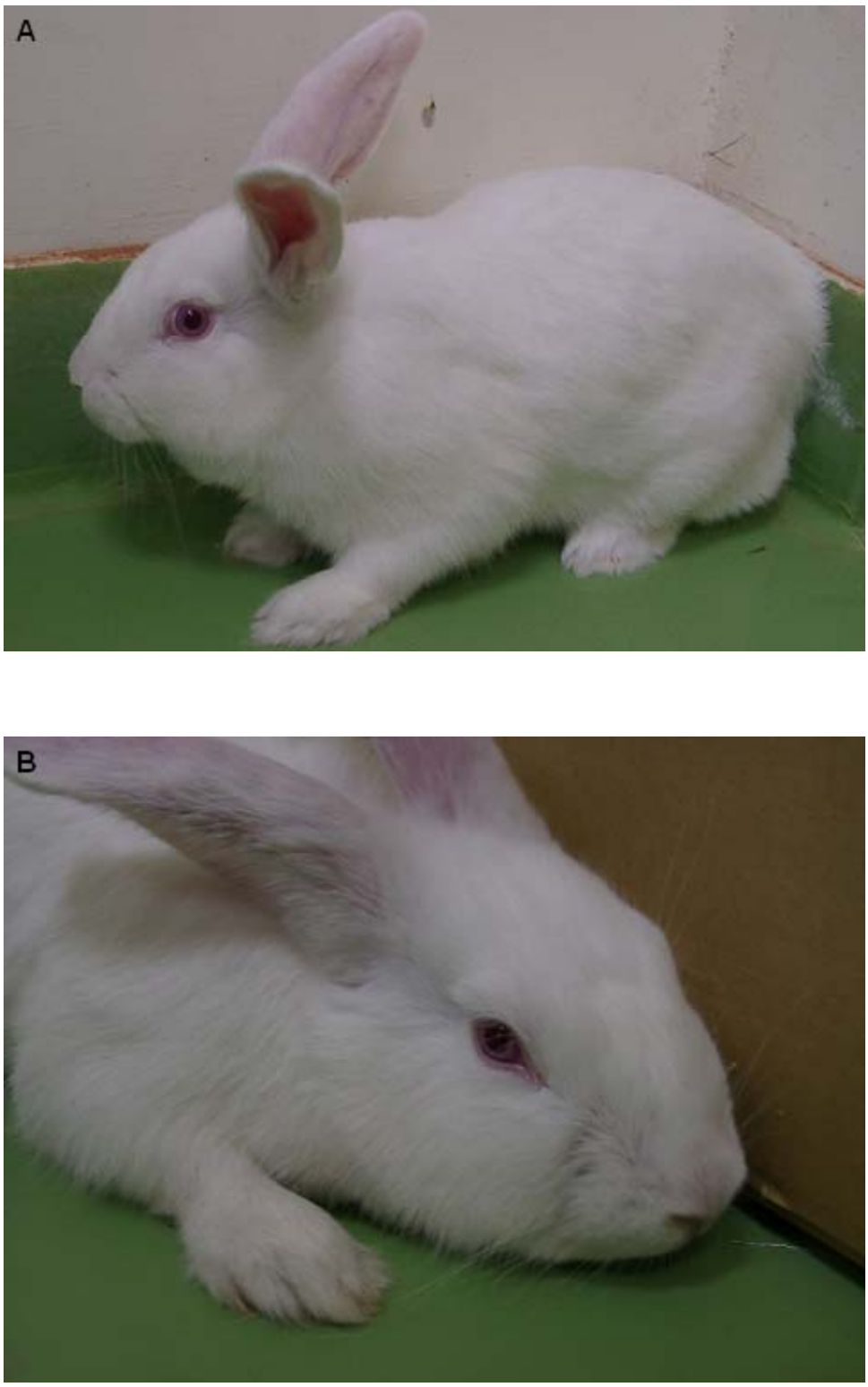


Fig. 1. The adult New Zealand White rabbit (*Oryctolagus cuniculus*).

A: The whole body picture. **B:** A close-up shot.

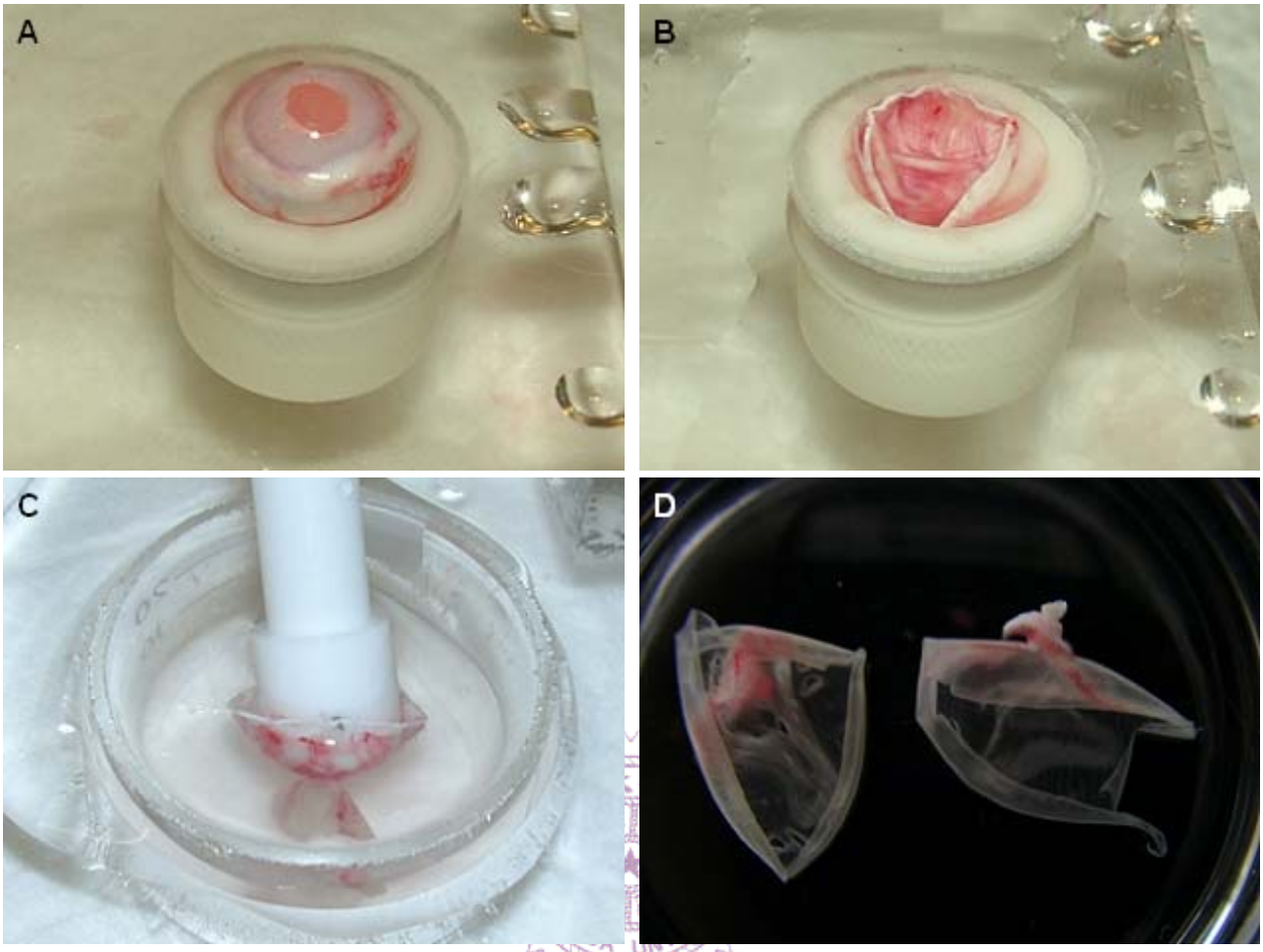


Fig. 2. Steps of the retina isolation.

A: An intact eyeball was enucleated and put on a dissection stage. **B:** The eyeball was hemisected. The anterior part and the vitreous were removed. **C:** The eyecup was nailed on a Teflon rod. The retina was then carefully detached from the retinal pigment epithelium and the sclera. **D:** The isolated retinae were transferred to a dish for further processing. The tissues were always immersed in the oxygenated simplified Ames' medium.

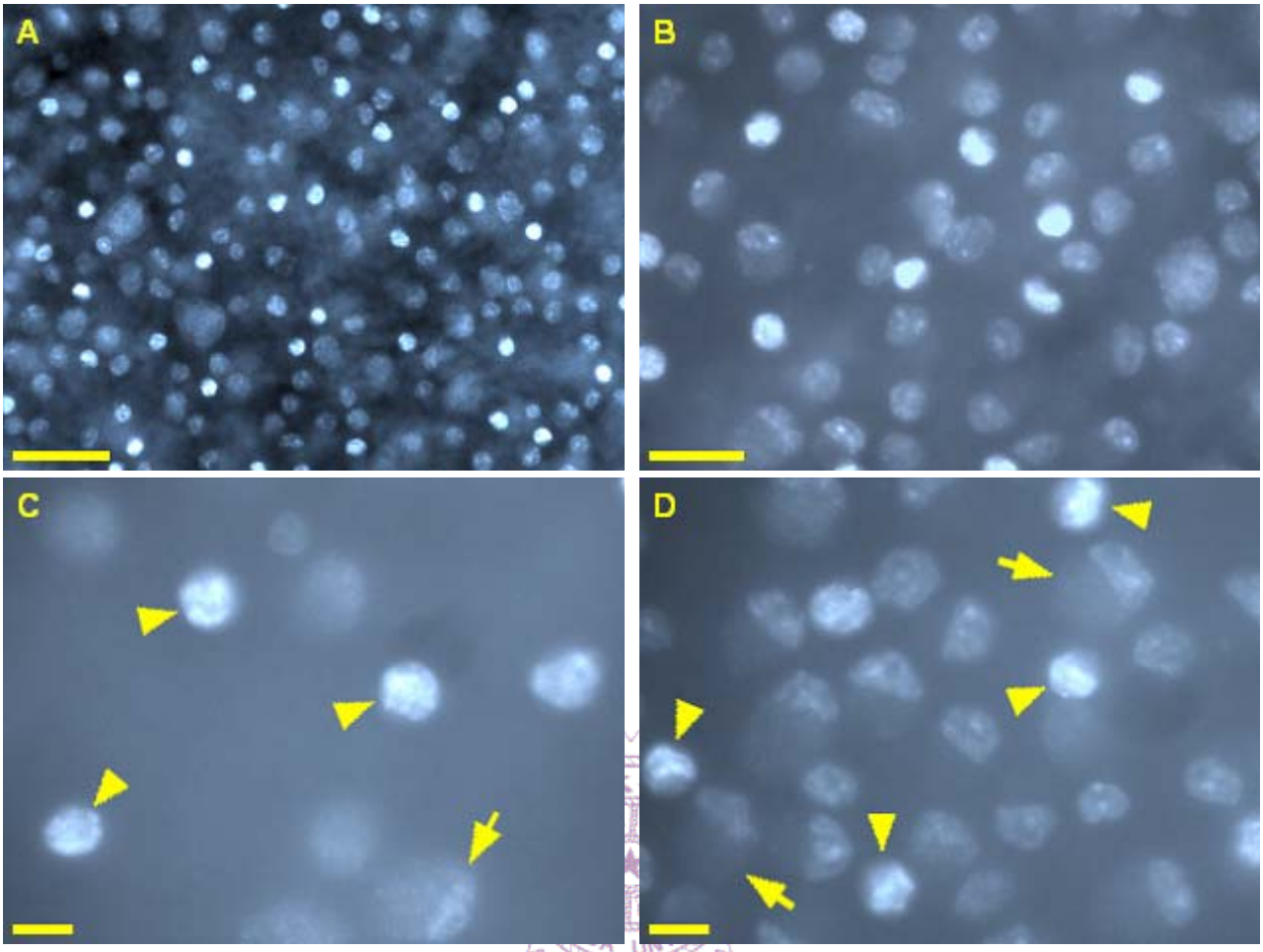


Fig. 3. DAPI labeling for retinal neurons in the ganglion cell layer (GCL).

A: Low magnification (20× objective lens) image on the GCL. **B:** Medium magnification (40× objective lens) image on the GCL. **C,D:** High magnification (63× objective lens) images for different regions in the GCL (C for periphery, whereas D for central retina), the cell density usually tends to be sparser at periphery. Arrowheads point to ON-starburst amacrine cells (SACs), and arrows indicates the potential direction selective ganglion cells (DSGCs). Scale bar: 50 μm for A, 25 μm for B, and 10 μm for C, D.

