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Supplemental Information

Müller Glial Cells Participate in Retinal Waves via Glutamate Transporters and AMPA Receptors

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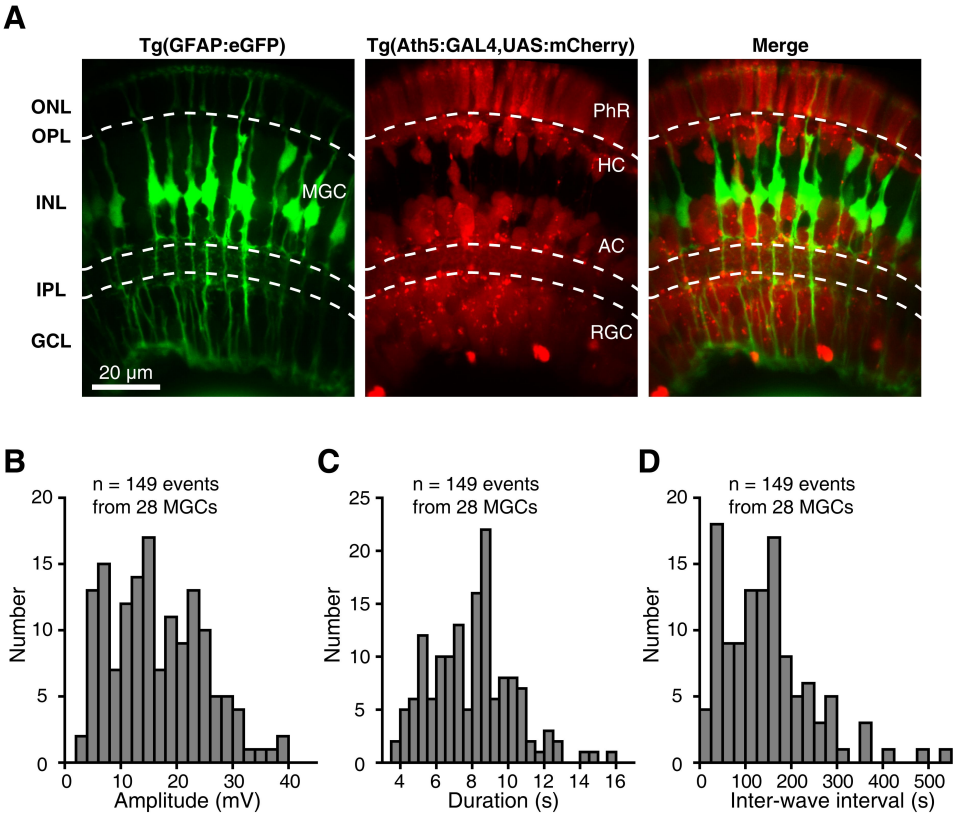

Figure S1. Morphology of MGCs and Characterization of MGCs' Wave-Like Spontaneous Electrical Activities in Zebrafish Larvae, Related to Figure 1.

(A) In vivo confocal images show MGCs and other retinal cells (including PhRs, HCs, ACs and RGCs) in a Tg(GFAP:eGFP,Ath5-gal4,UAS:mCherry) larva aged at 3 dpf. The top dash line indicates the location of the outer plexiform layer (OPL), and the bottom two lines indicates the boundaries of the inner plexiform layer (IPL). AC, amacrine cell; HC, horizontal cell; GCL, ganglion cell layer; INL, inner nuclear layer; MGC, Müller glial cell; ONL, outer nuclear layer; PhR, photoreceptor; RGC, retinal ganglion cell.

(B-D) Distribution of the amplitude (B), duration (C), and inter-wave interval (D) of spontaneous wave-like rhythmic electrical activities from 149 events in 28 MGCs at 3 dpf. Bin sizes: 2 mV (B), 0.5 s (C), 25 s (D).

