{10} = 0.206$, $p = 0.84$; figure 2*b*). Following exposure to the sheep faces, AD concentrations were significantly reduced compared with the pre-isolation period (baseline versus sheep: $t^{10} = 2.698$, $p = 0.022$). Overall there was a significant variation in the magnitude of AD changes across the three conditions ($F = 4.045$, d.f. 2, 10, $p = 0.034$). The increase in the triangle condition (233.6±74.85%, $p < 0.05$) was significantly higher than in the sheep (65.89±10.54%) or goat face (106.46±20.51%) conditions (figure 2*b*). There was no significant difference between the sheep and goat face conditions ($p > 0.05$). There were no significant changes in blood NA concentrations associated with either isolation or presentation of the pictures (data not shown).

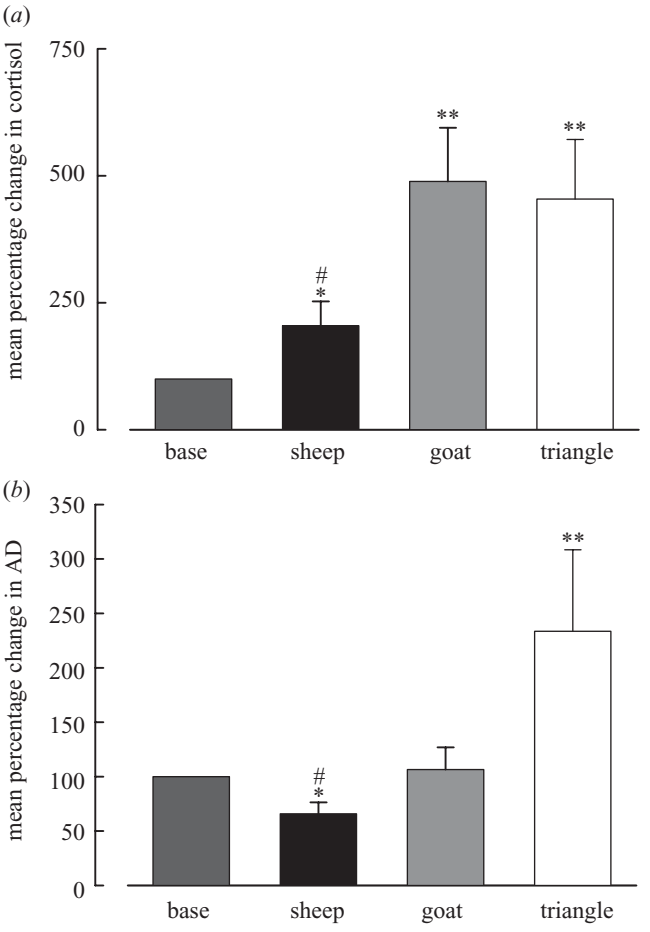

Figure 2. Histograms show mean + s.e.m. plasma concentrations of (*a*) cortisol and (*b*) AD before the period of isolation and immediately after it in the three different viewing conditions (triangle, sheep or goat face). Mean + s.e.m. basal concentrations of cortisol were 9.8±1.13 nM and for AD 3.5±0.65 nM. $^{**}p < 0.01$, $^{*}p < 0.01$ versus baseline and $^{\#}p < 0.05$ versus inverted triangle and goat face ($n = 11$ animals).

(c) *Altered* zif*/268 and c-*fos *mRNA expression*

When isolated sheep were exposed to sheep faces, *zif*/268 and c-*fos* mRNA expression increased significantly in face processing regions (the right medial and posterior inferior and superior temporal cortices, the medial prefrontal cortex and right basal amygdala) in comparison with animals exposed to the inverted triangles or goat faces (figures 3 and 4). By contrast, expression levels were significantly reduced in regions controlling fear (the right lateral and central amygdala nuclei) and stress (bilaterally in the PVN; figures 3*b* and 4*a*). There was also a significant increase in regions involved in emotional responses (the right lateral anterior cingulate cortex and the right orbitofrontal cortex). Significant changes in responses to goat faces were variable. They occurred in parts of the right medial and superior temporal cortices but not in the basal amygdala. Reduced *zif*/268 expression was seen in the right lateral amygdala in comparison with the triangle condition (figure 3*b*). Similar patterns of results were seen for optical