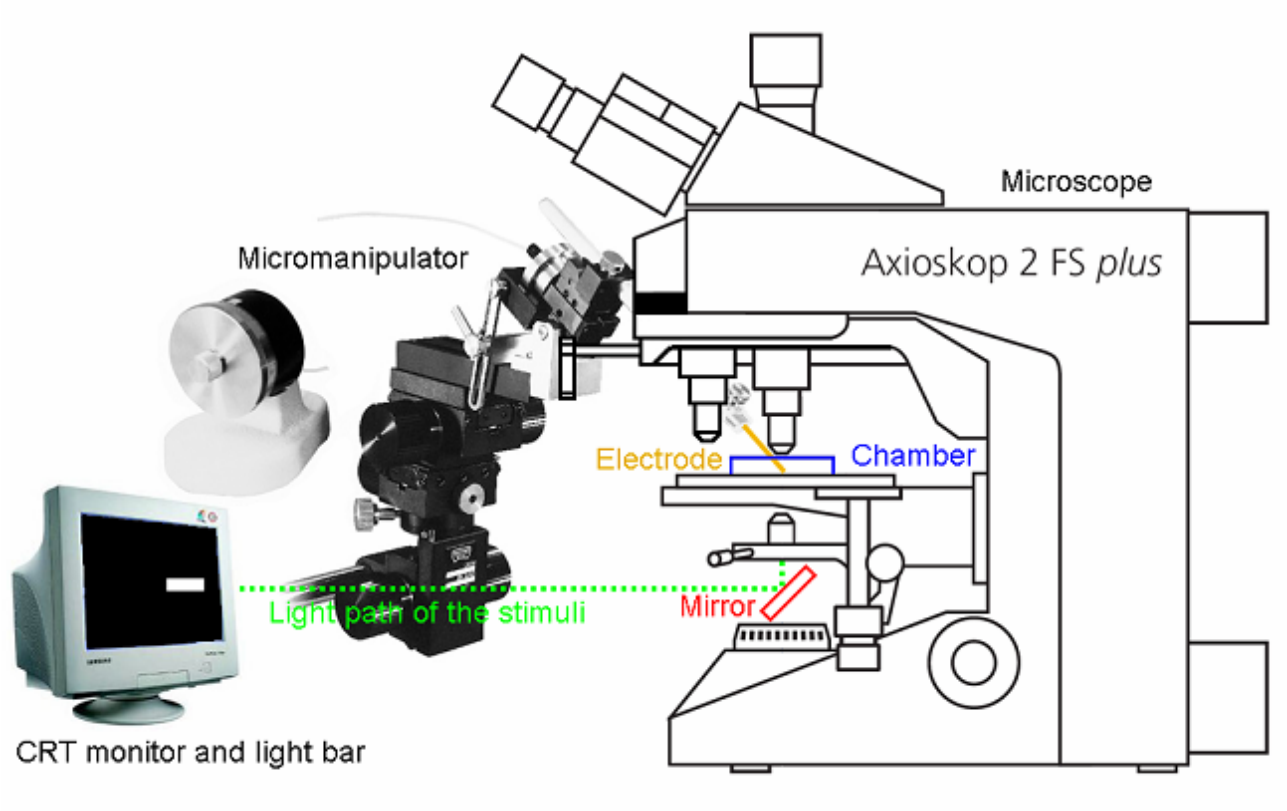


Fig. 4. Schematic diagram of the major experiment setup.

The fix-stage microscope was mounted on an air table. A CRT monitor which display the computer generated moving stimuli (white bar on the screen) was placed at the right side of the microscope. The screen images were projected through a mirror (red), an objective lens under the stage, and finally reach the retina in the chamber (blue). A micromanipulator was mounted at the left side of the microscope. An electrode (brown) was installed to perform the intracellular recording and microinjection.

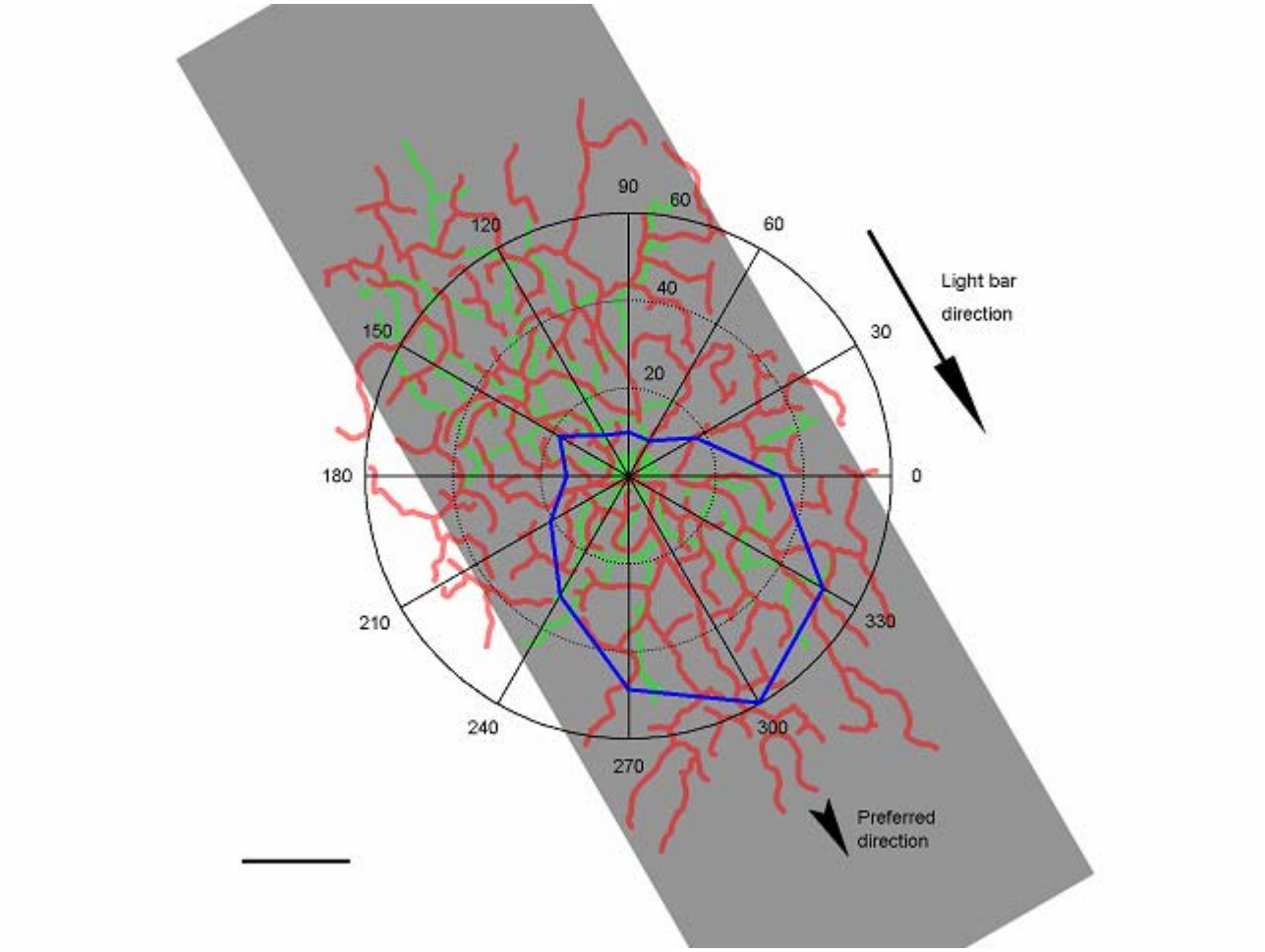


Fig. 5. A moving stimulus projected onto the retina and evoked responses of a direction selective ganglion cell (DSGC).

A DSGC was receiving a light bar stimulus (gray rectangle, 540 ×180 μm) which was moving in the preferred direction. The polar plot shows the number of spikes, and the morphology (green for the ON arbor, red for the OFF arbor) of this DSGC was depicted as well. Scale bar: 50 um.

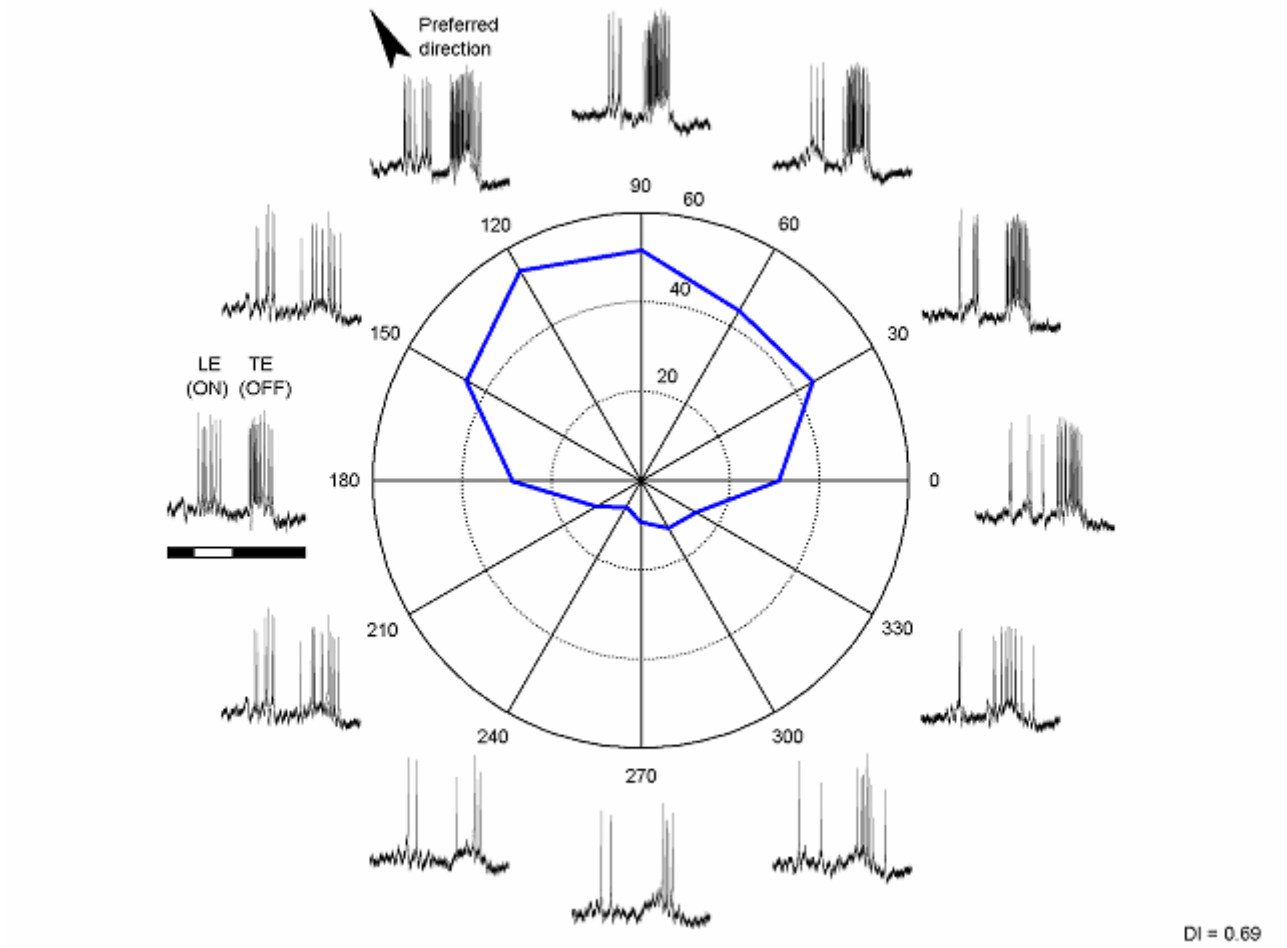


Fig. 6. Response of an ON-OFF direction selective ganglion cell (DSGC).

The polar plot shows the responses of a DSGC to a moving bar in 12 directions. Leading edge of the stimulus generates ON responses, whereas trailing edge evokes OFF responses. The cell exhibited plenty spikes when the stimulus moves in the preferred direction (arrowhead), but fired weakly in the opposite direction.

