Influence of prolonged optic flow stimuli on spontaneous activities of cat PMLS neurons

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Abstract **Changes in neuronal spontaneous activities after prolonged optic flow stimulation (using the three basic flow modes: translation, radiation and rotation) were investigated by extracellular single-unit recording in cortical area PMLS of the cat. The results showed that the evoked responses decreased with the prolongation of visual stimuli, and the spontaneous activities usually dropped to a lower level after the stimuli were withdrawn. Generally, the reduction in spontaneous activities was larger after adaptation in the preferred direction than in the non-preferred direction. This difference was much pronounced to translation stimuli, but relatively insignificant to radiation and rotation. These points suggest that non-specific fatigue may act as the key factor in adaptation to simple translation, while some kinds of more complicated, direction-specific mechanism may be involved in adaptation to the complex optic flow patterns. In addition, PMLS may play an important role in perception and adaptation to complex motion and the relevant motion after-effects.**

Continuous viewing of an object moving in a certain direction produces an illusion that the following stationary patterns appear to drift in the opposite direction. This phenomenon is usually termed motion after-effect (MAE)[1]. Sutherland[2] proposed that MAE could be generated upon the fatigue of direction-sensitive neurons in visual cortex, which won supports from a number of neurophysiological experiments. Barlow and Hill[3] found that the spontaneous activities of rabbit retinal ganglion cells decreased significantly after prolonged stimulation in the preferred direction, but changed very little after stimulation in the non-preferred direction. Therefore they suggested that the relative variations in neuronal spontaneous activities may serve as the physiological basis for MAE. Similar results were obtained in subsequent studies on the primary visual cortex of cat and monkey[4], but little has been done to check the variations in extrastriate cortical areas. It should be noted that some cortical cells displayed direction-specific adaptation in the experiments[4—6], indicating that

MAE could not be totally attributed to the fatigue of neurons[1].

On the other hand, many psychophysical experiments found that extrastriate cortex (e.g. area MT) is closely involved in the perception of MAE[7,8], and that MAEs caused by translation stimuli are different from those caused by radiation/rotation[9,10]. However, the relevant neurophysiological studies usually took moving bars, gratings or textures as stimuli (translation), but rarely the complex optic flow patterns (such as radiation and rotation) which have been widely used in psychophysics and brain imaging. It remains unclear whether there is any difference at neuronal level concerning the effects of different motion modes, which may lead to the above-mentioned perceptual phenomena. Moreover, previous studies indicated that adaptation to motion could occur at multiple sites in cortex, while the underlying information processing mechanism and the function of each cortical area still await further investigation[1].

In the posteromedial lateral suprasylvian area (PMLS) of the cat, most neurons were responsive to optic flow stimuli and somewhat sensitive to the mode and direction of flow motion[11]. PMLS is commonly considered as functionally analogous to primate MT[12] and thus a likely candidate subserving the generation and perception of MAE. In the present study, stimulus patterns simulating the three basic optic flow modes (translation, radiation and rotation) were employed to investigate the changes in spontaneous activities of PMLS neurons after prolonged stimulation. The effects of adaptation to different flow modes and different moving directions (preferred and non-preferred) were compared to explore the mechanism for MAE formation and the role of extrastriate cortex.

1 Materials and methods

Experiments were carried out on 8 normal adult cats (weighing 2.5—4.0 kg). The general procedures for animal preparation, cellular recording and data collection have been described in an earlier report[13]. The visual stimuli consisted of translation, radiation (expansion or contraction) and rotation (clockwise or counterclockwise) (the same as in ref. [11]). When a single unit was isolated and its receptive field had been qualitatively plotted, a series of optic flow stimuli were employed to determine the cell's preferred flow mode and moving direction. Then the effects of adaptation on neuronal spontaneous activities were tested with the prolonged stimulation, with one or two flow modes (translation and/or complex optic flow, in later case either radiation or rotation) which could elicit at least fair responses of the cells. Each sweep was composed of three continuous segments of data collection (30 s for each segment): ⅰ) No stimulation (blank screen), the spontaneous activity was recorded as control; ⅱ) the adapting stimulus was presented to record the evoked responses of the cell; and ⅲ) the stimulus was

removed, the change of the spontaneous activity was recorded. The adapting stimuli moved in either the preferred direction (P) or the opposite (non-preferred direction, N). The series of tests in preferred and non-preferred directions were pseudorandomly interleaved, each of them was presented 2—5 trials for averaging data.

