In primary visual cortex (V1) of macaque monkeys, motion selective cells form three parallel pathways. Two sets of direction selective cells, one in layer 4B, and the other in layer 6, send parallel direct outputs to area MT in the dorsal cortical stream. We show that these two outputs carry different types of spatial information. Direction selective cells in layer 4B have smaller receptive fields than those in layer 6, and layer 4B cells are more selective for orientation. We present evidence for a third direction selective pathway that flows through V1 layers 4Cm (the middle tier of layer 4C) to layer 3. Cells in layer 3 are very selective for orientation, have the smallest receptive fields in V1, and send direct outputs to area V2. Layer 3 neurons are well suited to contribute to detection and recognition of small objects by the ventral cortical stream, as well as to sense subtle motions within objects, such as changes in facial expressions.

The direction selective cells in primate area V1 are considered to be the basic units of motion processing (Livingstone & Hubel, 1988). Consistent with the perceptual importance of motion, it is a feature that has been used to distinguish the role of a dorsal cortical stream that processes motion and spatial relationships, from a ventral stream more concerned with colour and form (Ungerleider & Pasternak, 2003). The dorsal and ventral streams have their beginnings in V1, where direction selectivity first emerges in the path from the primate retina through the cortex (Fig. 1). Direction selectivity appears first in layer 4Cα (Hawken et al. 1988; Snodderly & Gur, 1995; Gur et al. 2005), and it initiates motion selectivity in the dorsal cortical stream through its projections to layer 4B and layer 6, both of which have many direction selective cells (Livingstone & Hubel, 1984; Orban et al. 1986; Hawken et al. 1988; Snodderly & Gur, 1995; Gur et al. 2005). Layers 4B and 6 project monosynaptically to MT (Movshon & Newsome, 1996; Nassi & Callaway, 2005), where almost all cells are direction selective (Fig. 1, left column).

In contrast to the well-recognized populations of direction selective cells in V1 that project to MT (Movshon & Newsome, 1996), awareness of motion selectivity in the superficial layers, which project to V2 (Fig. 1, right column) has been slow to develop. This situation persists, even though we and others have reported examples of direction selectivity in layer 2/3 (Hubel & Wiesel, 1968; Poggio et al. 1977; Livingstone & Hubel, 1984; Leventhal et al. 1995; Snodderly & Gur, 1995; Gur et al. 2005). We were motivated to re-examine the prevalence of direction selectivity in layer 3 when we discovered direction selective cells (Gur et al. 2005) in layer 4Cm – a thin layer between 4Cα and 4Cβ that projects directly to layer 3B (Yoshioke et al. 1994; Yabuta & Callaway, 1998; Boyd et al. 2000; Sawatari & Callaway, 2000). Such a projection implies that direction selectivity should be common in layer 3, which would make the organization of primate V1 more comparable to V1 of carnivores, where direction selectivity is ubiquitous in the upper cortical layers (Weliky et al. 1996; Roerig & Kao, 1999; Ohki et al. 2005; Li et al. 2006).

We find that nearly half the layer 3 cells are direction selective. Furthermore, they differ from direction selective cells in layers 4B and 6 in having smaller receptive fields. Our results imply a massively parallel analysis of motion in primary visual cortex, with each output layer providing information preferentially tuned to a different part of the spatial domain. As part of this system, layer 3 provides major outputs from V1 to V2 (Rockland, 1992) which, in turn, sends extensive outputs to V4 in the ventral cortical stream (reviewed by Sincich & Horton, 2005). Consequently, the direction selective cells of layer 3 are ideally placed to contribute to motion processing in the ventral stream for object recognition and discrimination.
Methods

Physiological procedures

Data were collected from six adult female monkeys (2 *Macaca fascicularis* and 4 *Macaca mulatta*). Monkeys were trained to fixate on a light emitting diode (LED) for water reward. To initiate training the animals were water-deprived for 24–36 h. During training and experiments they were allowed to work until their thirst was satiated. Once the monkey learned the task, a head-holding post, a cylinder that formed the recording chamber and a scleral search coil were implanted under deep anaesthesia. Initial anaesthesia was induced by an intramuscular (i.m.) injection of ketamine (10 mg kg\(^{-1}\)). The first three animals were then anaesthetized with intravenous sodium pentobarbital in a dose sufficient to suppress the corneal reflex. The last three animals were intubated and then anaesthetized with isoflurane (1–2%). For antibiotic coverage, the monkeys were given (i.m.) 600 000 units of penicillin G benzathine or equivalent. To assess the level of anaesthesia, the heart rate, indirect blood pressure, temperature, response to noxious stimuli and corneal reflex were monitored. At the end of the surgical procedure, buprenorphine (0.01 mg kg\(^{-1}\), i.m.) was administered for analgesia and repeated twice per day for up to 3 days as needed. On the third postoperative day, the monkeys were given a follow-up dose of 600 000 units i.m. of penicillin G benzathine or equivalent as prophylaxis against infection.

When the recording chamber was first implanted, the bone was left intact. After recovery from surgery and completion of training, a small craniotomy (2–3 mm) was made and the dura was slit while the animals were lightly anaesthetized with ketamine/xylazine (7/1 mg kg\(^{-1}\), i.m.) and sitting in the restraining chair. Electrodes were inserted into the craniotomy on subsequent days. Approximately 18 craniotomies per animal were performed, utilizing both cortices.

At the end of the experiments, two animals were killed by an intravenous injection of sodium pentobarbital (140 mg kg\(^{-1}\)). For three animals, anatomical preservation of tissues was optimized by deeply anaesthetizing them by pentobarbital sufficient to suppress reflex responses to touching the cornea or pinching the foot, and then perfusing them through the heart with aldehyde fixatives, instead of receiving a second (lethal) dose of anaesthetic (Snodderly & Gur, 1995). The fixative was only introduced after the animal was already insensate. One animal is still participating in experiments.

All procedures complied with National Institutes of Health guidelines and were approved by the Animal Care and Use Committees of the Schepens Eye Research Institute and the Medical College of Georgia.

Nerve-spike and eye-movement recording

Fibre electrodes made from quartz-insulated platinum–tungsten alloy (Reitboeck, 1983) with bare tip lengths ≤ 5 μm and impedance at 1 kHz of 3–4 MΩ were most frequently used to record single-unit activity. In some experiments, glass-insulated platinum–iridium electrodes (Snodderly, 1973) with a tip diameter of 1–1.5 μm, and bare tip length of 5–7 μm, were used. In the initial experiments, position of the dominant eye was monitored by a double Purkinje image eye tracker (2–3 minarc resolution; 100 Hz sampling rate) and recorded in a computer file, together with spike arrival times (0.1 ms time resolution) and spike shapes collected at 10–25 kHz (Gur *et al.* 1999). In recent experiments,
eye position was recorded with an implanted scleral coil (1–2 minarc resolution; 200 Hz sampling rate; Robinson, 1963; Judge et al. 1980). The trial started when the monkey correctly pressed the lever in response to illumination of the fixation LED and continued for 5 s provided that the gaze remained within a predefined window, between ±0.5 deg and ±1.5 deg.