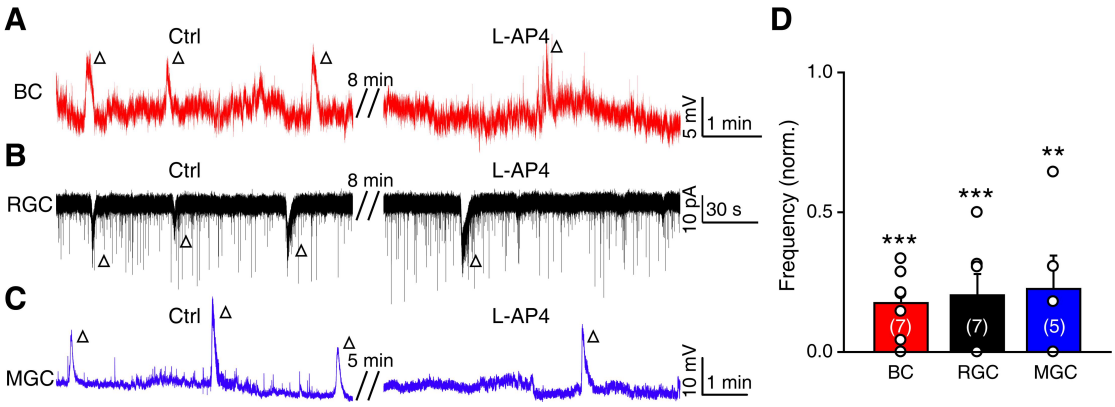

Figure S2. Activation of Type III Metabolic Glutamate Receptors Impairs Waves Activities in BCs, RGCs and MGCs, Related to Figure 3.

(A-C) Example traces showing spontaneous wave-like activities of a BC (A), a RGC (B), and a MGC (C) before (left) and after (right) bath application of L-AP4 (50 μM), an agonist of type III metabolic glutamate receptors. Each open arrowhead represents a wave-like event.

(D) Summary of data showing the effect of L-AP4 on the occurrence of waves in BCs, RGCs and MGCs. The wave frequency after L-AP4 application was divided by that before the drug application. Each dot indicates the data point from one cell.

The numbers on the bars indicate the numbers of cells examined. The two-tailed paired Student's *t*-test was used. **p < 0.01, ***p < 0.001. Data are represented as mean ± SEM.

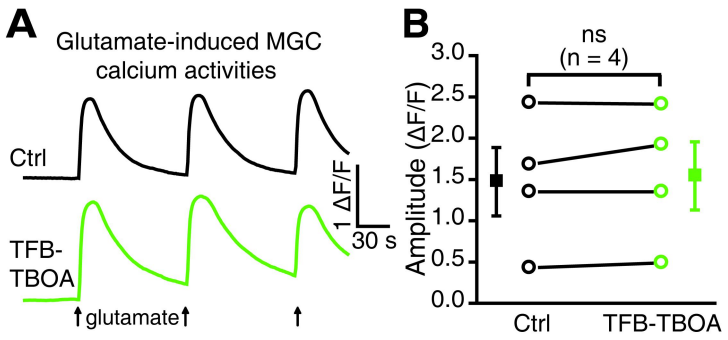

Figure S3. Calcium Waves of MGCs Are Not Dependent on Glial Glutamate Transporters, Related to Figure 5.

(A) Glutamate-induced calcium activities of MGCs before (top) and after (bottom) bath application of TFB-TBOA (1 μM).

(B) Summary of data showing the TFB-TBOA effect on glutamate-evoked calcium activities of MGCs.

The data obtained from the same larva are connected by a line, and the number in the brackets indicates the number of larvae examined. Two-tailed paired Student's *t*-test was used for data in (B). ns, not significant. Data are represented as mean ± SEM.