2 Results

A total of 104 PMLS neurons were tested for the adaptation and the changes in their spontaneous activities, of which 65 cells with translation stimuli, 82 cells with complex optic flow patterns (55 cells with radiation since they responded better to this mode, the other 27 cells with rotation). The cells were distributed in all layers of the cortex, without any obvious relationship between the density and the depth. Neighboring neurons, however, tended to present similar response properties. Two representative cells are shown in Fig. 1. For almost all the cells, the firing rate raised abruptly when the cells were exposed to stimulus moving in the preferred direction, but declined steadily with the prolongation of the stimulus, and reached a relatively stable plateau in several to over 10 s. Upon

withdrawal of the adaptation stimulus, the neuronal activities fell down rapidly from the plateau. For most cells (over 70%) the firing rate went below the original spontaneous level, some neurons even stopped firing to show a strong after-effect induced by adaptation. Afterward the spontaneous activities recovered slowly. Similar phenomenon was also observed with stimuli moving in non-preferred direction, though both the adaptation and the after-effect were relatively weaker, mainly due to the lack of good responses in that condition. In comparison with the data obtained after adaptation in the non-preferred direction, adaptation in the preferred direction normally resulted in larger decrement in the neuronal spontaneous activities and slower recovery. In addition, these differences in motion after-effects were dependent on the mode of optic flow stimuli used in the experiments (see Fig. 1), therefore they were subjected to further analysis.

First, the mean spontaneous activities of PMLS neurons after adaptation in the preferred and non-preferred direction (S_P and S_N) were compared for each flow mode. Since the recovery of spontaneous activities was usually
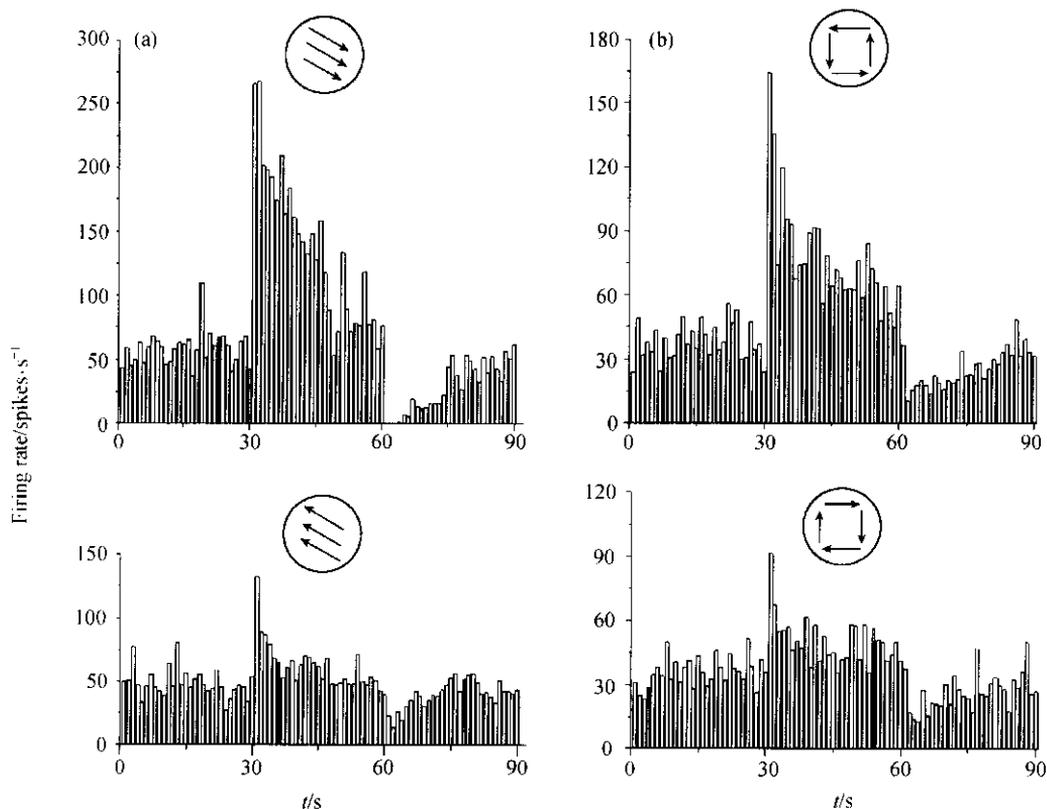

