2. MATERIALS AND METHODS

2.1 Retina preparation

New Zealand White rabbits of either sex, body weight between 1.2 kg ~ 1.8 kg, were used in this study. One day prior to the experiment day, the animal was anesthetized with the mixture of 75-100 mg/kg ketamine (Imalgene 1000; Merial Lyon, France) and 15-20 mg/kg xylazine (Chanazine; Chanelle Phatmaceuticals Manufacturing Ltd., Loughrea, Ireland) through an intramuscular injection. Two to three microliters (1μg/μl) of 4', 6-diamidino-2phenyl-indole (DAPI; Sigma, St. Louis, MO) were then injected intraocularly to label the ganglion cells and other retinal neurons. The animal was allowed to recover. On the experimental day, the animal was dark adapted for 1-2 hours before dissection. A deep anesthesia (double the dose of intramuscular injection used previously) and additional intravenous injection of 50 mg/kg ketamine was applied. A few drops of 0.5% proparacaine hydrochloride ophthalmic solution (Alcaine; Alcon-Couvreur, Belgium) was also used for local anesthesia.

After enucleating the eyes, the anterior segments and the vitreous were removed, and the posterior eyecups were immediately immersed in oxygenated (95% O_2 and 5% CO_2) simplified Ames' medium. The retinas were carefully isolated from the pigment epithelium, and put into a continually shaking bottle containing30-40 ml Ames' medium.

The retinas were kept at room temperature with exchanging refresh Ames' medium every hour . The anesthetized animal was euthanized by supplying 10-20 minutes of CO_2. All the surgery procedures were under dim red light illumination. All the experimental protocols were complied with the institutional Animal Care and Use Committee.

A small piece (5×5 mm) of retina, in the very center position of the ventral side (3-8 mm from the visual streak), was gently adhered, photoreceptor-side down, to a cover slip coated with CellTak (BD Sciences, Bedford, MA), and transferred to a chamber on the stage of a Zeiss Axioskop 2 FS Plus fluorescence microscope (Zeiss, Germany), perfuse with 34-37℃ oxygenated Ames' medium at 2.5-3.5 ml/min.

2.2 Visual stimuli

Visual stimuli generated by VisionWorks (Vision Research Graphics, Durham, NH), and driven by a Pentium 4 PC, were displayed on a CRT monitor (SyncMaster 757NF; Samsung, Korea) with 100 Hz refresh rate positioned 75 cm away from the microscope. A reflecting mirror under the 20X objective lens (A-plan, NA 0.45, Zeiss) reflected light through the objective lens and projected onto the retina. The objective lens was adjusted to focus the light onto the photoreceptor layer. For finding a DSGC, a 0.5 Hz, 180×180 μm^2 flash square was used to map the receptive field thus to locate the center of the area. In order to make sure the DSGC property, 12 directional moving bars (540×180 μm^2, 900

μm /sec = 5.14 deg/sec) were swept sequentially across the receptive field to determine the preferred direction of the DSGC (Fig. 1A).

2.3 Electrophysiology

DAPI labeled retinal ganglion cells were visualized under brief fluorescence illumination using a 40X water immersion objective lens (Achroplan, NA 0.8, Zeiss). The ganglion cell with crescent-shaped nucleus was selected to be a potential DSGC.

Single unit extracellular recording technique was applied. An tungsten-in-glass electrode (Levick, 1972) with impedance ranging between 0.5 – 1.5 MΩ was used to record the response of ganglion cells. The differential amplifier (ISO-80; National Instruments, Austin, TX) was used to amplify the signal 1000 folds. The signal was then sent into an analog-to-digital data acquisition card (NI 6040E; National Instruments, Austin, TX), sampled at 10k Hz, and recorded by a custom-written LabVIEW interface electrophysiology program. The raw data were stored in a Pentium 4 PC for later offline analysis.

2.4 Experimental design

To examine how the background property interacts with the receptive field center of the DSGCs, four different spatial characters were applied in the annulus surround

region：spatial coverage, immediate surround extend, spatial scale, and motion coherence. The center was continuously stimulated by a square wave grating at 1 cycle per receptive field center diameter in the preferred direction and velocity of 4 deg/sec. The diameter of the center stimulation was determined by the receptive field mapping result described above. A proper center size was used for different cells, generally in between 400-700 μm. In order to avoid underestimation of the receptive field, a black annulus in the immediate surround with annulus width of 1/4 center diameter was used to prevent the surround stimulus interference. In the beginning, a windmill pattern stimulus (four times the diameter of the center circle) with 8 vanes rotating clockwise in the surround annulus was used to characterize the motion surround suppression effect (Fig. 1B). The "random dots" were chosen as the background context with different properties in the following experiments.

Spatial Coverage (Fig. 1) The first issue is to ask how the cell responds to different percentage of random dots coverage in the surround. Surround coverage percentage is defined by the total area of the dots divided by the total surround area. Surround annulus outer diameter was four times the center diameter throughout the experiments. Dots size was 1/8 the diameter of the center area circle. Dots density was modulated to fit the coverage percentage used in this experiment: 0, 6.25, 12.5, 25, 37.5, 50, and 62.5 %. Dots were set to have 100% coherence moving in the same way as the preferred

direction of the DSGC, and the same velocity as the center grating.

Immediate Surround Extend (Fig. 2) The second part of the experiments is to ask how far the surround spreads would affect the center response. This was done by increasing the outer diameter of the uniform black annulus to reduce surround annulus area. Three different black annulus widths were used, which are 1/5, 1/3 and 1 fold of the center diameter. The random dot size again is set as 1/8 center diameter square, and the same velocity and 100% coherence were used.

Spatial Scale (Fig. 3) The third one is to ask how the surround spatial scale tunes the center response. To achieve this, different dot sizes were used at the same coverage percentage. After finishing the previous experiment, the dot size was changed to 1/4 and 1/2 of the center diameter to modulate the spatial scale, and accordingly the dots density was modified to fit the coverage percentage.

Motion Coherence The last of the interest is to test how randomness the DSGC can tell. All other experiments above were using 100% coherence of background moving dots, in this part the coherence was varied systematically (0, 25, 50, 75 and 100 %). Similar to previous experiment, the random dot size was 1/8 center diameter and the velocity was kept the same. A 37.5% of coverage percentage was used in this experiment.

2.5 Data analysis

All the data were analyzed off-line by MATLAB (The MathWorks Inc., Natick, MA). There are two indexes used in this thesis. Directional Selectivity Index (DSI) is used for indicating the strength of a ganglion cell having direction selectivity and is defined as: $DSI = \left| \frac{\sum \vec{v}_i}{\sum \vec{r}_i} \right|$, where ν_i are vectors pointing in the direction of moving stimulus and having length, r_i, equal to the averaged spiking rate. The vector sum of twelve directions points the preferred direction of DSGC (Taylor and Vaney, 2002). A ganglion cell having no direction selectivity, which responds equally in all directions, has a DSI value 0. In contrast, a ganglion cell with DSI value 1 indicates a sharp directional tuning.

Trend Index (TI) is defined by $TI = \frac{\sum_{i=1}^{n} i \times R_i}{\sum_{i=1}^{n} i}$, where i refers to the weighting of different coverage percentage and R_i is the normalized response at the ith coverage percentage condition. The indicator n is given by the number of coverage percentage we chosen in the experiment, $n = 7$ in Spatial Coverage experiment, for instance. Higher coverage percentage gets higher weighting, emphasizing the behavior of the high spatial coverage percentage.