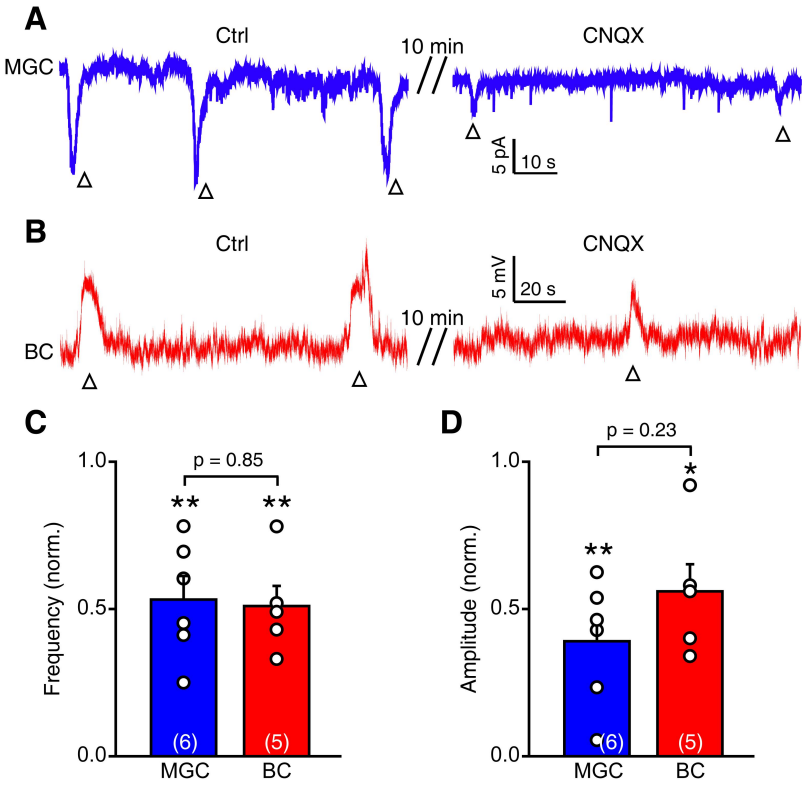

Figure S4. Blockade of AMPA Receptors Impairs the Electrical Activity of MGCs' and BCs' Waves, Related to Figure 4.

(A and B) Example traces showing spontaneous wave-like electrical activities of a MGC (A) and a BC (B) before (left) and after (right) bath application of CNQX (50 μM). Each open arrowhead represents a wave-like event.

(C and D) Summary of data showing the effects of CNQX on the frequency (C) and amplitude (D) of the electrical activities of MGCs' and BCs' waves.

The numbers on the bars indicate the numbers of cells examined. The two-tailed paired or unpaired Student's *t*-test was used for intra-group or inter-group comparison, respectively. *p< 0.05, **p< 0.01. Data are represented as mean ± SEM.

