the overexpression of Dsh[16] in the posterior compartment had exactly this phenotype (Fig. 3*a*, *b*). A further prediction of this model is that the ectopic expression of *wg* observed in *dsh*$^-$ clones should be reversed by the simultaneous loss of *Notch*; indeed, ectopic *wg* expression was not observed in *Notch*$^-$ *dsh*$^-$ clones (Fig. 1*l*). The mechanism by which Wg inhibits Notch activity is not known, but it has been suggested that this inhibition is mediated by the binding of Dsh to Notch[16]. One prediction of the Dsh–Notch-binding model is that removal of Wg signalling components downstream of Dsh should not affect Notch activity. Our evidence suggests that *Armadillo*, which like *dsh* is required for normal Wg signalling but which acts genetically downstream of *dsh*[25,26], is not required for Wg self-refinement (E.J.R., C.A.M., M. Halevy and S.S.B., manuscript in preparation).

That a narrow region of *wg* expression is normally retained along the margin, even after self-refinement, indicates that these cells are in some manner less sensitive to Wg signalling than cells more distant from the margin. This difference in sensitivity could be explained in two ways. First, some unknown factor specific to the dorsoventral boundary may render boundary cells less sensitive to Wg signalling. Recent evidence suggests that there are as yet uncharacterized signals organized around the dorsoventral boundary[27], and these could be responsible for localized biases in cell behaviour. A simpler hypothesis is that Notch activity at the dorsoventral boundary is initially higher and thus remains above levels required for *wg* expression (Fig. 3*c*). In support of this idea, it should be noted that both *Enhancer of Split* complex members and the *vestigial* second intron enhancer are expressed specifically along the margin, and that this expression depends upon the presence of Su(H) binding sites within their enhancers[6,7,27]; the Su(H) transcription factor is thought to mediate Notch signalling.

The self-refinement function of Wg may have parallels in other situations, including the vertebrate hindbrain. As in the wing margin, the boundary-specific domains of *Wnt-1* expression are initially sloppy, but become refined later in development; moreover, in *Wnt-1*^{sw} mutant mice many of the domains of *Wnt-1* hindbrain expression seem to be expanded when compared with the wild type[28]. □

Methods

wg^{ts} larvae were from *wg*^{IL}/*In(2LR) Gla Bc*^{1} X *wg*^{CX4}/*In(2LR) Gla Bc*^{1}; permissive and restrictive temperatures were 16.5 °C and 30 °C, respectively. Shifted discs and unshifted controls were marked and labelled in the same well. Antibody labelling was as described previously[3], using rabbit or rat (1/1000) anti-Wg (provided by R. Nusse), 1/1000 rabbit anti-Scute or 1/25 mouse anti-Ac (both provided by G. Panganiban), 1/400 rabbit anti-Dsh[15] (provided by R. Nusse), anti-Myc supernatant, and/or anti-β-galactosidase. *In situ* hybridization was as described previously[29] with dig-labelled *wg* complementary DNA (provided by F. M. Hoffmann). Clones were generated using the *FLP/FRT* system as described previously[3] with the following crosses: *wg*^{LacZ} *FRT*^{40A}/*CyO* X *y w FLP1*; *πM*^{21C} *πM*^{36F} *FRT*^{40A} (provided by A. Penton). *svb*^{YP17b} *dsh*^{v26} *FRT*^{101}/*FM7* or *y w dsh*^{75} *FRT*^{101}/*FM7* or *N*^{1081} *svb*^{YP17b} *dsh*^{v26} *FRT*^{101}/*FM7 X πM*^{5A} *FRT*^{101}; *FLP3, Sb/TM6, Tb*. *y w dsh*^{v26} *β*^{36a} *FRT*$^{9-2}$/*FM7 X ovo*^{D1} *FRT*$^{9-2}$; *FLP*^{38}/*FLP*^{38}. *y w dsh*^{75} *FRT*^{101}/*FM7; wg*^{IG22}/*Gla X ovo*^{D1} *FRT*^{101}; *FLP*^{38}/*FLP*^{38}. *y w dsh*^{75} *FRT101/+; hs-wg/+ X ovo*^{D1} *FRT*^{101}; *FLP*^{38}/*FLP*^{38}. *dsh*^{v26} and *wg*^{IG22} are protein nulls[15,30], whereas the sole phenotype of *ovo*^{D1} is female sterility. Larvae observed at late third instar (Fig. 1) were heat-shocked during second instar, and those reared to adulthood (Fig. 2) were heat-shocked during third instar. All were reared at 25 °C. Dsh overexpression was induced using the *GAL4/UAS* system as described previously[19], with *UAS-dsh* X *en-GAL4*. These larvae were reared at 22 °C.

