Signal Timing Across the Macaque Visual System

MATTHEW T. SCHMOLESKY, 1 YOUNGCHANG WANG, 1 DOUG P. HANES, 2 KIRK G. THOMPSON, 2 STEFAN LEUTGEB, 1 JEFFREY D. SCHALL, 2 AND AUDIE G. LEVENTHAL 1
1 Department of Neurobiology and Anatomy, University of Utah College of Medicine, Salt Lake City, Utah 84132; and 2 Department of Psychology, Vanderbilt Vision Research Center, Vanderbilt University, Nashville, Tennessee 37240

Schmolesky, Matthew T., Youngchang Wang, Doug P. Hanes, Kirk G. Thompson, Stefan Leutgeb, Jeffrey D. Schall, and Audie G. Leventhal. Signal timing across the macaque visual system. J. Neurophysiol. 79: 3272–3278, 1998. The onset latencies of single-unit responses evoked by flashing visual stimuli were measured in the parvocellular (P) and magnocellular (M) layers of the dorsal lateral geniculate nucleus (LGNd) and in cortical visual areas V1, V2, V3, V4, middle temporal area (MT), medial superior temporal area (MST), and in the frontal eye field (FEF) in individual anesthetized monkeys. Identical procedures were carried out to assess latencies in each area, often in the same monkey, thereby permitting direct comparisons of timing across areas. This study presents the visual flash-evoked latencies for cells in areas where such data are common (V1 and V2), and are therefore a good standard, and also in areas where such data are sparse (LGNd M and P layers, MT, V4) or entirely lacking (V3, MST, and FEF in anesthetized preparation). Visual-evoked onset latencies were, on average, 17 ms shorter in the LGNd M layers than in the LGNd P layers. Visual responses occurred in V1 before any other cortical area. The next wave of activation occurred concurrently in areas V3, MT, MST, and FEF. Visual response latencies in areas V2 and V4 were progressively later and more broadly distributed. These differences in the time course of activation across the dorsal and ventral streams provide important temporal constraints on theories of visual processing.

METHODS

The activity of 558 single units was recorded in the M and P layers of the LGNd, and in cortical visual areas V1, V2, V3, V4, MT, MST, and FEF in four paralyzed, anesthetized macaque monkeys using standard surgical and single-unit recording techniques consistent with Society for Neuroscience and National Institutes of Health guidelines (Leventhal et al. 1995). The areas studied for each monkey were V1, V2, and FEF in monkey 1; V2 in monkey 2; LGNd M and P layers, V2, V4, MT, and MST in monkey 3; and V2, V3, MT, and MST in monkey 4. Anesthesia was maintained via artificial ventilation with a mixture of nitrous oxide (75%) and oxygen (25%) containing halothane (0.25–1.0% as needed). The small variations made in halothane concentration did not appear to alter responsivity. Animals were studied for as long as stable, reliable recording was possible (2–9 days; see physiological criteria for data inclusion below). Optics were routinely checked, and deterioration was minimal in even the longest experiment. The proportion of cells meeting the data inclusion criteria did not appear to decrease over time. The order in which areas were studied was varied from animal to animal, thereby reducing the impact that this factor could have on any interarea latency differences found.

Visual stimulation

Flashing visual stimuli were generated on a Tektronix 608 display driven by a Picasso image synthesizer (Innisfree). The Picasso was controlled by a PC computer in conjunction with specially

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Spike train analysis

Times of onset of visually evoked activity were determined for each spike train using an adaptation of the Poisson spike train analysis originally described by Legendy and Salcman (1985) and modified by Hanes et al. (1995) and Thompson et al. (1996). Examples of the raster plots used to determine the visual onset response latencies of cells in the areas studied are shown in Fig. 1.

Histology and histochemistry

At the conclusion of each experiment, the animal was deeply anesthetized and perfused through the heart with 700 ml of lactated Ringer solution containing 0.1% heparin, followed by 1,000 ml of 1% paraformaldehyde and 2.5% glutaraldehyde in 0.1 M phosphate buffer at pH 7.4, followed by 600 ml of lactated Ringer solution containing 5% dextrose. Brains were removed, and the locations of the electrode tracks relative to specific sulci and gyri were determined. Portions of cortex containing electrode tracks were blocked, and alternating coronal sections (90–120 µm) were stained for cells bodies (Nissl) or myelin (Gallyas 1979). The surface position of electrode entrance (all electrodes were aligned perpendicular to the cortical surface), and/or the reconstruction of the electrode track itself was used to confirm the earlier classifications made of each cortical area based on comparisons of physiological recordings with well-documented RF properties (e.g., size, eccentricity, stimulus selectivity, progression of RFs relative to vertical meridian).

