

Finally, the results can explain the greater grazing effects on grassland species composition in South America compared with those in Africa. Subhumid grasslands of South America are invaded by exotic, mostly European, species when grazed by livestock and the importance of native grasses is drastically reduced¹⁷. In contrast, equivalent subhumid grasslands in East African game reserves are always dominated by native grasses, even under high grazing pressure¹⁸. A general model of the impact of grazing on community structure¹⁹ attributed this difference to the longer evolutionary history of grazing in African grasslands and savannas compared with those in South America, whose grazer and browser fauna almost totally failed to survive the Pleistocene extinctions of large herbivorous mammals. Our results show that grazing evolutionary history is not the only major difference between these systems. The much higher herbivore load supported by South American agricultural grasslands over the past two centuries may contribute to their different response to grazing. □

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- McNaughton, S. J., Oesterheld, M., Frank, D. A. & Williams, K. J. *Nature* **341**, 142–144 (1989).
- McNaughton, S. J., Oesterheld, M., Frank, D. A. & Williams, K. J. in *Comparative Analyses of Ecosystems: Patterns, Mechanisms and Theories* (eds Cole, J. J., Lovett, G. M. & Findlay, S. E. G.) 120–139 (Springer, New York, 1991).
- East, R. *Afr. J. Ecol.* **22**, 245–270 (1984).
- Ministerio de Economía Empadronamiento Nacional Agropecuario y Censo Ganadero (Buenos Aires, Argentina, 1974).
- Ministerio de Agricultura y Pesca *Censo General Agropecuario* (Montevideo, Uruguay, 1980).
- Production Yearbook 1986* (United Nations Food and Agriculture Organization, Rome, 1987).
- Lauenroth, W. K. in *Perspectives in Grassland Ecology* (ed. French, N. R.) 3–24 (Springer, New York, 1979).
- McNaughton, S. J., Sala, O. E. & Oesterheld, M. in *Biological Relationships Between Africa and South America* (ed. Goldblatt, P.) (Yale Univ. Press, New Haven, in the press).
- Scarnecchia, D. L. *J. Range Manage.* **43**, 553–555 (1990).
- Moen, J. & Oksanen, L. *Nature* **353**, 510 (1991).
- Darwin, C. *The Origin of Species* 6th London edn (Thompson & Thomas, Chicago, 1872).
- Errington, P. L. *Science* **124**, 304–307 (1956).
- Coe, M. J., Cumming, D. H. & Phillipson, J. *Oecologia* **22**, 341–354 (1976).
- Sharkey, M. J. *Mammalia* **34**, 564–572 (1970).
- McNaughton, S. J. *Am. Nat.* **124**, 863–886 (1984).
- Westoby, M. *Am. Nat.* **126**, 870–871 (1985).
- Sala, O. E., Oesterheld, M., Loin, R. J. C. & Soriano, A. *Vegetatio* **67**, 27–32 (1986).
- McNaughton, S. J. *Ecol. Monogr.* **53**, 291–320 (1983).
- Michener, D. G., Sala, O. E. & Lauenroth, W. K. *Am. Nat.* **132**, 87–106 (1988).
- Coughenour, M. B. *et al. Science* **230**, 619–625 (1985).
- Golley, F. B. *Ecology* **42**, 581–584 (1961).

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Time to collision is signalled by neurons in the nucleus rotundus of pigeons

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THROUGHOUT the animal kingdom, the sight of a rapidly approaching object usually signals danger and elicits an escape response^{1–6}. Gibson⁷ suggested that the symmetrical expansion of an object's image (looming) is the critical variable determining that the object is on a collision course with the observer. Similarly, large expanding flow-fields like those produced by locomotion may precipitate manoeuvres such as turning or landing^{8,9}. From such observations it has been shown that the optic flow parameter, τ , which specifies time to contact with the approaching object best fits the behavioural data^{10,11}. We describe a subpopulation of neurons in the nucleus rotundus of the pigeon brain that respond selectively to objects moving on a collision course towards the bird.