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Shared neural control of attentional shifts and eye movements

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We are able to move visual attention away from the direction of gaze, fixating on one object while attending to something else at a different location, within the region of peripheral vision. It has been widely assumed that the attentional neural systems are separate from the motor systems, but some studies challenge this idea[1–5]. It has now been suggested that the attentional system is part of the premotor processing in the brain[6]. This model proposes that attentional processes evolved as part of the motor systems, with isolated attentional shifts representing an artificial separation of a natural linkage. Here we test how attentional shifts might be linked to the preparations for making saccadic eye movements. We studied the superior colliculus in monkeys as they shifted their attention during different tasks, and found that each attentional shift is associated with eye-movement preparation.

Attention can be moved under voluntary or involuntary control[7–9]. A technique developed to study experimentally the dynamics of visual attention involves presentation of a cue, which indicates the target position before onset of that target[8]. This improves accuracy of target detection and decreases the time needed to detect or identify the target. Researchers refer to voluntary control of attention as endogenous and study it with symbolic cues. The involuntary control has been called exogenous (or reflexive), and is studied with cues directly priming a location.

Previous reports have proposed that the superior colliculus participates in shifting attention exogenously[10]. Patients suffering from tectal lesions often have deficits in the ability to shift attention[11]. The superior colliculus is also important in the generation of eye movements[12,13]. We have explored the relationship

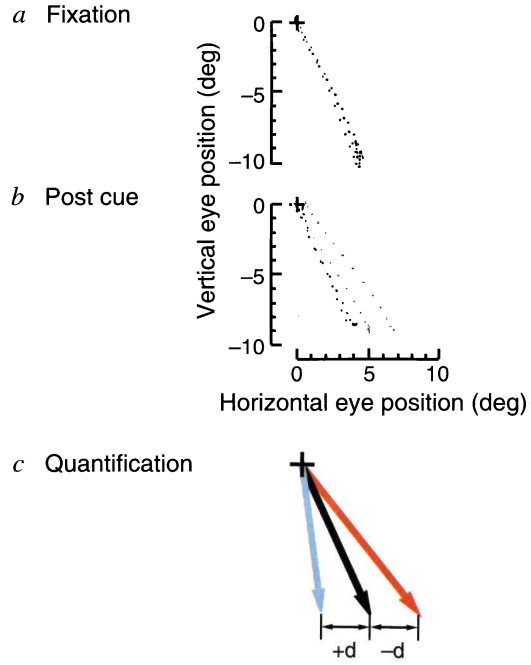

FIG. 1 *a*, Three fixed-vector saccades evoked during periods of fixation. *b*, Modified saccades after the cue onset. *X*–*Y* plots of the monkey's eye position; 2 ms between dots. Monkey fixated a central stimulus represented by +. The site of a burst cell, 1.7 mm from the collicular surface, was stimulated. In *b*, after the monkey maintained fixation for at least 1 s a cue appeared indicating the target location (12° to the left or right of the fixation point). At various random times after the cue onset, the superior colliculus was stimulated (five trials). The direction of the saccade evoked by collicular stimulation was different from that of the fixed vector saccade and shifted toward the cued location. Red traces were evoked after the right cue, blue were after the left cue, and black were evoked at the instant of the cue onset. *c*, Scheme showing how we analysed these data. Amount of shift of the evoked saccade was calculated as a distance (*d*) between end points of the averaged fixed-vector saccade and the saccade elicited after the cue onset. Positive values were arbitrarily assigned to modifications to the left.

between attention and eye movements by using the stereotyped saccadic eye movements evoked by collicular stimulation[12,14]. Our method was based on evidence that collicular stimulation applied during the preparation for a saccadic eye movement results in premature, imprecise saccades[15]. We analysed changes in the evoked saccades when the monkey moved its attention to the periphery before stimulation.

