Supplementary material

Methods

Electrophysiological recordings and surgical methods

Experiments were conducted in the right hemispheres of two healthy, adult monkeys (Macaca mulatta) weighing 12 and 16 kg. The studies were approved by the local authorities (Regierungspraesidium) and were in full compliance with the guidelines of the European Community (EUVD 86/609/EEC) for the care and use of laboratory animals. Recording chambers were positioned stereo-tactically with the aid of high-resolution magnetic resonance anatomical images. These methods have been described in detail previously 1.

Neural activity was recorded using custom-built tetrodes which were adjusted to achieve excellent single unit isolation. No pre-selection functional criteria were applied for the neurons. Neural activity was sampled at 32kHz, digitized, and stored using the Cheetah data acquisition system (Neuralynx, Tuscon, AR).

The animals were implanted with a scleral search coil 2 and their eye movements were monitored on-line. Data were also collected for off-line analysis using both the QNX-based data acquisition system at 200 Hz and the Cheetah data acquisition system at 2000 Hz.

Visual stimulation and behavioral task

Visual stimuli were displayed using a dedicated graphics workstation (TDZ 2000; Intergraph Systems, Huntsville, AL) with a resolution of 1280 x 1024, and an 85 Hz refresh rate, running an OpenGL-based stimulation program. The behavioral aspects of the experiment were controlled using the QNX real-time operating system (QSSL, Ontario,
Canada). After the monkey acquired fixation on a colored square target (0.2 degrees) for 300 ms, a field of drifting coherent random dots was presented on the monitor. The size of the field, size of the individual dots, color and location of the visual stimulus were selected so as to stimulate as many of the recorded neurons as possible. The center of the receptive field of the V4 neurons from both monkeys were located in the lower left visual field. The coherence of the drifting random dots was fixed at 60%.

During the classical stimulation paradigm a no-movement uniform intensity stimulus was presented during fixation, followed by a stimulation pattern moving in one of either 4 or 8 directions of motion equally distributed around the full 360°. Stimulation time was either 500 or 700 ms. During the testing phase of the adaptation stimulation paradigm the neurons were tested with the same directions of motion used in the classical stimulation paradigm. The motion stimuli were typically presented for a period of 700 ms. In this case however, the testing stimuli were preceded by another moving random dot pattern which was presented for approximately one second. Either two or four different directions of motion, one per trial and randomly interleaved across trials, were used as adapting stimuli. Note that at the point of transition from adapting to testing phase a different seed was used to generate the testing random dot kinematogram even when the direction of motion remained unchanged. This ensured that under all conditions there was always the same brief (1 frame equal to 11.8 ms) transient at the point of stimulus change. Ten different seeds were used to generate random dot kinematograms per direction of motion presented. Blocks of the classical and adaptation stimulation paradigm were interleaved.

We used a standard algorithm for generating random dot patterns. Briefly, during each movie frame a random subset of the dots (60%), defined as the “signal dots,” were displayed shifted by a specific vector from their preceding location. This vector determined
the direction and, together with the frame rate, the speed of the stimulus. Each movie frame was presented for two monitor frames (85Hz monitor refresh rate). The typical stimulus speed was 4 degrees per second. The remaining dots, defined as noise (40%), were displaced randomly and provided an incoherent motion signal.

The animals were trained to maintain fixation in a +/- 0.5 degree window. In a few early experiments monkey D was required to maintain fixation in a +/- 1 degree window. At the end of each successful trial the animals were rewarded with a drop of apple juice.

**Data Analysis**

Neurons were defined as visually responsive if their firing rate changed significantly from baseline (-200 to 0 ms relative to stimulus onset) for the direction of motion which elicited the maximum response (computed 100 to 500 ms after stimulus onset). Statistical significance was assessed by a t-test and set at an $\alpha$ value of 0.05. Significant activation of neurons was assessed in a similar fashion during the testing phase of the adaptation paradigm.

The significance of each neuron’s direction-of-motion selectivity was calculated using the Rayleigh test $^4$ applied to the number of spikes fired in a 400 ms window (from 100 to 500ms after a stimulus transition) across different testing directions of motion. The Rayleigh test is a test for circular uniformity. We quantified direction-of-motion selectivity across the population of neurons recorded from V4 by computing the magnitude of the *normalized resultant vector* of the pre-adaptation and post-adaptation tuning function of each neuron. This vector was calculated as $^4$: $R = \sqrt{\frac{\sum_{d} r_d e^{i\theta_d}}{\sum_{d} r_d}}$, where $r_d$ is the number of spikes fired by the neuron during presentation of a random dot pattern moving in direction.
\( \theta_d \) and \( d \) is an index labeling the different directions of motion tested. The angles tested, \( \theta_d \), were equally spaced from 0 to \( 2\pi \) and uniformly distributed (i.e. same number of trials carried out for each direction of motion). The magnitude of the normalized resultant vector is equivalent to one minus the circular variance and provides an index of selectivity ranging from 0 to 1, where 1 denotes maximum selectivity. This magnitude was also used to perform the Rayleigh test \(^4\).

The time of microsaccade onset was determined as the time when the eye movement speed exceeded 4 degrees per second. This was found to reliably indicate microsaccades. The speed of the eye movements was filtered using a fourth order, low-pass (20 Hz), bidirectional, zero-phase Butterworth digital filter. Neurons were defined as significantly modulated by micro-saccadic eye movements if their firing rate changed significantly when the spikes were aligned to the onset of the micro-saccadic eye movements (comparing the firing rate in a bin from -100 to 0 ms with the firing rate in a bin from 50 to 150 ms relative to the onset of the micro-saccadic eye movements). Statistical significance was assessed by a t-test and set to an \( \alpha \) value of 0.05.

References: