

Processing of first-order motion in marmoset visual cortex is influenced by second-order motion

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Abstract

We measured the responses of single neurons in marmoset visual cortex (V1, V2, and the third visual complex) to moving first-order stimuli and to combined first- and second-order stimuli in order to determine whether first-order motion processing was influenced by second-order motion. Beat stimuli were made by summing two gratings of similar spatial frequency, one of which was static and the other was moving. The beat is the product of a moving sinusoidal carrier (first-order motion) and a moving low-frequency contrast envelope (second-order motion). We compared responses to moving first-order gratings alone with responses to beat patterns with first-order and second-order motion in the same direction as each other, or in opposite directions to each other in order to distinguish first-order and second-order direction-selective responses. In the majority (72%, 67/93) of cells (V1 73%, 45/62; V2 70%, 16/23; third visual complex 75%, 6/8), responses to first-order motion were significantly influenced by the addition of a second-order signal. The second-order envelope was more influential when moving in the opposite direction to the first-order stimulus, reducing first-order direction sensitivity in V1, V2, and the third visual complex. We interpret these results as showing that first-order motion processing through early visual cortex is not separate from second-order motion processing; suggesting that both motion signals are processed by the same system.

Keywords: Primate, Physiology, V1, Luminance, Contrast

Introduction

We can perceive motion as a result of either the movement of luminance-defined spatial structures in a visual stimulus, or the movement of higher-order spatial structures, for example contrast envelopes or texture borders, (Badcock & Derrington, 1985; Derrington & Badcock, 1985; Chubb & Sperling, 1988). The two types of motion are called, respectively, first-order motion and second-order motion, (Cavanagh & Mather, 1989). First-order motion information abounds in our moving natural environment, often accompanied by second-order motion information. Second-order motion information can be used to break camouflage and evaluate movement when first-order motion information is unreliable or even absent.

There is substantial disagreement in the scientific literature whether first-order motion and second-order motion are processed separately. Motion is processed at multiple and distinct stages within the visual system (Zeki, 1990; Movshon & Newsome,

1996) and this have additionally confused the issue. In this study we address the question of whether first-order motion processing in V1, V2, and the third visual complex is influenced by an additional second-order motion signal.

Derrington and Badcock (1985) found that the discrimination of the direction of motion of first-order and second-order stimuli were different. Sensitivity to a moving second-order stimulus was lower and the necessary temporal resolution of the second-order stimulus was also lower than that for a moving first-order stimulus. The authors also found that second-order motion stimuli do not elicit a motion after-effect and proposed that the human visual system uses different mechanisms to process first- and second-order motion (Badcock & Derrington, 1985; Derrington & Badcock, 1985). Many other psychophysical studies have been used to argue that processing systems for first-order motion and second-order motion are largely independent (Harris & Smith, 1992; Mather & West, 1993; Ledgeway & Smith, 1994; Lu & Sperling, 1995; Nishida et al., 1997; Scott-Samuel & Georgeson, 1999). Separate processing of first-order motion and second-order motion has also received some support from physiological (Zhou & Baker, 1993, 1994; Mareschal & Baker, 1998a) and neurological studies (Vaina & Cowey, 1996; Greenlee & Smith, 1997).

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Psychophysical, physiological, and neurological evidence have been used to propose models in which first-order motion is processed in a separate cortical area to second-order motion (Wilson et al., 1992; Vaina & Cowey, 1996; Clifford & Vaina, 1999). Wilson et al. (1992) have suggested that V1 cells detect first-order motion information; a weighted sum of V1 cell responses provides an input to area MT/V5. In parallel, the filtered visual signal passes through a cortical non-linearity prior to the detection of second-order motion by V2 cells. A weighted sum of V2 cell responses would provide a second input to area MT/V5. MT/V5 neurons then calculate the maximum response of the weighted inputs *via* a method of competitive feedback.

Whereas there is agreement regarding the convergence of first-order motion information and second-order motion information at a later processing stage, it may not be necessary to have separate early processing systems (Taub et al., 1997) or even separate systems at all (Johnston et al., 1992). Studies have suggested that, at an early stage, first-order motion and second-order motion interact and might be processed by the same system (Cavanagh & Mather, 1989; Edwards & Nishida, 2004). Recent evidence using imaging (Nishida et al., 2003; Sofue et al., 2003) shows interactions between systems detecting first-order and second-order motion in humans.

The aim of this study is to determine whether first-order motion processing is separate from second-order motion processing in early primate visual cortex, areas V1, V2, and the third visual complex. Models suggesting separate processing systems predict that first-order motion processing in V1 should be unaffected by second-order motion signals. Additionally, second-order motion signals should have a significant influence on the response of neurons in V2, and possibly the third visual complex. If first-order motion and second-order motion are processed together, the responses of cells in V1, V2, and the third visual complex to first-order motion should be influenced similarly by the addition of second-order signals.

Materials and methods

Physiological subjects, recording, and reconstruction techniques

We recorded the action potentials of single neurons in areas V1, V2, and the third visual complex of the visual cortex using extracellular tungsten microelectrodes in nine anaesthetized and paralyzed marmosets (*Callithrix jacchus*). All work was carried out in accordance with the UK Animals (Scientific Procedures) Act, 1986. Surgical and physiological recording procedures have been described in detail previously (Felisberti & Derrington, 2001; Webb et al., 2002). Briefly, experiments were carried out on paralyzed marmosets (Vecuronium bromide) under N₂O/O₂ anesthesia supplemented with intravenous fentanyl citrate (20 µg/kg/h). Electrocardiogram, electroencephalogram, end-tidal CO₂, and body temperature were maintained at normal levels during the experiment. Penetrations were made with glass-coated tungsten microelectrodes (Merrill & Ainsworth, 1972) in areas V1, V2, and the third visual complex. Extracellular electrical signals were amplified and filtered between 300 Hz and 3 kHz before being sampled at 44 kHz by a Macintosh computer. The Macintosh, running bespoke software *Expo* (written by Peter Lennie), concurrently extracted the action potential waveform and generated the visual stimuli. Each eye was fitted with gas-permeable neutral contact lenses and any refractive error corrected with miniature

spectacle lenses chosen to maximize the response of an isolated simple cell to a high spatial-frequency grating.

