cytotoxic T cells (*15*). We speculate that intracellular targeting of WR4017/pMGB2 led to expression of CS antigen on the cell surface in association with class I MHC molecules and the induction of a cell-mediated immune response. This technique should be useful for precise mapping of critical T cell epitopes, and for construction of vaccines against other diseases in which cell-mediated immunity is important, such as leprosy, leishmaniasis, schistosomiasis, and infection with rickettsia or the human immunodeficiency virus.

Mutants of *S. typhi* have been used safely in humans as oral vaccines and elicit cellular immunity against *S. typhi* antigens (*9, 16*). Preliminary data indicate that galE (Ty21A) or aroA (541Ty) mutants of *S. typhi*, and *S. typhimurium* strain WR4017, express *P. falciparum* antigens when transformed with plasmids containing the *P. falciparum* CS gene. Thus it may be possible to develop an orally administered vaccine for the prevention of malaria.

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24. Mice were selected at random to receive sporozoite lysate or PBS in the right or left hind footpad. Lysate was prepared with sporozoites in PBS (1.2×10^6/ml) undergoing three freeze-thaw cycles. Lysate or PBS (25 μl) was injected with a Hamilton syringe repeating dispenser through a 27 gauge needle. Before and 24 hours after injection, footpad thickness to within 0.10 mm was determined with Schnelltaster calipers by an observer blinded to immunization group and test antigen. Mice immunized sc with WR4017/pMGB2 were not tested for DTH.
25. We thank H. Sidbery, C. Life, M. Gross, J. Egan, D. Gordon, I. Schneider, M. Dowler, U. Krzych, M. Chulay, and A. Shafferman.

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Increased Attention Enhances Both Behavioral and Neuronal Performance

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Single cells were recorded from cortical area V4 of two rhesus monkeys (*Macaca mulatta*) trained on a visual discrimination task with two levels of difficulty. Behavioral evidence indicated that the monkeys' discriminative abilities improved when the task was made more difficult. Correspondingly, neuronal responses to stimuli became larger and more selective in the difficult task. A control experiment demonstrated that changes in general arousal could not account for the effects of task difficulty on neuronal responses. It is concluded that increasing the amount of attention directed toward a stimulus can enhance the responsiveness and selectivity of the neurons that process it.

OUR PERCEPTUAL SYSTEMS DO NOT always work at their peak (*1*). As an extreme example, perceptions may seem to dull, then disappear, as we drift off to sleep. Likewise, within the visual system, neurons in the cerebral cortex of sleeping cats give weakened sensory responses that do not distinguish among incoming stimuli as well as neuronal responses in the awake animal (*2*). Moreover, neurons in certain cortical areas of awake monkeys show different degrees of responsiveness, depending on whether the monkey is idle, engaged in a detection task, or engaged in a discrimination task (*3*). It has not been clear from these physiological studies, however, whether neuronal responsiveness varies with changes in state, level of arousal, the specific task required of the animal, or the amount of attention devoted to the stimuli. To test specifically whether the amount of attention, or cognitive "effort," devoted to a stimulus affects how it is coded within the visual system, we studied the responses of visual neurons to stimuli presented within the same perceptual task at different levels of difficulty.

Neurons were studied in area V4 within the extrastriate cortex of monkeys. Area V4 is an intermediate station along the pathway from the primary visual cortex into the temporal lobe, a pathway critically involved in object recognition (*4*). It appears to be the first cortical area along this pathway in which neuronal responses are gated by spatially directed selective attention. Previously, it was found that when a monkey attends to a stimulus within the receptive field of a V4 neuron, the neuron's responses to nearby, but ignored, stimuli within the receptive field are greatly attenuated (*5*), possibly explaining why unattended stimuli are not normally perceived. Our goal in the present study was to determine if even the neuronal responses to attended stimuli are affected by "how much" attention, or effort, is devoted to them. To test this, we trained two rhesus monkeys to maintain fixation on a small spot and to discriminate the orientation or color of a stimulus presented within the receptive field of a neuron in V4. Every cell was tested with stimuli presented within the context of the same discrimination task at two levels of difficulty.

The task used was a modified version of matching-to-sample. While the monkey held a bar and gazed at a fixation spot, a sample stimulus appeared for 200 msec, and 400 to 600 msec later a test stimulus appeared for 200 msec at the same location (*6*). When the test stimulus was identical to the preceding sample (a "matching" trial), the animal was rewarded with a drop of water if it released the bar immediately; when the test stimulus differed from the sample (a "nonmatching" trial), the animal was rewarded only if it delayed release for 700 msec. Half the trials were matching and half nonmatching. The

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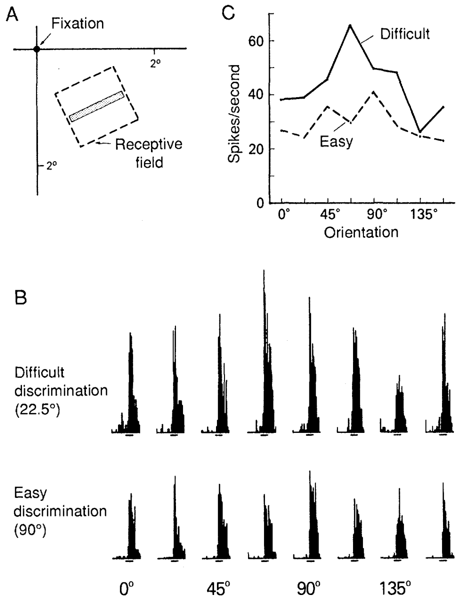

Fig. 1. Example of neuronal responses that were stronger and more selective when the animal was performing a difficult discrimination than when performing an easy discrimination on the same stimuli. (**A**) The stimuli were red bars varying in orientation. (**B**) The histograms show the neuronal responses to a set of stimuli when each was presented as the sample to be discriminated from a subsequently presented test stimulus differing by 90° of orientation (easy) or 22.5° (difficult). Responses were based on approximately 18 trials each. The horizontal bars indicate the 200-msec interval when the stimulus was on. (**C**) The tuning curve shows the firing rates to the different stimuli, averaged over a 300-msec interval beginning 60 msec after stimulus onset.

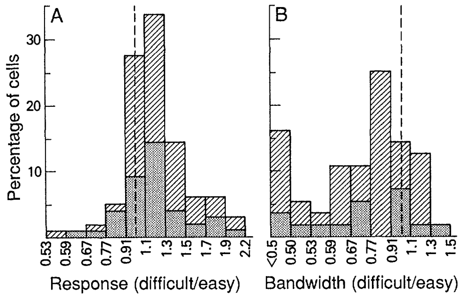

Fig. 2. Effects of task difficulty on all cells recorded in V4. (**A**) For each cell, the response to the best stimulus in the difficult condition was divided by the response in the easy condition. A ratio of 1.0 (dashed line) indicates that task difficulty did not affect the response. Most cells showed larger responses in the difficult condition (ratios >1.0). (**B**) The tuning bandwidth in the difficult condition was divided by the bandwidth in the easy condition. Most cells showed narrower bandwidths in the difficult condition (ratios <1.0). Hatched bars indicate cells studied with orientation as the relevant stimulus dimension, and shaded bars indicate cells studied with color.

stimuli were small colored bars generated on a computer display. Stimulus size and position were optimized for each cell studied. For some cells, all bars were of the same color but varied in orientation, whereas for other cells the reverse was true. On each trial of an experimental session, the sample and test bars were chosen from a list of four or eight different orientations or colors (*7*), and trials in a given condition were run until each of the four or eight stimuli had been presented as a sample at least 14 times. For the cells tested with eight different stimuli, a tuning curve was fit to the responses (Fig. 1C). The sample stimuli in the easy condition were identical to those in the difficult; the two levels of task difficulty were determined by the nature of the nonmatching test stimuli. In the easy condition, the nonmatching test stimuli differed from the samples by 90° of orientation or about 77 nm in wavelength. In the difficult condition, the nonmatching test stimuli varied from the samples by only 22.5° or 19 nm. Some cells were tested first in the easy condition, others in the difficult, and a few cells were held long enough to test in repeated conditions. We focused our analyses on the neuronal responses to the sample stimuli, since the sample stimulus presentation within a trial was identical across the two conditions. Neuronal data were accepted only for correctly performed trials.

The overall performance of the animals in the easy and difficult conditions was 93 and 73% correct, respectively, suggesting that the difficult condition was, indeed, more difficult. A difference in error rate alone, however, does not prove that the animals actually processed the stimuli differently in the two conditions. To examine this question, difficult nonmatching probe trials were inserted randomly on 6% of the trials within the easy task. The difficult probe trials were performed with far more errors when they were presented within the easy condition (48% correct) than when they were presented within the difficult condition (78% correct) (*8*). The corresponding matching trials were performed slightly better in the easy condition (96% correct) than in the difficult condition (91% correct). A signal detection analysis of these results indicated that in the difficult condition the animals adopted a stricter internal criterion for discriminating matching from nonmatching stimuli (likelihood ratio = 0.54 versus 0.22; $\chi^2 = 48$, $P < 0.01$) and also that the discriminability of the stimuli increased ($d' = 2.11$ versus 1.7; $\chi^2 = 9.7$, $P < 0.01$), that is, the animals' internal representations of the stimuli were better separated, independent of the criterion used to discriminate them (*9*). We interpret both of these results to mean that the animals devoted more attention to the stimuli in the difficult condition.