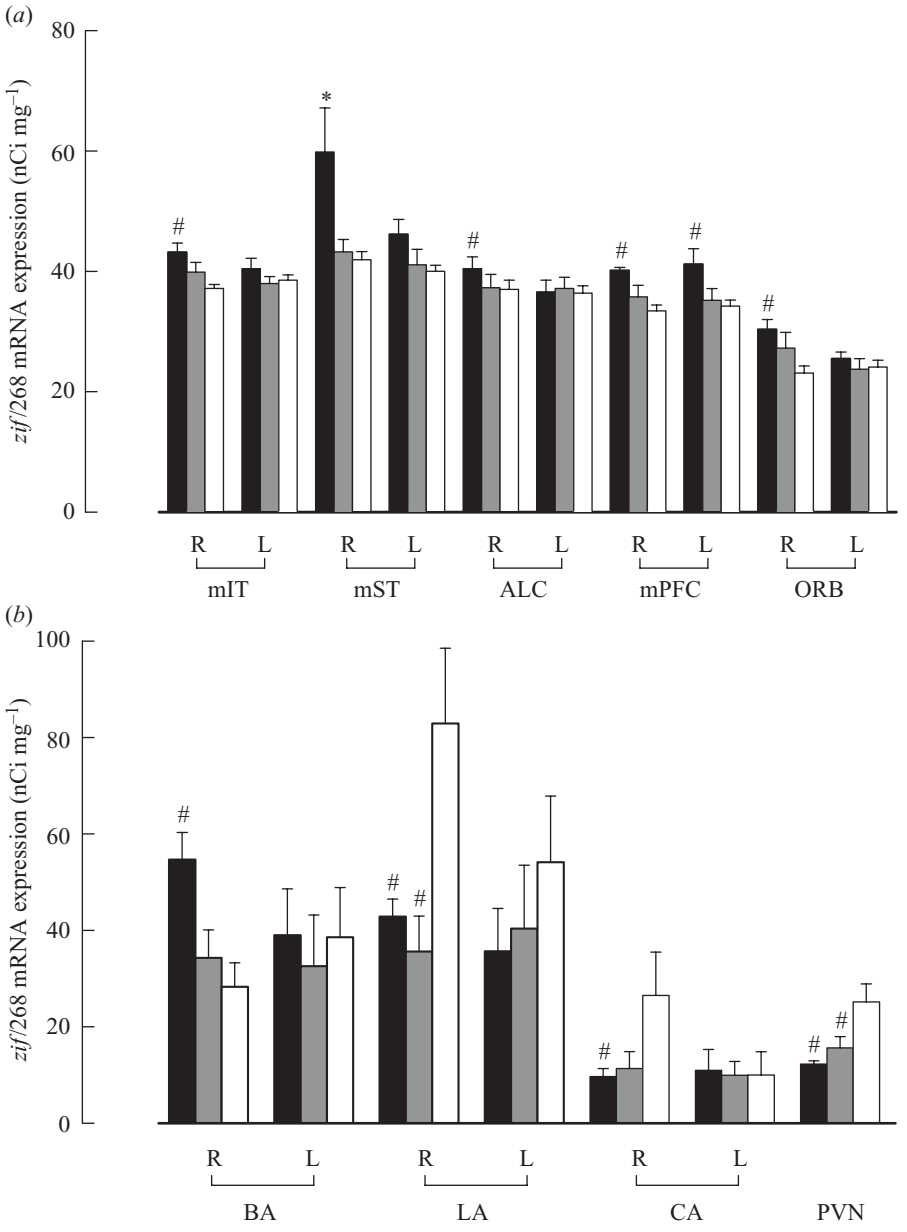

Figure 3. Histograms show mean + s.e.m. *zif*/268 mRNA expression (given as nCi mg^{-1} against ^{14}C standards) in right (R) and left (L) sides of (*a*) cortical regions and (*b*) amygdala and hypothalamus of the brain after 30 min of isolation while viewing pictures of inverted triangles (open bars), sheep (black bars) or goat (grey bars) faces. $^{\#}p < 0.05$ versus inverted triangle; $^{*}p < 0.05$ versus inverted triangle and goat face. mIT: medial inferior temporal cortex; mST: medial superior temporal cortex; ALC: anterior lateral cingulate cortex; mPFC: medial prefrontal cortex; ORB: orbitofrontal cortex; BA: basal amygdala nucleus; LA: lateral amygdala nucleus; CA: central amygdala nucleus; PVN: hypothalamic paraventricular nucleus ($n = 7$ animals per group).

density or grain counting measurements, and only optical density readings are represented in the figures.