**Stimulus presentation**

In the early experiments, bar stimuli were displayed on a Barco 7351 monitor at a 60 Hz non-interlaced frame rate, with a Truevision AT.Vista video graphics adapter. Most data (233/267 cells, 5/6 monkeys) were gathered under these conditions. The remaining 34 cells were studied with stimuli displayed on a Sony 500 PS monitor at a 160 Hz non-interlaced frame rate with a Cambridge Research Systems VSG2/3F video board. To obtain robust, consistent responses, bars were optimized for orientation, length, velocity, and colour (green or red), 0.3–1 log units brighter or darker than the background of 1–5 cd m$^{-2}$. Incremental (bright) bars were presented on a neutral grey background; decremental (dark) bars were presented on a background of a single colour (Snodderly & Gur, 1995).

After the ocular dominance was established, stimuli were viewed binocularly, unless responses during monocular viewing were substantially stronger. The eye position signal was added to the stimulus position signal at the beginning of each video frame (Gur & Snodderly, 1987, 1997a,b; Snodderly & Gur, 1995; Snodderly et al. 2001; Kagan et al. 2002; Gur et al. 2005). This was done to compensate for changes in eye position during intersaccadic intervals. Note that the maximum delay between shifts in eye position and subsequent corrections could be as long as 28 ms for the 60 Hz frame rate, and 10 ms at the 160 Hz frame rate; thus this procedure was not intended to compensate for the fast saccadic eye movements. Saccades were automatically detected using a velocity threshold of 10 deg s$^{-1}$ (Snodderly et al. 2001) and epochs where saccades occurred <100 ms before stimulus onset were excluded during data analysis so all data were collected only during intersaccadic, drift periods. Note that changes in position during eye drifts are typically <10 min s$^{-1}$ so that even for the largest delay (28 ms) in eye position, compensation would have caused an insignificant position error (<0.3 min). This procedure enabled us to accurately map receptive fields (Snodderly et al. 2001; Kagan et al. 2002; Gur et al. 2005) and to produce consistent measures of neuronal responses in alert monkeys (Gur et al. 1997; Gur & Snodderly, 2006).

**Assignment to layers**

Localizing single cells in the chronic alert preparation is a challenge, because most marking methods are not sufficiently long-lasting. To overcome this challenge, we developed a unique methodology where information from several sources – dye marking, electrode depth, and transitions between regions of multiunit spontaneous activity are combined. It is important to note that most of our penetrations were nearly normal to the cortical surface and they usually were not more than 1.5–2 mm long. It is easier to recognize the transitions between layers in these short, perpendicular penetrations than in longer tangential ones. Also, the fact that the distances were short helped to minimize the effects of any distortions that occur during tissue processing.

**Dye marking.** In a series of penetrations, electrodes were coated with fluorescent dye to mark the maximum depth of the penetration, and the track was anatomically recovered (Snodderly & Gur, 1995). In the early experiments, we recovered the dye marks by doing biopsies (11/79 cells). Once we determined that the dye marks were long-lasting, we discontinued biopsies and processed the intact brains after killing the animals to recover the marks. We have found that dye marks can survive for at least a year. The sequences of activity along these penetrations were studied to show that multiunit spontaneous activity can be used as a marker for layers rich in cytochrome oxidase (CytOx) – 4A, 4C and 6. A sample penetration is illustrated in the Results in Fig. 2.

**Electrode depth.** To utilize the micromanipulator depth readings we made penetrations that were nearly orthogonal to the surface, and to minimize dimpling and tissue drag, electrodes were slim, and gradually tapered. The cortical surface was visualized the day after the craniotomy and dura dissection, by using an operating microscope with a magnification of 40× or more. The electrode was inserted under visual control, and it was usually possible to see when the electrode touched the cortical surface. On subsequent days, we removed the fast-growing soft scar tissue, and for another day or so we could usually see the cortex when the electrode touched it. On later days, when we could not see the cortex, electrode depth was estimated, but it was not considered a sufficient criterion to locate cells by itself (see below). After a few days, we sealed the craniotomy and opened one in a new location, where the fresh dura could be dissected to visualize the cortex. To prevent infections, strict aseptic precautions were observed during these procedures.

**Sequence of transitions.** In alert monkeys, changes in multiunit properties as the electrode advances are clear and often quite dramatic. We have shown (Snodderly & Gur, 1995) that multiunit spontaneous activity is high in the CytOx rich layers (4A, 4Cα, 4Cβ, 6) and very low in the CytOx poor layers (2/3, 4B, 5). Independent data from
Table 1. Physiological characteristics of direction selective cells (DI = 0.5) assigned to V1 layers with confidence level 1 vs. cells assigned with confidence levels 2 and 3

<table>
<thead>
<tr>
<th>Confidence levels</th>
<th>Direction selectivity (DI)</th>
<th>Receptive field width (minarc)</th>
<th>Orientation half-bandwidth (deg)</th>
<th>Ongoing activity (spikes s(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2 + 3</td>
<td>1</td>
<td>2 + 3</td>
</tr>
<tr>
<td>V1 Layer 3</td>
<td>0.77</td>
<td>1</td>
<td>9</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>(11)</td>
<td>(11)</td>
<td>(11)</td>
<td>(11)</td>
</tr>
<tr>
<td>4Cm</td>
<td>1</td>
<td>1</td>
<td>12.5</td>
<td>11</td>
</tr>
<tr>
<td>4B</td>
<td>0.93</td>
<td>0.99</td>
<td>12.5</td>
<td>15.5</td>
</tr>
<tr>
<td></td>
<td>(10)</td>
<td>(34)</td>
<td>(10)</td>
<td>(34)</td>
</tr>
<tr>
<td>4Ca</td>
<td>0.81</td>
<td>0.86</td>
<td>29.5</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>(12)</td>
<td>(7)</td>
<td>(12)</td>
<td>(7)</td>
</tr>
<tr>
<td>6</td>
<td>0.65</td>
<td>0.83</td>
<td>40</td>
<td>63</td>
</tr>
<tr>
<td></td>
<td>(4)</td>
<td>(11)</td>
<td>(4)</td>
<td>(11)</td>
</tr>
</tbody>
</table>

Values are medians; numbers of cells in each category are in parentheses. All values for the two confidence groupings are not significantly different (P > 0.05; by Mann–Whitney U test, two-tailed) except for ongoing activity of cells in 4Ca (marked with \(\ast\)). There were not enough sample points for comparisons of layer 3 and 4Ca HBW and for all layer 6 characteristics.

Multiple laboratories support this view (Poggio et al. 1977; Livingstone & Hubel, 1984; DeYoe et al. 1995).

Other physiological indicators that help to identify specific laminae are the lack of orientation selectivity and small spike amplitude of multiunit in layer 4C\(\beta\) vs. the larger spike amplitude in the abutting layer 5, and the large receptive field size of multiunit activity in layer 6. These physiological indicators cannot by themselves pinpoint a specific layer but they contribute to the confidence rating that we give to the layer assignments.

In alert animals, response properties are very stable (Gur et al. 1997; Gur & Snodderly, 2006), and these transitions in physiological properties are consistent features of vertical penetrations through the cortex. To be able to record from the variety of cells that are found in V1, we make our own electrodes so that impedance and tip geometry are optimal.

**Combined criteria.** Depending on the information available for each penetration, the recording location was assigned one of three levels of confidence. Cells recorded in penetrations for which we had dye marking in addition to depth and transition information were assigned confidence level 1. When the tip of the electrode was clearly visualized touching the cortical surface, the depth estimate for layer 2 and layer 3 cells was a secure measure and those cells were also assigned confidence level 1. Thus cells for which the cortical surface had been visualized, and/or for which we had marking data, in addition to other criteria, were given confidence level 1.