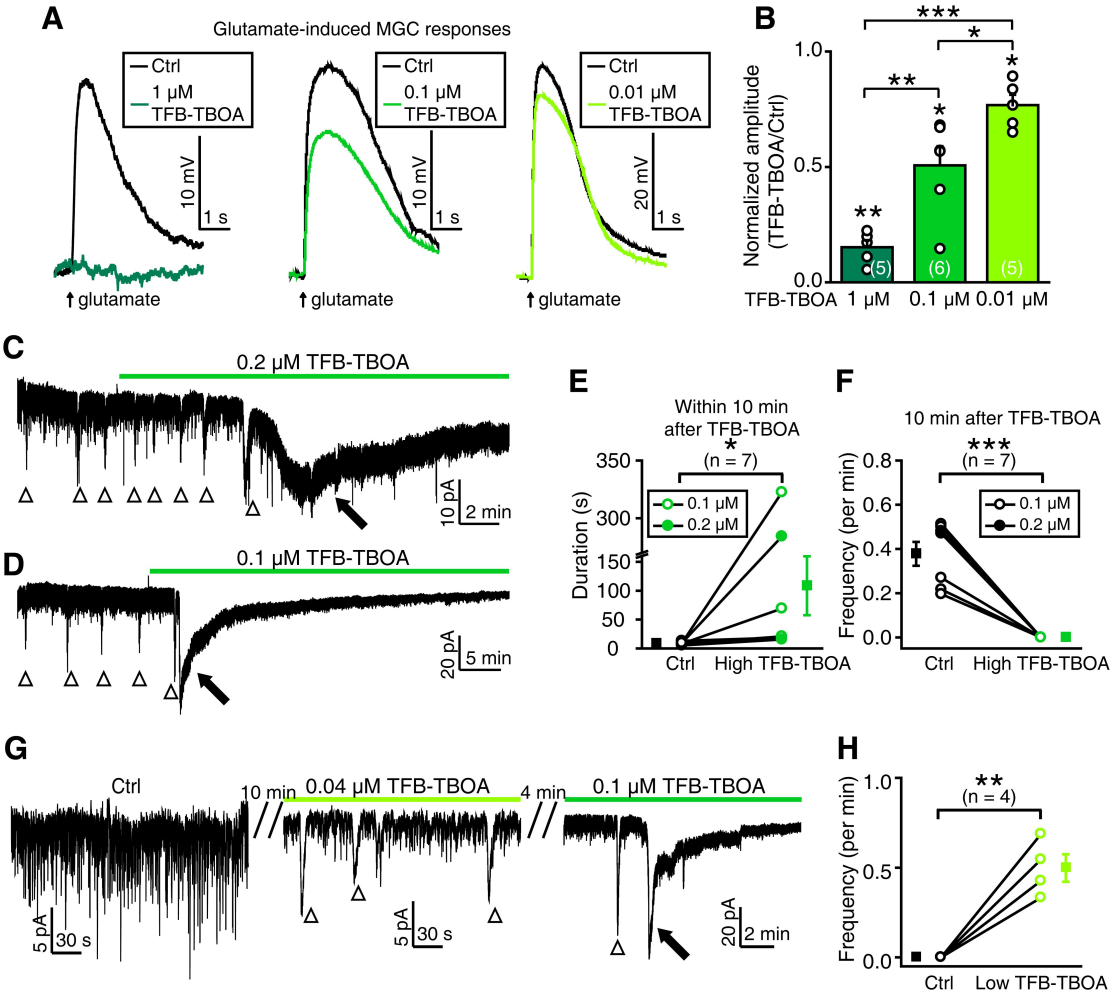

Figure S5. Different effects of High- and Low-Dose TFB-TBOA on Glutamate-Induced Responses of Müller Glial cells and Spontaneous Activities of RGCs, Related to Figure 6.

(A) MGC responses evoked by local puffing of glutamate to MGC processes in the IPL under different doses of TFB-TBOA (1 μM, 0.1 μM, and 0.01 μM). MGCs were recorded under current-clamp mode, and synaptic transmission was blocked by adding Co^{2+} in the external solution.

(B) Summary of data showing the effects of different doses of TFB-TBOA on glutamate-induced MGC responses. The data of 1-μM TFB-TBOA treatment was the same with those in Figure 4B.

(C and D) Effects of high-dose TFB-TBOA application (0.2 μM and 0.1 μM) on spontaneous wave activities of RGCs. Arrowhead, wave-like activity; filled arrow, drug-induced long-lasting inward current.

(E and F) Summary of data showing the change of RGC wave duration within 10 min after TFB-TBOA treatment (E), and the abolishment of RGC waves 10 min after TFB-TBOA application (F). The filled or open dots indicate 0.2- or 0.1-μM TFB-TBOA treatment, respectively.

(G) Example traces showing that low-dose TFB-TBOA (0.04 μM) induced wave-like electrical activities in the RGC with no native wave, while subsequent high-dose TFB-TBOA (0.1 μM) caused a long-lasting inward current (filled arrow). Notably, the scale bars are different among these traces.

(H) Summary of data showing that low-dose TFB-TBOA can facilitate the wave occurrence in RGCs with no native waves.

The numbers in the brackets and on the bars indicate the numbers of MGCs examined. The two-tailed paired or unpaired Student's *t*-test was used for intra-group or inter-group comparison in (B), respectively. The Mann-Whitney test was used for the data in (E), and the two-tailed paired Student's *t*-test was used for data in (F) and (H). *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. Data are represented as mean ± SEM.