No significant changes in *zif*/268 and c-*fos* mRNA expression were seen in the entorhinal, medial cingulate, lateral orbitofrontal, piriform and somatosensory cortices, diagonal band, dorsal striatum, dentate gyrus, hippocampus (CA1,2,3,4 sub-fields), medial amygdala nucleus or septum (data not shown).

4. DISCUSSION

These results provide behavioural, autonomic, endocrine and neural evidence that sheep exposed to face images of other sheep during a short period of isolation stress have significantly reduced levels of stress and fear. Similar exposure to faces of another related but unfamiliar species, the goat, was far less effective, although the latter often produced intermediate effects that were not significantly different from sheep faces. Thus sheep, like humans (Morton & Johnson 1991), may find the sight of familiar faces comforting in times of stress or, at the very least, a positive distraction. Exposure to the sheep faces increased activity (as evidenced by increased *zif*/268 and c-*fos* mRNA expression) in face processing regions of the brain (medial pre-frontal and inferior and superior temporal cortices and basal amygdala) and this was mainly restricted to the right brain hemisphere. Reduced expression of these same genes (implying decreased activation) was also seen exclusively in the right central and lateral amygdala nuclei, which are important for mediating fear responses, and in the cingulate

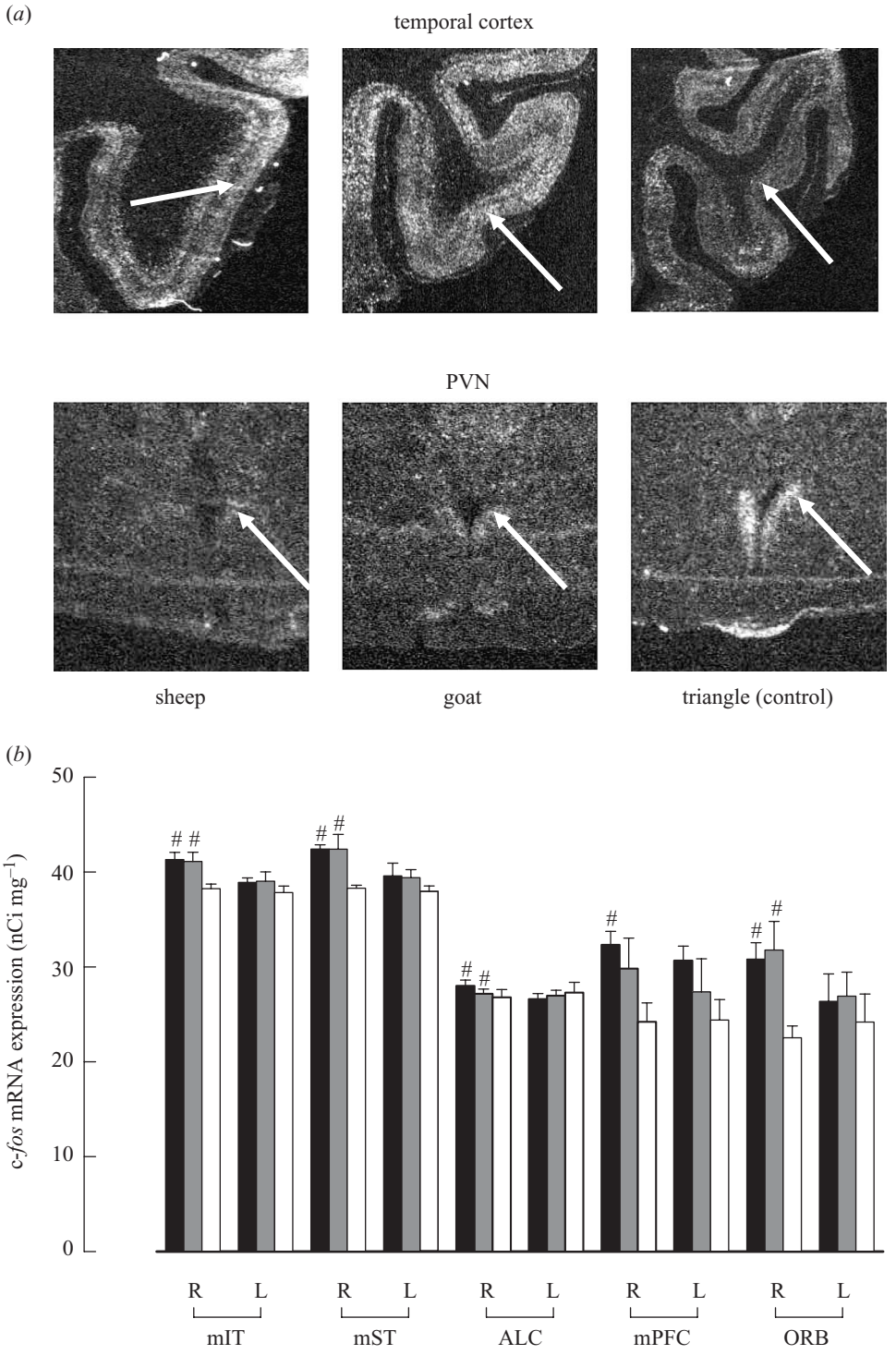

Figure 4. (*a*) Dark-field autoradiograms showing differences in *zif*/268 mRNA expression (white areas) in the temporal cortex and the paraventricular nucleus in isolated animals exposed to pictures of inverted triangles (control) or goat or sheep faces. Arrows indicate the locations of the different regions. (*b*) Histograms show mean + s.e.m. c-*fos* mRNA expression (given as nCi mg^{-1} against ^{14}C standards) in right (R) and left (L) sides of (*a*) cortical regions and (*b*) amygdala and hypothalamus of the brain after 30 min of isolation while viewing pictures of inverted triangles (open bars), sheep (black bars) or goat (grey bars) faces. $^{\#}p < 0.05$ versus inverted triangle face. Abbreviations as in figure 3 (assessments carried out on the same animals as for *zif*/268).