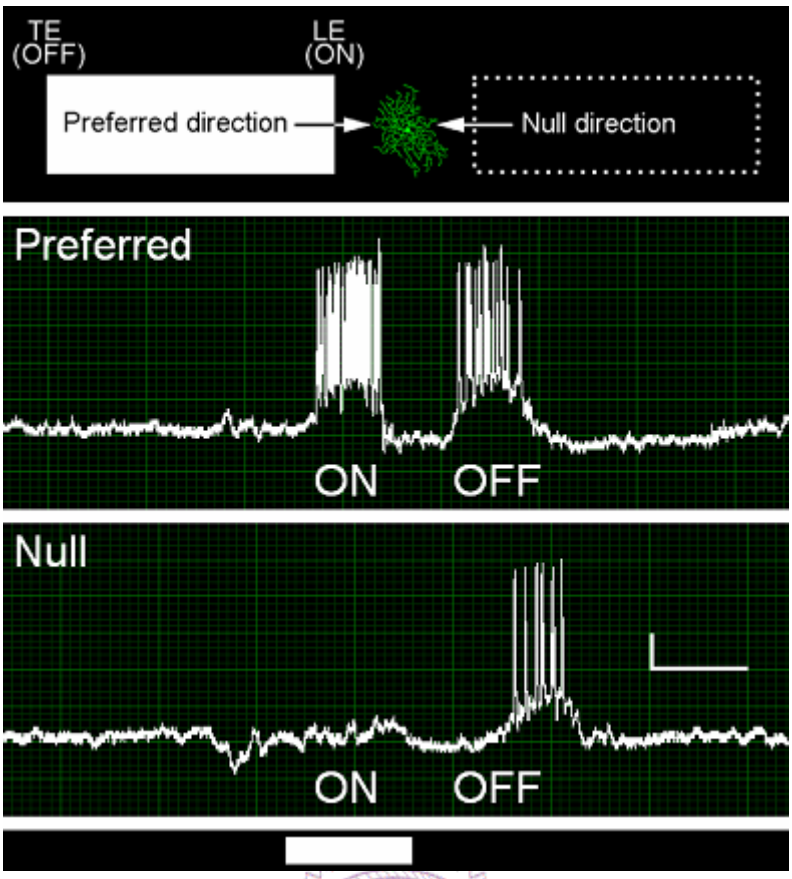
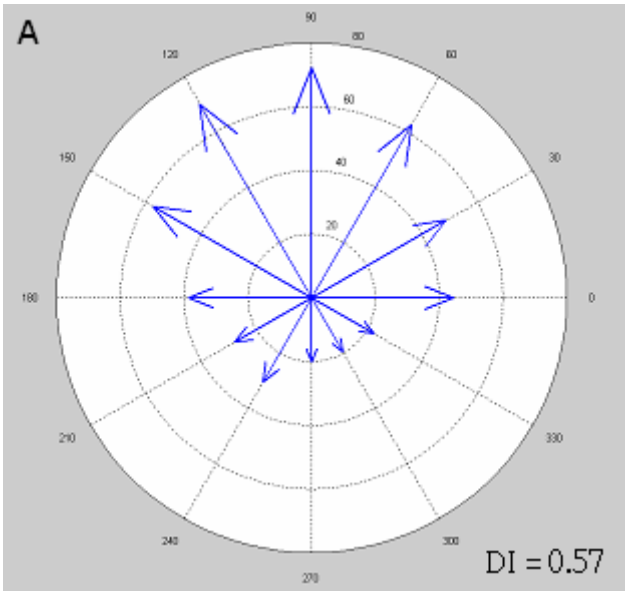
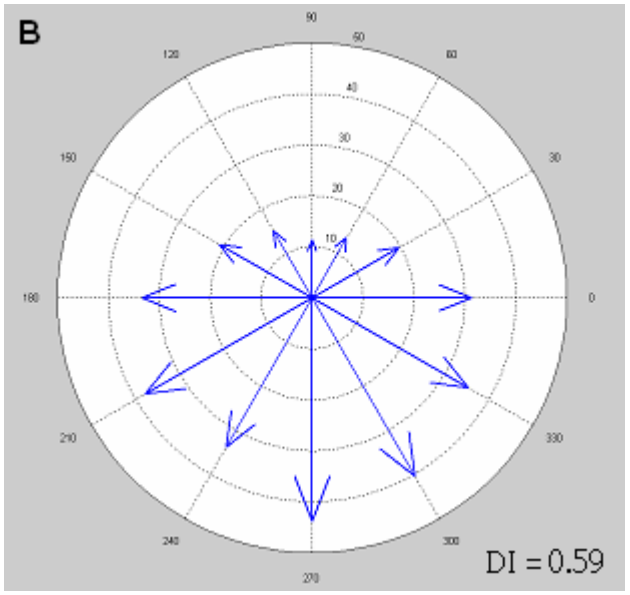
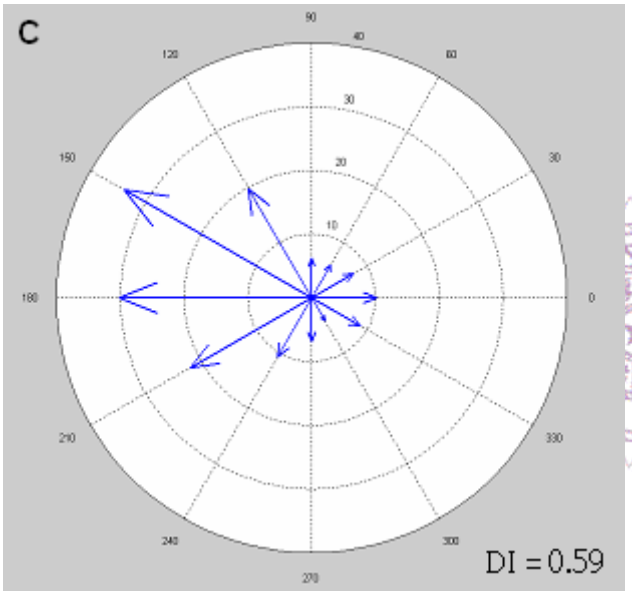


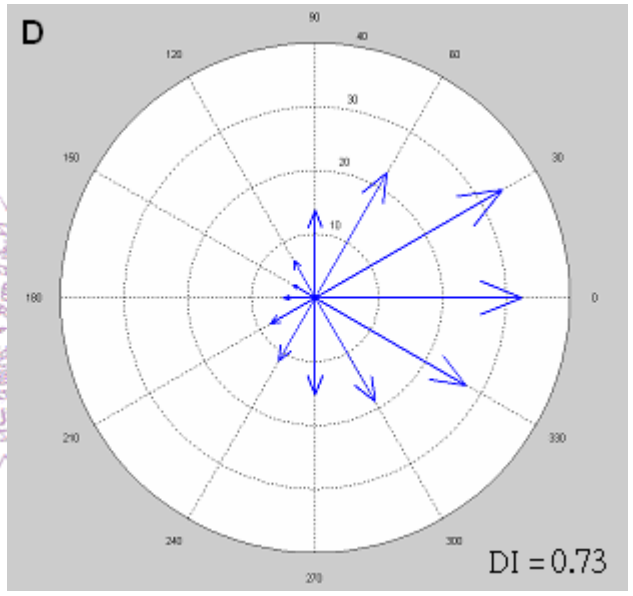
Fig. 7. Variation of the membrane potential corresponds to the preferred and null direction stimuli

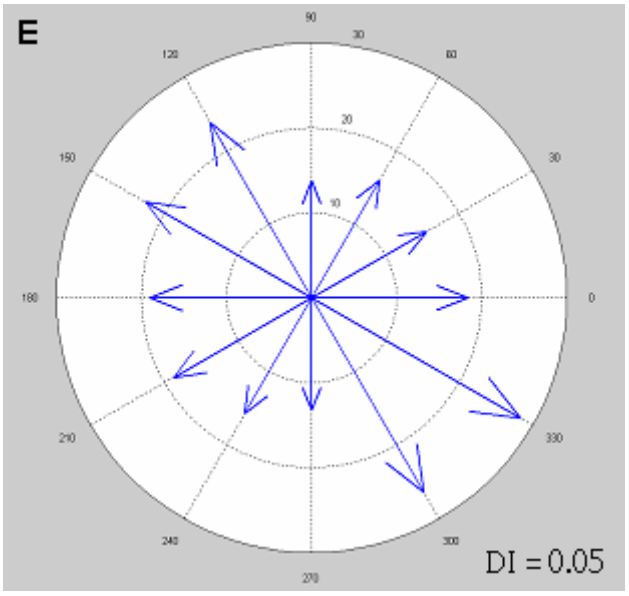
The schematic diagram illustrates the way a moving light bar (540 × 180 μm) projected onto a direction selective ganglion cell (DSGC). When the stimulus swept through the receptive fields of DSGCs (900 μm／s), both leading (LE) and trailing (TE) edges generate spikes. The top trace shows the strong ON and OFF responses of a DSGC when a light bar moved in the preferred direction. The middle trace shows the amount of action potentials remarkably declined when the cell was stimulated by null direction movement. Spike response of light ON was completely eliminated, whereas OFF remained some residual spikes. The bottom trace indicates the onset time of light ON and light OFF. The resting membrane potential was about -69 mV. Scale bar: 500 ms for X, 5mV for Y.


A
DI = 0.57


B
DI = 0.59


C
DI = 0.59


D
DI = 0.73


E
DI = 0.05

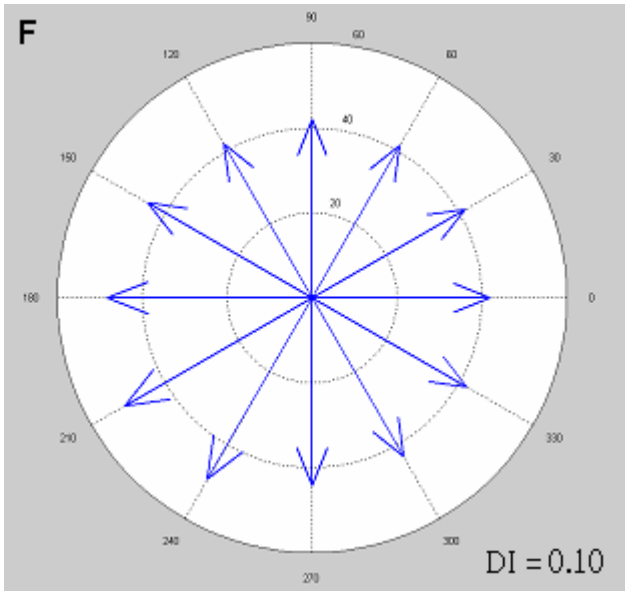

F
DI = 0.10

Fig. 8. Direction selectivity and other non-directional responses.

A-D: Spike responses of direction selective ganglion cells (DSGCs). Highly selectivity for directions was revealed. The measured preferred directions were usually fallen on one of the four cardinal directions. **E:** Spike response of an orientation selective ganglion cell (OSGC). They prefer two opposite directions (the preferred axis), rather than the perpendicular axis. **F:** Spike response of a non-DSGC. This cell exhibits almost equivalent responses in all directions.

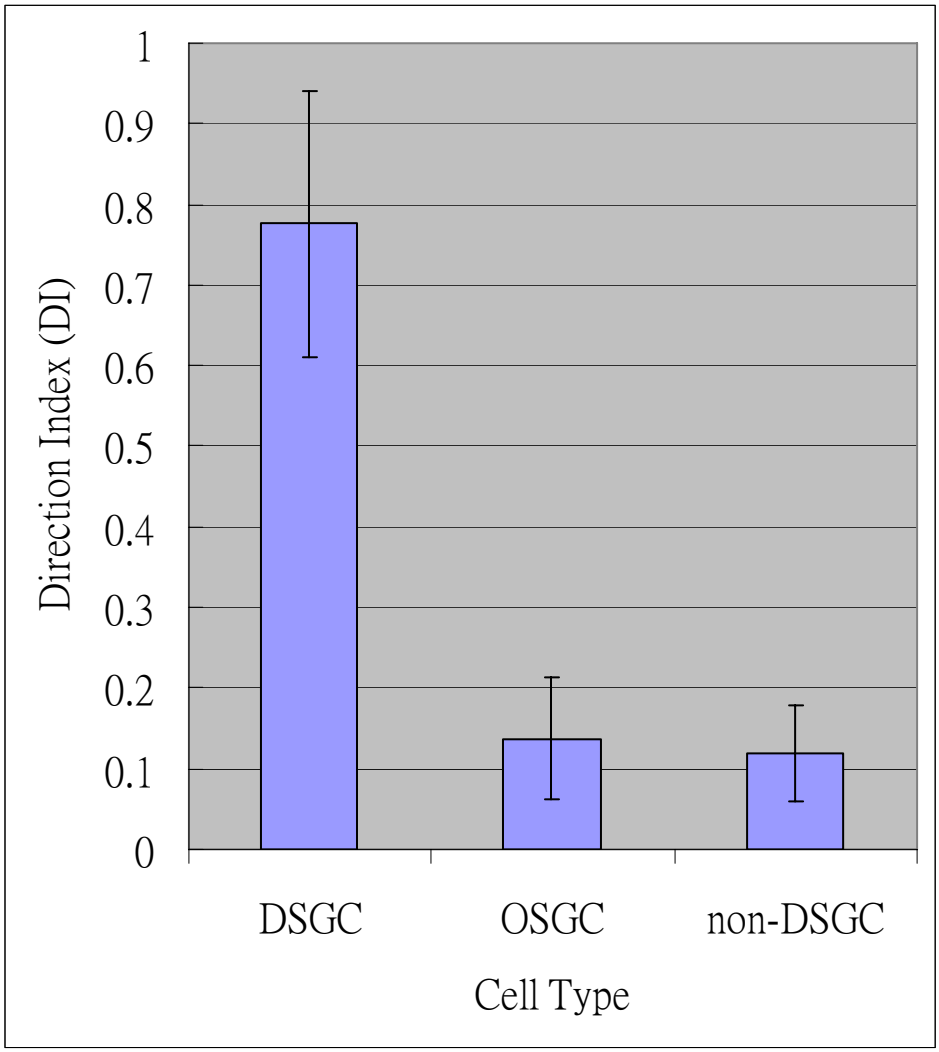


Fig. 9. Direction Indices (*DI*s) of different cell types.