When dye marking was not available, but depth and physiological data could lead to only one interpretation, confidence level 2 was assigned. For example, if there was a sequence of multiple transitions in the middle and deeper portions of the cortex that included orientation-selective 4C\(\alpha\)-like activity for 200 \(\mu\)m then a lull of spontaneous activity for 70–100 \(\mu\)m, then high, non-oriented spontaneous activity typical of layer 4C\(\beta\), followed by 100–150 \(\mu\)m traverse of a silent layer (layer 5) and finally a spontaneously active layer (layer 6), we assigned confidence level 2. When fewer transitions were available, say, the above-described penetration stopped before exiting what looked like 4C\(\beta\), we assigned confidence level 3 to cells localized in that penetration. With this approach, many penetrations do not yield sufficient information to assign cells to layers. For example, in a penetration without a dye mark, if clear transitions in multiunit properties were not observed and if absolute depth was not known then cells recorded on that penetration were not assigned to layers, and were not included in the sample for this paper.

We pooled data from the three confidence levels because repeated analyses of many physiological features such as spontaneous activity, receptive field size, orientation selectivity, direction selectivity, and colour opponency have shown that grouping according to layers was very similar across the three confidence levels (cf. Snodderly & Gur, 1995; Gur & Snodderly, 1997b; Gur et al. 2005). The latter publication is particularly relevant since Figs 10, 11, and 12 demonstrate that the laminar profiles for ongoing activity, receptive field size, orientation selectivity, and direction selectivity are virtually identical for cells assigned different confidence levels. For the present sample, we confirm the robustness of the assignment protocol by showing that properties of cells assigned to layers with confidence level 1 are not different from properties of cells assigned with confidence levels 2 and 3 (See Tables 1 and 2). An important strength of our assignment scheme is that it correctly places recording sites with high spontaneous...
Table 2. Physiological characteristics of non-directional cells (DI < 0.5) assigned to V1 layers with confidence level 1 vs. cells assigned with confidence levels 2 and 3

<table>
<thead>
<tr>
<th>Confidence levels</th>
<th>Direction selectivity (DI)</th>
<th>Receptive field width (minarc)</th>
<th>Orientation half-bandwidth (deg)</th>
<th>Ongoing activity (spikes s(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>V1 layer 3</td>
<td>0.16 (24) 0.1 (7)</td>
<td>14 13</td>
<td>16.5 (15) 11 (4)</td>
<td>0 0</td>
</tr>
<tr>
<td>4B</td>
<td>0.15 (7) 0.2 (16)</td>
<td>15 14</td>
<td>21.5 (4) 13.5 (10)</td>
<td>0 0.1</td>
</tr>
<tr>
<td>4C</td>
<td>0.14 (13) 0.13 (50)</td>
<td>20 29</td>
<td>35 (11) 40.5 (28)</td>
<td>6.9 7</td>
</tr>
<tr>
<td>6</td>
<td>0.22 (6) 0.16 (16)</td>
<td>30 33</td>
<td>33.5 (4) 38.5 (10)</td>
<td>13.6 9.7</td>
</tr>
</tbody>
</table>

Values are medians; numbers of cells in each category are in parentheses. All values for the two confidence categories are not significantly different \((P > 0.05\); by Mann–Whitney \(U\)-test, two-tailed). There were not enough measurements of HBW in layers 3, 4B, and 6 for comparisons between confidence levels, and there were only 2 non-directional cells assigned to layer 4Cm, so it is omitted.

multiunit activity in anatomical locations with high cytochrome oxidase activity, as shown independently by three different laboratories (Livingstone & Hubel, 1984; DeYoe et al. 1995; Snodderly & Gur, 1995).

Receptive field widths

The width and location of receptive-field activating regions (ARs) were estimated with increment (Inc) and decrement (Dec) bars swept forward and back across the receptive field in a direction orthogonal to the optimal orientation axis. During each trial several responses were usually recorded. The bar width was adjusted to generate strong responses and was typically smaller than the AR width for both RF mapping and derivation of orientation tuning.

Using eye position compensation and exclusion of epochs affected by saccades, average peristimulus time histograms (PSTH) of responses were constructed, and a cumulative curve was superimposed (Snodderly et al. 2001). The cumulative curve was manually divided into three quasi-linear sections by selecting the two points where the cumulative curve most rapidly changed slope. The optimal least-squares regression lines were then automatically fitted to each of the three sections. The AR width was measured as 95% of the region defined by intersections of the three least-squares lines for the response to motion in the preferred direction. If the cell responded to only one sign of contrast (i.e. only a light bar or a dark bar, but not both), the receptive field was defined as the width of the single AR. If the cell responded to both light and dark bars, the receptive field was defined as the outer limits of all ARs. These definitions are equivalent to the 'classical receptive field' of other authors (Kagan et al. 2002).

Orientation tuning

The orientation tuning of the cells was characterized by their orientation band-width (half-band widths at \(1/\sqrt{2}\) height). This measure was used because \(1/\sqrt{2}\) height is 1/2 power or \(-3\text{db}\) from the peak. Most of our orientation data were collected with drifting bars that changed in orientation in 10–20 deg angular steps. For some very narrowly tuned cells we used finer steps around the preferred orientation. Tuning curves were based on number of spikes generated by each sweep minus the ongoing activity. Several responses were averaged for each datum. Gur et al. 2005) for further details.

Ongoing activity

Ongoing activity was measured during trials with a blank grey screen, uniformly illuminated at 1–5 cd m\(^{-2}\).

Direction selectivity. A direction index (DI) was computed as 1 minus the ratio of the response in the non-preferred direction to the response in the preferred direction. We classified cells as direction selective when their DI was \(\geq 0.5\) (Snodderly & Gur, 1995 and references therein). For each cell studied, at least 10 responses in each direction contributed to the calculation of the DI.

Statistical analyses

Pairwise comparisons utilized the Mann–Whitney \(U\) test or the Wilcoxon matched-pairs signed-rank test. Values reported for individual parameters are medians. Proportions were compared using the chi-square test.
Results

Direction selectivity was measured for 267 V1 cells that could be assigned to individual layers. The number of cells per monkey was: 30, 47, 70, 9, 77, 34. Figure 2 shows results from a marked penetration that sampled a wide variety of cell types. The penetration was reconstructed (Snodderly & Gur, 1995) using a dye mark (Fig. 2, left panel) to establish the location and the maximum depth of the penetration relative to the cortical layers after viewing all sections containing the dye (Fig. 2, middle panel). Multunit ongoing activity and individual cells were placed along the penetration using the microdrive readings relative to the bottom of the penetration (Fig. 2, right panel). In the layers with multunit spontaneous activity, 4A, 4Co and 4Cβ (cells 2, 4 and 6, respectively) the individual cells were spontaneously active, classical receptive fields (CRFs) were fairly large, and orientation tuning was broad (4Co) or absent (4A, 4Cβ). In the silent layers (3, 4B, 4Cm) the cells were spatially selective; CRFs

Figure 2. Illustration of the sequence of events in a penetration (2776) in macaque V1 labelled with DiI (see Snodderly & Gur, 1995, for detailed methods)

