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Cells in Temporal Cortex of Conscious Sheep Can Respond Preferentially to the Sight of Faces

K. M. KENDRICK AND B. A. BALDWIN

To investigate whether the temporal cortex of a nonprimate species contains cells responsive to the sight of faces, a study was made in conscious sheep of the responses of neurons in this brain region to the sight of faces. Of 561 cells from which responses were recorded, 40 responded preferentially to faces. Different categories of these cells were influenced by dominance (presumably indicated by the presence and size of horns), breed and familiarity, and threatening faces such as those of humans and dogs. These results demonstrate that cells that respond preferentially to faces are present in the temporal cortex of a nonprimate species, and that the responses of these cells are influenced by factors relevant to social interaction.

IN MONKEYS, A REGION OF THE TEMPORAL lobe contains some cells that respond preferentially to monkey and human faces (*1*). These cells also respond selectively to specific faces (*2*) or facial expressions (*3*). Damage to this region is associated with social disturbance, including reduced aggression (*4*), impaired visual discrimination (*5*), and in humans facial recognition may be impaired (*6*). In primates, therefore, this region of the brain is thought to play a role in individual recognition and appropriate social behavior, although its function in nonprimate species is unknown.

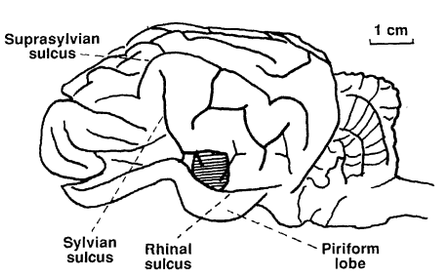

Fig. 1. Schematic drawing of a sagittal view of a sheep brain showing the location (shaded area) of cells that responded to the sight of faces.

Relatively little electrophysiological work has been done on the visual system of sheep but it has been shown, in anesthetized animals, that cells in their visual cortex respond to visual stimuli in the same way as similarly located cells in primates (*7*). As in primates, facial recognition is socially important to sheep since the face conveys breed and individual identity. Sheep prefer to interact socially with members of their own breed (*8*), and ewes visually recognize their own lambs by their faces rather than by other body features (*9*). Also, in horned sheep (*10*), and other horned ungulates (*11*), the size of the horns conveys information on both gender and position in the dominance hierarchy. The present study provides the first demonstration that cells showing preferential responses to the sight of facial stimuli are present in the sheep's temporal cortex. Further, different categories of these cells are distinguished by their responsiveness to dominance (the presence and size of horns), breed, familiarity, and stimuli associated with potential threat, such as humans and dogs.

Five Dalesbred sheep (a horned breed) were used. The methods for conscious single-unit electrophysiological recordings were as previously described (*12*) except that the temporal cortex was the brain region investigated. Recordings were made with the animals conscious and comfortably suspended in a canvas hammock. Head movement was prevented by anchoring the insensitive portion of the sheep's horns to the frame of the hammock. By means of a slide projector, visual stimuli were present binocularly with the use of a back-projection screen (0.6 m wide and 0.4 m high) placed with its center at eye level, 1 m directly in front of the animal. Approximately life-size stimuli were presented for 5 seconds, either stationary or slowly oscillating (0.2 Hz) horizontally. Although eye movements were not monitored the screen was positioned so that the stimuli fell on the area centralis of each eye when the sheep was looking forward. Since stimuli were presented in the dark, the screen represented the only major area of visual change for the animal and thus it probably focused on this for most of the

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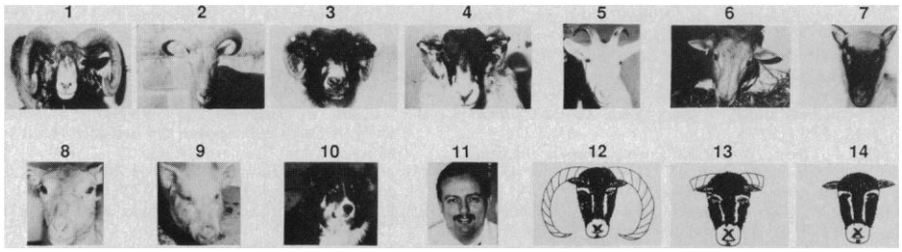

