

# Temporal response properties to second-order visual stimuli in the LGN of cats

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**Visual stimuli occurring naturally are rich in instances of objects delineated from the backgrounds only by differences in luminance, which is called first-order stimuli, as well as those defined by differences of contrast or texture, referred to as second-order stimuli. The neuronal mechanism for processing second-order stimuli is still unclear. In this study, we compared the responses of cat LGN (lateral geniculate nucleus) cells to second-order stimuli at five temporal frequencies to their responses to first-order stimuli. Our results showed that most LGN cells can be evoked by second-order stimuli, and their firing rates to second-order stimuli decreased relative to first-order stimuli as temporal frequency increased from 0.5 to 8 Hz; moreover the ratio of a nonlinear to linear factor had a higher value in the responses to second-order stimuli than to first-order stimuli. We also found that the responses of Y-cells to second-order stimuli were significantly higher than the responses of X-cells, suggesting the Y-cells have a more important role in the processing of second-order stimuli. All these results reveal that first-order and second-order signals might be processed in separate 'streams' of the visual system.**

first-order stimuli, second-order stimuli, temporal frequency, FFT, X/Y cell, LGN, cat

The perception of visual signals can arise from displacements in luminance (first-order stimuli) or from displacements in other physical features of an image, such as its contrast or texture, that are visible even when there is no change in mean luminance (second-order stimuli)<sup>[1]</sup>. First-order stimuli are widely used in vision research and the processing of these stimuli is usually thought to operate with a linear visual system, but this linear visual system cannot explain the visual perception of the second-order stimuli. Whether the processing of second-order stimuli involves neuronal mechanisms that are different from the processing of first-order stimuli is currently a focus of vision research. Many studies have been performed to answer this question.

Baker et al.<sup>[2–6]</sup> found single neurons in cat areas 17 and 18 that responded to both luminance and contrast-defined drifting periodically modulated stimuli. For a given neuron, the preferred contrast envelope of spatial

or temporal frequency was similar to, but often significantly lower than, the optimal spatial or temporal frequency for luminance gratings. In areas V1 (17) and V2 (18) of rhesus monkeys and cats, Leventhal et al.<sup>[7]</sup> found single neurons with consistent direction selectivity to bars defined either by luminance, or by texture on a gray background of the same luminance. There are also studies reporting that the neurons in primate area MT/V5 can respond to second-order stimuli; the response intensity to second-order stimuli are weaker than that of first-order stimuli for the same neurons, and the number of neurons responding to the second-order stimuli is also smaller than that to the first-order stimuli<sup>[8]</sup>. These studies show that responses to first-order and second-order stimuli have some different properties, and might be due to different processing mechanisms.

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In the psychophysics field, Henning et al.<sup>[9]</sup> first introduced contrast envelope stimuli in 1975, arguing that their detection could not be accounted for in terms of linear filter schemes. Some subsequent studies showed that adaptation to first-order or second-order stimuli selectively affects thresholds only for target stimuli of the same type, but that cross-adaptation is absent<sup>[10]</sup>, and that subthreshold facilitation is similarly specific to the type of stimulus<sup>[11]</sup>. But some other studies revealed that the spatial and temporal tuning functions were similar for first- and second-order stimuli detection tests. Whether the first-order and second-order signals are processed by different visual streams remains controversial.

Recently some researchers found out that retinal ganglion cells do respond to the spatiotemporal contrast modulations of the second-order motion stimuli<sup>[12]</sup>. This means the operation of second-order rectification might occur in the retina, prior to the ganglion cells, and the subcortex also plays an important role in the processing of the second-order signals. The LGN (lateral geniculate nucleus) is an important part of the visual subcortex, and should be involved in this processing. In this study, we recorded the responses of cat LGN cells to contrast modulated second-order stimuli at five temporal frequencies. Our results suggest the LGN cells have different temporal response properties to first-order and second-order visual stimuli.

## 1 Method

### 1.1 Animal preparation

Six normal adult cats (2.4–3.5 kg) were used in our experiment. The detailed methods for general preparation have been described in earlier publications<sup>[13]</sup>. Animals were initially anaesthetized with an intramuscular injection of ketamine (20 mg/kg), and then placed in a stereotaxic apparatus, with intravenous and tracheal cannula inserted. During the rest of the experiment, light anaesthesia was maintained by intravenous urethane infusion of 20 mg·kg<sup>-1</sup>·h<sup>-1</sup>, and the Gallamine triethiodide (10 mg·kg<sup>-1</sup>·h<sup>-1</sup>) was infused for paralysis. All the animals were artificially ventilated. The animal's body temperature, heart rate, EKG and end-tidal CO<sub>2</sub> were routinely monitored and kept within normal limits.

### 1.2 Visual stimuli and electrophysiological recording

All visual stimuli were programmed in Matlab with

Psychtoolbox extensions, generated in a P4 2.8 G PC, and displayed on 17" in Philips 107p monitor. The mean background luminance was set at 19 cd/m<sup>2</sup>, and the luminance GAMMA calibration was performed. The screen resolution was 1024 × 768 pixels with a frame rate of 85 Hz, and viewing distance was set at 57 cm (1 cm on screen equals to 1° of visual angle). The visual stimuli were first-order or second-order drifting grating stimuli, extending 3.0 visual degrees on the screen, demonstrated in Figure 1(a) and (c).

The first-order stimuli were sinusoidal luminance modulated gratings with additional static noise (same as the second-order stimuli noise carrier). The static noise was a 1-bit, spatially 2-d, random noise generated by assigning individual (single) screen pixels to be either 'white' or 'black' with equal probability to ensure that there was no spatial variation in luminance within each noise element. The luminance profile of the first-order stimulus is

$$I(x, y) = I_0 \times (1 + C_c \times F_c(x) + C_m \times \sin(2\pi (f_m x \pm \omega t) + \theta)), \quad (1)$$

where  $I_0$  is the mean luminance,  $F_c(x)$  and  $C_c$  are the function and contrast (0.5) of the static noise,  $C_m$  and  $f_m$  are the contrast (0.5) and spatial frequency of first-order signal.  $\omega$  is the temporal frequency, and  $\theta$  is the initial spatial phase. In Figure 1(a), the first-order motion stimuli are schematically shown.

The second-order stimulus was generated by the carrier contrast modulation, which consisted of the static noise carrier as described above whose contrast was modulated by the sinusoidal signal envelope. The schematic illustration is shown in Figure 1(c). And the luminance profile of the second-order stimulus is

$$I(x, y) = I_0 \times (1 + C_c \times F_c(x) \times (1 + C_m \times \sin(2\pi (f_m x \pm \omega t) + \theta))), \quad (2)$$

where  $I_0$  is mean luminance,  $C_c$  is contrast (0.5) of carrier and  $F_c(x)$  represents the carrier function,  $C_m$  and  $f_m$  are the modulation depth (0.5) and spatial frequency of envelop signal,  $\omega$  is the temporal frequency of motion, and  $\theta$  is the initial spatial phase.

For each LGN cell, we recorded response properties to both first-order and second-order stimuli at 5 temporal frequencies of 0.5, 1, 2, 4 and 8 Hz. The optimal spatial frequency of first-order stimuli was adopted as the spatial frequency to both the first-order and the second-order stimuli for each cell. The first-order and second-order stimuli lasted 10 cycles, and repeated 3 times

with intervals of 10–15 s. The sequence of temporal frequencies were randomized for first-order and second-order stimuli.

Extracellular recording technique was applied in this study. Glass-insulated tungsten electrodes were used, with impedances from 2 to 20 M $\Omega$ . Action potentials were amplified by an extracellular recording amplifier (Dagan 2400) and a preamplifier (KDS-1). Cells were determined to be X or Y type using methods reported previously<sup>[13]</sup>.

### 1.3 Data collection and analysis

Times of action potentials were collected by a computer with the Data Acquisition Device (NiDAQ PCI-6024E) and analyzed using software Igor 5.0. The sampling interval was set at 100  $\mu$ s.

The post-stimulus time histograms (PSTHs) of neuronal responses to the visual stimulus were compiled with a binwidth of 10 ms and a firing rate unit of Spikes/s. We integrated the PSTHs to obtain Mean, Peak, FFT1 (fast fourier transform), FFT2 and FFT2/FFT1 ratio of firing rate as a function of the temporal frequency. For each cell, a normalization was performed by dividing all firing rates by the firing rate response to a given temporal frequency of first-order stimuli, with 8 Hz for Mean, 4 Hz for Peak, and the response amplitude of FFT1 and FFT2 both normalized to the FFT1 value of

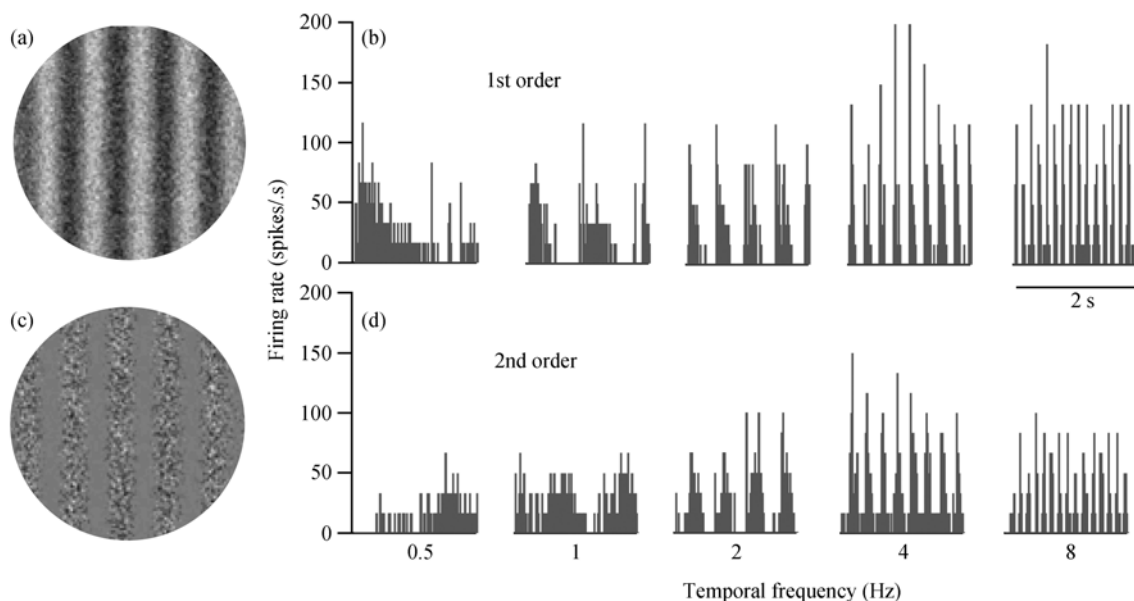
first-order stimuli at 8 Hz. The t-test and the within-subject ANOVA were used for statistical analysis, and the two factors in ANOVA were stimulus type (first-order and second-order) and temporal frequency.

## 2 Results

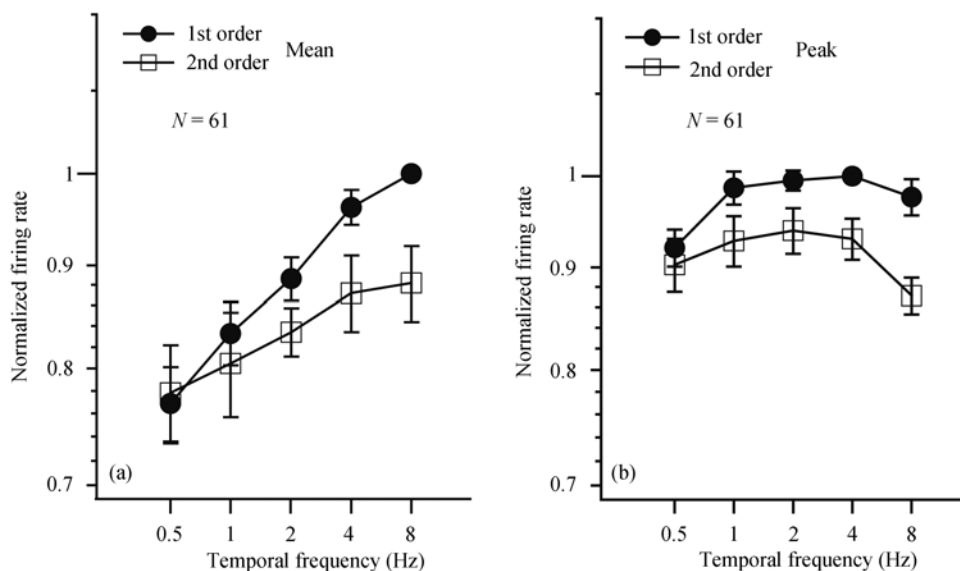
In our experiment, responses of 61 LGN cells were recorded, and most of them responded to both first-order and second-order grating stimuli. The PSTHs in Figure 1(b) and (d) illustrated the responses of a simple type cell to first-order and second-order stimuli. This LGN cell showed significantly modulated responses to second-order stimuli, whose strength changed with the temporal frequency. However, the responses to second-order stimuli were significantly lower than those to first-order stimuli.

### 2.1 Mean and peak firing rate to the first-order and second-order stimuli

The normalized temporal frequency tuning curves of Mean firing rate to first-order and second-order stimuli are shown in Figure 2(a). As the temporal frequency increased from 0.5 to 8 Hz, the difference between the responses of LGN neurons to second-order stimuli and first-order stimuli increased. The Mean spike frequencies for the two types of stimuli are significantly differ-



**Figure 1** Demonstration of layout and cell's response to the first-order and second-order stimuli. (a) An example of a first-order stimulus, a luminance-defined grating with added static random noise; (c) an example of a second-order stimulus, an envelope grating with modulated carrier contrast. (b) and (d) show the post-stimulus time histograms (PSTH) for a single LGN cell to the first-order and second-order stimuli at 5 different temporal frequencies.



**Figure 2** The normalized temporal tuning curves for the first-order stimuli (solid circle) and second-order stimuli (open square). (a) shows the Mean response and (b) the Peak response. Error bars represents SEs of the average response.

ent ( $F(1,60) = 7.54, P < 0.01$ ). Significant interaction of temporal frequency and stimulus type (first-order and second-order) was found in the ANOVA, and different temporal properties between the two types of stimuli were also revealed ( $F(4,240) = 21.8, P < 0.01$ ). The monotonic increase in Mean firing rate, as Figure 2(a) shows, suggested that the optimal temporal frequency was higher than 8 Hz for both the first-order or second-order stimuli. And the optimal temporal frequency to first-order stimuli was probably even higher than to the second-order stimuli, as the result of Peak firing rate shows in Figure 2(b).

The normalized temporal response tuning curve of Peak firing rate for the first-order and second-order stimuli are plotted in Figure 2(b). The Peak firing rate of second-order stimuli are significantly lower than that of first-order stimuli ( $F(1,60) = 6.80, P < 0.02$ ). And, similar to the mean values, the difference between the Peak response to first-order stimuli and second-order stimuli increased as the temporal frequency increased ( $F(4,240) = 3.16, P < 0.05$ ). The optimal temporal frequency was around 4 Hz for first-order stimuli, and 2 Hz for second-order stimuli. The significant difference between the responses to the two types of stimuli suggests different pathways for processing the two types of stimuli in LGN.

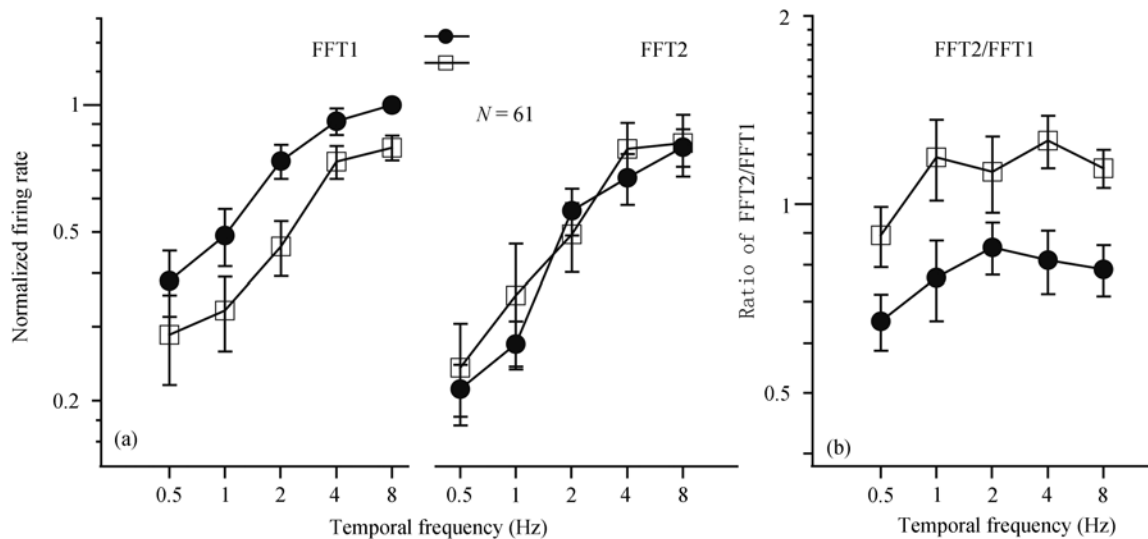
## 2.2 FFT1, FFT2 and FFT1/FFT2 ratio of firing rate to the first-order and second-order stimuli