A histogram shows the *DI*s of direction selective ganglion cells (DSGC), orientation selective ganglion cells (OSGC), and other ganglion cells (non-DSGC). Obvious difference were found, DSGCs: 0.78 ± 0.16 (mean ± s.d.), OSGCs: 0.14 ± 0.08, other non-DSGCs: 0.12 ± 0.06

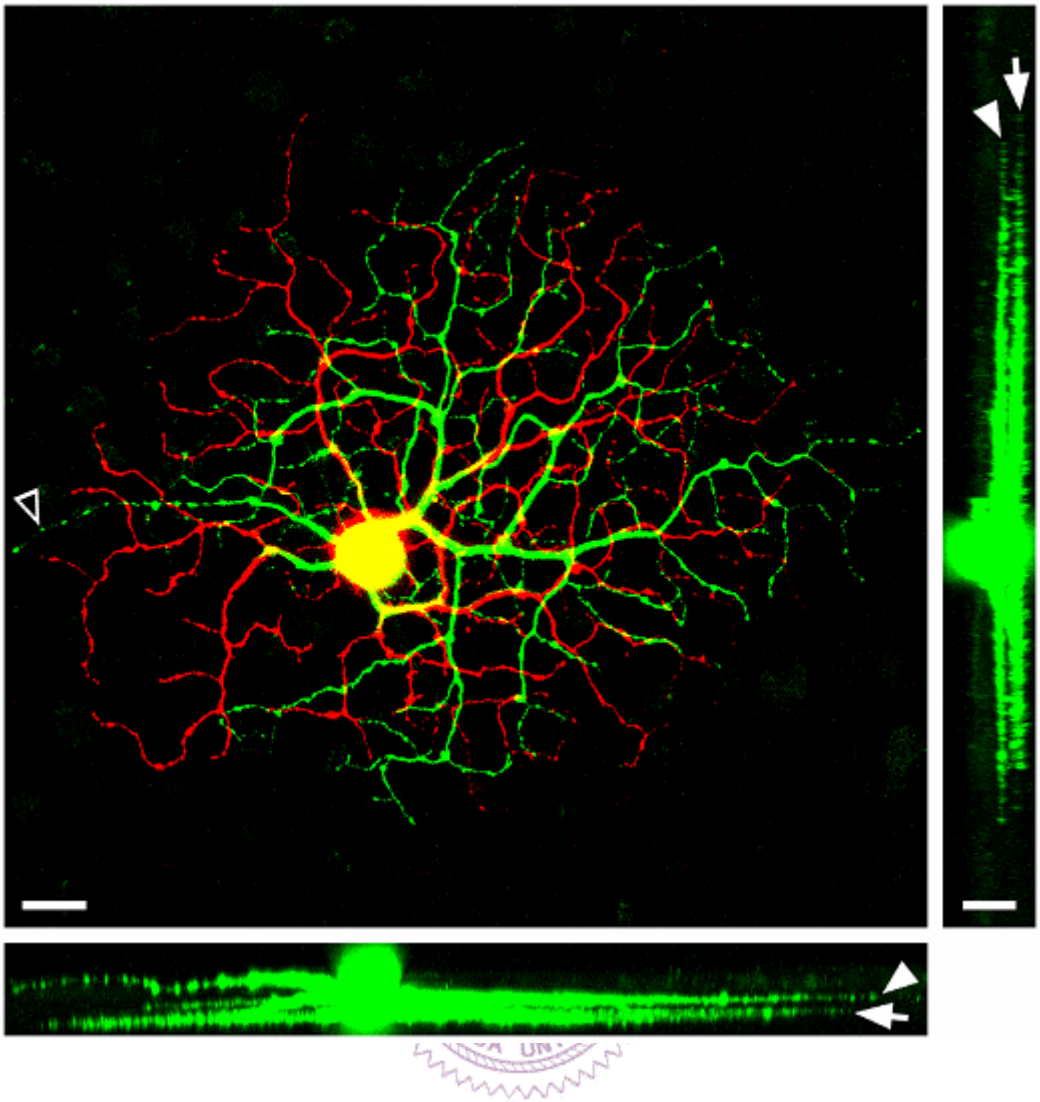


Fig. 10. Bi-stratification of a direction selective ganglion cell (DSGC) under horizontal and vertical views.

A typical DSGC possess ON and OFF dendritic arbors (green and red, respectively) stratified at different level of the inner plexiform layer (INL). Hollow arrowhead points to the axon. In the vertical view from two orthogonal directions, the bi-stratified appearance can be detected clearly (Arrowheads point to the ON arbor, whereas arrows point to OFF arbor). The horizontal view was merged from the images of different focal plane (axon, ON arbor, and OFF arbor). Scale bar: 20 μm for both XY and Z section.

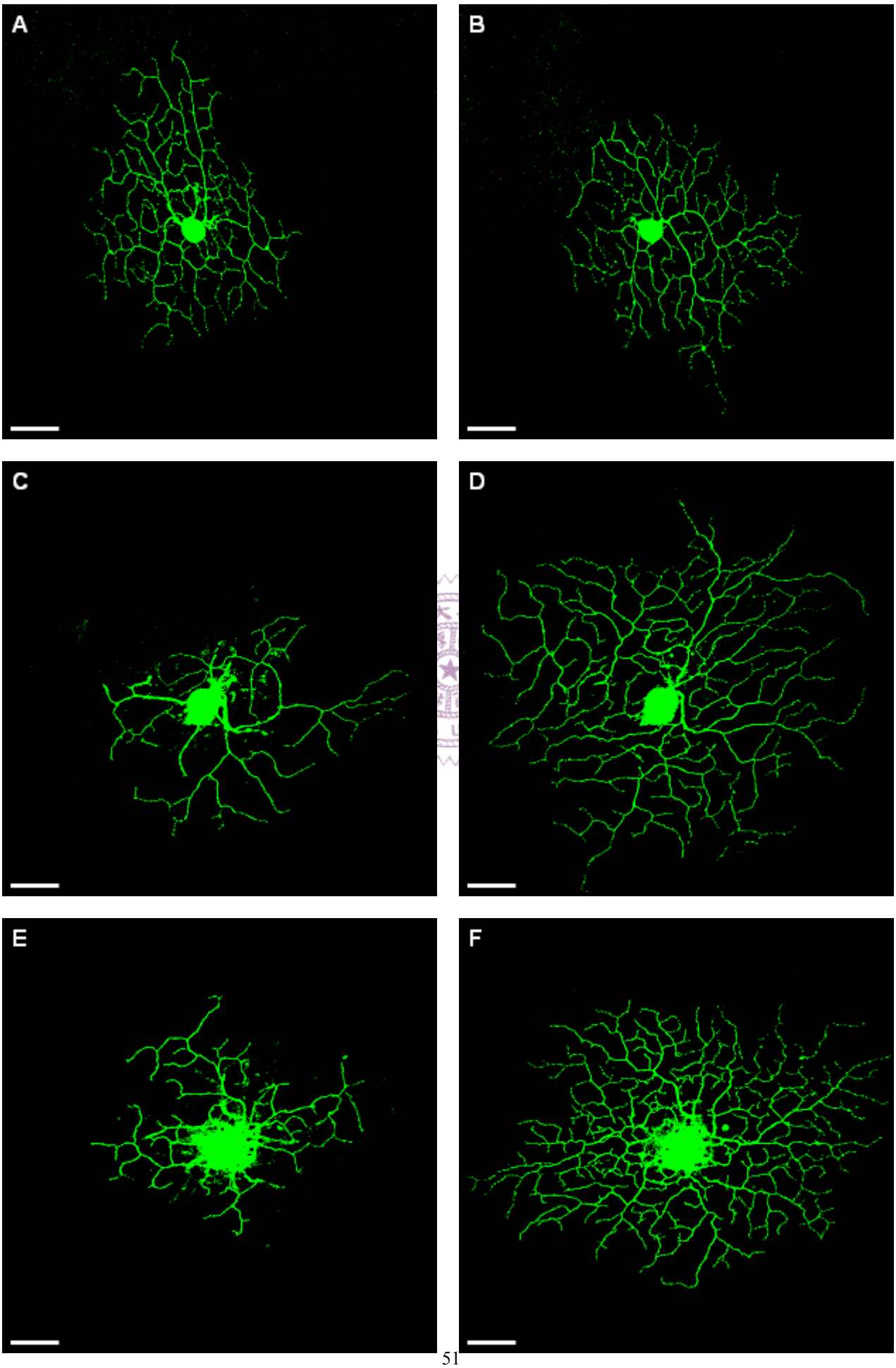

A
B
C
D
E
F

Fig.11. Ensembles of typical direction selective ganglion cells (DSGCs).

A,C,E: The ON arbors of three DSGCs. **B,D,F:** The OFF arbors of the same cell in A,C,E, respectively. The dendrites branch regularly and curve back. All images were taken at low magnification to reveal the whole dendritic arbors. Scale bar: 50 μm.

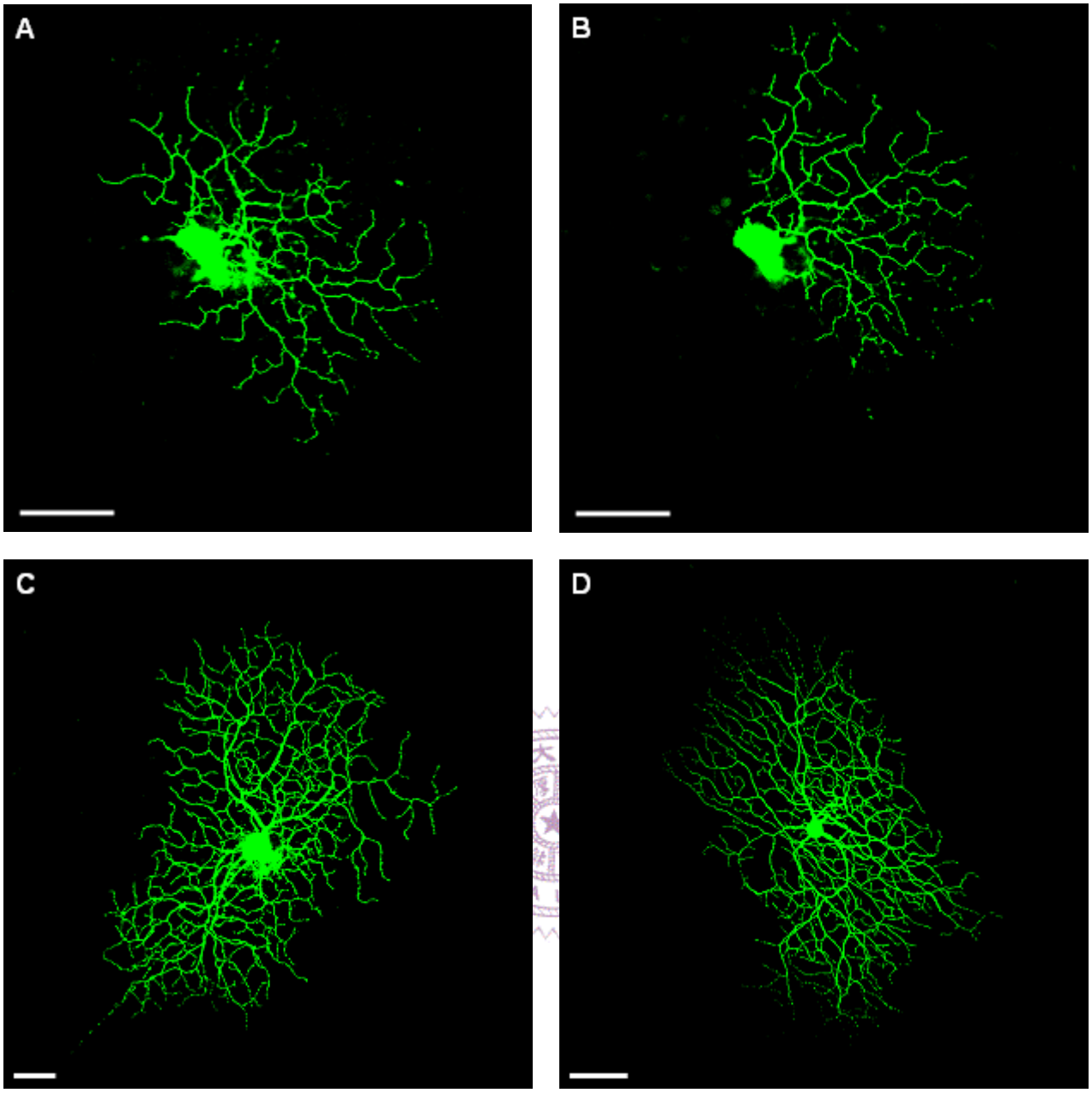


Fig.12. Morphology of direction selective ganglion cells at different eccentricities.

A,B: The ON and the OFF arbors of a DSGC in central retina, respectively. The dendritic field is relatively small and asymmetric. **C,D:** Two DSGCs located in periphery. Their dendrites spread equally, and were apparently larger. Scale bar: 50 μm in A-C, 100 μm in D

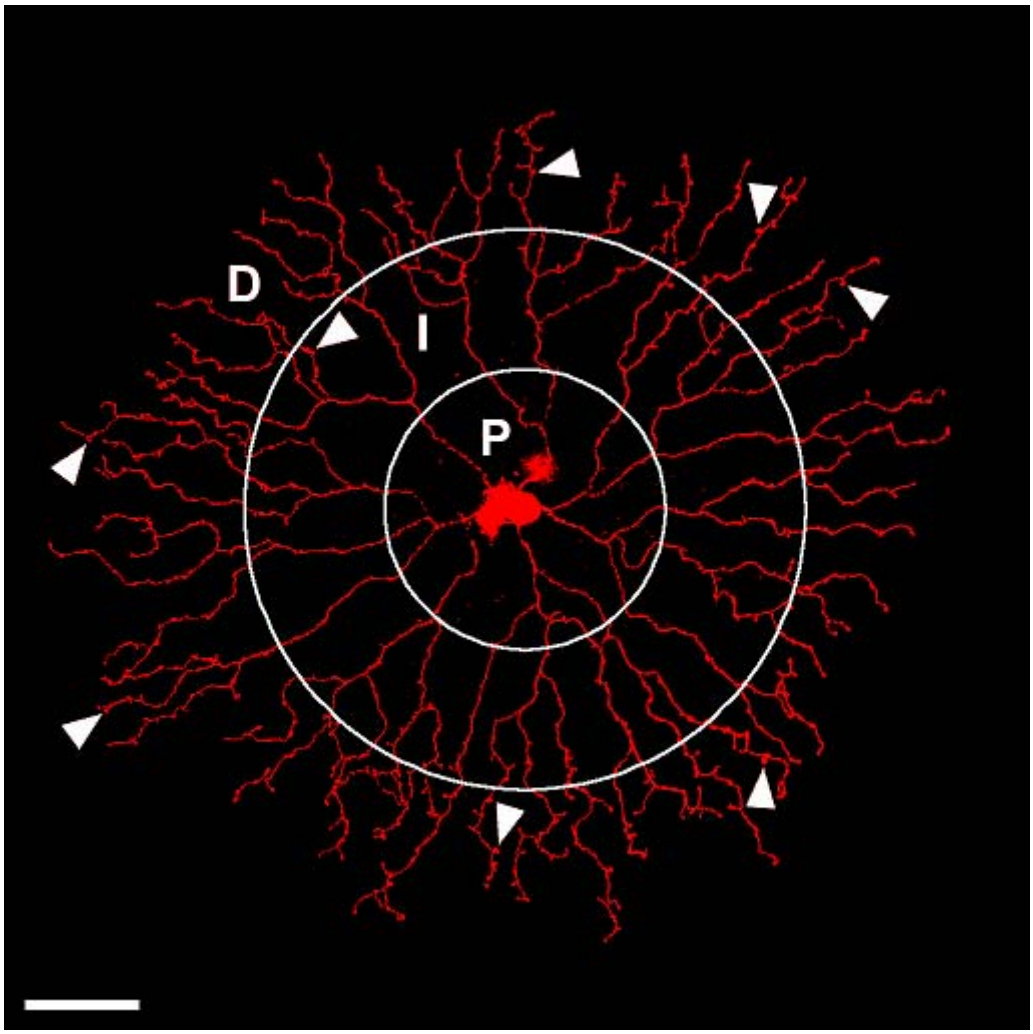


Fig. 13. A typical starburst amacrine cell (SAC).

Starburst amacrine cellls have highly symmetric dendritic arbors, which can be divided into three regions in according to the distance from soma. The most inner part is proximal zone (P), and then intermediate (I) and distal zone (D). Arrowheads point to some of the varicosities. Scale bar: 50 μm.

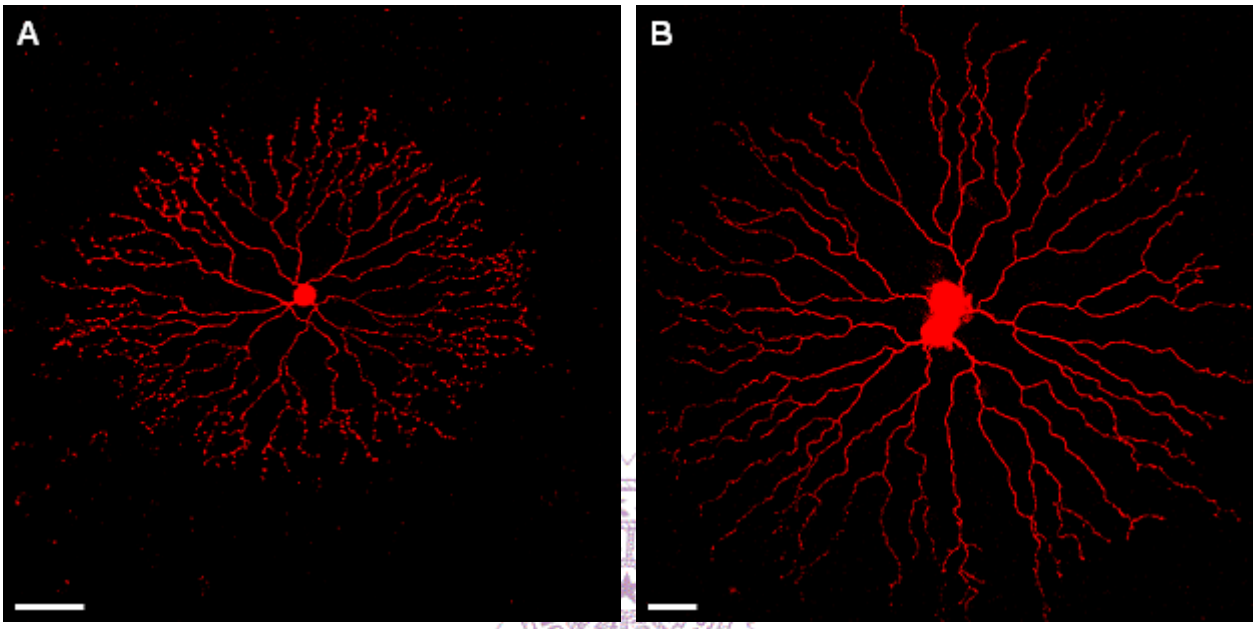


Fig. 14. Morphology and dendritic size of starburst amacrine cells (SACs) at different eccentricities.

A: When SACs locate near the central retina, their dendritic field size tends to be smaller. **B:** The dendritic arbors of SACs tend to be larger near the peripheral retina. Although dendritic field size of SACs varies for different retinal region, their unique feature remains the same. Scale bar: 50 μm.

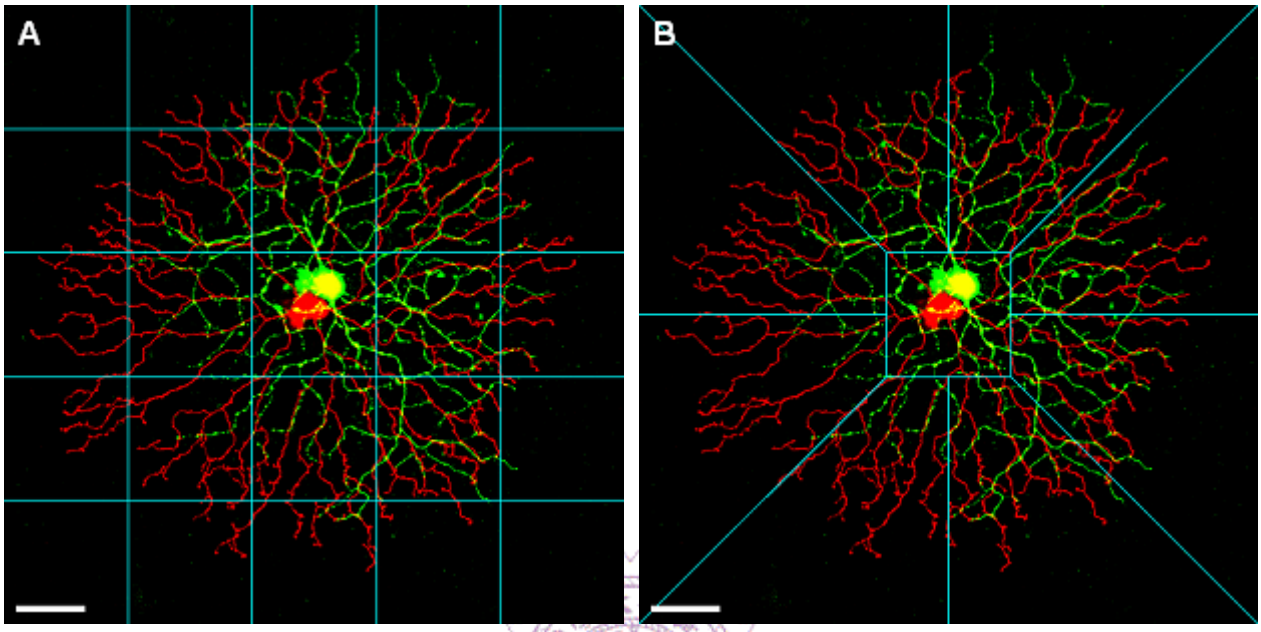


Fig. 15. Regions of a cell pair for scanning and analyzing

A: Twenty-five regions were demarcated after a low magnification scanning, and each region was individually obtained using a higher magnification scanning (except the empty ones). **B:** The scanned regions were divided into eight groups, which denote the directions sequentially for following processing. The center region was discarded because the spatial offset of the DSGC soma may lead to an erroneous estimation. Scale bar: 50 μm

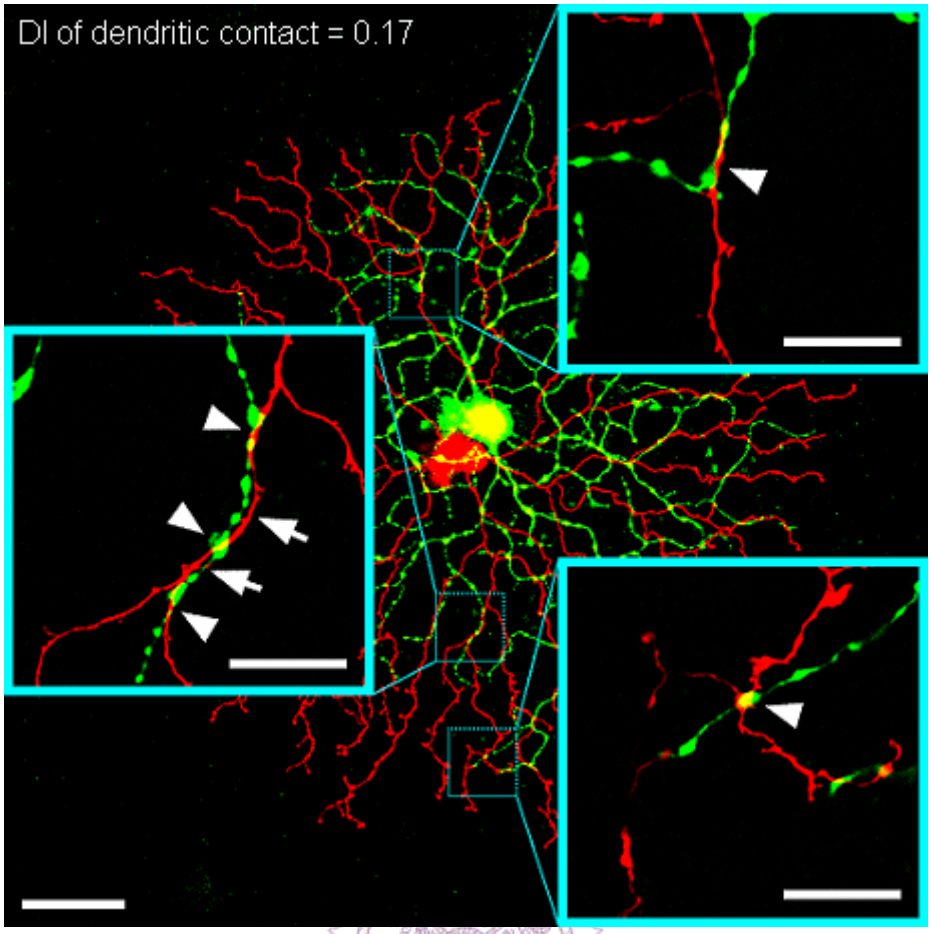


Fig. 16. Co-fasciculations and contacts between a direction selective ganglion cell (DSGC) and a starburst amacrine cell (SAC)

The co-fasciculations estimated under low magnification could be false in some cases. The contacted dendrites in the three small squares seemed to co-fasciculate with each other. However, in high optical magnification (the large squares), the contacts among them were not as many as the low magnification image. Arrowheads indicate the contact segments, whereas arrows point to the gaps. The arrowhead in the top-right square indicates a typical co-fasciculation, and the contact pointed in the bottom-right square is a crossing. Scale bar: 50 μm in low power photomicrograph, 10 μm in the enlarged image.