Fig. 2. Facial stimuli used: 1, Mouflon; 2, Barbary; 3, familiar Dalesbred; 4, unfamiliar Dalesbred; 5, Saanen goat; 6, Welsh Mountain; 7, Clun Forrest; 8, Finn; 9, pig; 10, sheepdog; 11, human; 12, drawing of sheep with large horns; 13, drawing of sheep with small horns; 14, drawing of sheep with no horns. Except for the drawings, colored stimuli were presented.

time. That the sheep were able to focus on the stimuli presented was evidenced by their occasional vocalizations following presentation of sheep faces or bowls of food. A Prontor shutter on the projector allowed automatic stimulus presentation as well as the recording of response latencies. For each cell, each stimulus was presented two to three times, and the firing rate for the 5 seconds immediately before stimulus presentation was compared with that during the 5 seconds when the stimulus was presented. A mean percentage change in firing rate was then calculated. In all cases where changes in firing rate were observed, the change in response to at least one stimulus was required to be statistically significant (t test comparing firing rate in ten 0.5-second periods before and during the stimulus). Histological localization of recording sites was as previously described (*12*). The region of the temporal cortex was as illustrated in an atlas of the sheep's brain (*13*) and the location of the cells that responded to faces is shown in Fig. 1.

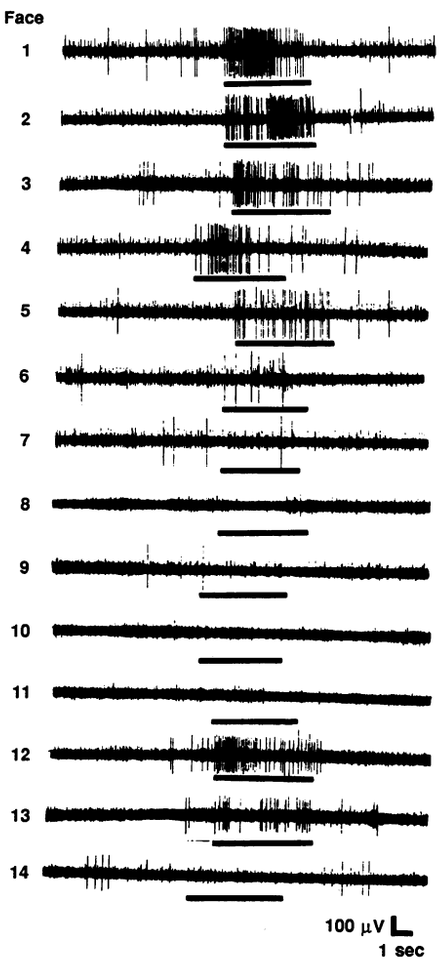

Fig. 3. Responses of a single cell to the 14 facial stimuli. Stimulus presentation is indicated by the black bar under each trace. A neuronal response was evoked by all the animals with horns (including the schematic drawings), although small horns (Welsh Mountain and schematic drawing) were less effective.

A cell was defined as responding preferentially to faces if it did not also respond to other arousing stimuli (loud noise or puff of air in the face), presentation of food, or the projection of either stationary or moving simple visual stimuli such as a checkerboard or grating. Lastly, such cells were required to be unresponsive to pictures of sheep that showed the animal's body but not its head. Cells fulfilling the above criteria were tested with a number of different faces (see Fig. 2). These included socially familiar (the sheep in the pen directly facing that of the experimental animal) and unfamiliar Dalesbred sheep (a number of different unfamiliar sheep were used); other horned sheep (Barbary, Mouflon, and Welsh Mountain) and goats (Saanen); nonhorned sheep (Clun Forrest and Finn); other animals such as pigs and dogs, and finally humans. Upside-down faces were also tested. Stimuli were presented randomly at 1-minute intervals with the room darkened.

