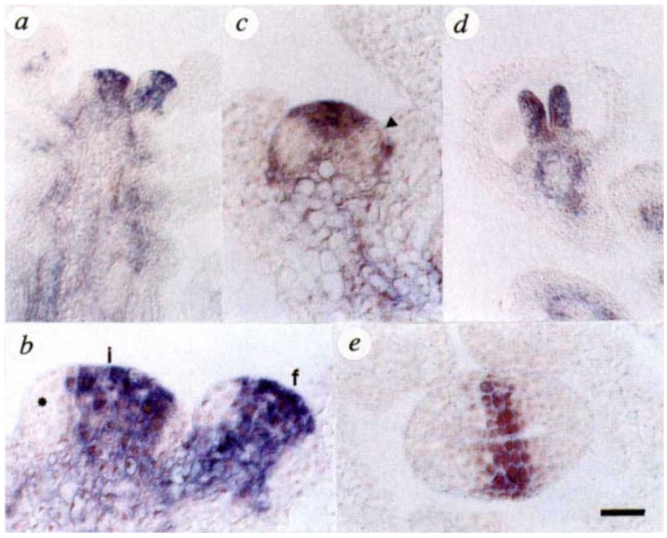

FIG. 4 Expression of *STM* during inflorescence and floral development. *a*, Inflorescence showing *STM* expression in central portions of inflorescence meristem and in central portion of stage 2 floral buds. *b*, Higher magnification of *a*. *STM* expression is downregulated in stage 1 floral buds (asterisk). Note lack of *STM* expression at position of incipient sepal primordia. i, Inflorescence meristem; f, stage 2 floral meristem. *c*, A stage 3 flower. *STM* expression is lacking at position of incipient medial stamen primordia (arrow). *d*, Stage 7–8 floral bud showing expression of *STM* in central portion of developing gyneocium. *e*, Transverse section through stage 7–8 gyneocium. Two ridges of expression are observed. Ovules will develop off the flanks of these ridges. Scale bar, 77 μm in *a* and *d* and 25 μm in *b*–*e*. Stages of flower development are as defined in ref. 20.

whereas *KN1* mRNA is not detectable in the maize L1 layer, *STM* mRNA is detectable in the *Arabidopsis* L1 layer.) It has been hypothesized that *KN1* functions to keep cells in an undifferentiated, undetermined, state[6,7]. This is similar to the proposed role for *STM* in SAM maintenance and suggests that these genes may be orthologous to one another. □

Received 3 August; accepted 20 October 1995.

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ACKNOWLEDGEMENTS. We thank P. Masson, C. Lincoln, S. Hake, J. Petrini and members of the Barton laboratory for helpful discussions and M. Evans and P. Masson for critical reading of the manuscript. R. Vierstra's laboratory kindly provided ubiquitin encoding plasmid p112. The *STM* sequence has been given GenBank accession number U32344 (Laboratory of Genetics paper no. 3441).

A role for glucagon-like peptide-1 in the central regulation of feeding

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The sequence of glucagon-like peptide-1 (7–36) amide (GLP-1) is completely conserved in all mammalian species studied, implying that it plays a critical physiological role[1]. We have shown that GLP-1 and its specific receptors are present in the hypothalamus[2,3]. No physiological role for central GLP-1 has been established. We report here that intracerebroventricular (ICV) GLP-1 powerfully inhibits feeding in fasted rats. ICV injection of the specific GLP-1-receptor antagonist, exendin (9-39)[4], blocked the inhibitory effect of GLP-1 on food intake. Exendin (9-39) alone had no influence on fast-induced feeding but more than doubled food intake in satiated rats, and augmented the feeding response to the appetite stimulant, neuropeptide Y. Induction of c-*fos* is a marker of neuronal activation[5]. Following ICV GLP-1 injection, c-*fos* appeared exclusively in the paraventricular nucleus of the hypothalamus and central nucleus of the amygdala, and this was inhibited by prior administration of exendin (9-39). Both of these regions of the brain are of primary importance in the regulation of feeding[6]. These findings suggest that central GLP-1 is a new physiological mediator of satiety.