The FFT analysis showed that the FFT1 values of firing

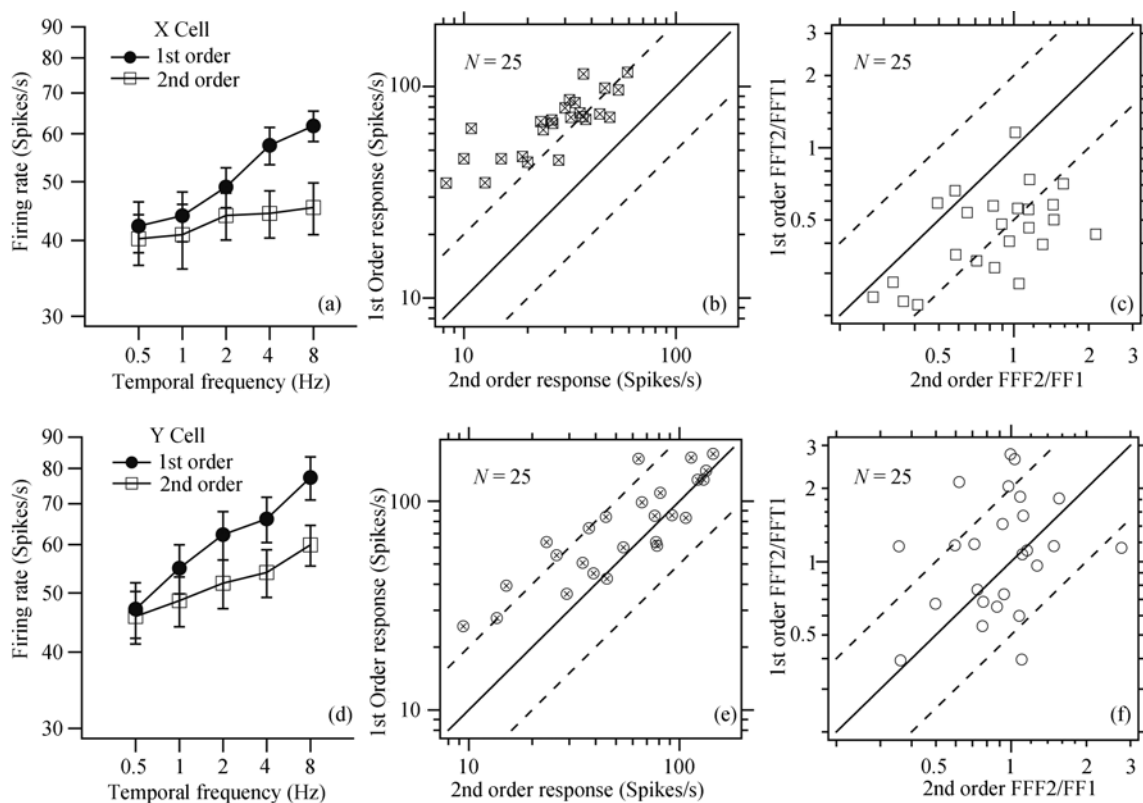
rate to the second-order stimuli were significantly lower than those to first-order stimuli at all five temporal frequencies ( $F(1,60) = 9.57, P < 0.01$ ), with the average difference of 28% (Figure 3(a)). The FFT2 values of firing rate to the second-order stimuli were close to those to the first-order stimuli. No significant difference was observed between them ( $F(1,60) = 1.14, P > 0.2$ ). The FFT1 value represented the linear factor of the response to stimuli, and the FFT2 value represented the nonlinear factor. Consequently, the FFT2/FFT1 ratio reveals the ratio of nonlinear to linear factors. As Figure 3(b) shows, the average ratios of FFT2/FFT1 were 0.80 and 1.1 for the first-order and second-order stimuli, respectively. The ratio for the second-order stimuli was significantly higher than that for the first-order ( $F(1,60) = 6.06, P < 0.02$ ). This result shows that in LGN cells, the responses evoked by the second-order stimuli contain larger proportions of nonlinear components than those evoked by first-order stimuli, which might be accounted for by the higher complexity of information contained in the second-order stimuli. This result also suggests that the first-order and second-order signals might be encoded differently in the LGN of cats.