Fig. 1. Adaptation of two PMLS neurons to prolonged optic flow stimuli (the period of 30—60 s) and their spontaneous activities before and after the adaptation. (a) Stimulated with translation; (b) stimulated with rotation. The direction of stimulation is shown with arrows in the circle above the histogram, and the upper and lower histograms correspond to the test in the preferred and non-preferred directions, respectively. It should be noted that, in comparison with cell (b), cell (a) displayed more obvious difference between its spontaneous activities after adaptation in the preferred and non-preferred directions.

quite slow and somewhat fluctuating, and in order to get   rid of the potential OFF-responses elicited by the sudden

withdrawal of stimuli, the activities during 0.5—5.0 s after adaptation was taken for averaging. Moreover, the period of 25.0—30.0 s after adaptation was also analyzed to check the recovery of spontaneous activities. The results showed that (see Fig. 2(a)), in the initial phase after adaptation to translation, the mean spontaneous level S_P was significantly lower than S_N (Wilcoxon test, $P < 0.00001$); but for radiation and rotation, S_P was only slightly lower than S_N ($P > 0.05$). In other words, translation in the preferred direction produced stronger after-effects and induced larger decrement in the neuronal spontaneous activities, in respect to that in the non-preferred direction; while the direction of radiation or rotation did not make such a clear difference. With the time passed by, the after-effects went down with the spontaneous activities recovered gradually, so that the differences between S_P and S_N almost disappeared in the later phase of the test (see Fig. 2(b)).

For a better clarification of the differences induced by different optic flow modes, the relative strengths of spontaneous activities (S_P/S_C and S_N/S_C) and an index quantifying the difference of adaptation in opposite directions ($DA = S_N/S_C - S_P/S_C$) were calculated for each cell, and the statistics on the whole sample are given in Table 1. A higher DA value indicates a higher spontaneous level after adaptation in the non-preferred direction, in comparison with the level after adaptation in the preferred direction.

It can be seen in Table 1 that, after adaptation to translation in the preferred direction, the neuronal spontaneous

neous activities decreased obviously during the first 5 s in respect to the control (prior to the adaptation), and the decrement was larger than that induced by radiation or rotation. On the contrary, when the adaptation stimulus was conducted in the non-preferred direction, the relative reduction of spontaneous activities induced by translation was smaller than that by radiation or rotation. These differences tended to disappear after a recovery period of over 20 s. As a result, in the initial phase after adaptation, the mean value of DA for translation was significantly above zero and larger than that for complex optic flow patterns (radiation and rotation) (U-test, $P < 0.01$). As the time passed by, all the mean DA values dropped close to zero, with the spontaneous activities recovered nearly to the control level regardless the flow mode and direction of the stimulation (see Fig. 3).

Since the differences between adaptation in the preferred and non-preferred directions might be related to the degree of direction selectivity, and previous study has found that PMLS neurons had higher direction indices (DI) to translation than to radiation and rotation[11], a further analysis on this issue turned out to be necessary. The results showed that the positive correlation between DI and DA was significant for translation ($r = 0.479, P < 0.00001$; i.e. the stronger the direction selectivity of a cell, the larger the difference between adaptation in the two opposite directions), but insignificant for radiation and rotation ($r = 0.173, P > 0.1$) though the neurons displayed fair direction selectivity to the stimuli. The mean DA value was only
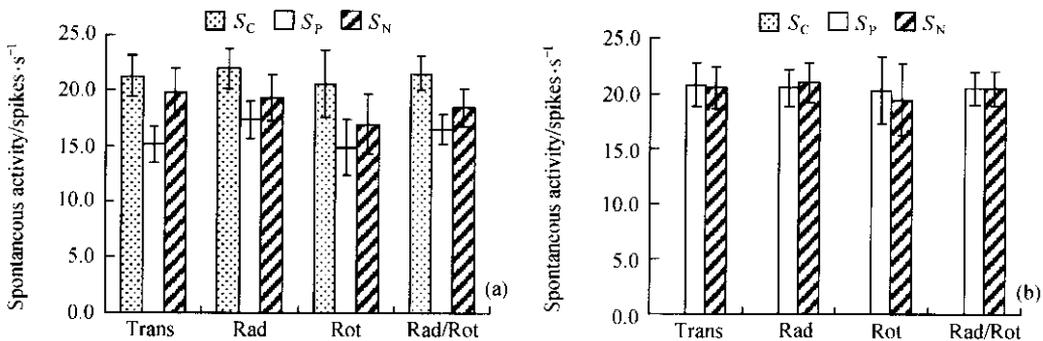