These neurons give their maximum response at a constant time before contact occurs, even when the size of the stimulus or its velocity is varied widely. We propose that these neurons are signalling the time to collision of approaching objects.

We examined the response properties of neurons in the pigeon nucleus rotundus, a major midbrain nucleus in the tectofugal visual pathway, to various computer-generated visual displays. Here we report the responses of neurons in the dorsal posterior zone of the nucleus rotundus, where preliminary qualitative observations had indicated some neurons might respond to motion in depth¹². A stimulus pattern resembling a soccer ball, consisting of a sphere with many alternating black and white panels, was generated by a graphics computer and projected onto a tangent screen 40 cm in front of the bird by a high-

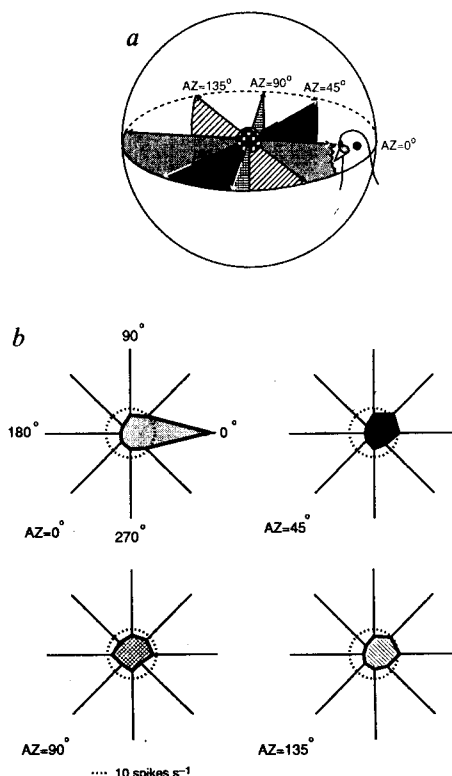


FIG. 1 A single neuron from the dorsal posterior zone of the nucleus rotundus exhibits clear selectivity for a looming visual stimulus. **a**, Diagram to illustrate the 4 planes along which a soccer-ball shaped stimulus pattern, consisting of black and white panels presented monocularly, was moved along 26 simulated three-dimensional trajectories 45° apart in spherical coordinates. (The 4 planes each with 8 directions total 32, but the vertical up and down directions are common, thus resulting in 26 directions.) **b**, Firing rate plots for the different directions of motion of the spherical stimulus within these planes. Each direction of motion was presented 5 times in a randomly interleaved sequence to a pigeon, anaesthetized with ketamine/xylazine (50 mg per kg, 5 mg per kg, respectively), and the values plotted represent the mean firing rate for each three-dimensional direction. An IRIS 4d/310 GTX graphics computer was used to generate the stimulus which was projected by a 1280 × 1024 pixel RGB projector. In the standard X–Y plot (tangent screen plane, or azimuth (AZ)=90°), there is no indication of directional preference, and firing rate is low. For the 0° azimuthal plane (z-axis) there is a strong preference for stimuli directly approaching the bird on a collision course. Polar tuning plots for directions specifying the azimuthal 45° and 135° planes similarly show no strong directional preference. This pattern of activity was typical of the 24 neurons studied. If the origin of the spherical coordinate system was moved to a different position in the receptive field, the direct collision direction again produced the maximal response in the looming-sensitive cells.

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resolution projector. This system allows realistic depiction of three-dimensional space and simulation of the motion of objects through this space or, alternatively, motion of the observer through this space.

Cells from the dorsal posterior zone (histologically confirmed) of the nucleus rotundus of 20 anaesthetized pigeons were studied quantitatively using standard extracellular recording techniques^{13,14}. Data were recorded for each cell during motion of the spherical stimulus pattern along 26 three-dimensional trajectories spaced 45° apart in spherical coordinates (Fig. 1a). Their monocular receptive fields, determined by hand-moved stimuli, were extremely large (approximately 110° of arc). Of 145 cells, 24 (16.5%) gave maximum responses to a looming stimulus moving towards the animal on a collision course (0° azimuth, 0° elevation), and very little response to motion in other directions through three-dimensional space, as illustrated by a typical cell in Fig. 1b. Finer grained tuning curves obtained for three typical cells exhibited extremely tight tuning, centred precisely on 0° azimuth with mean half-width at half height values of 3.3° and mean above baseline widths of 16° . Other neurons in this area responded best to stimuli moving in any direction (two-dimensional) through their receptive fields (motion cells; 47%), or preferred some two-dimensional directions over others (directional cells; 36.5%). For the monocular looming-sensitive cells, the directly approaching soccer-ball pattern elicited the same strong response over the entire receptive field, as long as it was on a collision course with the bird's head. Motion of the stimulus in other directions in simulated three-dimensional space produced very little or no increase in the cells' firing rate. The expanding flow pattern produced by the spherical stimulus could have been produced either by the motion of the ball toward the bird, or by the motion of the bird towards the ball. We therefore presented a computer-generated 'whole field' expanding chequerboard flow pattern which filled the whole tangent screen, simulating the approach of the bird toward a large stationary surface. This stimulus pattern produced minimal responses in the looming-sensitive cells. Other controls such as changes in illumination and the presentation of auditory

and somatosensory stimuli also failed to trigger these cells, and their response selectivity for looming stimuli was unaffected by contrast reversal. From these results we conclude that the looming-sensitive neurons are selectively responsive to object motion in depth.

Other experimental observations showed that looming-sensitive neurons were not only selective for object motion in depth, but also gave their maximum response at a constant time before the stimulus would contact the bird, signalling time to collision (Fig. 2). The range of values for time to collision obtained from the sample of 24 looming cells was 800–1,400 ms, with a modal value of 1,000 ms. The neurons exhibited a nearly identical visual response to different sizes of looming stimuli approaching along identical paths at a constant velocity (Fig. 2a), indicating that they are not triggered by the stimulus reaching a critical visual angle. Moreover, when the speed of the stimulus was systematically varied and the path and size kept constant, the cells' response onset and peak firing always occurred at constant times before the stimuli would have contacted the animal (Fig. 2b). Thus, initiation of firing must occur at more distant positions along the stimulus path as velocity is increased, providing strong evidence that these neurons are indeed signalling time to collision. As we have been unable to find neurons with similar characteristics in the optic tectum, which provides the main input to the nucleus rotundus, we conclude that the response properties of these looming cells probably result from patterns of neural connectivity in the rotundus.

It is generally acknowledged that natural or artificial threatening stimuli do not only elicit immediate overt defensive or avoidance behaviour, but also generate autonomic changes including a rapid increase in heart rate and blood pressure^{6,15}. To study these effects we recorded activity from seven looming-sensitive rotundal cells in six birds, while simultaneously recording their heart rate and the muscle activity (electromyograms) from their large pectoralis flight muscle (Fig. 3). The results show a tight correlation between the activity of the rotundal looming-sensitive cells, and the electromyogram and heart rate measurements. When the stimulus was on a collision course