We report that ICV administration of GLP-1 reduces food intake in fasted rats, with greater effect at higher doses (Fig. 1*b*). ICV injection of GLP-1 in rats at the beginning of the dark (feeding) phase also results in a profound decrease in feeding (Fig. 1*a*). When administered intraperitoneally up to a dose of 500 μg, GLP-1 did not affect early dark-phase feeding (data not shown), suggesting that the action of GLP-1 on food intake is through central rather than peripheral mechanisms. A reduction in locomotor activity is a well defined part of the satiety sequence and follows nutrient ingestion[7]. In a subgroup of the animals given ICV GLP-1 at the beginning of the dark phase, locomotor activity was monitored by the frequency of line-crossing[8]. A significant reduction in activity was seen after ICV administration of GLP-1 (10 μg; $41 \pm 7\%$ of control activity, $P < 0.05$: 100 μg; $32 \pm 9\%$, $P < 0.01$, $n = 8$ per group) compared to controls. Following ingestion of a palatable meal[9], the reduction in activity was similar to that observed following ICV injection of GLP-1 (10 μg) (palatable meal; $54 \pm 19\%$ of control activity, $P < 0.05$, $n = 6$). Although not assessed formally, the behaviour of the GLP-1-treated animals could not be distinguished, by observation, from those fed a palatable meal[10]. Fragments of GLP-1 are inactive peripherally[11]. To establish the specificity of GLP-1 on feeding,

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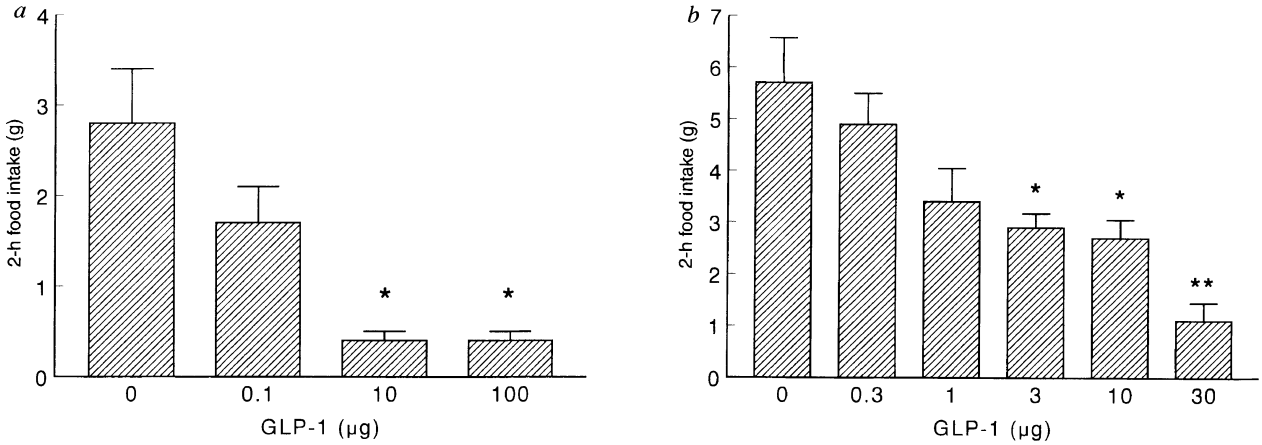