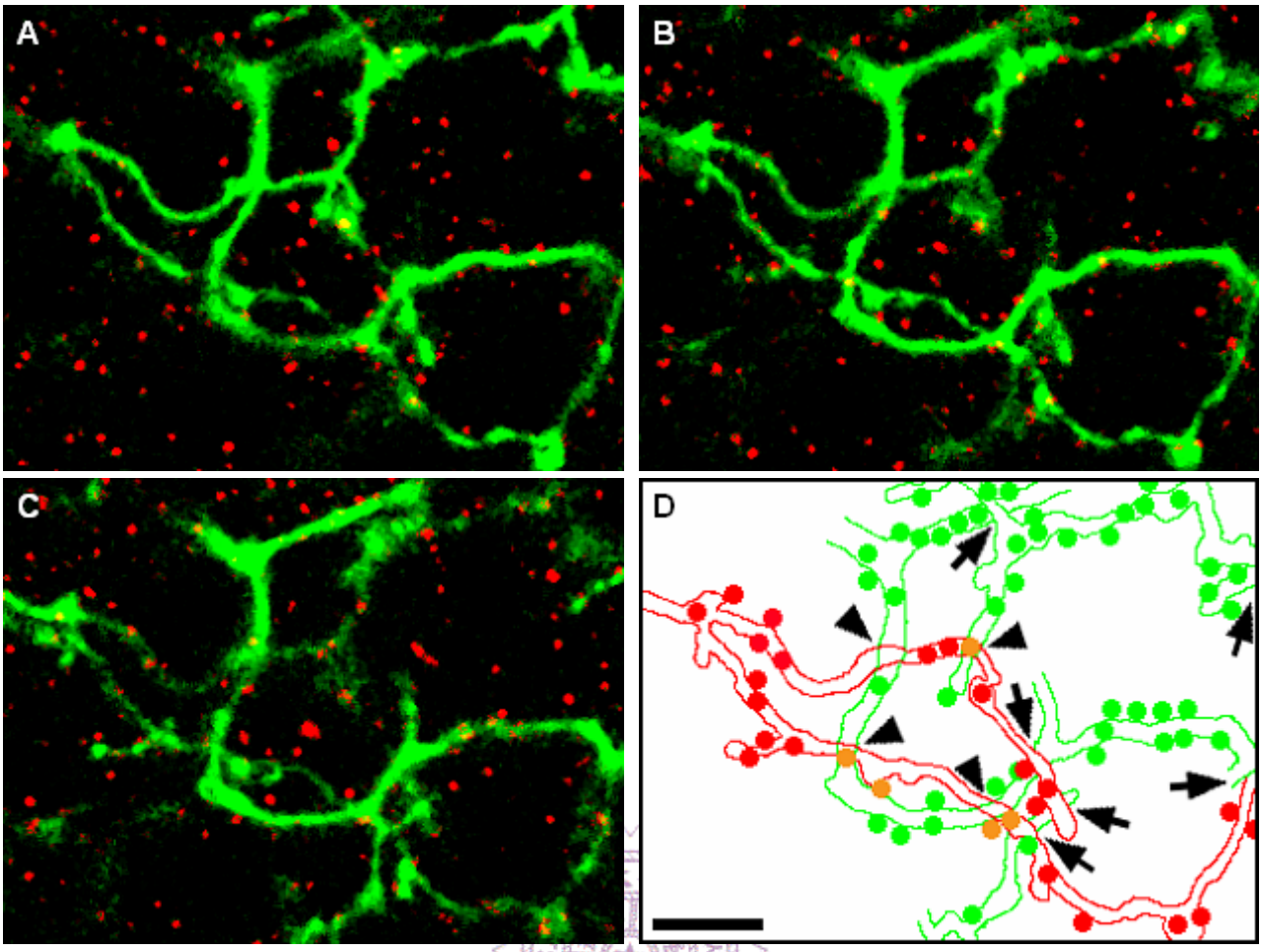
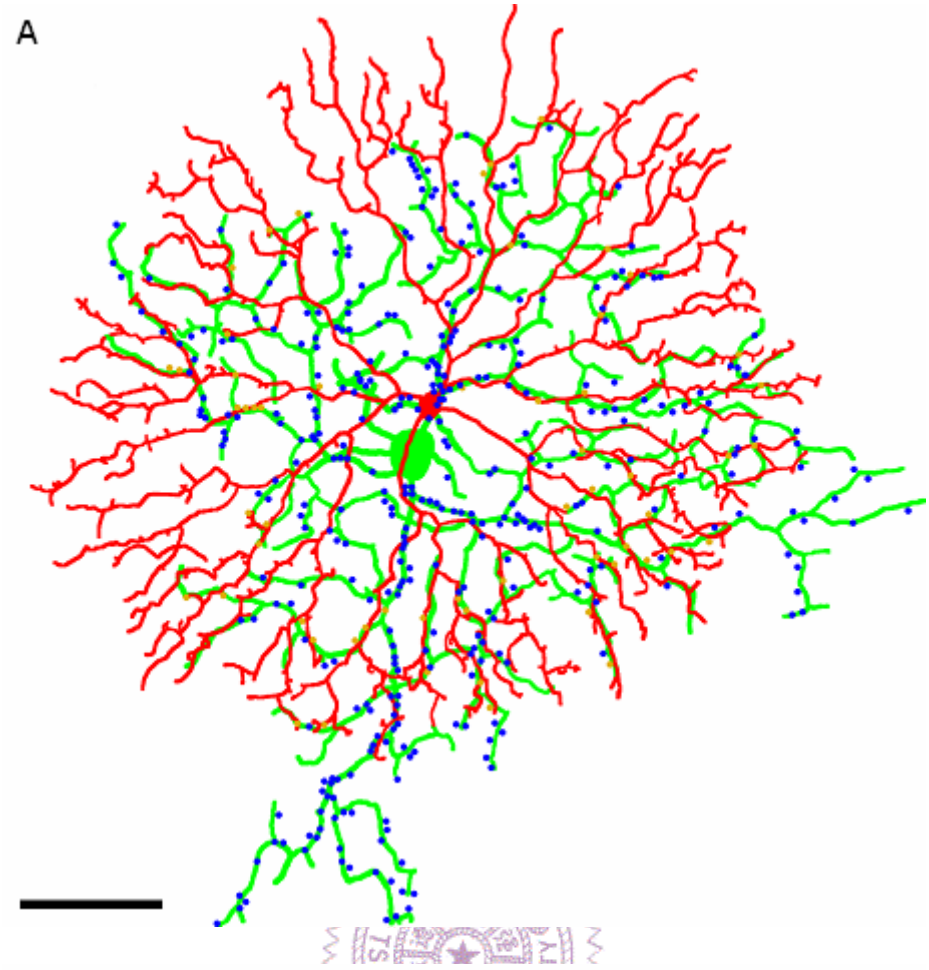
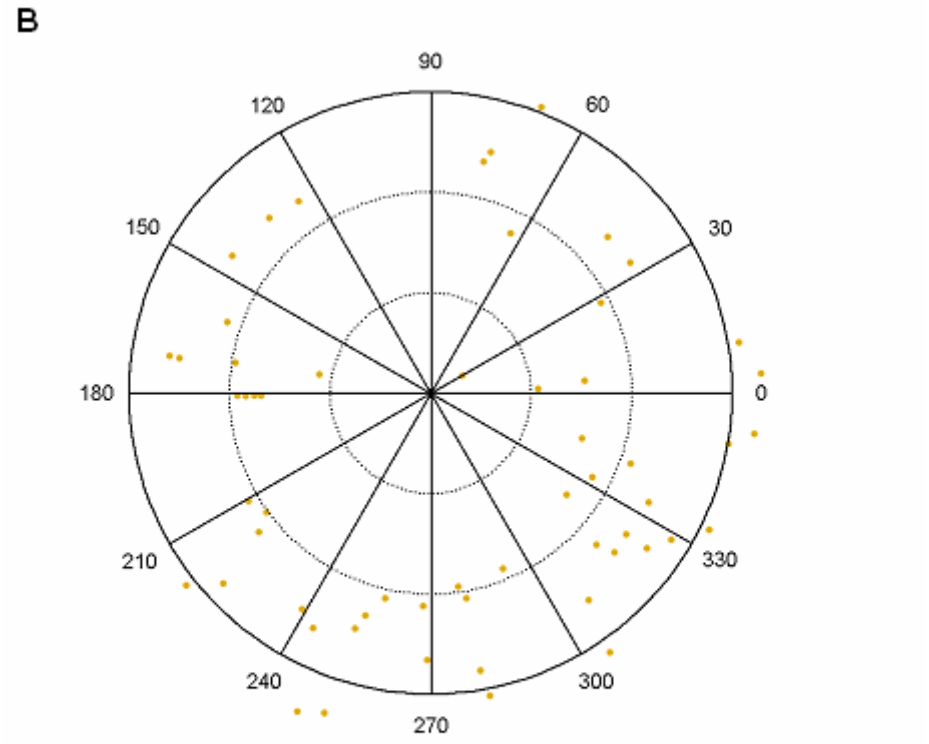


Fig. 17. Labeling for a direction selective ganglion cells (DSGC) and a starburst amacrine cells (SAC) together with γ-amino butyric acid-A ($GABA_A$) receptors.

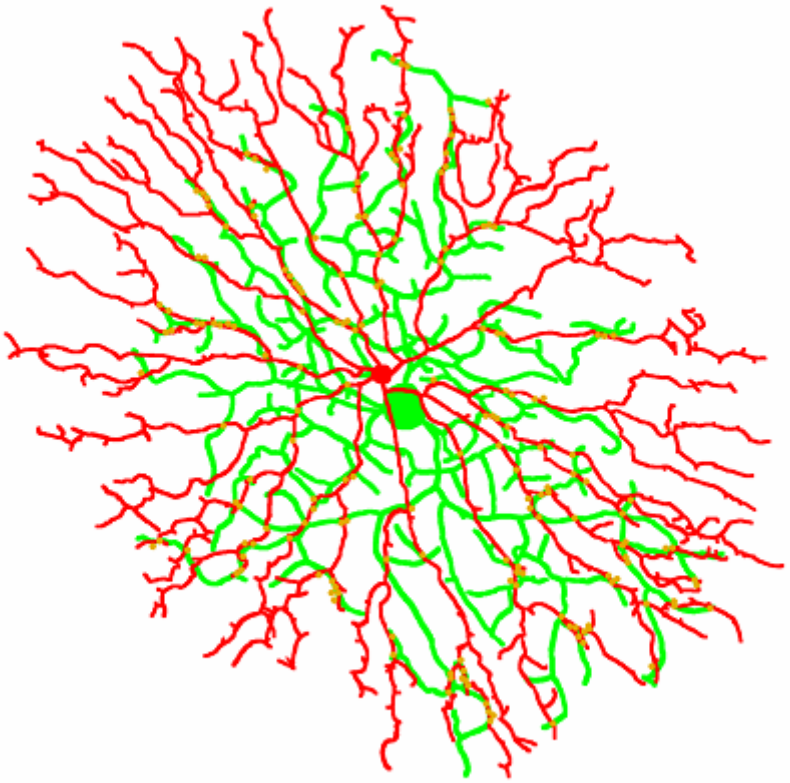
A-C: A segment of the dendrites of the cell pair (green) and $GABA_A$ receptor immunoreactivity (red) at different focal plane (0.6 μm interval). **D:** Tracing of cell dendrites and $GABA_A$ puncta. The processes of DSGC (green hollow lines) and SAC (green hollow lines) were separated carefully by hand, open ends indicate the processes extend beyond the focus. The $GABA_A$ puncta colocalized with DSGC (green circles), SAC (red circles) and both (brown circles) were marked. Arrowheads point to dendrites at the same focal plane (overlap with each other), whereas arrows indicate non-colocalized dendrites (one cover another). Scale bar = 5 μm in D (applies to A-C).