Microlesions (5 µA for 5 s, electrode negative) were made at different depths on each penetration; these were used to reconstruct each electrode track. At the conclusion of each experiment the animal was killed with an overdose of pentobarbitone (Sagatal, 60 mg/kg) and perfused trans-cardially with 4% formaldehyde in phosphate buffer. Brains were removed and sectioned parasagittally on a freezing microtome; sections were mounted on slides and stained with cresyl violet. The V1/V2 border was clearly identified as the edge between the darkly stained layer 4B in V1 and the lightly stained V2. The third visual complex was defined as a thin (2 mm) strip of cortex whose caudal border starts 2 mm laterally and 3 mm medially anterior to the V1/V2 border (Pessoa et al., 1992; Rosa et al., 1997, 2005; Lyon & Kaas, 2001). The position of the track with respect to the histologically defined border, the position in the visual hemifield, the sequence of changes in receptive field position over the surface of the cortex, and the cells' spatial and temporal frequency tuning was used to assign each cell to the areas V1, V2, or the third visual complex.

Visual stimuli

Stimuli consisted of computer generated sinusoidal gratings (gratings) or combinations of spatial frequency gratings (beats) to measure respectively the neural responses to first-order motion only and to combined first- and second-order motion. Contrasts of the stimuli are given as Michelson contrast $(L_{max} - L_{min})/(L_{max} + L_{min})$.

Beat patterns (c.f. Derrington & Badcock, 1985; Badcock & Derrington, 1985) were made by adding two component gratings of contrast 0.5:

$$L(x, t) = L_m(1 + 0.5 \sin[f_a x + g_a t] + 0.5 \sin[f_b x + g_b t]) \quad (1)$$

where L_m is the mean luminance, f_a and f_b are the spatial frequencies of the two component gratings (cycles/degree), x represents spatial position, g_a and g_b are the temporal frequencies of the two gratings (Hz) and t represents time.

Applying the trigonometric identity: $\sin(A) + \sin(B) = 2 \cos(A - B)/2 \cdot \sin(A + B)/2$ to Eq. (1) shows that the beat pattern can be expressed as the product of a high frequency carrier grating and a low frequency contrast envelope:

$$L(x, t) = L_m(1 + \cos([f_a - f_b]x/2 + [g_a - g_b]t/2) \cdot \sin([f_a + f_b]x/2 + [g_a + g_b]t/2)) \quad (2)$$

The spatial frequencies of each component grating were set to a value relative to a neuron's preferred spatial frequency (f), 1.5 f , and 2 f , respectively. The carrier appears as a first-order modulation in luminance. The spatial frequency of the carrier is half the sum of the frequencies of the two component gratings, 3.5 $f/2$. The contrast of the carrier is modulated by an envelope. The envelope is a cosine with a spatial frequency of half the difference between the component gratings, 0.5 $f/2$. The sign of the contrast is ignored, however, so it is effectively rectified and its apparent frequency is 0.5 f (Derrington & Badcock, 1985). The beat therefore appears as a second-order spatial modulation in the contrast of the carrier with a spatial frequency 0.5 f .

When the components of the beat pattern move, the temporal frequency of the carrier is calculated by averaging the component

temporal frequencies, see Eq. (2). The temporal frequency of the contrast envelope is calculated by subtracting the temporal frequencies of the components. The velocities of the carrier and the contrast envelope are dependent on the sign of their respective temporal frequencies. Therefore because the temporal frequency of the carrier is an average of the temporal frequencies of a stationary and moving component, it always moves in the same direction as the moving component. The sign of the temporal frequency of the contrast envelope is dependent on which component is moving. Consequently, by moving either the lower or the higher frequency component, the contrast envelope is made to move either in the opposite direction as the carrier (see Fig. 1a), or in the same direction (see Fig. 1b).

Stimulus presentation and testing procedure

Visual stimuli were generated and displayed using a Macintosh computer with a 10-bit Radius graphics card running *Expo* software. Initially, moving sinusoidal gratings were presented at a resolution of 9.0 pixels/deg on a tangent projection screen subtending approximately $87^\circ \times 67^\circ$ at a viewing distance of 57 cm, to map receptive field locations. Neuronal responses were tested for each eye; the eye that produced the weakest responses was occluded. The dominant eye could be ipsilateral or contralateral to the recorded hemisphere. The receptive field of the dominant eye was positioned using a front-surfaced mirror on the center of a CRT monitor. The CRT display (Sony Model No. GDM 200PST) subtended $31^\circ \times 23^\circ$ at a viewing distance of 57 cm; pre-test and experimental stimuli were presented at a resolution of 56 pixels/deg, and a frame rate of 120 Hz; mean luminance was approximately 50 cd/m². Stimulus luminances, derived from photometric measurements, were set using a lookup table to compensate for the nonlinear relation between luminance and applied voltage of the display.

Pre-testing of each neuron consisted of determining the spatial and temporal frequency tuning, optimal stimulus size and preferred

stimulus orientation using high-contrast drifting sinusoidal gratings. These values were used to set the spatial frequency (f) and the size and orientation of the test stimuli in the experimental phase. The preferred temporal frequency was used to determine the speed of movement of the moving gratings or moving components in the experimental stimuli. Neurons in V1, V2 are predominantly orientation-selective; some are also sensitive to the direction of movement of a grating of optimal spatial and temporal frequency and orientation. We ran our experimental test predominantly on neurons that showed a strong sensitivity to one direction of motion of the optimal first-order sinusoidal grating; we also tested several neurons showing only a mild direction bias. In all cases, the direction of movement of the optimal grating that produced the greatest response is referred to as the “preferred direction,” irrespective of the significance of the difference between responses to motion in the preferred and opposite (non-preferred) directions.

In the experimental phase each neuron was tested with stimuli consisting of moving gratings of spatial frequencies 1.5 f and 2 f , presented at maximum contrast, and beat patterns where the 1.5 f component or the 2 f component moved, referred to hereafter as the 1.5 f beat and 2 f beat, respectively. The single gratings and the moving component in the beat patterns moved at the preferred temporal frequency in both the preferred and non-preferred directions for the neuron. The carrier moved at half the preferred temporal frequency and the envelope at the preferred temporal frequency (see earlier). Each stimulus was presented for five seconds, six times (trials) in a pseudorandom order, with the constraint that no stimulus was presented for the $n + 1$ th time until each stimulus was presented n times.