FIG. 1 *a*, Reduction in 2-h food intake in rats ($n = 7–8$ per group) injected ICV with GLP-1 or 0.9% saline (10 μl) at the onset of the dark phase ($F(3,27) = 10.6$, $P < 0.001$), (GLP-1 versus control, $^*P < 0.001$). *b*, reduction in 2-h food intake in 24-h fasted rats ($n = 6–7$ per group) injected ICV with GLP-1 or 0.9% saline (10 μl) ($F(5,34) = 8.0$, $P < 0.001$), (GLP-1 versus control, $^*P < 0.05$, $^{**}P < 0.01$).
METHODS. Adult male rats (200–250 g) were maintained in individual cages under controlled conditions of temperature (21–23 °C) and light (13-h light, 11-h dark) with *ad libitum* access to chow and water. Rats were anaesthetized and stainless steel guide cannulae implanted into the third cerebral ventricle as previously described[17]. After a 5-day recovery period, rats (<10%) exhibiting a negative drinking response to ICV angiotensin II (AII) (150 ng 3 μl^{-1}) were excluded. Responders were sham injected before the study, and weighed and handled daily. Substances were dissolved in 0.9% saline and administered ICV as previously described[17]. Following injection, rats were returned to cages containing pre-weighed chow and observed. At the end of the study period, chow was reweighed and total food intake in grams (g) for each rat calculated. All injections were given at least 72 h apart, between 08:30 and 11:00. Cannula placement was verified at the end of the study by a drinking response to ICV AII, followed by ICV injection of dye, removal of the brain and visual examination of coronal slices. Those exhibiting a negative drinking response or absence of dye in the ventricular system were excluded. Results are shown as mean ± s.e.m. Comparisons between groups of data were made using *t*-tests or ANOVA with post-hoc Tukey's test.

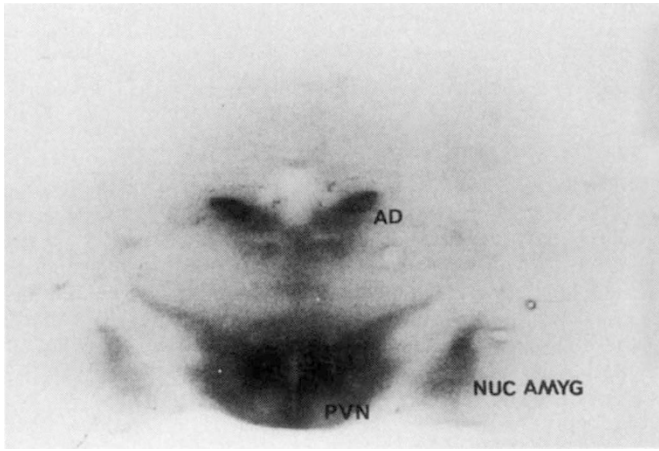

FIG. 2 Receptor autoradiography of GLP-1 binding sites in rat brain. Autoradiograph of a coronal section of rat brain incubated with 17 pM mono-^{125}I-GLP-1 alone, indicating total binding. No binding was observed in the presence of 200 nM unlabelled GLP-1 (nonspecific binding, not shown). High-density binding sites are visible in the hypothalamus (in particular the PVN), central nucleus of the amygdala (NUC AMYG) and anterodorsal thalamic nucleus (AD). No specific binding of GLP-1 was observed in the presence of 5 nM exendin (9-39) (not shown).
METHODS. ^{125}I-GLP-1 labelled by the chloramine-T method[3] was purified by reverse-phase HPLC (Waters C_{18} Novapak, Millipore). The specific activity of the label, determined by radioimmunoassay, was 60 Bq fmol^{-1}. Receptor autoradiography was carried out in a similar manner to that previously described[22]. Coronal brain sections (15 μm) were pre-incubated in 25 mM HEPES assay buffer, pH 7.4, containing 2 mM $MgCl_2$, 0.1% bacitracin, 0.05% Tween 20 and 1% BSA, for 20 min. Excess liquid was drained from the slides, which were then incubated in assay buffer containing ^{125}I-labelled GLP-1 (1,000 Bq ml^{-1}) for 90 min at room temperature. Non-specific binding was determined in the presence of 200 nM unlabelled peptide. The slides were washed four times for 30 s in ice-cold assay buffer, rinsed in ice-cold distilled water and dried overnight at 4 °C under vacuum. Autoradiographs of the slides were developed after 10 days exposure at −80 °C.