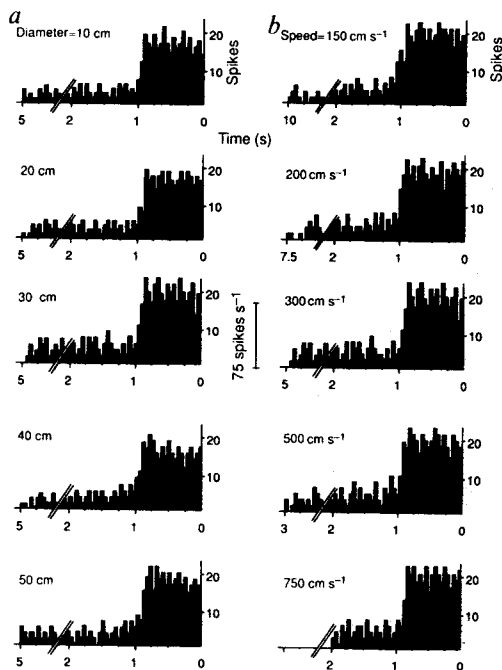
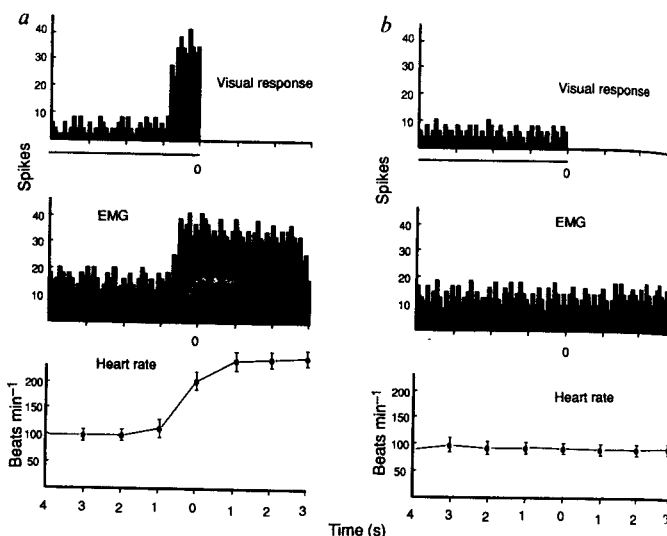


FIG. 2 Peri-stimulus time histograms for single looming-sensitive rotundal cell with a series of soccer-ball pattern stimuli of varying sizes (a), and velocities (b) swept along the direct collision course path toward the bird. Responses are the sum of five sweeps and are referenced to time zero, which is the time when the stimulus would contact the bird. The response remains invariant in amplitude and timing over substantial changes in size and velocity. The simulated path was 15 m long and the simulated size varied from 10 cm to 50 cm in diameter. Velocity in a, 300 cm s^{-1} ; size in b, 30 cm.

FIG. 3 Heart rate and pectoralis muscle electromyograms (EMGs), recorded concurrently with response rate from a single looming-sensitive rotundal neuron. **a**, Birds were surgically anaesthetized and very fine insulated stainless steel bipolar percutaneous electrodes were inserted into the pectoralis muscle¹⁶. After isolation of a looming-sensitive cell, the birds were allowed to recover from anesthesia just sufficiently to obtain EMGs from the muscle but not overt motion of the bird. No eye movements were discernible. Immediately following the EMG and heart rate recordings, birds were re-anaesthetized to the original level. The looming cell begins firing first, then the muscle response occurs and then heart rate increases dramatically as the soccer-ball moves toward the bird. **b**, No responses occur when the ball moves along the same path but in the opposite direction, directly away from the bird. Bars under the visual response histograms indicate the duration of the visual stimulus. Data collection for the looming-sensitive neuron was terminated with stimulus offset. The neuronal data and EMGs represent summed activity over 5 sweeps of the stimulus whereas the heart rate data represent the means and standard errors for the same 5 sweeps. Stimulated size of stimulus was 30 cm, path length 15 m, and velocity 375 cm s⁻¹.



with the bird, sharp increases occurred in firing rate in looming-sensitive cells as well as in heart rate and muscle activity. When the stimulus path deviated from the collision course by as little as 5°, indicating a near miss to either side of the bird, there was a marked reduction in all three responses. The peak muscle activity always occurred 50–100 ms after the onset of the looming-sensitive cell's response, and the heart rate increased about threefold when the stimulus was on a collision trajectory (Fig. 3a). When the stimulus was moving directly away from the animal all three responses remained at baseline levels (Fig. 3b).