Response analysis

Cell responses were analyzed offline using a Macintosh computer and *Expo* software. Mean responses were measured in a 5 s window starting at stimulus onset for each stimulus trial. Analysis of 1.5 f grating and 1.5 f beat responses was kept separate from analysis of 2 f grating and 2 f beat responses.

For each cell, the responses to the different stimuli were entered into two 2-factor ANOVAs, one ANOVA contained responses to the moving 2 f gratings and responses to the 2 f beat patterns (2 f ANOVA); the other ANOVA contained responses to the moving 1.5 f gratings and responses to the 1.5 f beat patterns (1.5 f ANOVA); [stimulus type (grating, beat) by movement direction (preferred, non-preferred) with trials as replicates]. By analyzing the responses to the 2 f gratings and 2 f beats separately from the responses to the 1.5 f gratings and 1.5 f beats, it was possible to examine the effect of the addition of a second-order signal on a first order motion response. When paired together like this the 2 f grating and the 2 f beat stimuli (likewise the 1.5 f grating and the 1.5 f beat stimuli) contain identical first-order signals, the difference between grating and beat stimuli lies in the beats' additional second-order signals. A main effect of stimulus type or interaction between stimulus type and movement direction indicated that the second-order signal had a significant influence on the cell response to first-order motion.

Direction indexes

Direction indexes were calculated from the responses to the four different stimulus types, 1.5 f and 2 f gratings, 1.5 f and 2 f beats, according to the formula:

$$(R_{pref} - R_{null}) / (R_{pref} + R_{null})$$

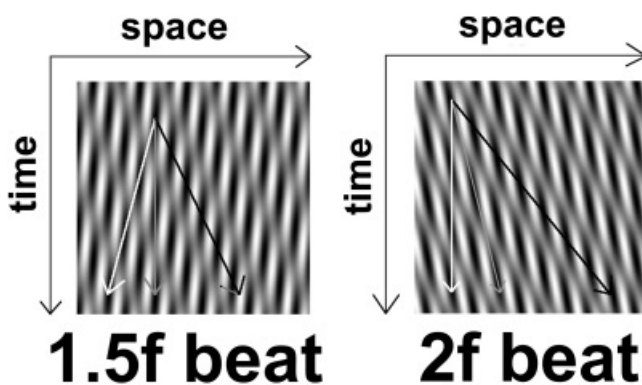


Fig. 1. Space-time plots of the two beat patterns. Arrows indicate the trajectory of each component; grey line shows the position of a dark region of the 2 f component over time, white line shows the position of a dark region of the 1.5 f component over time, black line shows the position of a high contrast region of the contrast envelope over time. (a) Beat pattern in which the 1.5 f component moves, the 2 f component remains stationary. The contrast-envelope moves in the opposite direction to the 1.5 f component and carrier. This pattern is referred to as the 1.5 f Beat. (b) Beat pattern in which the 2 f component grating moves, the 1.5 f component remains stationary. The contrast-envelope moves in the same direction as the 2 f component and carrier. This pattern is referred to as the 2 f Beat.

where R_{pref} is the mean response when the motion of the grating or the motion of carrier is in the preferred direction and R_{null} is the mean response when the motion of the grating or the motion of the carrier is in the non-preferred direction. Thus the direction indexes give a measure of the direction selectivity of the neuron when measured with first-order stimuli. A direction index of 0 indicates no direction selectivity; a direction index of ± 1 indicates very high direction selectivity. A positive direction index indicates that the response is greatest when the grating or carrier is moving in the preferred direction. As the preferred direction is determined using first-order stimuli, direction indexes calculated from the responses to the grating stimuli are all positive. For beat stimuli, containing both first- and second-order motion, the response can be greatest when the grating or carrier is moving in the non-preferred direction, creating a negative direction index. The significance of each direction index was calculated by analyzing the simple main effect of direction at each level of stimulus type within each ANOVA using Fisher's protected least significant difference test (PLSD). A comparison between the direction indexes calculated from the responses to the grating stimuli and the direction indexes calculated from the responses to the beat stimuli was used to characterize the influence of the addition of a second-order signal on first-order direction sensitivity.

Results

Ninety-three neurons were recorded in V1, V2, and the third visual complex of nine monkeys. Of those 93 neurons, 62 were recorded in V1 in seven animals, 23 in V2 in three animals, and eight in the third visual complex in two animals. Of the 93 cells recorded, 67 (72%) showed that the first-order motion response was significantly ($P < 0.05$) influenced by the addition of a second-order signal, tested by ANOVA. The first-order motion response of 45/62 (73%) of the neurons in V1, 16/23 (70%) of the neurons in V2 and 6/8 (75%) of the neurons in the third visual complex were influenced by the addition of a second-order signal. Processing of first-order motion was thus influenced by the addition of a second-order signal in the majority of cells tested in V1, V2, and the third visual complex.

Fig. 2 shows an example neuron whose response to a first-order signal is significantly ($P < 0.05$) influenced by a second-order signal. The cell showed large responses to the 1.5 f and 2 f gratings moving in the preferred direction (14.5 spikes/s and 12.8 spikes/s, respectively) and small responses to the 1.5 f and 2 f gratings moving in the non-preferred direction (4.8 spikes/s and 3.3 spikes/s, respectively), indicating direction sensitivity to first-order signals. The cell showed large responses to the 2 f beat pattern when the carrier and the envelope are moving in the preferred direction (8.2 spikes/s) and a small response when the carrier and the envelope are moving in the non-preferred direction (3.0 spikes/s). In contrast, the response to the 1.5 f beat pattern was greater when the envelope moved in the preferred direction and the carrier moved in the non-preferred direction (12.3 spikes/s), than when the carrier moved in the preferred direction, and the envelope in the non-preferred direction (7.3 spikes/s). The response of the cell was greatest when the envelope was moving in the preferred direction, thus this neuron's response was dominated by the second-order signal.