We studied six colliculi in three macaque monkeys (*Macaca mulatta*). Our data include a total of 23 penetrations into the colliculi and 20 sites which we recorded from and stimulated. When the monkeys performed the peripheral-cue eye-movement task, targets that had been cued in the correct location (valid cue) were associated with faster eye-movement reaction times than these that were cued in the incorrect hemifield (invalid cue) (Table 1). Thus, each cue caused a shift in visual attention to its locus and facilitated saccadic eye movements to that location[16]. We next tested whether this shift of attention had any effect on the oculomotor systems. When the monkeys simply fixated on a target, repeated stimulation evoked uniform fixed-vector saccades (Fig. 1*a*). However, when we stimulated the superior colliculus after the onset of cues, there were shifts in the direction of the evoked saccades (Fig. 1*b*). We measured the distance between the end of a fixed-vector saccade and the end point of the saccadic eye movement evoked at various times after the cue onset (Fig. 1*c*)[17].

For validly cued targets, the saccadic eye movements evoked by stimulation of the superior colliculus were deviated in the direc-

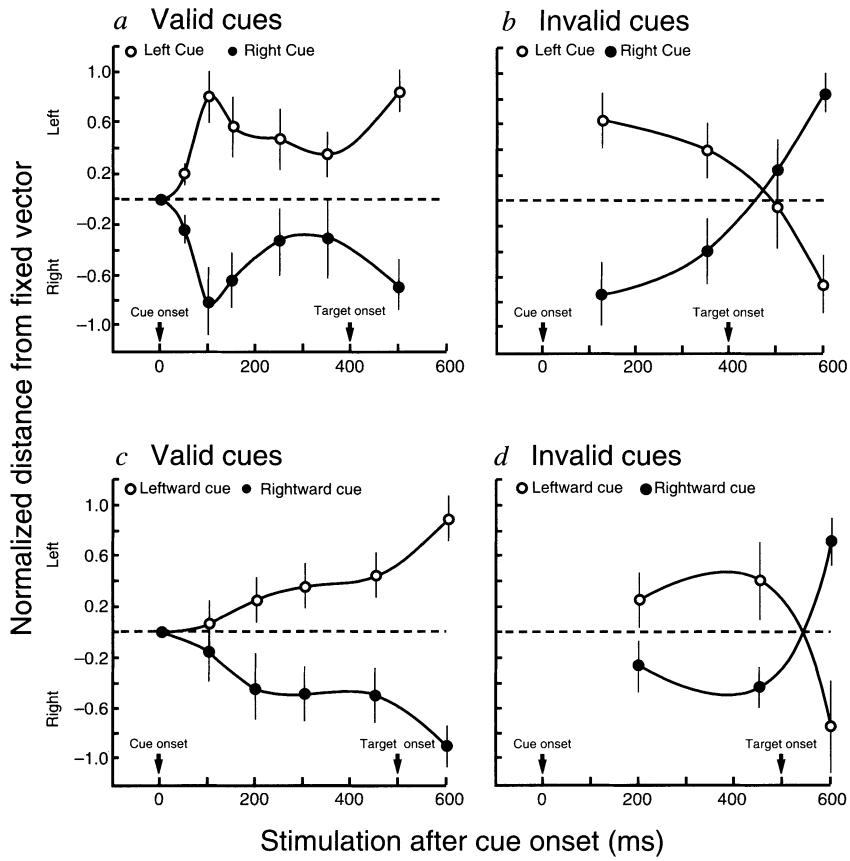

FIG. 2 Effects of peripheral cueing (*a*, *b*) and symbolic cueing (*c*, *d*) in eye-movement tasks on the shift of the evoked saccade. For all plots, the vertical axis is the normalized distance between the end points of the fixed-vector saccades and task-evoked saccades (see Fig. 1). Data from each experiment were normalized using the formula: $(d - d_o/(d_{max} - d_o))$, where d_{max} is the maximal shift of saccades evoked from that site and d_o is for saccades evoked at cue onset. Vertical bars, s.d. The horizontal axis is the time of stimulation after cue onset for the one cue–target interval illustrated. The 80% valid (*a* and *c*) and 20% invalid (*b* and *d*) cueing trials, cue–target intervals, and sides were randomly interleaved. The curves were generated from spline fitting of the data. The data in *a* and *b* represent the average change for 10 different collicular sites; those in *c* and *d* come from 11 loci studied with symbolic cueing.