A, left, a fluorescence photograph of the electrode track at its deepest point. Part of the tapered portion of the electrode was coated with DiI (Molecular Probes) up to 40 μm from the tip, and the tip remained at the deepest point of the penetration for 20 min. The black dashed line is the border of the fluorescent mark derived by thresholding the intensity values of the image. The fluorescent mark is widest where the amount of dye crystallized on the electrode was greatest, and the bottom of the mark is a reliable indicator of the maximum depth of the penetration (after correcting for the distance from the end of the dye coating to the tip of the electrode; Snodderly & Gur, 1995). We have previously shown that the penetration depth indicated by the dye is consistent with values derived from electrolytic lesions made on the same penetration (Snodderly & Gur, 1995). B, middle, white arrows point to vessels that served as histological landmarks allowing registration of the dye mark with the same section stained at a later date for cytochrome oxidase. C, right, physiological results obtained on this penetration, with the borders of the middle layers of the cortex indicated. Multunit ongoing activity (grey bars) was recorded in layers 4A, 4Co, and 4Cβ. Cells selective for orientation are indicated by black rectangles whose widths are proportional to the widths of the classical receptive fields (CRFs) and whose lengths are proportional to the length of the stimulus bar that elicited a strong response from the cell. An arrow perpendicular to the bar indicates direction selectivity. Non-oriented cells are indicated by black circles with diameters proportional to CRF widths. Quantitative characteristics of the individual cells were as follows. Cell 1 (layer 3): CRF width 10 min; stimulating bar length 23 min; direction index (Di) 0.9; ongoing activity 1 s⁻¹. Cell 2 (layer 4A; colour opponent, R⁺B⁻): CRF width 30 min; bar length 125 min; Di 0.05; ongoing activity 43 s⁻¹. Cell 3 (layer 4B): CRF width 15 min; bar length 100 min; Di 0.05; ongoing activity 0.8 s⁻¹; Half-bandwidth (HBW) 11.5 deg. Cell 4 (assigned to layer 4Co based on multunit spontaneous activity): CRF width 22 min; bar length 92 min; Di 0.14; ongoing activity 5.5 s⁻¹; HBW 27 deg. Cell 5 (layer 4Cm): CRF width 12 min; bar length 100 min; Di 1; ongoing activity, 2 s⁻¹; HBW, 17 deg. Cell 6 (layer 4Cβ): CRF width, 42 min; bar length 100 min; ongoing activity 45 s⁻¹.
were small and orientation tuning was narrow. Note that the layer 3 and layer 4Cm cells were strongly directional (DI = 0.9, and 1, respectively). In assigning cells to layers, we used a system of three levels of confidence. For a marked penetration like this one, we assigned confidence level 1 to all cells in the penetration. In unmarked penetrations, we also assigned confidence level 1 to cells in layer 3 if, and only if, the tip of the electrode was clearly visualized touching the cortical surface at the beginning of the penetration so that absolute depth in the cortex was securely known. Half (11/22) of the direction selective cells in layer 3 were assigned confidence level 1. We had dye marking data for 3 of the 11 cells, and absolute depth for 8 (for 2 cells we had both depth and dye data). For the other 11 cells assigned to layer 3 for penetrations where we did not have absolute depth or dye marking, 9 were given confidence level 2, and 2 cells were assigned confidence level 3 based on combinations of physiological and depth information (see Methods). As shown in Tables 1 and 2, properties of cells – both direction selective and non-selective – assigned to layers with confidence level 1 were not significantly different from cells assigned confidence levels 2 and 3. The consistency of the results justified pooling data from cells assigned to individual layers with different levels of confidence, as we have done in subsequent figures (see also Gur & Snodderly, 2005). In Fig. 3 we show the direction index for 29 layer 3 cells as a function of absolute cortical depth. If direction selective cells in layer 4B were being mistakenly assigned to layer 3, one might expect direction selective cells to cluster at the bottom of layer 3, but this did not occur.

Figure 4 compares records from representative direction selective cells in the newly recognized motion selective pathway flowing from 4Cα through layers 4Cm and 3. The layer 4Cα and layer 3 cells (Fig. 4A and B) were not completely directional and fired occasionally when the bar moved in the non-preferred direction. The layer 4Cα cell, unlike the cells in the other two layers, was spontaneously active, and a few spikes unrelated to the stimulus timing can be seen as well as a burst (around 300 ms into the trial; Fig. 4B) generated by a saccade. The 4Cm cell (Fig. 4C) was completely directional; no spikes were generated when the bar moved in the null direction.

**Degree of direction selectivity in V1 layers**

The distributions of the direction index for the main layers of V1 containing direction selective cells are illustrated in Fig. 5.

**Layer 4Cα.** Direction selectivity in the primate visual system is first found in layer 4Cα, which is the principal recipient of magnocellular inputs from the lateral geniculate nucleus (LGN). Unlike the LGN afferents, nearly one-quarter of the cells in this layer (23%) were selective for direction of motion. The degree of direction selectivity was nevertheless moderate, with only 21% of the selective cells failing to give some response to the non-preferred direction of movement (DI < 1).

**Layers with direct outputs to MT: 4B and 6.** Layer 4B receives its major input from Layer 4Cα, and it transforms this input to attain greater selectivity. In 4B, most cells (66%) were directional and 59% of those were completely unidirectional or nearly so (DI > 0.9). Layer 6 also receives an input from 4Cα and 39% of the cells were direction selective, with 40% of the directional cells having DI > 0.9. These new data, from a large sample of V1 neurons recorded in alert monkeys, are consistent with earlier, smaller samples (Hawken et al. 1988; Snodderly & Gur, 1995; Gur et al. 2005) showing strong direction selectivity in layer 4B and somewhat weaker selectivity in layer 6. Both of these layers feed area MT and the dorsal cortical stream. There are also less prominent projections from layers 4B and 6 to area V2 (Fig. 1), which could play a role in motion processing in the ventral stream as well.

**Layers in a parallel pathway projecting heavily to V2: 4Cm and 3.** Layer 4Cm was defined by anatomical data (Yoshioka et al. 1994; Yabuta & Callaway, 1998; Boyd et al. 2007; Yabuta, 2007). Directional selectivity in 4Cm is strong, and many layers with direct outputs to MT: 4B and 6. Layer 4B receives its major input from Layer 4Cα, and it transforms this input to attain greater selectivity. In 4B, most cells (66%) were directional and 59% of those were completely unidirectional or nearly so (DI > 0.9). Layer 6 also receives an input from 4Cα and 39% of the cells were direction selective, with 40% of the directional cells having DI > 0.9. These new data, from a large sample of V1 neurons recorded in alert monkeys, are consistent with earlier, smaller samples (Hawken et al. 1988; Snodderly & Gur, 1995; Gur et al. 2005) showing strong direction selectivity in layer 4B and somewhat weaker selectivity in layer 6. Both of these layers feed area MT and the dorsal cortical stream. There are also less prominent projections from layers 4B and 6 to area V2 (Fig. 1), which could play a role in motion processing in the ventral stream as well.