We recorded from 98 neurons and found that 81% responded more strongly to the optimal sample stimulus when it was presented in the difficult condition than when the same stimulus was presented within the easy condition ($\chi^2 = 18$, $P < 0.01$) (*10*). The median change in response in the difficult condition was an 18% increase (Figs. 1 and 2A). To determine whether this increase in responsiveness reflected only an increase in the "gain" of the sensory response or whether it also reflected an improved selectivity of the cells, we examined the tuning curve bandwidths of the 42 cells tested with bars at eight orientations and 14 cells tested with eight colors (*11*). Overall, 77% of the cells showed a narrowing of their bandwidths in the difficult task ($\chi^2 = 7.5$, $P < 0.01$), indicating an improvement in selectivity. The median orientation and color bandwidths in the easy task were 81° and 59 nm, respectively, compared to 53° and 52 nm in the difficult condition. The median change in bandwidth was a 20% decrease (Fig. 2B). Qualitatively similar but smaller effects were found for responses to test stimuli.

We considered two possibilities to explain the improvement in neuronal responses in the difficult condition. The first was general arousal, which might cause an improvement in the responsiveness of all the cells in V4. The second was a restricted effect on only the cells whose receptive fields contained the discriminanda, that is, the stimuli the animal was attending. To decide between these possibilities, we tested 41 of the above cells in another condition: the task stimuli were placed outside of the receptive field of the recorded neuron, while irrelevant stimuli were presented inside the receptive field. On any given trial, a single irrelevant stimulus was presented simultaneously with the relevant sample stimulus. Both were chosen from the same set of stimuli that were used when the task stimuli were located inside the receptive field. Since no test stimulus was presented at the irrelevant location, the animal could not perform its task on the stimuli at that location. Neuronal responses to the unattended stimuli were measured when the animal was performing the easy and difficult versions of the task in response to the stimuli outside the recorded neuron's receptive field (*12*). We reasoned that if the difficult task increased the responsiveness of all the neurons in V4 because of arousal, then responses to even the unattended stimuli inside the receptive field should be enhanced in the difficult condition. The results showed that when the discriminanda were placed outside the field of the recorded neuron, the difficult task no longer caused any overall enhancement of responses or sharpening of tuning curves for the stimuli located inside the field. In fact, there was a slight but insignificant tendency in the opposite direction: the median change in response in the difficult condition was a 7% decrease, and the median change in bandwidth was a 6% increase. These results are inconsistent with the explanation of general arousal.

Both our behavioral and neurophysiological results indicate that increasing the amount of effort needed to perform a perceptual task can affect how information is processed within the visual system. When an animal is challenged by a difficult task, it appears to "rise to the occasion" by concentrating its attention, two neural correlates of which appear to be enhanced responses and sharpened selectivity of the neurons that are processing the stimuli. Because these neuronal changes are specific to the neurons activated by attended stimuli within their receptive field, the changes presumably result from a different physiological mechanism than the one that regulates the changes in neuronal responsiveness that occur in conditions of sleep and wakefulness (*2*). Furthermore, although these changes are spatially localized, their mechanism may also be different from that for spatial attention, since spatial attention appears to cause suppression of responses to unattended stimuli in V4 (*5*), whereas increased effort appears to cause enhancement of responses and sharpened selectivity for attended stimuli.

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7. Orientation ranged from 0° to 157.5° in eight steps of 22.5°. Dominant wavelengths ranged from 468 to 603 nm in eight steps of an average of 19.3 nm. The specific wavelength of each stimulus was adjusted so that the animals made about equal errors in discriminating adjacent colors throughout the spectrum.
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10. All statistical tests were based on χ^2.
11. Bandwidth equals the full width of the tuning curve at a distance of $1/e$ (0.368) from the peak.
12. This control experiment was possible because it had been previously shown that attention to a stimulus outside the receptive field of a V4 neuron does not suppress responses to ignored stimuli within the receptive field. Responses to ignored stimuli are suppressed only when the attended and ignored stimuli are both located within the receptive field (*5*).
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"I can't possibly see anyone at the moment."