In contrast, Fig. 3 shows the responses of a neuron whose response to a first-order stimulus was unaffected by the addition of a second-order signal. There was no significant difference between the first-order response, with or without a second-order signal

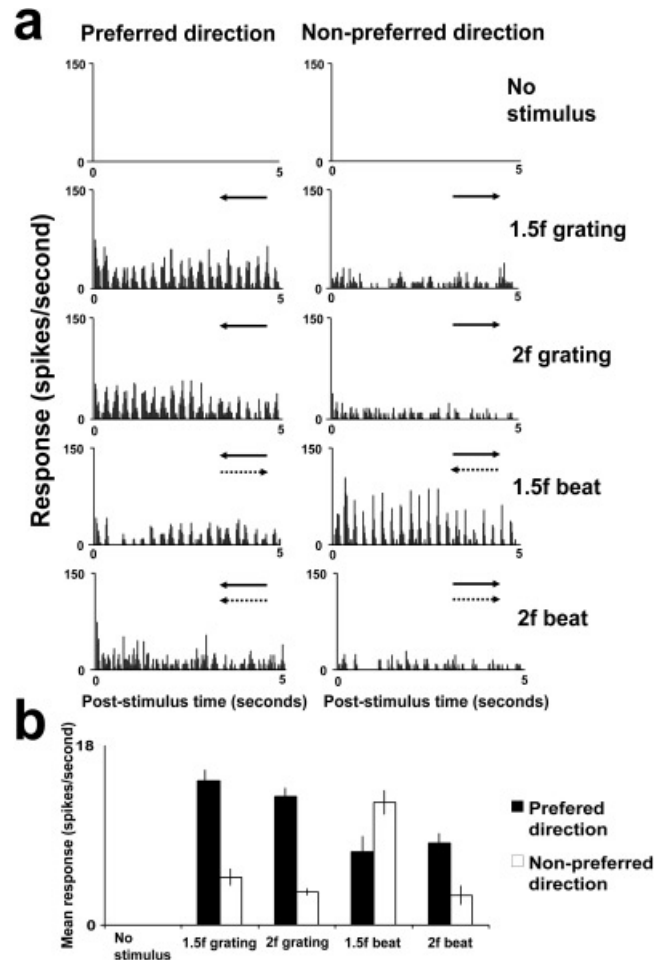


Fig. 2. Cell showing responses dominated by a second-order signal. (a) Post-stimulus time histograms (PSTHs) showing the response of a V2 neuron to each stimulus; the stimulus is described on the right. The left column shows the responses when the grating or the carrier is moving in the preferred direction, and the right column shows the responses when the grating or the carrier is moving in the non-preferred direction. Arrow indicators on each plot show the direction of first-order motion (solid arrow) and second-order motion (dotted arrow) in each stimulus. For illustrative purposes the preferred direction is indicated to the left and the non-preferred direction to the right. (b) Mean cell responses ($\pm$ sem) to each stimulus when the grating or carrier is moving in the preferred (solid) or non-preferred (open) direction. The first-order response is significantly influenced by the addition of a second-order signal (2f ANOVA: stimulus type $F(1,20) = 8.50$, $P < 0.005$; stimulus type $\times$ movement direction $F(1,20) = 6.38$, $P < 0.05$). (1.5 f ANOVA: stimulus type $F(1,20) = 0.02$, $P > 0.05$; stimulus type $\times$ movement direction $F(1,20) = 35.8$, $P < 0.001$).

($P > 0.05$). The cell showed large responses to the 1.5 f and 2 f gratings moving in the preferred direction (110.2 spikes/s and 102.7 spikes/s, respectively) and small responses to the 1.5 f and 2 f gratings moving in the non-preferred direction (1.9 spikes/s and 5.6 spikes/s respectively); like the cell illustrated in Fig. 2, this cell showed direction sensitivity to first-order signals. The cell showed large responses to the 1.5 f and 2 f beat patterns when the carrier moved in the preferred direction (92.7 spikes/s and 93.9 spikes/s, respectively) and small responses when the carrier moved in the non-preferred direction (8.3 spikes/s and 3.3 spikes/s, respectively). The response was greatest when the carrier moved in

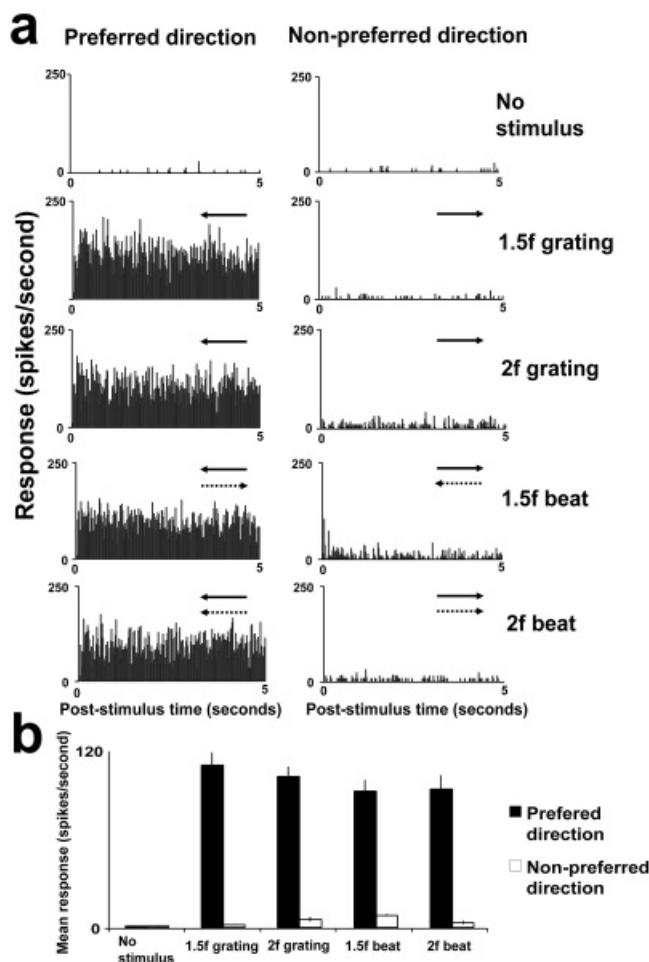


Fig. 3. Cell showing responses dominated by a first-order signal. (a) Post-stimulus time histograms (PSTHs) showing the response of a neuron in the third visual complex to each stimulus; the stimulus is described on the right. All conventions as for Fig. 2. (b) Mean cell responses ($\pm$ sem) to each stimulus when the grating or carrier is moving in the preferred (solid) or non-preferred (open) direction. The first-order response is not significantly influenced by the addition of a second-order signal [2 f ANOVA: stimulus type $F(1,20) = 0.91$, $P > 0.05$; stimulus type $\times$ movement direction $F(1,20) = 0.31$, $P > 0.05$]. [1.5 f ANOVA: stimulus type $F(1,20) = 0.95$, $P > 0.05$; stimulus type $\times$ movement direction $F(1,20) = 0.42$, $P > 0.05$].

the preferred direction even when, as in the 1.5 f beat, the envelope moved in the opposite direction to the carrier; thus the cell's response was dominated by the first-order signal.