**Figure 3. Direction selectivity index (DI) vs. absolute cortical depth for 29 layer 3 cells recorded in penetrations where the cortical surface was visualized.**

There was a 15 μm gap (371–386 μm; 2 dashed lines) within which no cells were recorded so we found it convenient to use the 386 μm depth as the upper boundary of layer 3. This depth is consistent with anatomical data (cf. Poggio et al. 1977; Fitzpatrick et al. 1985; Lund & Wu, 1997). Cortical depth of 700 μm was used as layer 3 lower boundary based on the average depth of the layer 4A as determined by anatomical and physiological criteria (Snodderly & Gur, 1995).
2000; Sawatari & Callaway, 2000) as a thin layer lodged between the LGN-recipient layers 4Cα and 4Cβ. Dendrites of cells in this layer are in a position to receive a variety of inputs including parvocellular and magnocellular LGN afferents, as well as inputs from V1 layers 4Cα and 4Cβ (Yabuta & Callaway, 1998). We assigned cells to this layer using the sequence of spontaneously active layers and other multiunit properties (see Methods) encountered in the penetration, in combination with the electrode depth. This sequence of multiunit activity was determined independently of the properties of the individually recorded cells. For 11 of 26 cells in 4Cm we also had anatomical dye marking. The relative depth within 4C of all cells assigned to subdivision 4Cm is shown in Fig. 6. The cells assigned to 4Cm were physiologically distinct from cells in 4Cα and 4Cβ, which is consistent with considering it a separate layer, as implied by the anatomy. While only about a quarter of 4Cα cells were direction selective, in 4Cm almost all cells (24/26, 92%) were direction selective and most direction selective cells (79%) were nearly unidirectional (DI > 0.9). In fact, 4Cm may be the most direction selective layer in the primary visual cortex.

Layer 4Cm outputs to layer 3B, which projects in turn to layer 3A (Yoshioka et al. 1994). Cells in layer 3 receive a complex mixture of inputs from other cortical layers that include strong influences from both the magnocellular and parvocellular pathways of the LGN (Sawatari & Callaway, 2000). Here we present data from 53 layer 3 cells. In our physiological recordings we assigned cells to layer 3 that

![Experimental records and orientation tuning curves from three direction selective cells in V1 layers 3, 4Cα and 4Cm](image)

**Figure 4.** Experimental records and orientation tuning curves from three direction selective cells in V1 layers 3, 4Cα and 4Cm

Complete action potential data from one behavioural fixation trial for each cell are shown. Graphs depict occurrence times for each nerve impulse (short vertical lines). The arrow-pairs on the left hand side indicate direction of motion. The sweeping bar icons in A, B, and C are scaled in proportion to the actual size of the stimulus. Orientation tuning curves depict average number of spikes/sweep at various orientations. A, an orientation selective, silent cell (2477A002, trial 8; 1.3 deg eccentricity) in layer 3 with a receptive field activating region 7 min wide stimulated by a 4 × 29 min, red bar tilted at 150 deg sweeping forward and back at 3.3 deg s⁻¹ across the receptive field orthogonal to its long axis. The stimulus reversed direction every 500 ms. Thick lines on the horizontal axis denote sweep duration in one direction. B, an orientation selective, spontaneously active (6.5 spikes s⁻¹) cell (2980A002, trial 15; 3.1 deg eccentricity) in layer 4Cα with a receptive field activating region 12 min wide stimulated by a 9 × 125 min, red bar tilted at 45 deg sweeping forward and back at 2.9 deg s⁻¹ across the receptive field orthogonal to its long axis. The stimulus reversed direction every 700 ms. C, an orientation selective, silent cell (1594A007, trial 9; 1.3 deg eccentricity) in layer 4Cm with a receptive field activating region 6 min wide stimulated by a 4 × 76 min, red bar tilted at 60 deg sweeping forward and back at 2.7 deg s⁻¹ across the receptive field orthogonal to its long axis. The stimulus reversed direction every 500 ms.
Figure 5. Distribution of the direction index, DI, for 267 cells in the main layers of V1 containing direction selective cells
Receptive fields were at eccentricities of 4.1 ± 1.9 deg. DI = 1 minus (response to movement in the non-preferred direction divided by response in the preferred direction). Values are percentages within each layer; the number of cells recorded in each layer is indicated separately.

were located from 385 to 695 μm (Fig. 3) below the cortical surface. For 11 of 55 cells we had anatomical dye marking in addition to the other criteria we used to assign cells to layer 3. We did not attempt to subdivide the layer into 3A and 3B. Nearly half the cells assigned to layer 3 were direction selective (42%; Fig. 5). We show below that direction selective cells of layer 3 are a special group that are more selective spatially than the direction selective cells in other layers.

Characteristics of direction selective cells in individual layers of V1
Receptive field size: mostly large in layer 6, but small in layers 4B and 3. We use the term ‘receptive field’ to indicate the region of the visual field within which localized bright or dark stimuli cause the cell to fire. This region is also called the ‘classical receptive field’. In layer 6 there was a population of direction selective cells with large receptive fields that had no counterparts in the other layers (Fig. 7, left panels; Table 3). These large receptive fields are known to contribute to the properties of MT neurons (Movshon & Newsome, 1996; Fig. 1) and may be useful in sensing optic flow associated with movement of the organism through the environment. However the median receptive field width of layer 6 cells was still less than 15% the average width reported for MT receptive fields at this eccentricity (Orban, 1997).

MT neurons also receive monosynaptic inputs from smaller V1 receptive fields in layer 4B. The convergence of large receptive fields from layer 6 and small receptive fields from layer 4B may facilitate extraction of information about relative motion in the dorsal stream.

Both layer 4Cm and layer 3 cells had small receptive fields, but receptive fields of direction selective cells in layer 3 were narrower than those of direction selective cells in 4B (P < 0.01). Thus layer 3 is distinctive in sending especially...
Table 3. Characteristics of direction selective (Dir, DI ≥ 0.5) and nondirectional (Non-dir, DI < 0.5) neurons in V1 layers

<table>
<thead>
<tr>
<th></th>
<th>Direction selectivity (DI)</th>
<th>Receptive field width (minarc)</th>
<th>Orientation half-bandwidth (deg)</th>
<th>Ongoing activity (spikes s⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dir</td>
<td>Non-dir</td>
<td>Dir</td>
<td>Non-dir</td>
</tr>
<tr>
<td>V1 layer 3</td>
<td>0.78</td>
<td>0.14</td>
<td>9</td>
<td>13</td>
</tr>
<tr>
<td>4B</td>
<td>0.97</td>
<td>0.17</td>
<td>15</td>
<td>16</td>
</tr>
<tr>
<td>4Cα</td>
<td>0.82</td>
<td>0.16</td>
<td>29</td>
<td>30</td>
</tr>
<tr>
<td>6</td>
<td>0.83</td>
<td>0.16</td>
<td>60*</td>
<td>34*</td>
</tr>
</tbody>
</table>

Values are medians. *Values for directional and non directional neurons in the indicated layers are significantly different (*P < 0.05; by Mann–Whitney U test, one-tailed because the null hypothesis was based on previous data; Gur et al. 2005). For differences between layers, comparisons between layers 4B, 4Cα, and 6 were one-tailed, based on previous data, and comparisons between layer 3 and other layers were two-tailed because there were no previous data on which to base the null hypothesis. No corrections were made for multiple comparisons. For receptive field width of non-dir cells (column 5), layer 6 is not different from 4Cα. Both 6 and 4Cα receptive fields are larger than those in 4B and 3 (P < 0.002). Layer 3 receptive fields are smaller than those in 4B (P < 0.01). For receptive field width of dir cells (column 4), layer 6 receptive fields are larger than all other layers (P < 0.01–0.001) and 4Cα receptive fields are larger than those in 4B, 4Cm, and 3 (P < 0.001). Layer 3 receptive fields are smaller than those in 4B (P < 0.01). For orientation selectivity of both dir and non-dir cells (columns 6 and 7), those in layers 3, 4Cm, and 4B had narrower orientation bandwidths than cells in 4Cα and 6 (P < 0.001).