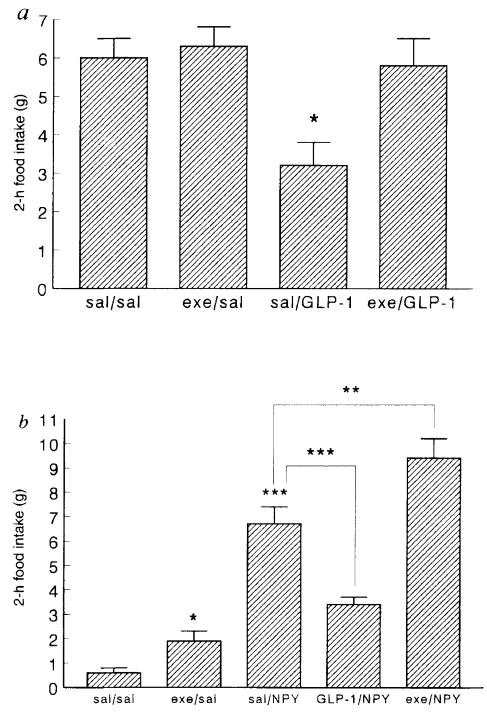

FIG. 3 Two-hour food intake in *a*, 24-h fasted rats ($n = 12–14$ per group) injected ICV with 0.9% saline (sal) (5 μl) or exendin (9-39) (exe) (100 μg 5 μl^{-1}), immediately followed by 0.9% saline (5 μl) or GLP-1 (3 μg 5 μl^{-1}) ($F(3,48) = 6.2$, $P < 0.005$; GLP-1 versus control, $^*P < 0.05$). *b*, Rats fed *ad libitum* ($n = 6–9$ per group) injected ICV at the start of the light phase with 0.9% saline (5 μl) (sal) or NPY (10 μg 5 μl^{-1}), immediately followed by either 0.9% saline (5 μl), GLP-1 (10 μg 5 μl^{-1}) or exendin (9-39) (exe) (100 μg 5 μl^{-1}). Both exendin (9-39) and NPY significantly increased food intake ($^*P < 0.05$ and $^{***}P < 0.001$, respectively, compared to control). GLP-1 significantly reduced ($^{***}P < 0.001$) whereas exendin (9-39) significantly increased ($^{**}P < 0.01$) the feeding response to NPY. Methods as for Fig. 1.