Of 561 cells recorded from the temporal cortex of the 5 sheep, 40 responded preferentially to facial stimuli (7 cells were inhibited and 33 excited). Cells responsive to facial stimuli could be divided into four distinct types. The predominant type (21 cells) responded best to faces of horned species (Figs. 3 and 4A). This type of neuron did not respond to nonhorned animals and responded significantly more to animals with large horns (Mouflon) than to those with small horns (Welsh Mountain). Such neurons also had significantly stronger responses to drawings of sheep with large horns compared with small or no horns. These cells did not, however, respond to pictures of horns per se; they responded only when the horns were presented in context with a face. A second type (eight cells) had significantly greater responses to the face of a familiar compared with that of an unfamiliar Dalesbred sheep and responded only slightly, if at all, to the other animal faces (Fig. 4B). For three cells, this familiarity effect was confirmed with pictures of an additional one or two different pairings of familiar and unfamiliar Dalesbred sheep. A third type (nine cells) responded significantly more to

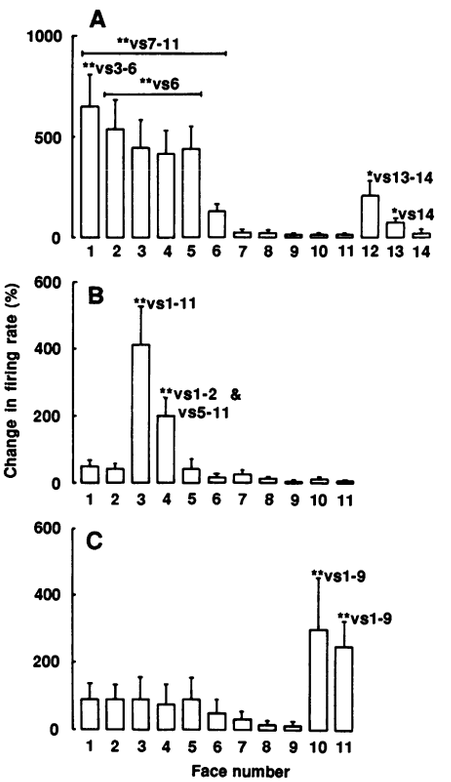

Fig. 4. Histograms show mean (± SEM) percentage changes in firing rate of cells responding to faces. The mean ± SEM spontaneous firing rate of these cells was 3.5 ± 0.5 Hz. Analysis of variance (Friedman test) was used to determine overall significance, and the Wilcoxon test was used to determine significant differences between individual facial stimuli. ** $P < 0.01$; * $P < 0.05$ compared to the number of the facial stimulus indicated to the right of the asterisks. (**A**) Data for 16 out of the 21 cells showing responses to horned animals and drawings of sheep faces (6 out of 16 cells—stimuli 12 to 14). (**B**) Same as (A) but for eight cells responding to Dalesbred sheep, particularly familiar animals. (**C**) Same as (A) but for seven out of the nine cells responding to pictures of dogs and humans. Although these cells also responded slightly to horned species the overall mean percentage change was less than 100% and not normally significant. Cells responding only to Dalesbred sheep or to dogs and humans were not tested with the sheep face drawings.

faces of humans and sheepdogs than to sheep or other animal faces (see Fig. 4C). There was also a slight tendency for these cells to respond to horned animals. Lastly, two cells responded similarly to a number of different faces including those of animals such as pigs. All cells responding to faces responded much less, or not at all, to upside-down faces. On a few occasions facial profiles of sheep were shown and these also evoked similar, although generally smaller, responses compared with the faces shown frontally. The response latencies of these cells responding to faces were short: between 80 and 180 msecs (median 120 msec) and the responses did not generally outlast the period of stimulus presentation. A total of 56 other visually responsive cells were found in the temporal cortex that responded to movement, usually in a specific direction, of any object either in the contralateral visual field (46 cells) or in both ipsi- and contralateral fields (10 cells). A further 25 cells responded to auditory cues and 10 cells responded to somatosensory cues (touching of areas of the head and face). None of these cells, or those responding to faces, were polysensory, however.