Figure 7. Distributions of physiological properties of the same cells illustrated in Fig. 5

Black squares represent cells with DI = 0.5. Grey diamonds represent cells with DI < 0.5. Numbers of cells in each category are indicated under the layer numbers as (n = cells with DI = 0.5/ cells with DI < 0.5). Black and grey triangles underneath the horizontal axes indicate the respective median values of each distribution. Note that the median symbols are located at their exact numerical values rather than the centres of the bins. Left column, distribution of receptive field widths in individual layers. Middle column, distribution of orientation selectivity (half-band widths at 1/√2 maximum height). Right column, distribution of ongoing activity (activity of cell during presentation of a blank screen). Other conventions same as Fig. 5.
fine-grained information about motion to more central areas. The tiny (median = 9 min) receptive field sizes that we have measured are doubtless overestimates because of residual errors in compensation for eye movements and placement of stimuli (Tang et al. 2007). Thus layer 3 is probably even more distinctive than our results imply.

**Orientation selectivity: enhanced in the output layers.**
There was a trend for direction selective cells to have smaller orientation bandwidths than non-directional cells (Fig. 7, middle panels). The greater selectivity of direction selective cells compared to non-directional ones was particularly evident and statistically significant in layer 4Cα (Table 3). The small difference in orientation bandwidths between cells in 4Cα and those in layer 6 was not significant with this sample size. However, the differences in orientation selectivity between both directional and non-directional cells in layers 4Cα and 6, and their counterparts in layers 3, 4B and 4Cm were highly significant (Tables 3, P < 0.001). Selectivity for orientation in both directional and non-directional cells increased as information flowed from the input layers to the output layers of V1.

**Ongoing activity: higher in non-directional cells.** In layers 4B and 3, while ongoing activity for both direction selective and non-directional cells was very low (Fig. 7, right panels) it was even lower for direction selective cells; more direction selective cells were completely silent (layer 3–95%; layer 4B – 81%) than non-directional ones (layer 3–74%; layer 4B – 65%). A similar pattern occurred in the two spontaneously active layers, 4Cα and 6 – cells with high ongoing activity were found almost exclusively among non-directional cells (Fig. 7), with lower ongoing activity in direction selective cells.

**Length selectivity in V1 direction selective cells**

To select optimal stimuli for measuring orientation tuning and receptive field width we searched for the most robust response while varying stimulus size, orientation, colour, velocity, etc. When possible, we used a long bar because it made responses less vulnerable to residual position errors introduced by eye movements and because long bars generated more precise orientation tuning curves. Thus when a long bar (> 60 min) was used it indicates that the cell gave robust responses and was not selective for short bars. This does not rule out the possibility that some cells would have shown end-inhibition if we had used very long bars. Conversely, when a short bar (< 60 min) was used, the cell was usually end-inhibited and selective for short bars. In Fig. 8 we show the number of directional (DI = 0.5) cells for which we used a bar longer or shorter than 60 min to stimulate cells in each V1 layer.

**Layers 4Cα, 6 and 4B – pathway with direct outputs to MT.** Long bars were quite effective stimuli for layer 4Cα and layer 6 cells. For layer 6, only long bars (> 66 min) were used, and for layer 4Cα, bars > 70 min were used for all but 3 cells (Fig. 8). For layer 4B, 25% of the cells required a stimulus shorter than 60 min, indicating a mixture of cells selective for different lengths of stimuli.

**Layers 4Cm and 3 – pathway projecting heavily to V2.** The clearest difference in response properties between layer 4Cm cells and the layer 3 cells that are their targets was the selectivity for short stimuli in layer 3. In 4Cm, only 27% of the cells required a short bar (length < 60 min) while in layer 3, 45.5% required short stimuli. Direction selective and non-directional cells in layer 3 were about equally selective for stimulus length (not shown). The selectivity for shorter stimuli in layer 3 compared to layer 4B (P < 0.01 by chi-square, one tail), along with the
smaller receptive field width suggests a greater emphasis on fine-scale motion and feature analysis in layer 3. The strong end-stopping in the layer 3 cells may also help to increase the efficiency of neural coding by limiting redundancy in cortical responses evoked by natural images (Zetzsche et al. 1993).

**Discussion**

In this paper we establish that direction selectivity is common in layer 3, not just an occasional occurrence. While some earlier studies identified direction selective cells in V1 upper layers (Hubel & Wiesel, 1968; Poggio et al. 1977; Livingstone & Hubel, 1984; Leventhal et al. 1995; Snodderly & Gur, 1995), this fact has largely been ignored because direction selectivity has been considered to be primarily a property of other layers, specifically ones ignored because direction selectivity has been considered to be primarily a property of other layers.

In a previous publication (Gur & Snodderly, 2005) we presented preliminary data on 12 mid-layer 4C (4Cm) cells for which we had orientation tuning curves. Here we presented data for a larger sample of cells \( n = 26 \); many of which were localized relative to dye marks that were consistent with assigning the cells to 4Cm (Figs 2 and 6). Layer 4Cm cells are quite different from cells in 4Cα and 4Cβ, the two adjacent layers. 4Cm cells are silent in the absence of deliberate stimulation, generate larger spikes, and are much more spatially selective. We interpret these characteristics as the physiological counterpart of the anatomically established 4Cm (Yoshioka et al. 1994; Yabuta & Callaway, 1998; Boyd et al. 2000; Sawatari & Callaway, 2000).

We show here, for the first time, the distribution of direction selectivity index, DI, for all V1 layers containing direction selective cells (4Ca, 4B, 6, 4Cm and 3). We also show, for the first time, physiological characteristics (spontaneous activity, classical receptive field width, orientation selectivity, length selectivity) of direction selective cells in all these layers. Recognition of the physiological differences between direction selective cells in different layers is what enables us to follow functional transformations of neuronal properties from input (4Cα), through intermediate (4Cm) to output (4B, 6, 3) layers, and to suggest possible contributions of direction selective cells to more than one aspect of perception.

Taken together, the new findings presented in this paper are evidence for three parallel pathways in V1 with different types of motion-selective cells. Two sets of direction selective cells, one in layer 4B and the other in layer 6, send parallel direct outputs to area MT in the dorsal cortical stream. The cells in these layers differ in their orientation tuning and receptive field sizes. The unexpectedly high prevalence of direction selective cells in layers 4Cm and 3 forms a third direction-selective pathway through V1 with direct outputs to area V2. Cells in layer 3, the output layer of this pathway, are very orientation selective, and have the smallest receptive fields in the primary visual cortex. Because layer 3 has extensive parvocellular inputs (Sawatari & Callaway, 2000; Sincich & Horton, 2005), there is presumably opportunity for motion selectivity to be based on more than the magnocellular system, even at the level of V1.

**Assigning cells to layers 3 and 4Cm**

Our findings of direction selective cells in layer 3 and a distinct population of direction selective cells in layer 4Cm are critically dependent on the correct anatomical assignment of our physiological recordings. Here we consider the likelihood of mistakenly assigning direction selective cells that actually reside in other layers.

**Layer 3.** The nearest layer containing large numbers of direction selective cells that could have been wrongly assigned to layer 3 is 4B, which is located at least 800–850 \( \mu \)m from the brain surface (cf. Hawken et al. 1988; Snodderly & Gur, 1995). However, all direction selective cells assigned to layer 3 for which we had absolute depth measurements \( n = 11 \), confidence level 1) were recorded well away from layer 4B (recall Fig. 3). The remaining layer 3 direction selective cells \( n = 11 \), confidence levels 2 + 3) were assigned after identifying the location of layers 2, 3, 4B, 4A and 4C by multiunit activity and relative electrode depth readings (see Methods). The fact that the physiological properties of cells with confidence level 1 were identical with the rest of the cells assigned to layer 3 (Table 1) is consistent with a correct assignment.