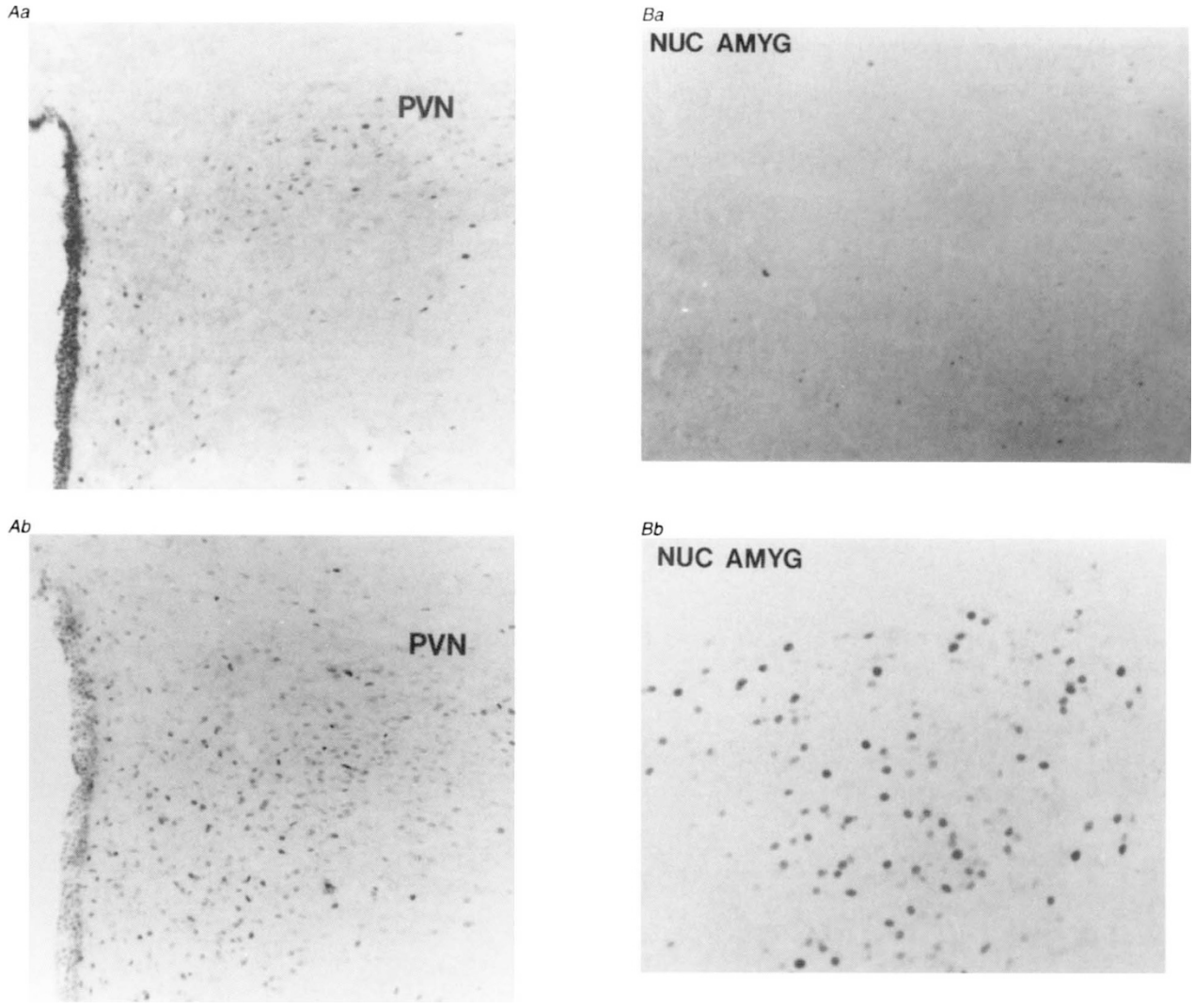

FIG. 4 Activation of c-*fos* following ICV injection of: *Aa*, 0.9% saline (10 μl) (control), demonstrating standard basal expression in the PVN (21 ± 2.2 positive cells per 10^4 μm^2, identical to that observed following ICV exendin (9-39) (100 μg 10 μl^{-1}) (not shown); and *b*, GLP-1 (10 μg 10 μl^{-1}). Significant activation is observed in the PVN (57 ± 2.3 positive cells per 10^4 μm^2, $P < 0.001$ compared to control). *Ba*, 0.9% saline (10 μl) demonstrating standard basal expression in the central nucleus of the amygdala (4 ± 0.5 positive cells per 10^4 μm^2); and *b*, GLP-1 (10 μg 10 μl^{-1}). Significant activation is observed in the central nucleus of the amygdala (NUC AMYG) (13 ± 0.5 positive cells per 10^4 μm^2; $P < 0.001$ compared to control).