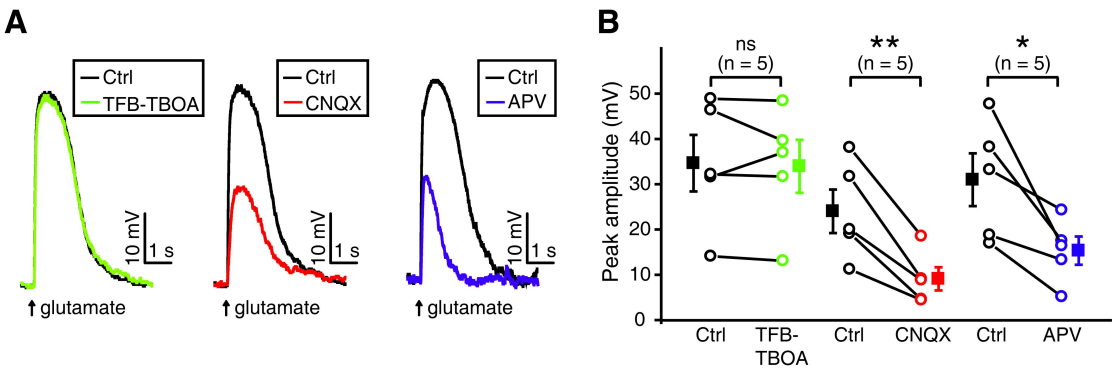

Figure S6. Glutamate-Induced Responses of RGCs Are Mediated by Ionotropic Glutamate Receptors But Not Glutamate Transporters, Related to Figure 6.

(A) RGC responses evoked by local puffing of glutamate to RGC dendrites in the IPL under control (black), bath application of TFB-TBOA (1 μM) (green), CNQX (50 μM) (red), or APV (50 μM) (blue). MGCs were recorded under current-clamp mode, and synaptic transmission was blocked by adding Co^{2+} in the external solution.

(B) Summary of data. The data obtained from the same RGC are connected by a line. The numbers in the brackets indicate the numbers of cells examined. The two-tailed paired Student's *t*-test was used for the data in (B). ns, not significant; *p< 0.05, **p< 0.01. Data are represented as mean ± SEM.

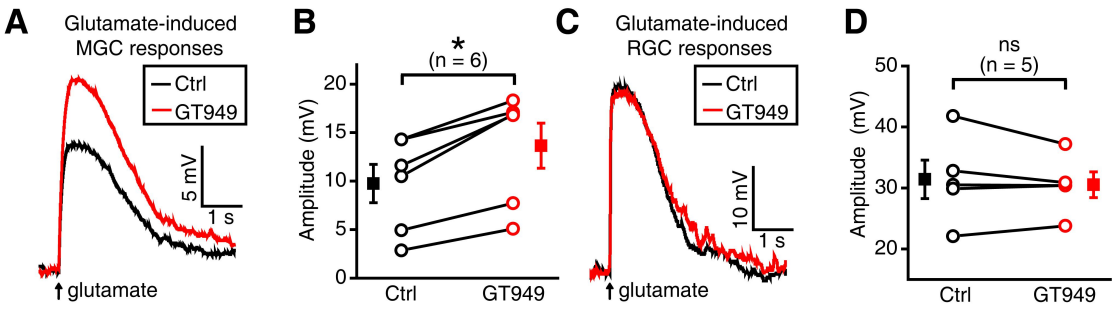

Figure S7. Effects of the Glial Glutamate Transporter Modulator GT949 on Glutamate-Induced Responses of MGCs and RGCs, Related to Figure 6.

(A) Effect of GT949 (0.1 μM) on glutamate-evoked electrical activities in MGCs. Here, CNQX was added in the bath to abolish the activation of AMPA receptors.

(B) Summary of data. The data obtained from the same MGC are connected by a line.

(C) Effect of GT949 (0.1 μM) on glutamate-evoked electrical activities in RGCs.

(D) Summary of data. The data obtained from the same RGC are connected by a line.

The numbers in the brackets indicate the numbers of cells examined. The Mann-Whitney test was used for the data in (B), and the two-tailed paired Student's *t*-test was used for data in (D). ns, not significant; *p< 0.05. Data are represented as mean ± SEM.