and orbitofrontal cortices which are important for the control of emotional responses (Le Doux 2000). Thus sheep may, like us, process and respond to negative emotional experiences using the right brain hemisphere (Davidson 1995; Markowitsch 1998; Perry *et al.* 2001).

We exposed sheep to only a single short-term psychological stress condition in this study since physiological and autonomic responses to such an acute stress are both strong and robust. This also reflects a type of stress to which these animals could be exposed during normal husbandry conditions. Whether the face pictures would be as effective in reducing fear and stress in a situation of longer-term isolation would be more difficult to assess since many of the brain, behavioural, autonomic and endocrine

changes would be less easy to quantify. It would seem likely that the stress-reducing effects of the face pictures would gradually reduce over time if no direct social contact with another animal occurred. However, in other experimental contexts we have confirmed that periods of social isolation of up to 5 h can be well tolerated, as evidenced by few, if any, protest vocalizations and other signs of an absence of stress such as rumination, when the animals are viewing a number of different sheep face pictures (K. M. Kendrick, unpublished observation). In the absence of these face stimuli signs of distress are exhibited throughout the period of isolation. From a practical viewpoint therefore, simple exposure of domestic or wild animals to familiar face pictures may be an effective way of reducing the levels of fear and stress that they experience in the context of unavoidable short-term social isolation.

Our current finding that exposure of sheep to conspecific faces increases both *zif*/268 and c-*fos* mRNA expression in right brain hemisphere regions associated with face recognition supports our previous findings in a face discrimination task (Broad *et al.* 2000). Whereas our electrophysiological findings have shown that both the right and left temporal cortices have cell populations that respond preferentially to sheep faces, we have also found that response latencies in the left hemisphere are up to 400 ms slower than in the right hemisphere (Peirce & Kendrick 2002). We have suggested therefore that the face-sensitive cells in the two hemispheres have different functional roles.