METHODS. Adult male rats (200–250 g) were maintained in individual cages under controlled conditions of temperature (21–23 °C) and light (13-h light, 11-h dark) with *ad libitum* access to chow and water. Rats were anaesthetized and stainless steel guide cannulae were implanted into the lateral cerebral ventricle as previously described[23]. Rats were handled for 10 min each day following surgery, to familiarize them with infusion procedures and to minimize stress, which can readily activate c-*fos*[24]. Substances were dissolved in 0.9% saline and administered ICV as previously described[23]. Following injection, rats were returned to their cages for 1 h with no access to food. Rats were anaesthetized and perfused transcardially with 0.1 M PBS followed by 4% paraformaldehyde solution. The brains were removed and stored in 20% sucrose solution before being processed for detection of c-*fos* as previously described[23]. Coronal sections (40 μm) were washed and incubated with antisera and processed using the Vectastain ABC kit (Vector Laboratories, USA). Sections were immersed in 0.1% diaminobenzidinetetrahydrochloride solution and mounted on slides. Expression of c-*fos* was then quantified as previously described[23]. Results are expressed as mean ± s.e.m. of positive cells per 10^4 μm^2. Comparisons between groups of data were made using *t*-tests.

GLP-1 (8–36), (9–36) and (11–36) amide were therefore tested by ICV injection in fasted rats. At doses of up to 100 μg, these peptides had no effect on food intake or locomotor activity compared to controls (data not shown).

Exendin (9-39) has sequence homology to GLP-1 (ref. 12). It is a highly selective antagonist at GLP-1 receptors *in vitro*[4]. Furthermore, we have shown that exendin (9-39) specifically abolishes GLP-1-induced insulin secretion in the rat at a ratio of 15 : 1 (ref. 13), and *in vitro* in a clonal β-cell line at a ratio of 10 : 1 (ref. 14), indicating blockade of β-cell GLP-1 receptors. We therefore used a dose of 100 μg of exendin (9-39) to antagonize a dose of 3 μg GLP-1 *in vivo*. Autoradiography of GLP-1 binding sites in the brain shows dense specific binding of GLP-1 in the hypothalamus, especially the paraventricular nucleus (PVN); the central nucleus of the amygdala and the anterodorsal thalamic nucleus (Fig. 2). Addition of 5 nM exendin (9-39) blocks GLP-1 binding in these regions (not shown).

ICV administration of exendin (9-39) (100 μg) blocks the inhibitory effect of GLP-1 (3 μg) on food intake in fasted rats (Fig. 3*a*), but exendin (9-39) alone does not affect food intake in this circumstance (Fig. 3*a*). When administered by ICV injection to rats fed *ad libitum* at the start of the light phase (satiated), exendin (9-39) (100 μg) more than doubled food intake (Fig. 3*b*). The profound effect of exendin (9-39) in satiated animals without effect in fasted animals suggests a role for endogenous GLP-1 as a central satiety factor[15].

Neuropeptide Y (NPY) is the most powerful stimulant of feeding known[16,17]. In animals fed *ad libitum*, at the start of the light phase, ICV administration of GLP-1 (10 μg) immediately

before NPY (10 μg) greatly reduced food intake (Fig. 3*b*). Furthermore, ICV administration of exendin (9-39) (100 μg) immediately before NPY (10 μg) significantly increased food intake, compared to treatment with NPY (10 μg) alone (Fig. 3*b*). Hypothalamic NPY messenger RNA was assessed, as previously described[18], following repeated ICV administration of 0.9% saline or GLP-1 (100 μg) in both 72-h fasted and *ad libitum* fed rats. ICV injections were given during this 72-h period at 48, 24, 12 and 4 h before being killed. No change in NPY mRNA was found following ICV GLP-1 injection (data not shown). This suggests that GLP-1 does not act by altering hypothalamic NPY synthesis. The increase in food intake following blockade of GLP-1 receptors by exendin (9-39) and the augmented NPY response with coadministration of exendin (9-39) supports a physiological role for central GLP-1 in the regulation of feeding.