## 2.3 Response properties of X/Y cells to the first-order and second-order stimuli

Identification of X/Y types was performed on 50 of the 61 LGN cells we recorded, and the analysis shows that there were 25 X-cells and 25 Y-cells. Figure 4(a) plots the temporal frequency tuning curves of the mean re-



**Figure 3** Proportions of FFT1 and FFT2 for the first-order and second-order stimuli in LGN cells. (a) Normalized average FFT1 values (left) and average FFT2 values (right) for the first-order and second-order stimuli at 5 temporal frequencies; (b) normalized average FFT2/FFT1 ratios for the first-order and second-order stimuli at 5 temporal frequencies. The responses to the first-order stimuli are represented by solid circles; the responses to the second-order stimuli are represented by open squares. Error bars represent  $\pm$  SD error.



**Figure 4** Response properties of X-cells (a)–(c) and Y-cells (d)–(f) to the first- and second-order stimuli. (a) and (d) Temporal frequency tuning curves to the two types of stimuli for a single X- and Y-cell; (b) and (e) distribution of optimal response strength to the first-order stimuli versus second-order stimuli for the 25 X-cells and 25 Y-cells. Solid line depicts a unity ratio of response strength to the first-order and second-order stimuli, and dashed lines depict the double response strength (upper) and one-half response strength (lower) ratio. (c) and (f) The distribution of average FFT2/FFT1 ratio for X-cells and Y-cells to the two types of stimuli across all 5 temporal frequencies.

responses to the first-order and second-order stimuli for a single X-cell; Figure 4(d) plots a similar curve for a single Y-cell. As the two figures indicate, X- and Y-cells

show a similar response pattern in which the responses to the second-order stimuli were lower than to the first-order stimuli, and this difference became increas-

ingly greater as the temporal frequency increased. These figures also show that the responses of X-cells to second-order stimuli were poorly tuned, and that at high temporal frequencies, the differences between the responses to the first-order and second-order stimuli of X-cells were greater than those of Y-cells.

The distribution of response strength to first-order stimuli versus second-order stimuli for X-cells is shown in Figure 4(b), with each data point corresponding to a given LGN neuron's optimal response to the two types of stimuli. Figure 4(e) illustrates a similar distribution of responses for Y-cells. In Figure 4(b) and (e), the solid lines depict the equality ratio when the responses for the two types of stimuli have the same strength, and the dashed lines depict the ratio when the strength of responses to first-order stimuli is double or one-half of the strength of responses to second-order stimuli. As shown in Figure 4(b), the strength of responses of X-cells to first-order stimuli was significantly greater than to second-order stimuli ( $T(24) = 14.4$ ,  $P < 0.01$ ), in which 19 out of the 25 X-cells showed higher responses to first-order stimuli than the double strength of responses to second-order stimuli. Figure 4(e) shows that the response strength of Y-cells to first-order stimuli were also greater than to second-order stimuli ( $T(24) = 3.29$ ,  $P < 0.01$ ), but not so much as in X-cells, with only 6 out of the 25 Y-cells having higher responses to first-order stimuli than the double strength of responses to second-order stimuli. From the two figures, we learn that the responses of X-cells to first-order stimuli were comparable to the response of Y-cells ( $T(48) = 1.38$ ,  $P > 0.1$ ). But the responses of X-cells to second-order stimuli were significantly lower than the responses of Y-cells ( $T(48) = 4.23$ ,  $P < 0.01$ ).

Figure 4(c) and (f) plot the values of FFT2/FFT1 ratio to first-order stimuli versus second-order stimuli for X-cells and Y-cells, where each data point represents the average FFT2/FFT1 ratio for one LGN cell to both first-order and second-order stimuli across all the 5 temporal frequencies. Figure 4(c) shows that the values of the FFT2/FFT1 ratio (0.47 on average) in X-cells' responses to first-order stimuli were significantly lower than the values (1.0 on average) to second-order stimuli ( $T(24) = 5.16$ ,  $P < 0.01$ ), with 22 out of 25 X-cells located at the right bottom half of the figure. For Y-cells in Figure 4(f), the values of FFT2/FFT1 ratio in the response to the two types of stimuli had no significant dif-

ference ( $T(24) = 1.49$ ,  $P > 0.1$ ), and the average values were around 1.1 in both cases. From the two figures, we learn that the values of FFT2/FFT1 ratio for first-order stimuli in X-cells were significantly lower than in Y-cells ( $T(48) = 5.44$ ,  $P < 0.01$ ), while the FFT2/FFT1 values for second-order stimuli had no significant difference between X- and Y-cells ( $T(48) = 0.68$ ,  $P > 0.4$ ).