The present study suggests that, in sheep, the consequences of recognizing a familiar type of face may preferentially influence right-hemisphere structures controlling emotional responses because face recognition is also carried out on this side. In humans, recognition of face emotion can activate either the left or right amygdala or both depending upon the type of expression and the mode in which it is presented, as well as the face recognition areas in the right fusiform gyrus (Morris *et al.* 1996; Zald 2003). Interestingly, recent human imaging studies using face pictures to study romantic and maternal love have found deacti-vations in the amygdala under these circumstances, which parallels the situation we have seen when the isolated sheep see face pictures of familiar animals (Bartels & Zeki 2000, 2004). Whereas this amygdala deactivation does not happen when humans view pictures of friends as opposed to loved ones, it is possible that this is simply a reflection of the differential intensity of attraction in the two cases. If studies had been carried out on socially isolated humans, where any face might have been perceived as being more attractive than normal, it is possible that a reduced amygdala response could have occurred in the same way as we have seen in sheep under these circumstances. Some general support for this idea of emotional state influencing face emotion perception is gained from children with anxiety disorders, who show exaggerated activation of the amygdala to fearful faces whereas depressed individuals show reduced activation (Thomas *et al.* 2001).

There have been reported several examples of right-hemisphere dominance in the processing of emotion cues in animal species, including lower vertebrates (Andrew & Watkins 2002). However, it is unclear whether this reflects the results of perceptual bias or a true lateralization of emotional responses. Consistent with this idea of perceptual bias are the results of experiments with rodents, where simple visual or auditory fear-eliciting stimuli, which would normally activate sensory systems bilaterally, seem mainly to cause bilateral activation in the central and lateral amygdala (Le Doux 2000).

While the stress-reducing effects of faces on cortical and limbic system structures involved in face recognition and the control and expression of emotional responses are lateralized in the right brain hemisphere, effects on hypothalamic (PVN) structures controlling endocrine stress responses are bilateral. The most likely explanation for this is that the visual stimuli from faces do not influence the PVN directly but are instead caused indirectly by reduced levels of arousal, or even feedback from reductions in adrenocortical hormones.

Overall, from these experiments we can conclude both that simple representations of visual social stimuli like faces can reduce the psychological stress of social isolation and that sheep, like humans, show evidence for hemispheric specialization both in the processing face stimuli and their emotional salience. We are not aware of any previous systematic studies investigating whether similar exposure to face pictures can alleviate separation anxiety in humans, although it is common practice to carry pictures of loved ones when separated from them either temporarily or as a result of death. One method of relieving separation anxiety in young children may also be to give them pictures of their parents to carry.

This work was supported by a BBSRC project grant to K.M.K. Experiments were carried out on a project licence in strict accordance with the Animals (Scientific Procedures) Act 1986.

REFERENCES

Albert, D. J. & Walsh, M. L. 1984 Neural systems and the inhibitory modulation of agonistic behaviour: a comparison of mammalian species. *Neurosci. Biobehav. Rev.* **8**, 5–24.

Andrew, R. J. & Watkins, J. A. S. 2002 The nature of lateralization in tetrapods. In *Comparative vertebrate lateralization* (ed. L. J. Rogers & R. J. Andrew), pp. 70–93. Cambridge University Press.

Bartels, A. & Zeki, S. 2000 The neural basis of romantic love. *NeuroReport* **11**, 3829–3834.

Bartels, A. & Zeki, S. 2004 The neural correlates of maternal and romantic love. *NeuroImage* **21**, 1155–1166.

Broad, K. D., Mimmack, M. L. & Kendrick, K. M. 2000 Is right hemisphere specialization for face discrimination specific to humans? *Eur. J. Neurosci.* **12**, 731–741.

Broad, K. D., Hinton, M. R., Keverne, E. B. & Kendrick, K. M. 2002 Involvement of the medial prefrontal cortex in mediating behavioural responses to odour cues rather than olfactory recognition memory. *Neuroscience* **14**, 715–729.

da Costa, A. P., Broad, K. D. & Kendrick, K. M. 1997 Olfactory memory and maternal behaviour-induced changes in c-*fos* and *zif*/268 mRNA expression in the sheep brain. *Mol. Brain Res.* **46**, 63–76.

Davidson, R. J. 1995 Cerebral asymmetry, emotion and affective style. In *Brain asymmetry* (ed. R. J. Davidson & K. Hugdahl), pp. 361–387. Cambridge, MA: MIT Press.

Edwards, J., Jackson, H. J. & Pattison, P. E. 2002 Emotion recognition via facial expression and affective prosody in schizophrenia: a methodological review. *Clin. Psychol. Rev.* **22**, 789–832.