Influence of second-order signals on first-order motion processing in V1, V2, and the third visual complex

For all 93 cells tested in V1, V2, and the third visual complex, first-order direction indexes were calculated from the responses to the 1.5 f and 2 f gratings and from the 1.5 f and 2 f beats. Direction index values were entered into a 3-way mixed ANOVA [stimulus type (grating, beat) by frequency (1.5 f, 2 f) by area (V1, V2, third visual complex), with cells as replicates].

The results from the three-way ANOVA revealed two statistically significant differences between direction indexes. First, di-

rection indexes calculated from beat stimuli were significantly lower than direction indexes calculated from grating stimuli, (ANOVA: stimulus type, $F(1,90) = 26.27$, $P < 0.001$). Thus direction sensitivity was greatest to first-order stimuli alone; when a second-order signal was added, direction sensitivity was significantly reduced.

Second, direction indexes calculated from different brain areas were significantly different (ANOVA: area, $F(2,90) = 6.82$, $P < 0.005$). Post-hoc testing (Scheffe) showed that direction indexes calculated from V1 responses were significantly ($P < 0.05$) larger than direction indexes calculated from V2 or third visual complex responses; there was no difference ($P > 0.05$) between direction indexes calculated from V2 responses and third visual complex responses. Thus the mean direction sensitivity of V1 cells was greater than the mean direction sensitivity of V2 or third visual complex cells. There were no other significant main effects or interactions between stimulus type, frequency, or brain area.

For each cell, the average of the direction indexes for the 1.5 f and 2 f gratings gave the mean direction sensitivity to a first-order stimulus alone, and the average of the direction indexes for the 1.5 f and 2 f beats gave mean direction sensitivity to the combined first- and second-order stimuli. Mean direction indexes ($\pm$ sem) for each of the three visual areas are plotted in Fig. 4. Direction sensitivity measured with only first-order motion was different from direction sensitivity measured with combined first-order and second-order motion in V1 (paired t -test, $t_{[61]} = 3.99$, $P < 0.001$), V2 (paired t -test, $t_{[22]} = 2.99$, $P < 0.05$) and the third visual complex neurons (paired t -test, $t_{[7]} = 2.65$, $P < 0.05$). The mean V1 direction index, 0.60 (sem ± 0.04), was reduced to 0.51 (sem ± 0.04), the mean V2 direction index, 0.45 (sem ± 0.06), was reduced to 0.28 (sem ± 0.08) and the mean third visual complex direction index, 0.30 (sem ± 0.11), was reduced to 0.09 (sem ± 0.06).

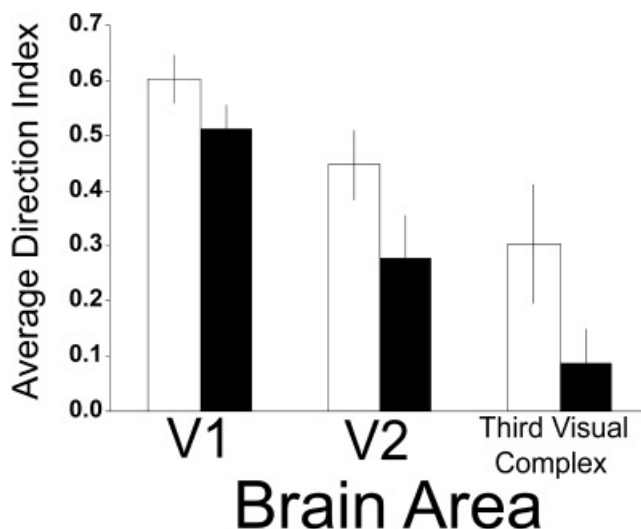


Fig. 4. Mean direction indexes of cells in V1, V2, and the third visual complex. For each cell the direction indexes calculated from the 1.5 f and 2 f gratings are averaged and the direction indexes calculated from the 1.5 f and 2 f beats are averaged to provide a mean direction index to grating stimuli and a mean direction index to beat stimuli. These values are averaged for each brain area and plotted here ($\pm$ sem): open, mean direction index to gratings (first-order only) and solid, mean direction index to beats (first-order and second-order).

Influence of envelope direction on first-order motion responses

Responses to the 1.5 f grating and 1.5 f beat were analyzed separately from the responses to the 2 f grating and the 2 f beat, in order to examine the effect of the direction of movement of the envelope on the first-order motion response. Only those cells whose first-order responses were significantly ($P < 0.05$) influenced by a second-order signal were examined. Of the 93 cells tested 52 showed a significant difference ($P < 0.05$) in the first-order response when a second-order signal was added, when tested with the 1.5 f ANOVA. Of the 93 cells tested 42 showed a significant difference ($P < 0.05$) in the first-order response when a second-order signal was added, when tested with the 2 f ANOVA.

On the left of Fig. 5, the direction indexes calculated from the response to the 1.5 f gratings are plotted against the direction indexes calculated from the response to the 1.5 f beats for the 52 cells where the second-order signal significantly ($P < 0.05$) influenced the first-order response, tested by the 1.5 f ANOVA. Histograms of the mean direction indexes for these cells are shown in Fig. 7a. Direction indexes calculated from the gratings gave a measure of the direction sensitivity to a first-order stimulus alone, and were always positive; they are illustrated on the x-axis. The direction indexes calculated from the beats, plotted on the y-axis,