**Layer 4Cm.** If we were seriously in error, we might assign layer 4B or layer 6 direction selective cells to 4C. However, for 11/26 cells assigned to 4Cm we had anatomical dye marks that enabled us to securely locate these cells in layer 4C (Fig. 6). Second, all cells were localized within 4C only if we could identify the upper and lower boundaries of layer 4C. As noted above (see Methods) the unique multiunit characteristics of these layers are fairly easy to recognize (cf. Livingstone & Hubel, 1984). We stress that we always require a sequence of transitions between silent and active layers and a correct relative electrode distance before concluding that layer 4C was encountered.

The most serious objection to assigning cells to a sublayer within 4C is that the cells could actually be in layer 4Cα. The reason we distinguish layer 4Cm cells from layer 4Cα cells is not based solely on anatomical marking since no known technique that can be applied to alert monkeys has such refined anatomical resolution. An important criterion is that the 4Cm cells lie between the bands of spontaneous activity that presumably represent...
the parvo- and magno-cellular LGN inputs. When these criteria are satisfied, we find that the cells assigned to 4Cm have a host of physiological properties that are not found in 4α cells (large spikes, lack of spontaneous activity, small receptive fields, strong orientation- and very strong direction-selectivity). Finally, the location of the 4Cm cells is compatible with the anatomically defined layer 4Cm (Fig. 6).

Characteristics of neurons in the direction selective pathway directed to area v2

Nowhere in the primary visual cortex does one find a more dramatic transformation of physiological properties than that between layers 4α and 4β and their immediately adjacent layer, 4Cm. Cells in 4α and 4β are very spontaneously active, with a range of receptive field sizes and spike size around 0.15–0.6 mV. 4Cβ cells have small spikes (~0.15–0.3 mV) and are not orientation selective while 4α cells have larger spikes (~0.2–0.6 mV), are moderately orientation selective, and some are direction selective (Hawken et al. 1988; Snodderly & Gur, 1995; Gur et al. 2005). In contrast, layer 4Cm cells have larger spikes (0.5–2.2 mV), are silent, have small receptive fields, are very orientation selective and most are unidirectional. If the classic transformation between the non-oriented, non-directional LGN input and the moderate orientation and direction selectivity in 4α is impressive, the transformation to the refined selectivity of 4Cm cells is equally dramatic. We assume that this transformation is achieved in part by strong intracortical inhibition that competes with the spontaneous excitatory input and shapes the spatiotemporal receptive fields of the cells.

The next processing stages are in layer 3, which receives inputs from the 4Cm cells. At a depth compatible with layer 3 we recorded large spikes (median 1.2 mV) that we assumed were from large pyramidal cells (Gur et al. 1999). These cells were silent and spatially selective, with small receptive fields and high orientation selectivity. Many cells in this layer were also direction selective and end-stopped. These results are consistent with earlier measurements of spatial properties of layer 3 neurons in anaesthetized macaques by Sceniak et al. (2001). These authors fitted a difference-of-Gaussians model to spatial summation data and found that the excitatory space constant, which should be the major determinant of size selectivity, was smallest in layer 3.

Finding the optimal stimulus for cells with the combination of end-stopping, small receptive fields, and high selectivity for orientation and direction of movement (Table 3) represents a challenge for the experimenter. When studying alert monkeys, the challenge is intensified by the fixational eye movements that move the receptive fields in unpredictable trajectories during the recordings. The misleading discharges introduced by these movements can be confusing — especially when first encountering a cell — and the variability introduces a strong bias against acquiring sufficient data to include these selective cells in the final sample. We have found that on-line compensation for fixational eye movements (Snodderly & Gur, 1995) reduces sampling bias because it reduces the time necessary to determine the receptive field location and the orientation preference of the cell. Therefore the measurement protocol can be completed in the limited time available for study of each cell.

A belated recognition of direction selectivity in the upper layers of macaque V1

Although direction selectivity is widely recognized to be common in the upper layers of carnivore V1 (Weliky et al. 1995; Roerig & Kao, 1999; Ohki et al. 2005; Li et al. 2006) it is often described as being restricted to the deeper layers 4B, 4α and 6 in primate V1 (Dow, 1974; Livingstone & Hubel, 1984; Orban et al. 1986; Hawken et al. 1988; Shipp & Zeki, 1989; Movshon & Newsome, 1996). We believe that the sampling bias resulting from the multidimensional selectivity of layer 3 cells is one reason why direction selectivity has not been recognized as a common attribute of primate layer 3. Other authors (e.g. Jones et al. 2001; Sceniak et al. 2001) have noted difficulty stimulating cells in layers 2 and 3, perhaps because their stimuli were not small enough. Similarly, Hawken et al. (1988) were unable to include end-stopped cells in their study, and they did not find direction selectivity in the upper layers of macaque V1. Nevertheless, modest numbers of direction selective cells in the upper cortical layers have been found in multiple studies (Hubel & Wiesel, 1968; Poggio et al. 1977; Livingstone & Hubel, 1984; Leventhal et al. 1995; Sato et al. 1995; Roe et al. 2005), which did not distinguish layer 2 from layer 3 as we have done. Because layer 2 cells are not direction selective (Gur & Snodderly, 2005), this practice tends to obscure the high prevalence of direction selectivity that is revealed when layer 3 is analysed separately.

A limitation of our study, and of most single-unit studies, is a lack of information about the tangential organization of the cortex. Clearly there is a range of direction selectivity in layer 3 of macaques (Fig. 5), and future research may find a systematic organization in orderly arrays similar to those described in V1 of ferrets (Weliky et al. 1996) and in area 18 of cats (Ohki et al. 2005).

Potential contributions of v1 layer 3 neurons to perception

The small receptive field size, strong end-stopping, and direction selectivity of layer 3 cells should enable them...
to contribute to several visual functions important for perception. One such function is to resolve the ambiguity between orientation and direction of motion of an elongated contour known as the 'aperture problem'. Direction selective cells that are not end-stopped respond to contours that extend beyond their receptive fields, and they can only measure the component of motion that is perpendicular to the contour. However, V1 end-stopped cells respond preferentially to the endpoints of contours and they can accurately signal the direction of motion (Pack et al. 2003). It has been suggested that this capability could contribute to the ability of MT neurons in the dorsal stream to solve the aperture problem (Pack & Born, 2001).

With regard to V2, which has strong outputs to V4 and the ventral stream (Yukie & Iwai, 1985; Sincich & Horton, 2005), we expect that the same computational analysis of motion needs to take place, and layer 3 cells are obvious candidates to contribute to solving the aperture problem on a very fine scale. For example, face perception is considered to be one of the more sophisticated operations of the object recognition system of the ventral stream (e.g. Desimone et al. 1984; Perret et al. 1985; Kanwisher et al. 1997). Although most experiments on this subject employ static pictures or drawings of faces, it has been shown that recognition of subtle differences in facial expressions is much improved by small motions of the facial features (Ambadar et al. 2005). Very refined spatial information is clearly utilized because we are able to discriminate between faces that are only ∼10 min wide (M. Gur, unpublished results). Furthermore, some ventral stream neurons in area IT are selective for such subtle differences between faces as the direction of gaze (Perret et al. 1985). To support these sophisticated functions of the ventral stream, layer 3 can provide the needed fine-scale information to discriminate motions of small elements within objects, such as facial features.