Fig. 2. The mean spontaneous activities of PMLS neurons after adaptation to various optic flow modes. (a) 0.5—5.0 s after adaptation; (b) 25.0—30.0 s after adaptation. The average spontaneous level before adaptation (S_c) is also shown in (a) as a control.

Table 1 The mean relative strengths of neuronal spontaneous activities after adaptation to stimuli of various optic flow modes (S_P/S_C, S_N/S_C) and the index showing the difference of adaptation (DA)

Mean± SE	0.5—5.0 s			25.0—30.0 s		
	S_P/S_C	S_N/S_C	DA	S_P/S_C	S_N/S_C	DA
Translation ($n=65$)	0.681±0.043	0.943±0.044	0.262±0.053	0.944±0.036	0.968±0.030	0.024±0.050
Radiation ($n=55$)	0.811±0.060	0.886±0.051	0.074±0.057	0.961±0.032	0.960±0.033	−0.001±0.042
Rotation ($n=27$)	0.724±0.051	0.833±0.053	0.109±0.059	1.001±0.060	0.920±0.047	−0.081±0.076
Rad/Rot ($n=82$)[a]	0.782±0.044	0.868±0.039	0.086±0.043	0.974±0.029	0.947±0.027	−0.027±0.038

a) Since the difference was insignificant between the results obtained with radiation and rotation stimuli, the two rows of data are combined into group "Rad/Rot" for a better comparison with translation mode.

slightly above 0 for radiation and rotation. Therefore, the significant difference between DA values for translation

and radiation/rotation could not be completely attributed to the relatively strong direction selectivity of PMLS neu-

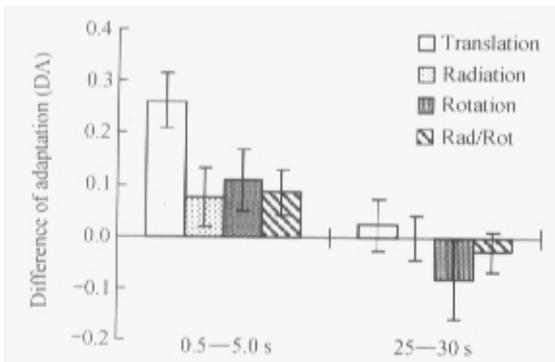rons to the former stimulus.

Fig. 3. The mean indices showing the difference of adaptation to various optic flow modes, with the relative changes in spontaneous activities of PMLS neurons.

3 Discussion

In the present study, the adaptation and after-effects of cat PMLS neurons to prolonged optic flow stimuli were investigated, and it was found that the neuronal responsiveness normally decreased with the continuation of visual stimuli of various flow modes. After the period of adaptation, the spontaneous activities of the cells dropped in general, but the extent of reduction was different after adaptation in the preferred and non-preferred directions, and the difference was relevant to the mode of optic flow stimuli.