To establish the neuronal population(s) activated by central GLP-1, expression of the immediate early gene c-*fos*, a well established marker of neuronal activation[5], was mapped following ICV injection of GLP-1. Administration of exendin (9-39) (data not shown) or 0.9% saline (Fig. 4*Aa*, *Ba*) did not activate c-*fos* in any region of the brain. In contrast, rats injected with GLP-1 exhibit dense expression of c-Fos in the PVN (Fig. 4*Ab*) and central nucleus of the amygdala (Fig. 4*Bb*), but not the remainder of the hypothalamus or anterodorsal thalamic nucleus. The induction of c-*fos* following ICV GLP-1 injection was inhibited by prior administration of exendin (9-39) (data not shown).

In conclusion, central GLP-1 is a new physiological regulator of feeding in the rat. Many neuropeptides have been proposed as central satiety factors[6], but few have an established physiological role. GLP-1 may be the most potent inhibitor of feeding yet identified in the rat. Its role in man remains to be determined. The interaction of GLP-1 with other established regulators of food intake, such as leptin (Ob protein)[19–21] and NPY, also needs investigation. Understanding the function of these centrally acting regulators should ultimately lead to new and more effective agents for the management of appetite disorders. □

Received 2 October; accepted 3 November 1995.

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ACKNOWLEDGEMENTS. We thank P. Byfield for the synthesis of all peptides used in these studies and P. Phillips for his expert technical assistance.

Rapid colour-specific detection of motion in human vision

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THE human visual system is much better at analysing the motion of luminance (black and white) patterns than it is at analysing the motion of colour patterns[1–4], especially if the pattern is presented very briefly[5] or moves rapidly[6]. We report here that observers reliably distinguish the direction of motion of a colour pattern presented for only 17 milliseconds, provided that the contrast is several times the threshold value (the contrast needed to detect the presence of the pattern). A control experiment, in which a static luminance 'mask' is added to the moving colour pattern, proves that discrimination of the direction of motion of these brief stimuli is colour-specific. The mask drastically impairs discrimination of the direction of motion of a luminance pattern, but it has little effect on a colour pattern. We conclude that the human visual system contains colour-specific motion-detection mechanisms that are capable of analysing very brief signals.

The prevailing view is that the visual system's primary analyses of colour and movement occur in parallel in different areas of the brain[7–9]. An important observation supporting this view is that the motion of many patterns defined solely by colour is undetectable[1] or hard to see[4]. The colour of such a pattern must be analysed before its motion, so if motion and colour are analysed in parallel, motion analysis must inevitably be compromised.

To demonstrate that colour contributes directly to the analysis of motion, it is important to show that motion discrimination is not secondary to analysis of position[10] or to the production of eye movements that track the stimulus. The only way to be sure of this is to use a very brief presentation so that the stimulus disappears before an eye movement can be initiated. Previous demonstrations that colour contributes distinctively to motion analysis have always used long presentations[11,12]. Thus, even though direction-discrimination thresholds for colour stimuli may be lower (in cone contrast) than those of luminance stimuli[6], we cannot yet exclude the possibility that the motion of such stimuli is analysed by conscious monitoring of changes in position[10].

Figure 1 shows that the motion of colour gratings can be discriminated reliably even when the grating is only present for 17 ms, about 5 times less than the time taken to initiate an eye movement[13]. Discrimination of such brief stimuli could only be mediated by a mechanism whose primary function is the analysis of motion.

We must ensure that the motion of colour gratings shown in Fig. 1 depends on a mechanism selective for colour and is not caused by inadvertent stimulation of mechanisms sensitive to luminance patterns[14]. Accordingly, we retested motion discrimination using a red–green colour grating to which a static luminance mask had been added. The test depends on the assumption that if the colour grating's motion is detected by a luminance-sensitive mechanism, then we can treat it as equivalent to the luminance grating that would support the same direction-discrimination performance. The exact position of the luminance grating relative to the colour grating (its phase) is unknown because it depends on the nature of the hypothetical artefact that causes the colour grating to stimulate a luminance-sensitive mechanism.

The basis of the masking test is shown in Fig. 2, which illustrates the effect of static masks added to a moving luminance grating in different phases. In the absence of the mask (lower left-hand