For many animal species, including humans and monkeys, responses to a rapidly expanding (looming) image are very similar to those elicited by a real approaching object^{1–6}. It follows that neural mechanisms must extract path and velocity information from dynamic retinal images (including motion in depth) to allow these behaviours to occur. There is considerable evidence that the tectofugal pathway is specialized to respond to the spatial position and motion characteristics of moving objects^{13,14} but ignores self-induced visual motion. The looming-sensitive neurons described here seem well suited to provide information about time to collision of approaching objects. We cannot conclude that rotundal looming-sensitive cells are directly responsible for providing information specifically used to initiate and control avoidance behaviour elicited by such stimuli as there may be other visual structures with similar processing capabilities, but the nucleus rotundus is a strong candidate for this functional role. □

New mammalian chloride channel identified by expression cloning

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ION channels selectively permeable to chloride ions regulate cell functions as diverse as excitability and control of cell volume^{1–5}. Using expression cloning techniques, a complementary DNA from an epithelial cell line has been isolated, sequenced and its putative structure examined by site-directed mutagenesis. This cDNA, encoding a 235-amino-acid protein, gave rise to a chloride-selective outward current when expressed in *Xenopus* oocytes. The expressed, outwardly rectifying chloride current was calcium-insensitive and was blocked by nucleotides applied to the cell surface. Mutation of a putative nucleotide-binding site resulted in loss of nucleotide block but incurred dependence on extracellular calcium concentration. The unusual sequence of this putative channel protein suggests a new class of ion channels not related to other previously cloned chloride channels^{6–11}.

We used an expression cloning strategy using the Madin Darby canine kidney (MDCK) epithelial cell line as a source of messenger RNA. MDCK cells contain chloride channels dependent on cyclic AMP, calcium, volume and voltage^{12–15}. *Xenopus* oocytes were injected with water or with polyadenylated mRNA isolated from MDCK cells. Injected oocytes were then assayed for chloride channel activity by two-electrode voltage clamp¹⁶ (Fig. 1). Oocytes injected with native MDCK mRNA (50 nl at 1 ng nl⁻¹) displayed a large chloride-selective current.

A cDNA library in the plasmid vector pcDNA1 was constructed (see legend to Fig. 1). RNA transcribed *in vitro* was prepared from pools of 25,000 independent cDNA clones, injected into oocytes and assayed for the expression of activity. A positive

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- Schiff, W. *Psychol. Monographs* **78**, No. 11, (1965).
- Hayes, W. N. & Schiff, E. I. *Anim. Behav.* **15**, 102–106 (1967).
- Schiff, W., Caviness, J. A. & Gibson, J. J. *Science* **136**, 982–983 (1962).
- Swanson, M. T. & Gogel, W. C. *Percept. Psychophys.* **39**, 309–326 (1986).
- Martino, C. & Dellus, J. D. *Biol. Cybern.* **63**, 127–134 (1990).
- Dean, P., Redgrave, P. & Westby, G. W. M. *Trends Neurosci.* **12**, 137–147 (1989).
- Gibson, J. J. *The Ecological Approach to Visual Perception* (Houghton Mifflin, Boston, 1979).
- Cogshall, J. C. *J. exp. Biol.* **57**, 401–413 (1972).
- Borst, A. & Bahde, S. *Naturwissenschaften* **75**, 265–267 (1988).
- Lee, D. N. & Reddish, P. E. *Nature* **283**, 293–294 (1981).
- Wagner, H. *Nature* **287**, 147–148 (1982).
- Rezin, A. M. *Brain Behav. Evol.* **3**, 195–204 (1970).
- Frost, B. J., Wyllie, D. R. & Wang, Y.-C. *Vision Res.* **30**, 1677–1688 (1990).
- Frost, B. J. & Nakayama, K. *Science* **220**, 744–745 (1983).
- Hilton, S. H. *J. exp. Biol.* **100**, 158–174 (1982).
- Loeb, G. E. & Gans, C. *Electromyography for Experimentalists* (Chicago University Press, 1986).

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