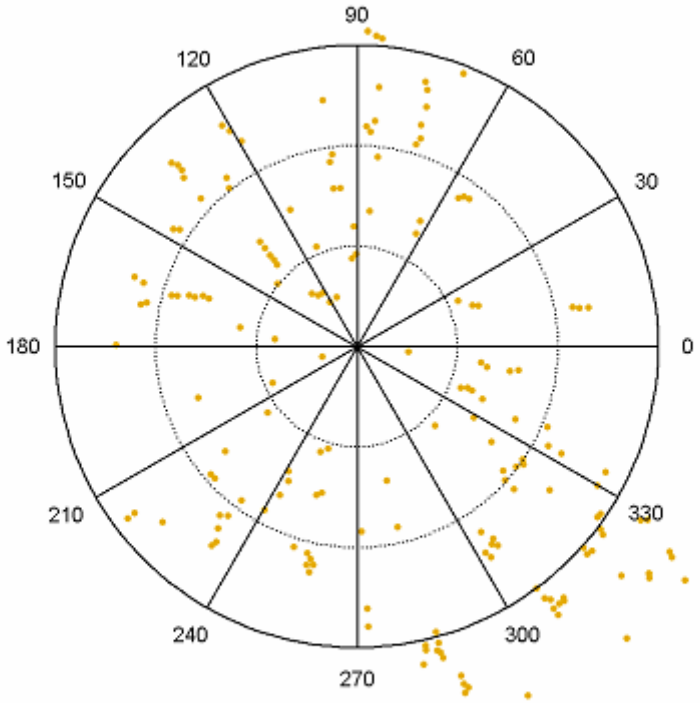


DI of synaptic connection = 0.07

C

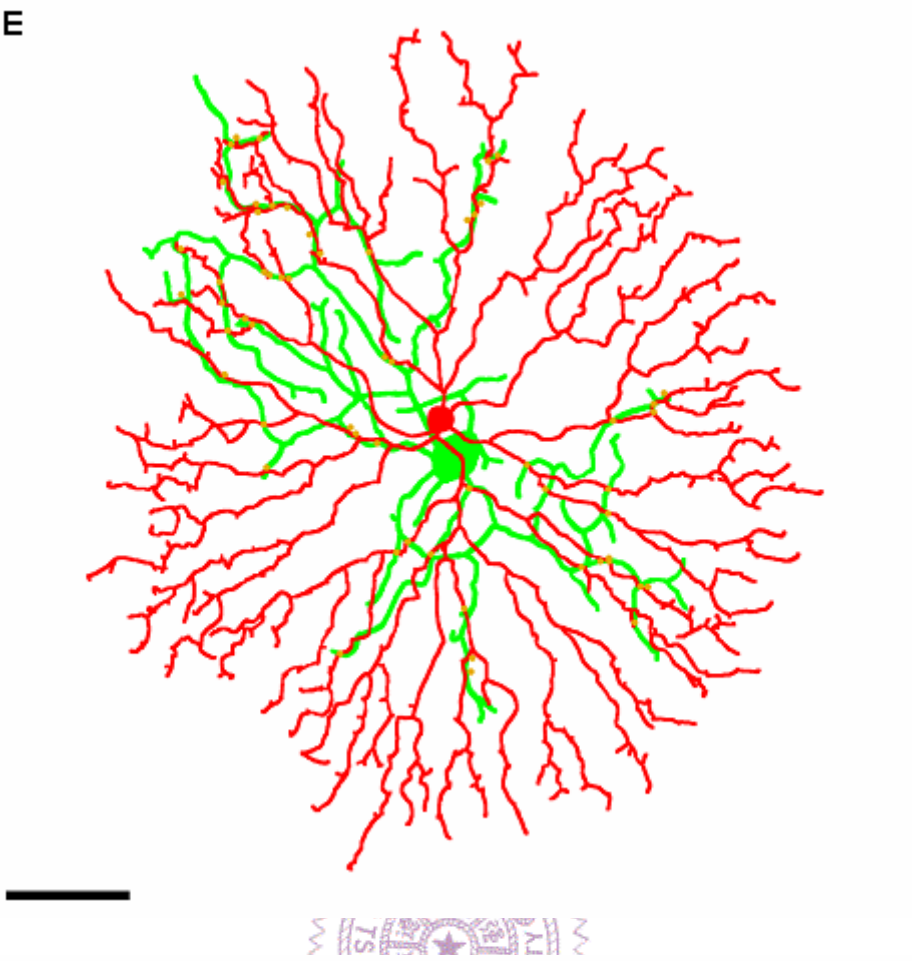


D

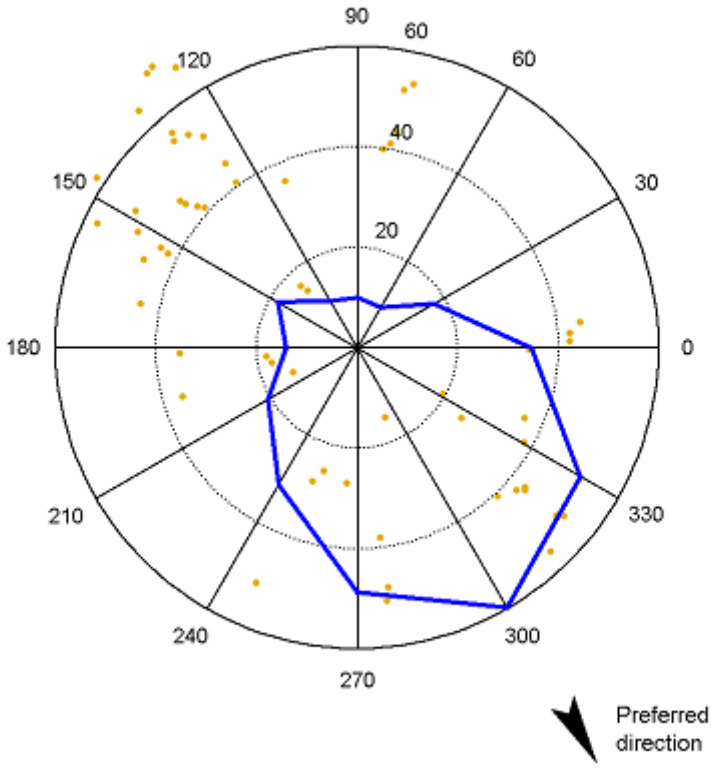


DI of synaptic connection = 0.02

E



F



DI of synaptic connection = 0.14

Fig. 18. The distribution patterns of the inhibitoy synaptic inputs between a direction selective ganglion cell (DSGC) and a starburst amacrine cell (SAC).

A,C,E: A hand drawing from a digital photomicrograph, both the ON arbor of DSGC (green) and the SAC dendrite (red) were depicted. The immunolabeled γ-amino butyric acid-A ($GABA_A$) receptors were represented as dots. $GABA_A$ receptors colocalized with the dendrites of both DSGCs and SAC were viewed as the effective inhibitoy synaptic inputs (brown dots) for the two cells. **B,D,F:** Clear representations of the inhibitory synaptic inputs correspond to A, C, E, respectively. The $GABA_A$ receptors were separated from the dendrites, thus the distribution pattern can be easily seen. Notably, other irrelevant $GABA_A$ receptors on the DSGC dendrites were also marked (blue dots) in A, and the physiological results was included in F. The polar plots represent the averaged spike numbers, and the arrowhead indicates the preferred direction. Scale bar = 50 μm

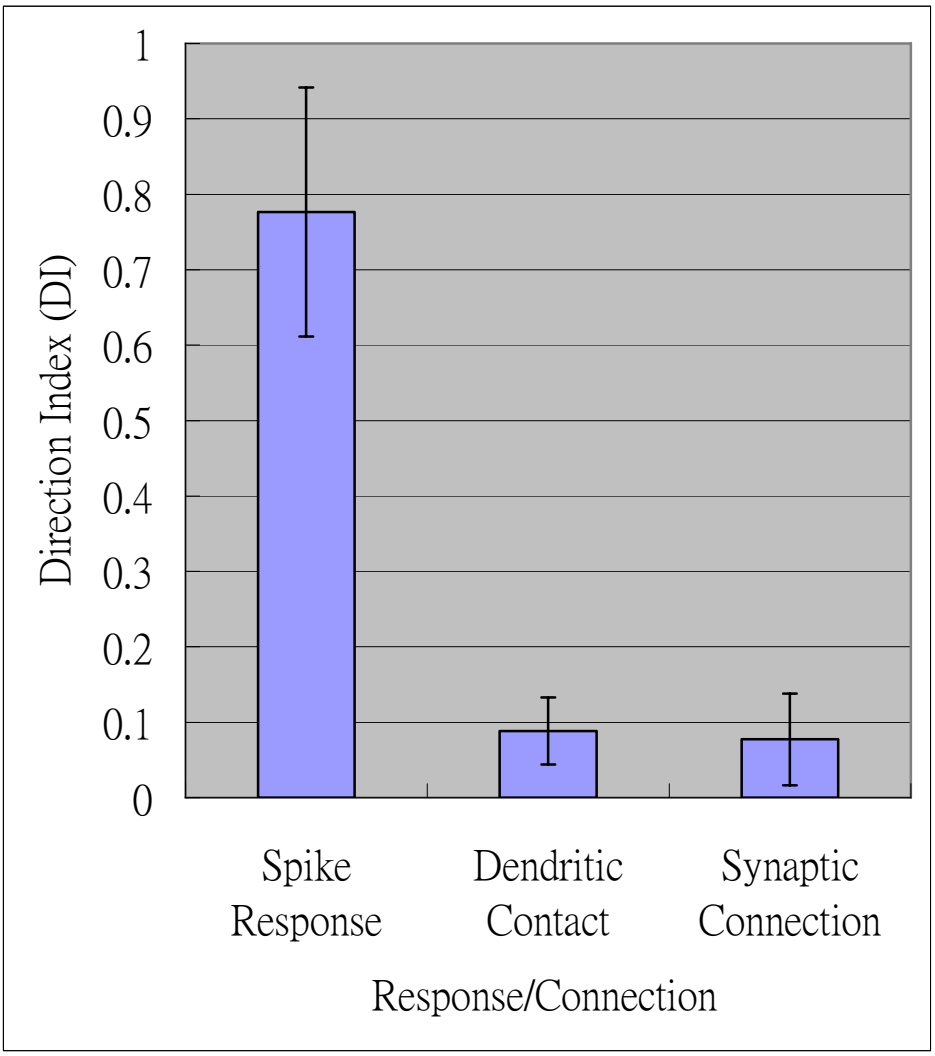


Fig. 19. Direction Indices (*DI*s) of spike response, dendritic contact, and synaptic connection.

A histogram shows the *DI*s of the spike response, the dendritic contact, and the synaptic connection. The dendritic contact (0.09 ± 0.04, mean ± s.d.) and the synaptic connection (0.08 ± 0.06) obviously express a symmetric pattern comparing with the spike response (0.78 ± 0.16).

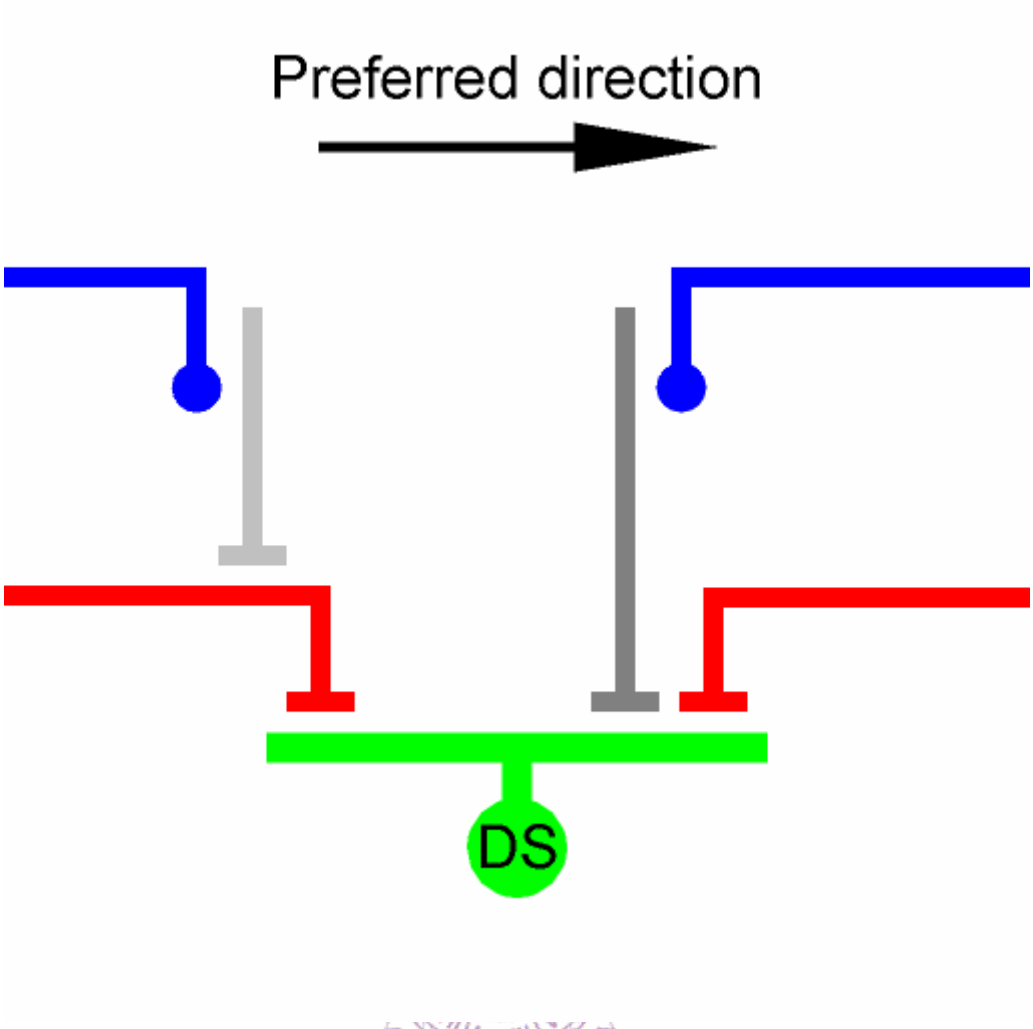
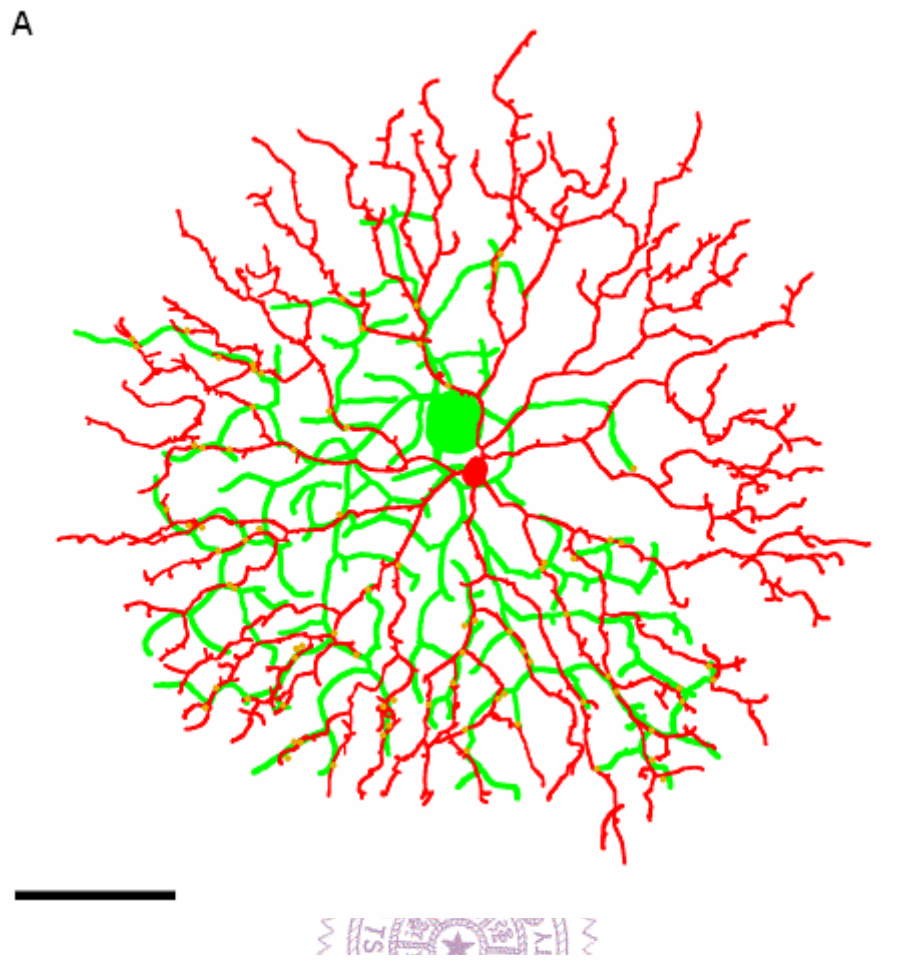
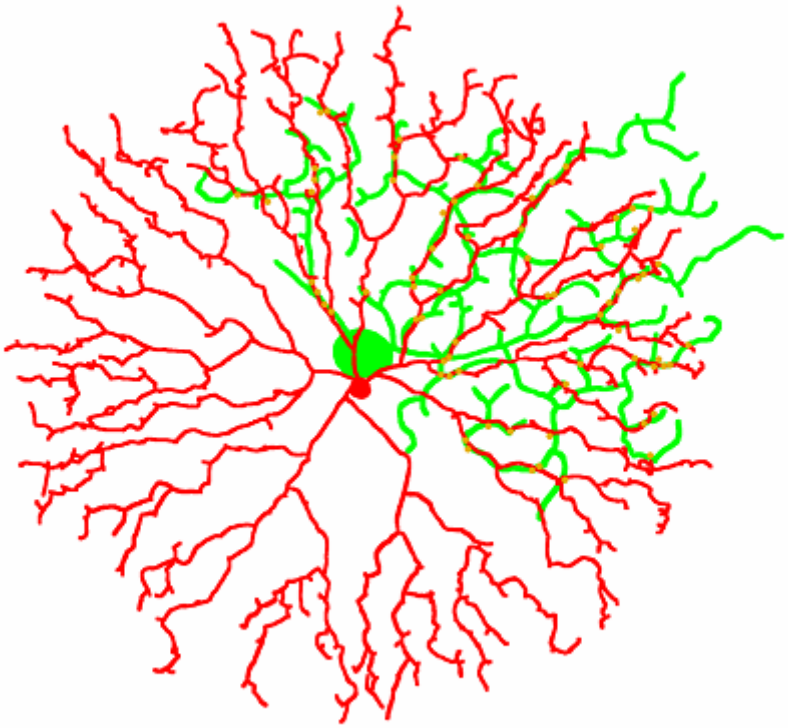