Results

The areas studied include the M (n = 52) and P (n = 78) layers of the LGNd, and cortical areas V1 (n = 74), V2 (n = 61), V3 (n = 100), V4 (n = 29), MT (n = 79), MST (n = 59), and FEF (n = 26). A number of V1 cells were classified as 4C (n = 13) or 4Cβ (n = 9) based on penetration depth and response characteristics (e.g., nonoriented, small RF, etc.). The peripheral RF eccentricity of the FEF cells studied suggests a correspondence to area 8Ac (Schall et al. 1995). For areas that were studied in more than one monkey, interanimal comparisons of average response latencies taken from relatively equal and large sample sizes did not reveal statistically significant differences.

The earliest visual responses measured were in the M layers of the LGNd (see Figs. 1 and 2) in which there was very little latency spread (33 ± 3.8 ms, mean ± SD). P cells in the LGNd exhibited longer, more variable latencies, ranging from 31 to 76 ms (50 ± 8.7 ms). The modal latencies of M and P LGNd cells did not overlap and were, in fact, 10 ms removed; M cell 25–75 percentile was 31–34 ms, P cell 25–75 percentile was 44–56 ms (see Fig. 2).

The shortest latencies in visual cortex were found in layer 4Cα of V1. These cells had latencies as short as 34 ms. Even though the number of cells we identified as being in layer 4C was small, the latencies of 4Cα cells were, on average, significantly shorter than those for 4Cβ, t (1, 20) = 2.66, P = 0.02. Thus the latency difference found between M and P LGNd layers is maintained in the genticulo-recipient layers of V1. Overall, the latencies of V1 cells ranged from 34 to 97 ms (66 ± 10.7 ms). V2 cells exhibited latencies with an average of 82 ms and a large variance (SD, 21.1 ms). Previous research has shown that V2 latencies increase from thick to pale to thin bands (Munk et al. 1995) and when all three subdivisions are included, as is the case here, a large latency spread is to be expected. Figure 1 gives examples of responses of individual neurons showing how the putative M (A, C, and E) and P (B, D, and F) streams could pass staggered but parallel signals with 10- to 15-ms delays between each stage of activation. V4 cells exhibited the longest and most varied latencies of any area recorded from in this study (104 ± 23.4 ms).

The latencies of cells recorded in areas associated with the dorsal stream of visual processing were shorter and more uniform. The latencies of V3 cells ranged from 55 to 101 ms (72 ± 8.6 ms). Cells in area MT had an identical average
FIG. 1. Representative responses of sampled neurons. Activity is displayed as a raster in which each vertical tick mark indicates the time of an action potential. Horizontal lines above the tick marks indicate periods of significant activation identified by the spike train analysis. Panel above each raster plot is the average spike density. The spike density was derived using a kernel shaped like an excitatory postsynaptic potential (Thompson et al. 1996). Each division along the ordinate is equal to 50 Hz. Indicators of the phase of visual stimulation are shown above A and B. Black region represents the time the stimulus was on, and the white region represents the time the stimulus was off. Stimulus onset is at 0 ms and ends at 500 ms in each case. Arrow beneath each raster plot indicates the visual response latency of the cell, and the exact value is displayed in the top right corner. A–F: putative temporal hierarchy of the early stage M and P stream areas, where a 10- to 20-ms delay occurs between each stage. G–J: simultaneity of average activation in the middle tier dorsal stream areas. LGNd, dorsal lateral geniculate nucleus.
FIG. 2. Cumulative distributions of visually evoked onset response latencies in the LGNd, striate and extrastriate visual areas as labeled. Percentile of cells that have begun to respond is plotted as a function of time from stimulus presentation. The V4 curve is truncated to increase resolution of the other curves; the V4 range extends to 159 ms.

Latency of $72 \pm 10.3$ ms. The MT latency data gathered by Maunsell (1987) were obtained from awake monkeys in response to very different stimuli (high contrast square wave gratings) and were presented in a population response format that does not provide a range of latencies. Thus a comparison of the Maunsell (1987) data with our own is difficult. However, note that the earliest response reported by Maunsell (1987) was 39 ms, 10 ms faster than the earliest MT latency reported here. This difference is most likely due to lack of anesthesia and/or differences in stimulus presentation and data analysis. The only other study of macaque MT visual onset latencies (Raiguel et al. 1989) cites a much slower median latency (94 ms) and a particularly wide range of latencies (35–272 ms). Because the V1 latencies reported by Raiguel et al. (1989) are also considerably longer and more varied than our own or those reported by others (Celebrini et al. 1993; Knierim and Van Essen 1992; Maunsell and Gibson 1992; Nowak et al. 1995), the MT data differences are likely due to the use of moving, as opposed to flashing, visual stimuli in the Raiguel et al. (1989) study or differences in analysis techniques. The onset latency data presented are the first reported for V3 and the first reported for MT under anesthetized preparation, flash stimulus conditions and indicate coincident activation timing in the two areas.