TABLE 1 Reaction times from target onset to saccade

	Eye movement task						Manual task					
	Peripheral cues			Symbolic cues			Peripheral cues			Symbolic cues		
SOA	100	300	500	100	300	500	100	300	500	100	300	500
Valid reaction times	249 ± 5	233 ± 11	267 ± 5	247 ± 6	211 ± 5	206 ± 5	420 ± 7	362 ± 8	352 ± 8	396 ± 7	382 ± 5	375 ± 6
Invalid reaction times	295 ± 17	315 ± 11	310 ± 19	283 ± 12	244 ± 10	211 ± 19	413 ± 8	403 ± 10	398 ± 8	406 ± 7	409 ± 6	407 ± 5
Validity effect	46*	82**	43*	36*	33**	5	−7	41**	46**	10	27**	32**

Behavioural data collected outside recording sessions, presented as means ± s.e.m.. SOA, stimulus onset asynchrony. Validity effect is the difference between the reaction time means of valid and invalid cue conditions. $*P \leqslant 0.05$, $**P \leqslant 0.01$, significance of validity effect as calculated by the Wilcoxon test.

tion of the cue and target. Figure 2*a* shows the shift with the variable time of stimulation in relation to the onset of the cue. In contrast, when the cue appeared in one visual field, but the target appeared in the opposite field (invalid trials), the evoked eye movements were initially shifted towards the cued side. After the onset of the target, this shift began to reverse (Fig. 2*b*). As can be seen for all these data, the parameters that determine the degree of rotation are the time delay between the cue onset, target onset and moment of collicular stimulation. Whereas others have found that the motor preparation to make an eye movement can modify the electrically evoked saccade[18], we show that the shift of attention also leads to modifications of the evoked saccades.

We also used a 'central symbolic cueing' task, in which the animals translated the colour of a foveal cue into the spatial location of the forthcoming target. Again we obtained appropriate effects of cueing on the reaction times for eye movements (Table 1). As can be seen in Fig. 2*c* and *d*, symbolic cueing altered the stimulation-evoked eye movements.

From these results, we cannot rule out the possibility that changes might be due to the preparation for making an eye movement. Therefore we trained one of the previously tested monkeys and a totally naive animal on new peripheral- and symbolic-cueing tasks. Here the monkeys had to maintain fixation on the central stimulus throughout the trial and were trained to respond only with hand movements. Typical reaction-time data in Table 1 show attentional effects. These manual tasks produced similar deviations of the eye movements evoked from stimulation of the superior colliculus (Fig. 3). With peripheral cueing, there is strong and stable modification of the evoked movement, which reversed directions after invalid cues (Fig. 3*a*, *b*). The effects in the symbolic cueing task appear more gradually (Fig. 3*c*, *d*), just as the translation of the coloured signal must be more gradual than for a peripheral flashed cue. These data show that attentional shifts that are independent of eye movements to the targets still lead to modifications of the evoked saccades.

'Build-up' cells in the intermediate layers of the superior colliculus have progressive increases in activity during the preparation of saccadic eye movements[19]. Moreover, such cells can modulate with anticipation of an eye movement towards a specific spatial location[19,20]. In the monkeys that performed the attention tasks with eye-movement responses, we found that build-up cells responded in a time-locked fashion when the peripheral cue appeared in the receptive field (Fig. 4*a*). Importantly, build-up cells responded to the symbolic cueing (Fig. 4*b*). The timing and level of activity were significantly changed with the timing of stimulation ($F = 3.654$, $P < 0.01$) and also differed from the peripheral cueing task. Even