Fig. 20. Schematic diagram of inhibition asymmetry of direction selectivity

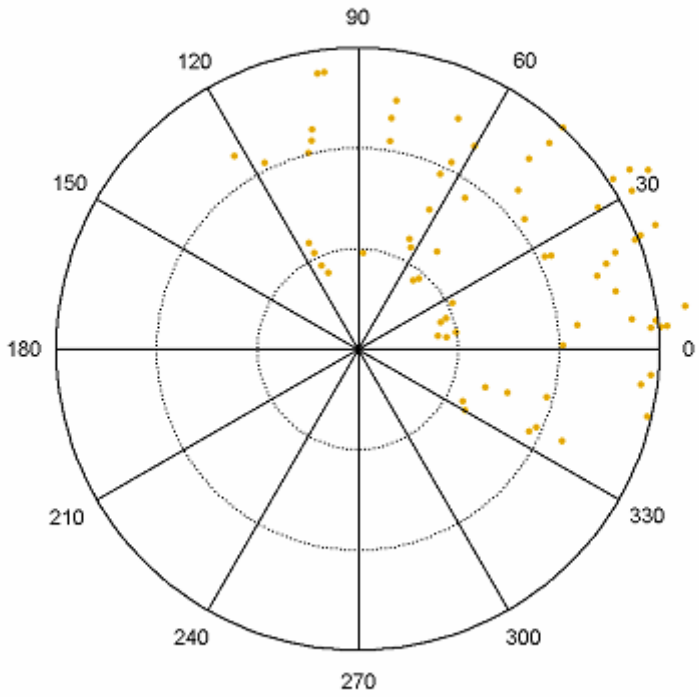
A DSGC (green) receives equal GABAergic inhibitions from the SAC dendrites (red) which projected from either the preferred side or the null side. An upstream driving factor (light gray) may suppress the GABA release from those SAC dendrites which projected from the preferred side. This suppression is probably mediated by the cholinergic input (blue). There is no such suppression onto the SAC dendrites originated from the null side. Instead, an additional direct inhibitory input (dark gray) mediated by the cholinergic drive may be involved. Direction selectivity can be generated by this asymmetric inhibition.

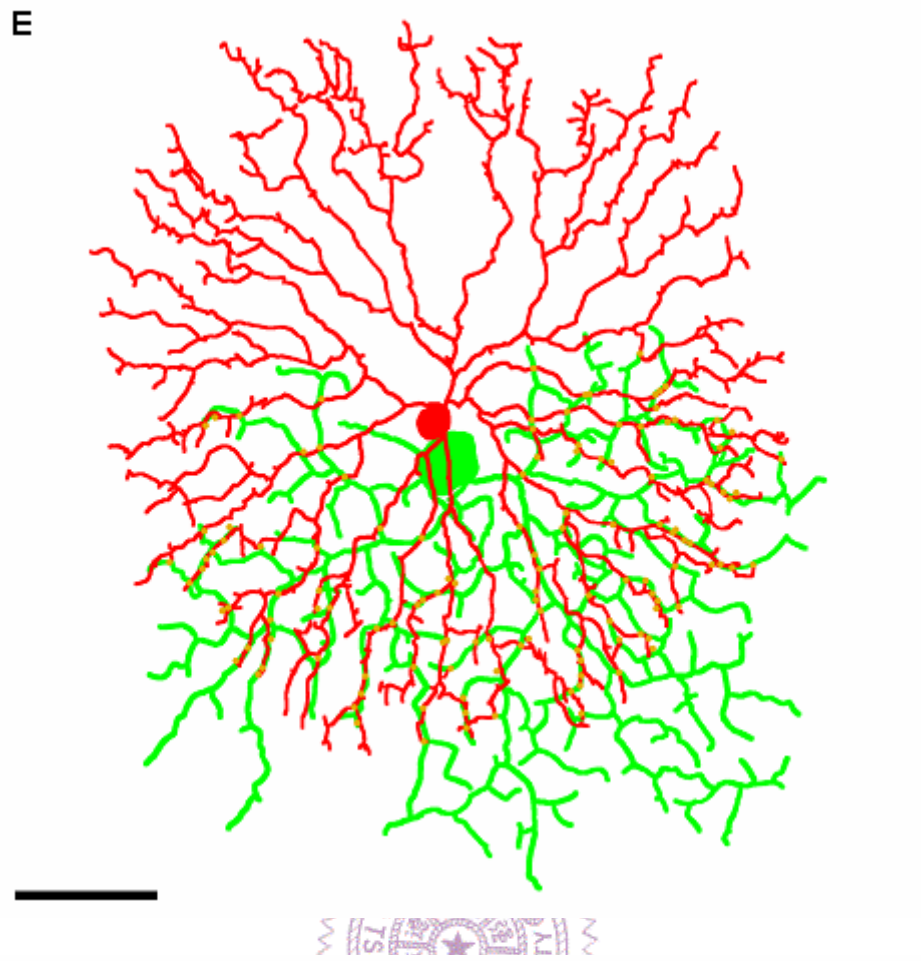

A
B
90
120
60
150
30
180
0
210
330
240
300
270

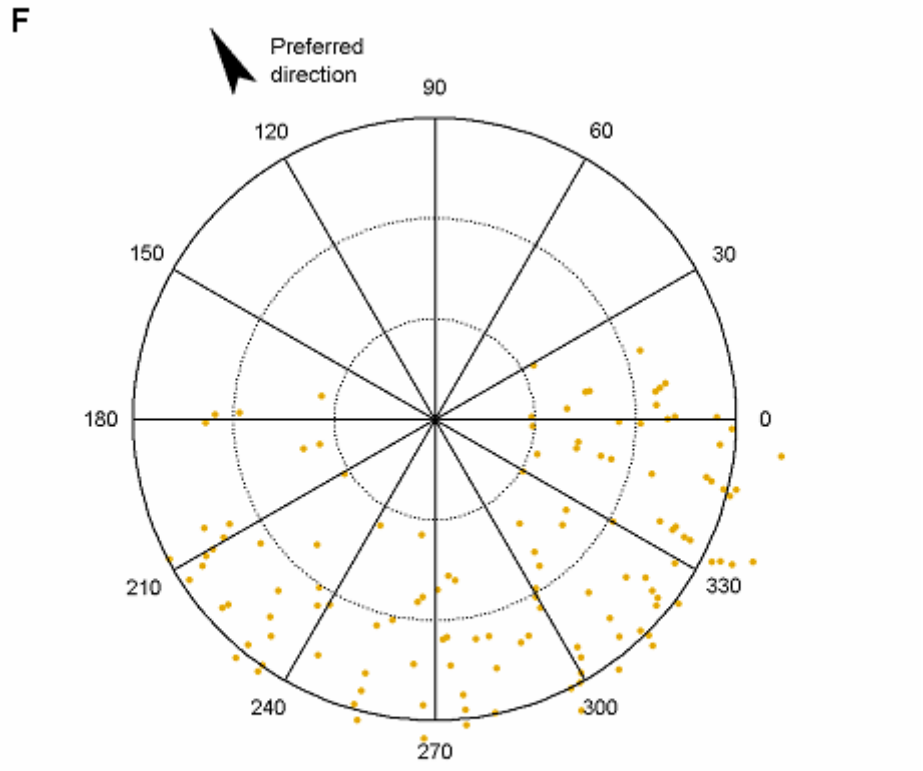
C



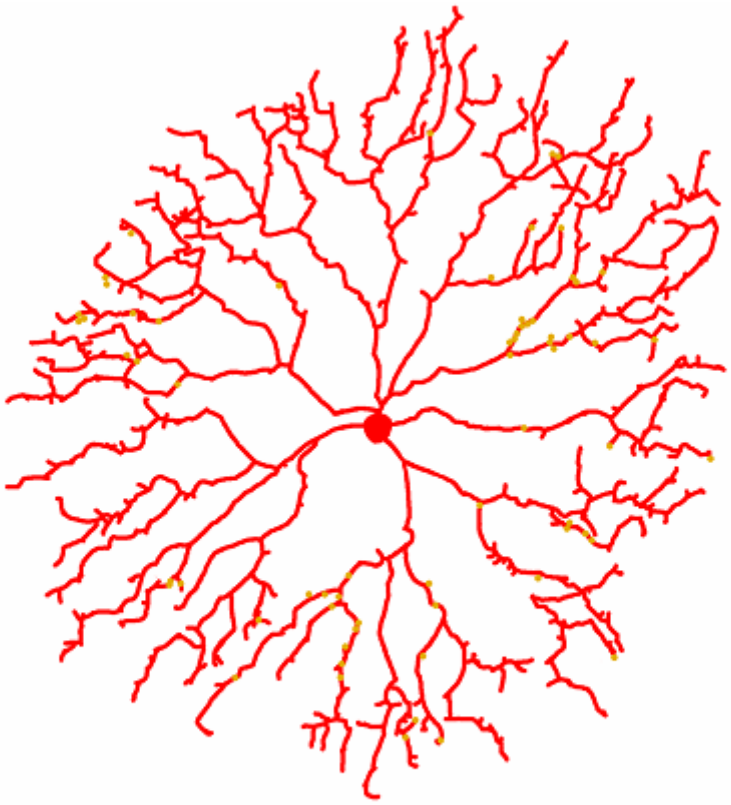
D




E


F
Preferred
direction
90
60
30
0
330
300
270
240
210
180
150
120

G



H

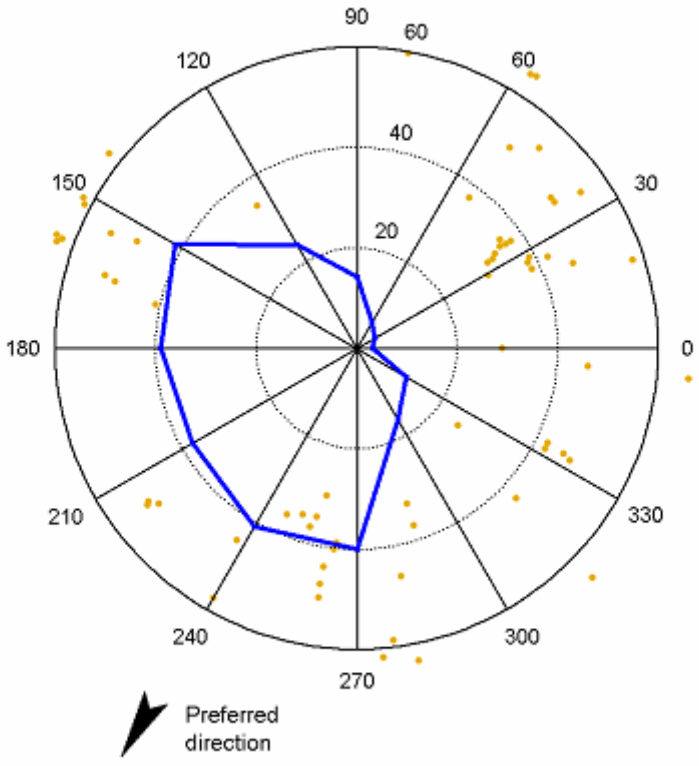

90
60
60
120
40
30
150
20
180
0
210
330
240
300
270
Preferred
direction

Fig. S1. The unqualified cell pairs for calculating Direction Index (*DI*) of synaptic connection.

The SAC dendrites were not completely covered by the dendrites of DSGC in some cell pairs, thus these pairs can not be quantified (the DSGC in G were absent because the dendrites of this cell undulated up and down, made it difficult to trace). However, the $GABA_A$ puncta in the regions where the DSGC dendrites covered still exhibit a random and even distribution pattern. **A,C,E,G:** A hand drawing from a digital photomicrograph, both the ON arbor of DSGC (green) and the SAC dendrite (red) were depicted. The immunolabeled γ-amino butyric acid-A ($GABA_A$) receptors were represented as dots. $GABA_A$ receptors colocalized with the dendrites of both DSGCs and SAC were viewed as the effective inhibitoy synaptic inputs (brown dots) for the two cells. **B,D,F,H:** Clear representations of the inhibitory synaptic inputs correspond to A, C, E, G, respectively. The $GABA_A$ receptors were separated from the dendrites, thus distribution pattern can be easily seen. Notably, the physiological results were included in F and H. Polar plot represent the averaged spike numbers, and arrowheads indicate the preferred direction (in F, only the preferred direction was marked, because the membrane potential of this cell was not stable to record). Scale bar = 50 μm