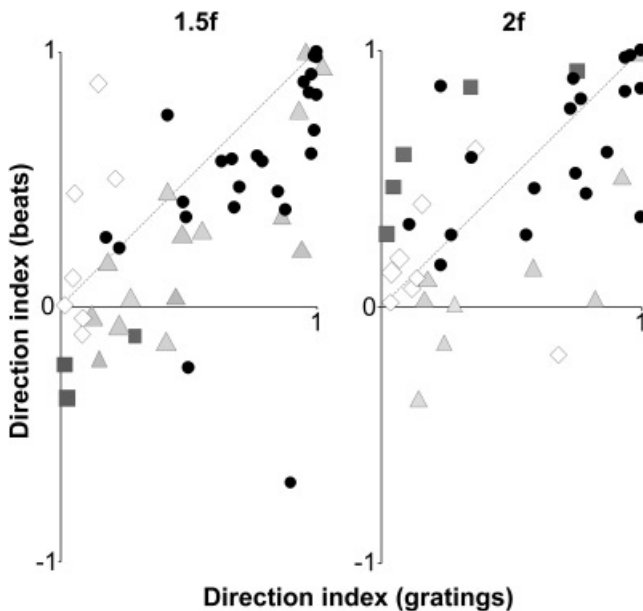


Fig. 5. Comparison of direction indexes in cells where a second-order signal significantly influences responses to first-order stimuli. The left side of the figure shows a plot of the direction indexes calculated from the responses to the 1.5 f gratings and 1.5 f beats in the 52/93 cells whose first-order motion response was significantly ($P < 0.05$) influenced by a second-order signal, tested with the 1.5 f ANOVA. The right side of the figure shows a plot of the direction indexes calculated from the responses to the 2 f gratings and 2 f beats in the 42/93 cells whose first-order motion response was significantly ($P < 0.05$) influenced by a second-order signal, tested with the 2 f ANOVA. Dotted lines on both graphs indicate where the direction index calculated from the beats would equal the direction index calculated from the gratings. Light grey triangles: grating direction index is significant; dark grey squares: beat direction index is significant; black circles: both grating and beat direction indexes are significant; white diamonds: neither direction index is significant, (see Materials and methods).

gave a measure of the direction sensitivity to a first-order stimulus when the envelope moved in the opposite direction. Domination of the first-order signal by the second-order signal resulted in a reversal in the direction signaled, and thus a negative direction index, this occurred in 11/52 (21%) cells; V1, $n = 3$; V2, $n = 5$; third visual complex, $n = 3$; this negative direction index was significant in 5/11 cells. In the majority of cells 44/52 (85%) movement of the envelope in the opposite direction reduced the direction sensitivity as shown by their position later the dotted line.

On the right of Fig. 5, direction indexes calculated from the response to the 2 f gratings are plotted against direction indexes calculated from the response to the 2 f beats for the 42 cells where the second-order signal significantly influenced the first-order response, tested by the 2 f ANOVA. Histograms of the mean direction indexes for these cells are shown in Fig. 7b. For these cells, the direction index calculated from the beats, plotted on the y-axis, gave a measure of the direction sensitivity to a first-order stimulus when the envelope moved in the same direction. In 22/42 (52%) of the cells movement of the envelope in the same direction reduced the direction sensitivity; for 4/42 (9%) cells (V1, $n = 2$; V2, $n = 2$) the direction signaled was reversed, although this was not significant. For 5/42 (12%) cells movement of an envelope in the same direction as a first-order stimulus significantly improved direction sensitivity (black squares, right side, Fig. 5). The same cells plotted in Fig. 5 are plotted in Fig. 6 to illustrate the comparative direction indexes for each cortical area. Cells that showed a significant influence of the moving envelope on first-order direction sensitivity were found in V1, V2, and the third visual complex.

In order to see the effect of the envelope moving in the same or different direction to the first-order stimulus, for the cells we tested in V1, V2, and the third visual complex where the envelope has a significant influence, mean direction indexes are plotted in Fig. 7. Fig. 7a shows mean direction indexes for a first-order stimulus

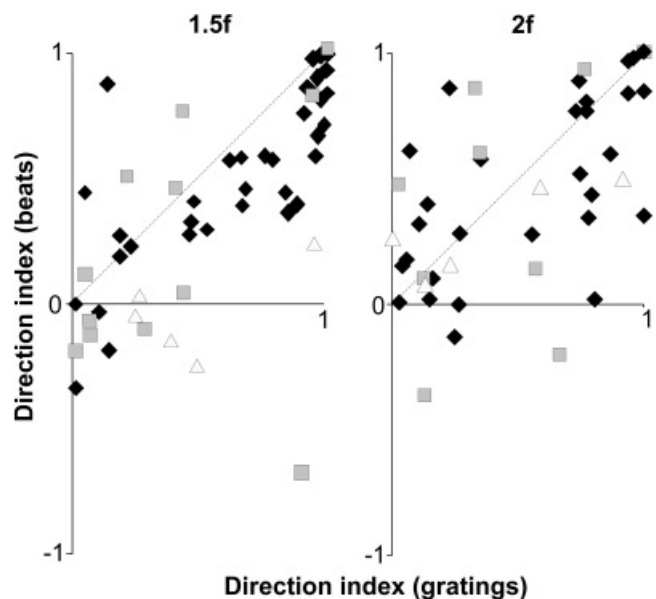


Fig. 6. Direction indexes in cells—coded for cortical area. As in Fig. 5 the same cells are plotted on the same axes. Cells are however coded differently, where black diamonds: V1 cells; grey squares: V2 cells; white triangles: third visual complex cells.