George, M. S., Huggins, T., McDermut, W., Parekh, P. I., Rubinow, D. & Post, R. M. 1998 Abnormal facial emotion recognition in depression: serial testing in an ultra-rapid-cycling patient. *Behav. Modif.* **22**, 192–204.

Haxby, J. V., Hoffman, E. A. & Gobbini, M. I. 2000 The distributed human neural system for face perception. *Trends Cogn. Sci.* **4**, 223–233.

Kendrick, K. M. & Baldwin, B. A. 1987 Cells in the temporal cortex of conscious sheep respond preferentially to the sight of faces. *Science* **238**, 1497–1499.

Kendrick, K. M., da Costa, A. P., Leigh, A. E., Hinton, M. R. & Peirce, J. W. 2001 Sheep don't forget a face. *Nature* **414**, 165–166.

Le Doux, J. E. 2000 Emotion circuits in the brain. *A. Rev. Neurosci.* **23**, 155–184.

MacDonald, H., Rutter, M., Howlin, P., Rios, P., Le Conteur, A., Evered, C. & Folstein, S. 1989 Recognition and expression of emotional cues by autistic and normal adults. *J. Child Psychol. Psychiat.* **30**, 865–877.

Markowitsch, H. J. 1998 Differential contribution of right and left amygdala to affective information processing. *Behav. Neurol.* **11**, 233–244.

Morris, J. S., Frith, C. D., Perrett, D. I., Rowland, D., Young, A. W., Calder, A. J. & Dolan, R. J. 1996 A differential neural response in the human amygdala to fearful and happy facial expressions. *Nature* **383**, 812–815.

Morton, J. & Johnson, M. 1991 CONSPEC and CONLEARN: a two process theory of infant face recognition. *Psychol. Rev.* **98**, 164–181.

Parrott, R. F., Misson, B. H. & De la Riva, C. F. 1994 Differential stressor effects on the concentrations of cortisol, prolactin and catecholamines in the blood of sheep. *Res. Vet. Sci.* **56**, 234–239.

Parrott, R. F., Hall, S. J. G. & Lloyd, D. M. 1998 Heart rate and stress hormone responses of sheep to road transport following two different loading procedures. *Anim. Welf.* **7**, 257–267.

Peirce, J. W. & Kendrick, K. M. 2002 Functional asymmetry in sheep temporal cortex. *NeuroReport* **13**, 2395–2399.

Peirce, J. W., Leigh, A. E. & Kendrick, K. M. 2000 Configurational coding, familiarity and the right hemisphere advantage for face recognition in sheep. *Neuropsychologia* **38**, 19–35.

Perry, R. J., Rosen, H. R., Kramer, J. H., Beer, J. S., Levenson, R. L. & Miller, B. I. 2001 Hemispheric dominance for emotions, empathy and social behaviour: evidence from right and left handers with frontotemporal dementia. *Neurocase* **7**, 145–160.

Ross, E. D., Orbelo, D. M., Cartwright, J., Hansel, S., Burgard, M., Testa, J. A. & Buck, R. 2001 Affective-prosodic deficits in schizophrenia: profiles of patients with brain damage and comparison with relation to schizophrenic symptoms. *J. Neurol. Neurosurg. Psychiat.* **70**, 597–604.

Thomas, K. M., Wayne, C. D., Dahl, R. E., Ryan, N. D., Birmaher, B., Eccard, C. H., Axelson, D., Whalen, P. J. & Casey, B. J. 2001 Amygdala response to fearful faces in anxious and depressed children. *Arch. Gen. Psychiat.* **58**, 1057–1063.

Vellucci, S. V., Parrott, R. F., da Costa, A. C., Ohkura, S. & Kendrick, K. M. 1995 Increased body temperature, cortisol secretion, and hypothalamic expression of c-*fos*, corticotrophin releasing hormone and interleukin-1 β mRNAs, following central administration of interleukin-1 β in the sheep. *Mol. Brain Res.* **29**, 64–70.

Zald, D. H. 2003 The human amygdala and the emotional processing of sensory stimuli. *Brain Res. Rev.* **41**, 88–123.

As this paper exceeds the maximum length normally permitted, the authors have agreed to contribute to production costs.