According to the classical fatigue theory, motion in the preferred direction induces the strongest responses of a cell, and the excitability of the cells would decrease dramatically with the ongoing fatigue caused by continuous firing under prolonged stimulation. On the contrary, since the cell usually responds less to motion in the non-preferred direction, the fatigue caused by the long-term stimuli is relatively weak, and would result in less adaptation and after-effect[2,3]. The present results demonstrate that, if PMLS neurons were stimulated with prolonged translation, the decrement of spontaneous activities was much larger after adaptation in the preferred direction than that after adaptation in the non-preferred direction, and the difference was significantly correlated to the degree of direction selectivity of the cells. Moreover, our recent study found that, following prolonged translation stimulus, the responsiveness of PMLS neurons to test stimulus was only dependent on the direction of adaptation stimulus, i.e. adaptation in the preferred direction would always induce larger reduction in the responses to test stimulus than adaptation in the non-preferred adaptation, regardless of the direction of test stimulus[13]. Therefore it is plausible to suggest that non-specific fatigue may act as an important and probably key factor in adaptation to long-term translation stimuli. By such a mechanism, the neurons with

stronger direction selectivity would respond more differently to translation in the preferred and non-preferred directions, which leads to a larger difference between the extents of fatigue after prolonged stimuli in the opposite directions, then a larger difference between the spontaneous activities after adaptation, and finally, a higher index for difference of adaptation (DA).

On the other hand, the present results show that, when PMLS neurons were exposed to the complex optic flow patterns (radiation/rotation), there was little difference between the changes in spontaneous activities induced by adaptation in the preferred and non-preferred directions (Wilcoxon test, P 0.02), and the correlation between DA and DI was very weak, indicating that the decrement of spontaneous activities was, to a great extent, independent of the strength of visual responses. Furthermore, our previous study found that the adaptation of PMLS neurons to radiation/rotation was clearly direction-specific, which was dependent on whether the test stimulus matched the prior adaptation stimulus in the moving direction (if the two stimuli moved in the same direction, the responses to test stimulus decreased significantly; otherwise, little change was observed)[13]. It turns out that a single fatigue mechanism cannot fully explain such after-effects induced by long-term complex optic flow stimuli, which instead, may require the substantial involvement of more complicated and direction-specific mechanisms. Recent findings indicated that fatigue could arise from hyperpolarization at the level of single neurons, while specific adaptation may come from some kind of neural network or the interaction of synapses[14]. Therefore, one may propose that the processing of complex optic flow information in area PMLS should be related to complicated interactions of many neurons.

All the above-mentioned results suggest that different mechanisms are possibly involved in adaptation to simple translation and complex optic flow patterns in the extrastriate cortex. Previous studies proposed that the visual system of higher mammals may analyze optic flow information of various modes and degrees of complexity in a hierarchical structure with a combination of parallel and serial processing[15,16]. Our findings indicate that PMLS of the cat may play an important role in perception and adaptation to complex motion and the relevant motion after-effects, analogously to the function of primate MT as revealed by some psychophysical experiments[7—9].

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Efficient expression of human factor IX cDNA in liver mediated by hydrodynamics-based plasmid administration

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Abstract Hydrodynamics-based administration via tail vein was used to deliver naked plasmid with human factor IX (hFIX) cDNA in 2.2 mL Ringer's solution into mice within 7 s. The peak level of expression of hFIX was 2921 ng/mL in mouse plasma. The hFIX cDNA expression increased with increasing the amount of plasmid DNA injected. The peak level of gene expression declined after repeated injection of plasmid (1459 ng/mL). The hFIX cDNA was detected in various organs, but the highest level of gene expression appeared in liver. Transaminase levels and liver histological results showed that rapid intravenous plasmid injection into mice induced transient focal acute liver damage, which was rapidly repaired within 3—10 d. These results suggested that high-level expression of hFIX cDNA can be achieved by hydrodynamics-based plasmid transfer and this method is now further used for gene therapy and gene function study in our lab.

Keywords: hFIX, gene transfer, naked plasmid, hydrodynamics-based transfection.

The transfection of plasmid DNA into mammalian cells is an indispensable tool in the study of gene transfer and gene function. Since the original report in 1990 on the successful expression of a reporter gene in muscle[1], plasmid DNA injection has been widely used to mediate gene transfer study. As a gene transfer vector, naked DNA has many obvious advantages. It is easy and cheap to be prepared and more safe after transfection. But the plasmid mediated gene expression is low because of the less efficiency of gene transfer. It is critical to improve the efficiency of gene transfer and the level of gene expression. There have been numerous *in vivo* studies demonstrating successful transgene expression using the methods like