These results demonstrate that facial recognition cells in the temporal cortex are not unique to primates. As in the monkey (*1*), these cells have short response latencies suggesting that they are primarily involved in sensory processing rather than in motor responses to these stimuli. However, unlike the monkey (*1*), they do not respond to upside-down faces, which seems reasonable since, unlike monkeys, sheep do not usually view other sheep upside down. Different groups of facial recognition cells code for different features known from behavioral studies to be socially significant. One group of cells codes selectively for the presence and size of horns, possibly allowing for a rapid estimation of the perceived animal's sex or position in the dominance hierarchy. A second group of cells may code for animals of the same breed and for familiarity. A third group of cells appears to code for potential threatening stimuli, such as humans and dogs. These results provide an important insight into the way the brain can rapidly code complex visual information in terms of appropriate behavioral responses.

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A Small Gold-Conjugated Antibody Label: Improved Resolution for Electron Microscopy

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A general method has been developed to make the smallest gold-conjugated antibody label yet developed for electron microscopy. It should have wide application in domainal mapping of single molecules or in pinpointing specific molecules, sites, or sequences in supramolecular complexes. It permits electron microscopic visualization of single antigen-binding antibody fragments (Fab') by covalently linking an 11-atom gold cluster to a specific residue on each Fab' such that the antigenic specificity and capacity are preserved. The distance of the gold cluster from the antigen is a maximum of 5.0 nanometers when the undecagold-Fab' probe is used as an immunolabel.

THE USE OF ANTIBODIES TO MARK molecules or tissues specifically has been an important tool in structural biology. Visualization of these antibody labels in the electron microscope (EM) has usually required conjugation either with an electron-dense moiety such as ferritin (*1*) or colloidal gold (*2*). Although these procedures are adequate for some studies, these labels are generally too large for submolecular mapping, which is unfortunate since monoclonal antibodies to various determinants on a molecule are available. The antibody molecule (*3*) is ~15 nm in diameter, and the Protein A–colloidal gold complex is ~12 to 35 nm in diameter or larger, so that a conjugated label (*4*) has a diameter of ~27 to 50 nm. An antibody label 1/5 to 1/10 the size of those currently available has now been synthesized. It is formed by coupling an antigen-binding antibody fragment (Fab') to an undecagold (Au_{11}) complex such that antigenic binding capacity is retained. The Fab' fragment is 50 kD and is 5.0 by 4.0 by 3.0 nm in size (*5*). The undecagold complex has a core that is 0.8 nm in diameter and is composed of 11 gold atoms (with an organic shell that is ~0.6 nm thick). The core is easily visible in the scanning transmission electron microscope (STEM). The maximum dimension of the

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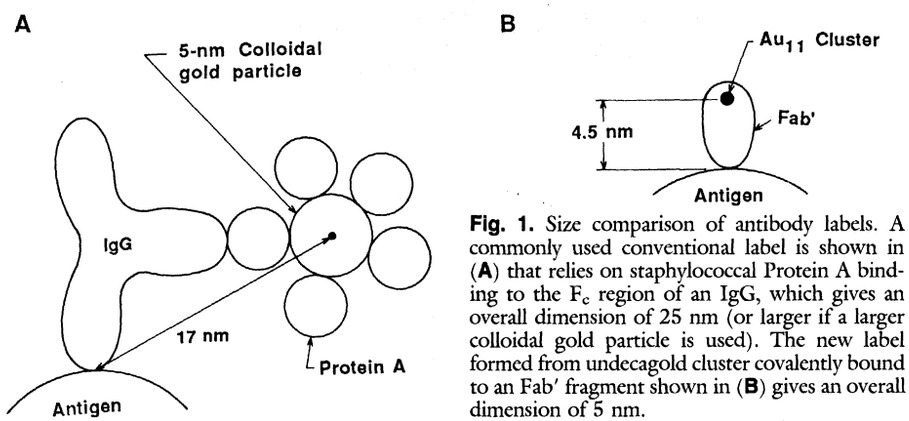

Fig. 1. Size comparison of antibody labels. A commonly used conventional label is shown in (**A**) that relies on staphylococcal Protein A binding to the F_c region of an IgG, which gives an overall dimension of 25 nm (or larger if a larger colloidal gold particle is used). The new label formed from undecagold cluster covalently bound to an Fab' fragment shown in (**B**) gives an overall dimension of 5 nm.