In addition to analysis of visual motion, end-stopped layer 3 cells are well suited to contribute to segregation of objects by occlusion cues (Peterhans & von der Heydt, 1991; Heider et al. 2000) and detection of curved boundaries (Dobbins et al. 1987), which are important elements of form perception (for references see Pasupathy & Connor, 1999). In fact, macaque V4 cells, which receive both monosynaptic and disynaptic inputs from V1 layer 3 (Yukie & Iwai, 1985) are reported to respond better to curved boundaries than to straight ones (Pasupathy & Connor, 1999).

Properties of the dorsal and ventral cortical processing streams inherited from V1

It is well accepted that many characteristics of motion selectivity in area MT of the dorsal cortical stream are inherited from its V1 inputs. These include the preferred direction of motion, speed tuning, and the minimum distance of travel over which motion can be sensed (Born & Bradley, 2005). Here we show that layer 4B and layer 6 differ strongly in their inputs to MT; layer 4B direction selective cells have small, strongly orientated receptive fields and are not spontaneously active while layer 6 cells have very large receptive fields, are not as sharply tuned for orientation, and are spontaneously active. Future research should show the contribution of these two input types to MT cells’ organization and functionality.

We suggest that a parallel assimilation of direction selectivity and end-stopping of V1 layer 3 neurons occurs in V2. Using the usual criterion for direction selectivity (DI = 0.5), 21–39% of cells in the different compartments of V2 are reported to be direction selective (Peterhans & von der Heydt, 1992; Gegenfurtner et al. 1996), and around one-quarter of V2 cells are end-stopped or inhibited by large stimuli (Hubel & Livingstone, 1987; Peterhans & von der Heydt, 1992; Gegenfurtner et al. 1996).

A question of great interest is how the layer 3 outputs from V1 might contribute to the ventral cortical stream. The ventral stream flows from V1 layer 3 through the pale and thin stripes of V2 to area V4 to reach the inferotemporal cortex (Ungerleider & Pasternak, 2003). Thus, if the properties of V1 layer 3 and V2 pale and thin stripes appear in V4, it is reasonable to propose that they probably were inherited from the earlier stages. In fact, 34% of cells in the pale and thin stripes of V2 (Peterhans & von der Heydt, 1992; Gegenfurtner et al. 1996), and 24–31% of cells in V4 are reported to be direction selective (Desimone & Schein, 1987; Ferrera et al. 1994). Furthermore, end-stopping or inhibition by large stimuli is especially frequent or especially strong in the pale stripes of V2 (Hubel & Livingstone, 1987; Peterhans & von der Heydt, 1992; Gegenfurtner et al. 1996), which project to V4. With bar stimuli like those we have used, 59% of V4 cells respond best to a bar that is shorter than the length of the receptive field, indicating antagonism within the receptive field (Desimone & Schein, 1987). This is the outcome that would be expected if the large V4 receptive fields are constructed from smaller, end-inhibited receptive fields of V1 and V2 neurons.

Summarizing the parallel pathways

Figure 9 summarizes the properties of the different stages of motion selectivity in V1 and the major direct outputs to more central cortical areas. For simplicity, the less prominent outputs (see Fig. 1) are not shown, but they are discussed below. Direction selectivity in the dorsal cortical stream (magenta) begins in the spontaneously active layer 4Co and is elaborated in layers 6 and 4B. Layer 6 cells are spontaneously active and have large receptive fields with moderate orientation selectivity; they
project directly to area MT in the dorsal stream. We suggest that their large fields contribute to sensing optic flow as the organism moves through the environment. Layer 4B cells are silent and have small receptive fields, along with refined orientation selectivity and moderate length selectivity. They also project directly to area MT as well as to other cortical areas. The combination of large and small receptive fields in the dorsal stream could contribute to figure–ground discrimination and to detection of objects by relative motion. The dorsal stream icons indicate analysis of self motion, object motion, and spatial location in three dimensions (Ungerleider & Pasternak, 2003).

An indirect and less prominent V1 input to MT and the dorsal stream is relayed via the thick stripes of V2 (Fig. 1). In principle, this pathway could supply motion information from layer 3 direction selective cells to the dorsal stream. However, cooling V2 and V3 impairs disparity selectivity in MT while leaving motion sensitivity relatively intact (Ponce et al. 2006), which suggests that the indirect pathway may be more involved in depth perception than motion perception.

The other major direct pathway from V1 carrying motion information (cyan) also begins in the spontaneously active layer 4Cα and it is refined in layers 4Cm and 3, where direction selective cells are silent until stimulated. Layer 4Cm cells have small receptive fields and tight orientation selectivity and they project in parallel with connections from 4Cα to layer 3. Layer 3 provides the most numerous V1 outputs to more central cortical areas, including the ventral stream via area V2. Both direction selective and non-directional layer 3 cells are narrowly tuned for orientation. Furthermore, the direction selective cells have the smallest receptive fields in V1. The small size, end-stopping, and direction selectivity of layer 3 cells makes them well suited to contribute to detection of complex boundaries and small, subtle motions such as changes in facial expressions (icons).

Area V2 receives a less prominent input from layers 4B and 6, in addition to the massive input from layer 3 (Fig. 1). It is possible that these sparser connections provide additional motion information for the ventral stream, which would give it access to motion information at multiple spatial scales.

The realization that motion processing is an integral part of the ventral stream is important because it simplifies

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**Figure 9. Motion-selective pathways in V1 and their relationships to the dorsal and ventral cortical streams of information processing**

The dorsal and ventral streams are indicated on a drawing of a lateral view of a monkey brain. Rectangular coloured boxes represent median values of physiological characteristics of direction selective cells in individual V1 layers. Box width is proportional to measured receptive field width (Table 3). Box height is proportional to the percentage of cells responsive to a bar longer than 60 min (Fig. 8). The symbol for included angle is proportional to the measured half bandwidth of the orientation tuning curve (Table 3). Layer 4Cα feeds layer 4B and layer 6 in the pathway leading to the dorsal stream (magenta). Layers 4B and 6 send outputs from V1 through area MT to the parietal areas (magenta arrow) responsible for sensing object motion and location, including relations in depth. Layer 4Cα also feeds layers 4Cm and layer 3 in a separate direct pathway to V2, which projects to the ventral stream (cyan) responsible for object recognition, including faces. The small dimensions of layer 3 receptive fields (narrow widths as well as preferences for very short stimuli) are well suited to sense subtle motions within objects such as changes in facial expressions.
thinking about natural vision. Consider the need of a monkey to identify small prey or the head of a snake when it is camouflaged by vegetation. The slightest movement will break the camouflage. To be fast and efficient it is likely that the same ventral stream that is able to identify extremely small objects will also be able to sense movement within objects that may be a fraction of the object’s size. Our results lead to the parsimonious proposal that for object-related motion, there is no need to assume that motion is analysed separately by the dorsal processing stream and later combined with shape processing in the ventral stream (Oram & Perret, 1994; Shipp, 2006; Vuong et al. 2006). Instead motion processing is already built in to the ventral stream for object recognition.

References


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**Author’s present address**

D. M. Snodderly: Department of Human Ecology/Nutritional Sciences, Institute for Neuroscience, and Center for Perceptual Systems, University of Texas, Austin, TX 78712, USA.