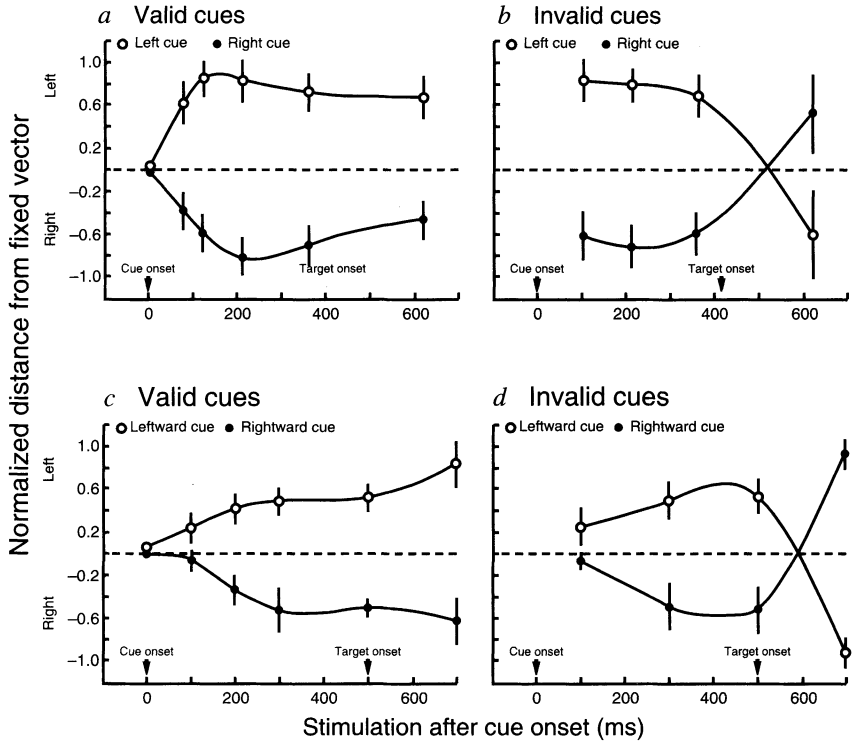

FIG. 3 Effects of peripheral cueing (*a*, *b*) and symbolic cueing (*c*, *d*) on the shift of the evoked saccade during a hand-response task. The format of the figure is as Fig. 2. The data in *a* and *b* represent the average of 8 different collicular sites, whereas those in *c* and *d* come from 7 loci.

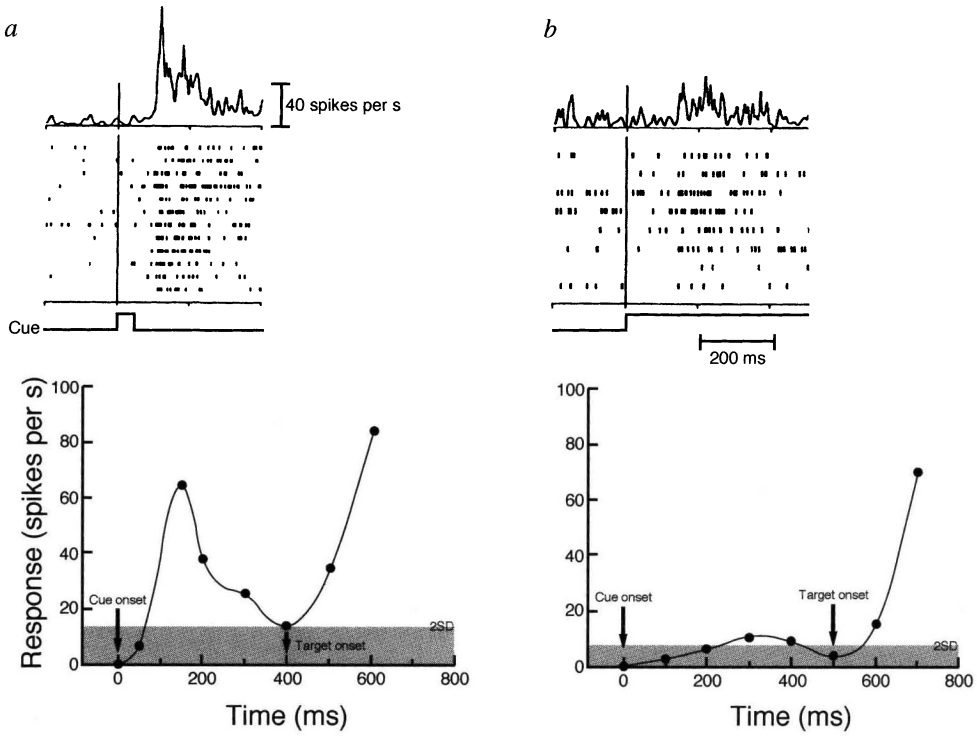

FIG. 4 Activity of build-up neurons during peripheral (*a*) and symbolic (*b*) cueing tasks. Top panels: responses of the cells to the cue onset while the animal fixated a central spot of light. Each horizontal row represents one trial and each dot corresponds to a single spike. Spike-density curves are shown at the top. Vertical line represents cue onset. Bottom panels: cell activity was calculated as the number of spikes in 50- or 100-ms intervals and normalized against its baseline activity during simple fixation. The shading at the bottom of each graph represents two standard deviations from the mean background activity of the cell. Each data point on the curve shows averaged activity of 8 (*a*) and 6 build-up cells (*b*) at different times after the cue or target onset, indicated by arrows. Similar data were obtained at other cue–target intervals (not illustrated). Smooth lines are spline-approximation curves. Note a sharp increase in cell discharge after the target appearance, which is the pre-saccadic burst response.

when no stimulus appeared in the receptive field of a build-up cell, the central cue induced a build-up response. This means that the translation of the symbolic cue occurred within the saccadic system (possibly in parietal cortex), reached the superior colliculus, and was used to prepare a saccade.

Stimulation of the superior colliculi of monkeys during fixation leads to stereotyped eye movements. When cues (peripheral visual or foveal symbolic) shift attention, there is a consistent shift in the direction of the stimulation-evoked eye movement. This indicates that shifts of attention might be associated with the preparation to make an eye movement towards the attended location. Recordings from build-up neurons in the superior colliculus reveal responses to the attentional cueing and modulation with the intention to look at the specified location. These observations indicate that the build-up cells in the superior colliculus might be participating in the shift of attention as well as the preparation to make an eye movement, and they differ from the cells in the collicular superficial layers which are not modulated by endogenous attentional shifts[10]. However, the initiation of a saccade in such experiments can be under voluntary suppression which involves other neural centres[5,21]. Our data are consistent with the hypothesis that shifts of attention, however evoked, are tightly coupled with the preparation to make oculomotor responses to the attended area.

The strength of the effects of peripheral cueing on stimulation-evoked saccades supports the idea that the superior colliculus is involved in reflexive attentional shifts. On the other hand, symbolic cueing does require an analysis of the meaning of the sensory input (voluntary attention). It has been suggested that such analysis might be done in posterior parietal cortex[22]. Parietal neurons send strong projections to the superior colliculus[23,24], and the effects we report here may be mediated by parietal cortex. □

Methods

Techniques have been described previously[17,25]. In the peripheral cueing task, the monkeys fixated a central spot, and at some variable time later, a large light was flashed briefly (16 or 50 ms) in one hemifield[8,16]. Between 100 and 500 ms after cue onset, a target was turned on at the cued location (valid cue), the fixation point was simultaneously extinguished, and the monkeys then made a saccadic eye movement to fixate that target. On 20% of the experimental trials, the cue and target were in opposite visual fields (invalid cue).

In the symbolic cueing task, the monkeys fixated centrally, and a large, coloured cue overlapped the fixation point. The colour encoded the location where the monkey would make an eye movement. A red cue symbolized that the target would appear in the right hemifield; a green foveal cue symbolized a left target. Valid cues were presented on 80% of the trials. In manual versions of the cueing tasks, cues and targets were presented as before, and the monkey released a bar with the right hand after right targets and with the left hand after left targets. No eye movements were allowed. Targets were dim (6% brighter than background) and briefly flashed.

The superior colliculus was stimulated during fixations and at various times after cue onsets. Stimulus trains were of 40 ms duration, at 400 Hz, consisting of 0.1-ms pulses at twice threshold amplitude. To prevent the monkeys from adapting to stimulation, 20% of all trials were made without it. Visual target positions were selected to be roughly orthogonal to the stimulation-evoked saccades.

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