### 3 Discussion

In this study, we compared the temporal frequency properties of responses to first-order and second-order stimuli in LGN of cats, and found that the response of LGN cells can be evoked by second-order stimuli, though significant lower than that evoked by first-order stimuli, and the difference between these two stimulus types became greater as the temporal frequency increased. Our result also showed the ratios of FFT2/FFT1 for second-order stimuli were significantly higher than that for first-order stimuli in LGN cells, which suggests that the responses to second-order stimuli include a higher nonlinear component than the responses to first-order stimuli, and different encodings might exist in these two stimulus types. Hence, the visual system probably processes the two types of signals in different pathways.

It is generally accepted by recent studies that the second-order signals are processed through a 'filter-rectify-filter' sequence in the visual system<sup>[14,15]</sup>. But it remains unknown where the rectifying occurs. Many studies have focused on the rectification of second-order stimuli in the visual cortex. However, Demb et al.<sup>[12]</sup> recorded the response of *in vitro* Y retinal ganglion cells of guinea pig to second-order signals. Their results suggested that the rectification might start in retinal ganglion cells. According to Demb et al.<sup>[12]</sup>, when the contrast of the carrier signal changes, the local luminance varieties are captured by photoreceptors, then these changes of signal are further processed and rectified by excitatory subunits (bipolar cells) and inhibitory subunits (amacrine cells), and these subunits output the signal to the retinal ganglion cells. In this way, the retinal ganglion cells could respond to the change of contrast. Other morphology studies demonstrated that amacrine cells synapse with most rod bipolar and 45% cone bipolar cells. Moreover, the amacrine cells pass information to bipolar cells and ganglion cells through inhibitory synapses. All these studies suggest that processing of nonlinear signals may occur as early as in the retina due to the unique connec-

tion between the retinal cells. Our experiment shows that, as the destination of projection from retinal ganglion cells, LGN cells inherit the responsive properties of retinal ganglion cells to second-order (nonlinear) stimuli. The LGN cells show similar responses as retinal ganglion cells in that LGN cells respond significantly to second-order stimuli, but weaker than to first-order stimuli. These results further indicate the rectification for second-order stimuli might first occur in the retina.

In areas 17 and 18 of cat visual cortex, the optimal temporal frequency and cut-off frequency to second-order stimuli were lower than those to first-order stimuli<sup>[3,5]</sup>, which was consistent with our observation in LGN cells. The temporal response property of LGN cells, in which the optimal temporal frequency to second-order stimuli was lower than to first-order stimuli, might serve as the basis for this response property in visual cortical cells. Nevertheless, the optimal temporal frequency for cortical cells in area 17/18 for second-order stimuli was slightly lower than that for LGN cells, which suggests further processing of second-order stimuli are performed in cortex. All these experiments show that the two types of signals are kept separate throughout the early stages of visual system, and they might be passed through two different pathways from retina to

visual cortex.

By comparing the responses of X-cells and Y-cells in LGN to the two types of signals, we found that Y-cells might play a more important role in transmitting second-order stimuli, because they respond more significantly to this type of stimulus. And the X-cells, with a higher ratio of FFT2/FFT1 for second-order stimuli than for first-order stimuli, might be more sensitive to the carrier which represents the high frequency components of the second-order stimuli; hence, the carrier of second-order stimuli is probably transmitted by X-cells to the visual cortex.

The processing of second-order stimuli is one part of the nonlinear analysis in the visual system. Many researchers have proposed different models in attempt to explain the origin and mechanism of the nonlinearity of the visual system<sup>[6,14,16]</sup>. In our experiment, we measured the responses of LGN cells to second-order stimuli, and provide evidence that the rectification of second-order stimuli occurs in the sub-cortical structures. Our results show that these two types of stimuli are probably processed by two separate ‘streams’ or mechanisms.

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