Cells in MST exhibited latencies effectively equivalent to V3 and MT averaging at $74 \pm 16.1$ ms. FEF cell response latencies have not been recorded previously in the anesthetized monkey. We found that cells were visually responsive in arcuate frontal cortex of the anesthetized, paralyzed monkey and that the latencies of a sample of FEF cells gave an average of $75 \pm 13.0$ ms. This distribution of FEF cell visual onset latencies (Raiguel et al. 1989) cites a much slower median latency (94 ms) and a particularly wide range of latencies agrees with the distribution of latencies measured in awake, behaving monkeys using approximately equal strength stimuli (Schall 1991; Thompson et al. 1996). A Kruskal-Wallis one-way analysis of variance (ANOVA) on ranks was performed to statistically compare the distributions of latencies across the layers of the LGNd and the different visual areas. Significant variation in latency was confirmed $[H(8,488) = 336.9, P < 0.001]$. The results

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<th>TABLE 1. Mann-Whitney rank sum tests</th>
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<td>Values in Area 1 and Area 2 are means $\pm SD$ with medians in parentheses. Multiple two-group comparisons of visual areas carried out by Mann-Whitney rank sum tests. The Bonferroni correction for 13 planned comparisons yields a significance criterion of 0.004. Probability values are reported. Marginally significant results are marked by &quot;ms&quot; and nonsignificant results are marked by &quot;ns.&quot; Because Mann-Whitney rank sum tests are carried out on median values, rather than means, both values are included. Standard deviations are based on means. M, magnocellular; P, parvocellular; MT, middle temporal area; MST, medial superior temporal area; FEF, frontal eye field.</td>
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of multiple Mann-Whitney two-way rank sum comparisons corrected by the Bonferroni method are shown in Table 1. These results suggest that, based on response onset latency alone, there is a functional sequence in the ventral stream, wherein LGNd P layers, V1, V2, and V4, demonstrate successively longer latencies. In contrast, although the dorsal stream does show progressively longer latencies from LGNd M cells to V1 to V2, there is simultaneous onset of firing in V3, MT, MST, and FEF (see Fig. 1, G–J for representative responses).

**DISCUSSION**

Within the limits of methodology and analysis, our findings are generally consistent with estimates of response latencies throughout the visual system (Givre et al. 1995; Maunsell and Gibson 1992; Nowak et al. 1995; Schroeder et al. 1991; for review see Nowak and Bullier 1998). However, the fact that the present data from multiple stations of the visual system were collected in individual monkeys using common stimulus presentation and analysis techniques significantly improves the reliability of conclusions drawn about the relationships between the latencies of cells across the visual system.

**Latency differential between M and P streams**

One salient finding of the present study was that the onset latency distribution of the M and P layers of the LGNd are almost entirely separated with the P cells being nearly 20 ms slower. There are only two other studies of macaque exhibit almost completely overlapping latencies (see Fig. 2). Shown is a 10- to 20-ms latency difference between Y and X that V2 fast latency pale bands and V2 slow latency thin bands (range 33 ± 108 ms) and color opponent (range 23 ± 80 ms) cells (1994 for IT latencies). The V2 and V4 latencies still do not significantly improve the reliability of conclusions drawn about the relationships between the latencies of cells across the visual system.

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**Anatomic and functional hierarchies**

Anatomic evidence has been employed to argue for a hierarchy of visual areas (e.g., Felleman and Van Essen 1991; Hilgetag et al. 1996). However, examination of the present data as well as published results from different laboratories for individual areas reveals a number of inconsistencies. For example, FEF is at level 8 of the Felleman and Van Essen (1991)
anatomic hierarchy. However, cells from this area exhibit visual latencies comparable with those in V2 (level 2), V3 (level 3/4), MT (level 5), and MST (level 7), and sometimes even as early as some cells in V1 (level 1). Conversely, many V2 cells exhibit longer response latencies than most MT or MST cells. These inconsistencies are not resolved by alternative hierarchical schemes (Hilgetag et al. 1996).

Clearly an anatomic substrate for the time course of visual activation must exist. Our data simply indicate that the rules of connectivity used to produce the anatomic hierarchies fail to account for the initial flow of signals in the visual system and therefore may not accurately represent the “functional” hierarchy of the visual system (see also Nowak and Bullier 1998). In fact, the results indicate that in many cases the short latencies of cells in higher tier areas can only be accounted for if multiple tiers of processing are bypassed entirely during the transfer of initial information from V1. Anatomic studies do support many bypass routes (e.g., V1 to MT to FEF) (Maunsell and Van Essen 1983; Ungerleider and Desimone 1986), but hierarchical models rarely weigh such paths heavily when assigning areas to tiers. Thus the sequence of neural activation in different areas highlights the limitations of interpretations provided by hierarchical schemes derived solely from anatomic data. Continued studies of the timing of information processing in different cortical areas, layers, and functional cell types are necessary to expand our understanding of the mechanisms of visual perception.

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Address for reprint requests: A. G. Leventhal, Dept. of Neurobiology, University of Utah School of Medicine, Salt Lake City, UT 84132.

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