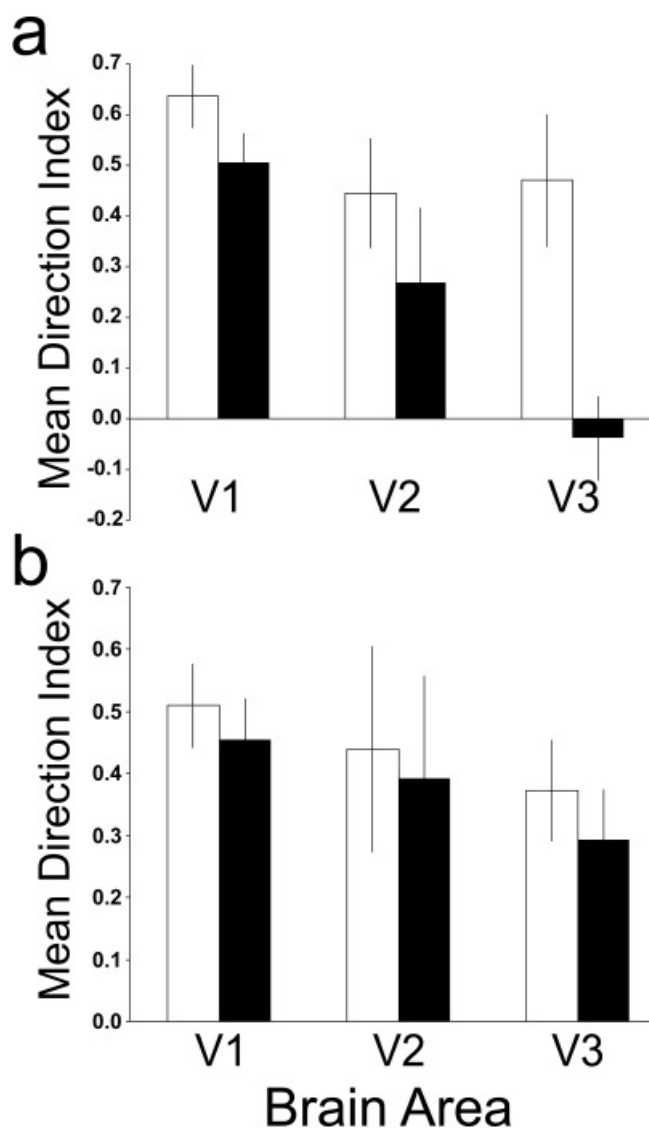


Fig. 7. Mean direction indexes of cells in V1, V2, and the third visual complex to 1.5f stimuli and 2f stimuli. (a) Mean direction indexes ($\pm$ sem) calculated from responses to the 1.5 f gratings (open) and 1.5 f beats (solid) of the 52 cells (V1, $n = 34$; V2, $n = 13$; third visual complex, $n = 5$) where a second-order signal significantly ($P < 0.05$) influences the first-order response, tested by the 1.5 f ANOVA. (b) Mean direction indexes ($\pm$ sem) calculated from responses to the 2 f gratings (open) and 2 f beats (solid) of the 42 cells (V1, $n = 28$; V2, $n = 9$; third visual complex, $n = 5$) where a second-order signal significantly ($P < 0.05$) influences the first-order response, tested by the 2 f ANOVA.

with and without an envelope moving in the opposite direction. When a second-order signal was added in the form of an envelope moving in the opposite direction to the first-order stimulus, the direction sensitivity was significantly reduced in V1 (paired t -test, $t_{[33]} = 3.25$, $P < 0.005$) and our sample of cells in the third visual complex (paired t -test, $t_{[4]} = 4.79$, $P < 0.01$). The direction sensitivity of V2 cells appeared reduced, but this was not significant (paired t -test, $t_{[12]} = 1.33$, $P > 0.05$).

When the envelope moved in the same direction to the first-order stimulus (Fig. 7b), the direction sensitivity appeared reduced

but was not significant in V1 (paired t -test, $t_{[27]} = 0.96$, $P > 0.05$), V2 (paired t -test, $t_{[9]} = 0.3$, $P > 0.05$) or our sample of cells in the third visual complex (paired t -test, $t_{[4]} = 0.75$, $P > 0.05$).

Neurons were recorded in the supragranular, granular, and infragranular layers of V1 in 7 monkeys, the supragranular, granular, and infragranular layers of V2 in three monkeys and from the infragranular layers of the third visual complex in two monkeys; neurons were recorded from nine monkeys in total. The third visual complex is likely to consist of the medial area DM and the lateral area VLP, a marmoset homologue of area V3 seen in other mammals (Rosa & Manger, 2005). There is, however, still some controversy regarding the delineation of this region of cortex (see Lyon & Kaas, 2001; Rosa et al., 2005). Our recording sites in the third visual complex are likely to be within the area VLP, because they were made more than 5 mm from the midline (more lateral than area DM) and all receptive fields covered the lower visual quadrant. Cells showing a significant influence of a second-order signal on first-order motion processing were found within all sampled layers. The receptive fields of the recorded cells were all parafoveal; mean eccentricity of sampled V1 cells was 3.47 deg (sem ± 0.26), V2 cells 4.45 (sem ± 0.79), and sampled third visual complex cells 8.46 (sem ± 0.66).

Discussion

We measured the direction sensitivity of neurons in V1, V2, and the third visual complex with first-order stimuli and with combined first- and second-order stimuli. Our principle findings were (1) in the majority of neurons tested in V1 (73%), V2 (70%) and the third visual complex (75%) first-order motion responses were influenced by the addition of a second-order signal; (2) in each brain area the addition of a second-order signal significantly reduced mean first-order direction sensitivity; and (3) reduction in the first-order direction sensitivity was significant when a contrast-envelope moved in the opposite direction to the first-order stimulus. In cells where the second-order signal had a significant influence, the reduction in first-order direction sensitivity was prevalent in 85% of cells when the movement of the envelope was in the opposite direction to the first-order signal.

Our results are consistent with an early investigation of macaque V1 and cat area 17 neural responses to contrast-defined motion (Albrecht & De Valois, 1981). Albrecht and De Valois, however, assumed that the two species' visual systems were equivalent and thus the data from both animal types were combined. It is therefore difficult to determine any differences that may exist between the cat and macaque systems' performance. Albrecht and De Valois (1981) found that cells in V1 and area 17 did not show excitatory responses to moving contrast-defined patterns, but such patterns interfered with the processing of first-order motion, reducing the first-order response. Consistent with these results, we found that V1 direction sensitivity was significantly reduced by the addition of a second-order signal.

Our results parallel those of Zhou and Baker (1993, 1994) and Mareschal and Baker (1998a, 1998b) who showed that neurons recorded in areas 17 and 18 of cat cortex were sensitive not only to first-order motion but also to second-order motion. There are, however, some important differences between our results and those detailed in the cat studies, which may be partially explained by species difference. It is also important to be careful when comparing the cat and primate physiology data. Cat areas 17 and 18 are roughly equivalent to primate V1 and V2, however cat area 18 receives substantial non-linear input from Y-cells in the lateral

geniculate nucleus (LGN) (LeVay & Gilbert, 1976; Lee et al., 1998). The primate LGN contains very few Y-like neurons (Derrington & Lennie, 1984), and there is also very little direct input from the LGN to extrastriate cortex (Kaske et al., 1991; Levitt et al., 1996). Thus there are at least two routes for subcortical visual input to reach cat cortex directly, whereas primate cortex receives the majority of its subcortical input into V1.

In agreement with studies of cat cortical responses (Zhou & Baker, 1993, 1994; Mareschal & Baker, 1998a, 1998b), we found that cells in striate and extrastriate cortex are responsive to first-order motion are influenced by second-order motion. In 39/94 area 17 and area 18 cells tested, Zhou and Baker (1993) found that second-order motion had a significant influence on cell responses. For each cell, however, Zhou and Baker (1993) found that responses to second-order motion were never larger than first-order motion responses. We found that first-order responses could be dominated by second-order signals in 11/52 cells ($V1, n = 3$) when the envelope moved in the opposite direction to the first-order stimulus. Our finding that some V1 cells respond to second-order stimuli is consistent with previous studies showing that V1 cells will respond to movement of stimuli defined by cues other than luminance (Chaudhuri & Albright, 1997; Bourne et al., 2002, 2004).

The major difference between our results and the previous studies in the cat lies in our higher proportion (73%) of cells in V1 significantly *influenced* by second-order signals. It is difficult, however, to compare this proportion with these previous studies in the cat because of significant differences between Zhou and Baker's (1993, 1994) and Mareschal and Baker's (1998a, 1998b) experiments and ours. First, the carrier in our beat patterns fell within the tested cells' spatial band-passes, thus our recorded cells were responding to combined first-order and second-order signals. Zhou and Baker's (1993) second-order motion signal was an amplitude modulation of a stationary carrier grating. In order to measure responses to a moving contrast-modulation, the first-order components of their pattern did not fall within the spatial bandpass of their recorded neurons. Zhou and Baker's unpublished observations, suggesting that moving carriers increased neuronal responses to second-order motion, might explain some of our recorded cells' sensitivity to second-order signals. Second, we did not optimize the frequency of the envelope in our stimulus and did not measure the response profile of marmoset cortical neurons to moving envelopes. It is therefore conceivable that there is an even greater influence of second-order signals on first-order processing in marmoset V1, V2, and third visual complex neurons than observed here. Third, unlike the previous studies of cat cortical responses to second-order stimuli alone, our stimulus was designed to test the *influence* of a second-order signal on first-order direction sensitivity. Zhou and Baker (1993, 1994) found that the majority of cells in area 18 sensitive to first-order motion were also sensitive to the movement of a contrast-defined stimulus; a minority of first-order sensitive cells in area 17 also responded to contrast-defined motion. Our results show, similarly across all cortical areas tested, that a higher proportion of first-order motion responses (V1, 73%; V2, 70%; third visual complex, 75%) were influenced by second-order signals. We attribute the differences between our results and previous studies to the sensitivity of our testing procedure and differences between the primate and cat visual systems.

Two processing streams or one?

The results described here do not appear to fit with models suggesting two separate processing streams for first-order motion

and second-order motion (Wilson et al., 1992; Vaina & Cowey, 1996; Clifford & Vaina, 1999). Wilson et al.'s (1992) model predicts that second-order motion is processed in V2 separately from first-order motion processing in V1. We found that processing of first-order motion in V1, V2, and the third visual complex was influenced by second-order signals. These results suggest that there is no separate processing stream for first-order motion, and that first-order motion and second-order motion are processed together in all these early visual areas.

Models that argue for a separate processing stream for second-order motion propose that there is a narrowly tuned pre-filter situated in V1 (Wilson et al., 1992; Chubb & Sperling, 1988; Zhou & Baker, 1993; Mareschal & Baker, 1998a). First, we find cells in infragranular, granular, and supragranular layers of V1 whose first-order response is influenced by second-order signals. Finding such cells so early on in visual cortex suggests that any pre-filtering might be performed subcortically. Second, narrowly tuned pre-filtering would render neurons that respond to second-order motion incapable of responding to first-order motion at similar frequencies. Such neurons have still to be found in either cat or primates. Both our methodology and those of other physiological studies (e.g., Zhou & Baker, 1993; Mareschal & Baker, 1998a) search for cells using first-order stimuli and are therefore unsuitable for finding cells responsive only to second-order motion. Thus, we cannot be certain that pure second-order selective cells do not exist in visual cortex. The results described here show that moving envelopes of spatial frequency 0.5 f/2 influence neurons that respond to first-order motion at 1.5 f and 2 f. This suggests that pre-filtering in these cells is broad enough to allow first- and second-order signals through, inconsistent with the proposal in some models of motion processing of a narrowly tuned pre-filter within a separate second-order system (e.g., Wilson et al., 1992; Chubb & Sperling, 1988).

A prediction of broad pre-filtering would be that neurons that are direction selective for first-order motion would also be direction selective for second-order motion. If the first- and second-order signals opposed (as in our 1.5 f beat patterns), then a cell sensitive to both signals, would not register a coherent direction of movement. The response to a first-order signal in the preferred direction would be reduced, and in the non-preferred direction would be increased, by the second-order signal in the opposite direction. We would therefore predict that the measured first-order direction sensitivity would thus be reduced; this was seen in most of our tested cells. Although not directly measured here, in principle, similar cells might show second-order direction sensitivity influenced by the addition of a first-order signal of similar frequency moving in the opposite direction. Johnston and Clifford (1995) found that an oppositely moving carrier reduced the perception of the speed of the moving envelope in an amplitude-modulated grating. They show that their multi-channel gradient model (Johnston et al., 1992) consisting of a single motion processing mechanism can better explain this effect than models proposing two or more processing systems with narrow pre-filtering stages (e.g., Chubb & Sperling, 1988; Wilson et al., 1992). In our cells where movement of the envelope in the opposite direction significantly influences the first-order response, it is to reduce the direction sensitivity in 85% of those cells, consistent with similar direction selectivity for first- and second-order motion, a broad pre-filtering stage and multi-channel gradient models proposing one motion-processing pathway (e.g., Johnston et al., 1992). Previous studies, for example Zhou and Baker (1996) and Mareschal and Baker (1998a), did not see an interaction between

the directions of first- and second-order signals. Their stimuli, however, were deliberately designed to position the carrier frequency outside the cells' luminance spatial-frequency ranges, and thus the carrier signal would have been filtered out.

The influence of second-order motion over first-order motion processing in all recorded layers of V1, V2, and the third visual complex suggests that there is not a formal structure for processing second-order motion. Rather in the majority of neurons in striate and early extrastriate cortex second-order motion might be processed to differing extents. Differences seen in the human visual system's behavior when presented with first- and second-order motion could be because of differences in neuronal tuning to first- and second-order motion, for example see Zhou and Baker (1996). Second-order signals rarely occur in natural scenes unaccompanied by first-order signals. It would therefore seem unlikely that a cortical stream devoted to second-order motion would have evolved. Our results reported here show that in cells in V1, V2, and the third visual complex, both types of motion are processed together; this may be more efficient and is consistent with models proposing one motion pathway (Johnston et al., 1992; Taub et al., 1997).

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