Inhibition to Excitation Ratio Regulates Visual System Responses and Behavior in vivo

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Abbreviated title: I/E Regulates Visual Function

Figures: 12

Tables: 2

Keywords: Inhibition/Excitation ratio; Temporal precision; Receptive field; γ-aminobutyric acid; GABA_A Receptor; Synaptic transmission; Inhibition; Excitability; Visual avoidance behavior; First spike latency; Xenopus; retinotectal; homeostasis

Acknowledgements: We thank Massimo Scanziani, Carlos Aizenman and members of Cline lab for helpful discussions. This work was supported by the NEI (EY011261), the Nancy Lurie Marks Foundation, an endowment from the Hahn Foundation and DNS, LLC to HTC.
Abstract

The balance of inhibitory to excitatory synaptic inputs is thought to control information processing and behavioral output of the CNS. We sought to test the effects of decreased or increased inhibition/excitation (I/E) ratio on visual circuit function and visually-guided behavior in *Xenopus* tadpoles. We selectively decreased inhibitory synaptic transmission in optic tectal neurons by knocking down the \( \gamma_2 \) subunit of the GABA\(_A\) receptors (GABA\(_A\)R) using antisense morpholino oligonucleotides or by expressing a peptide corresponding to an intracellular loop of the \( \gamma_2 \) subunit, called ICL, that interferes with anchoring GABA\(_A\)R at synapses. Recordings of mIPSCs and mEPSCs showed that these treatments decreased the frequency of mIPSCs compared to control tectal neurons without affecting mEPSC frequency, resulting in a ~50% decrease in the ratio of inhibitory to excitatory synaptic input. ICL expression and \( \gamma_2 \) subunit knockdown also decreased the ratio of optic nerve evoked synaptic inhibitory to excitatory responses. We recorded visually-evoked responses from optic tectal neurons in which the synaptic I/E ratio was decreased. Decreasing the synaptic I/E ratio in tectal neurons increased the variance of first-spike latency in response to full field visual stimulation, increased recurrent activity in the tectal circuit, enlarged spatial receptive fields and lengthened the temporal integration window. We used the benzodiazepine, diazepam, to increase inhibitory synaptic activity. Diazepam increased optic nerve evoked inhibitory transmission, but did not affect evoked excitatory currents, resulting in an increase in the I/E ratio of ~30%. Increasing the I/E ratio with diazepam decreased the variance of first-spike latency, decreased spatial receptive field size and lengthened temporal receptive fields. Sequential recordings of spikes and excitatory and inhibitory synaptic inputs to the same visual stimuli demonstrated that decreasing or increasing I/E ratio disrupted input/output relations. We assessed the effect of altered I/E ratio on a visually-guided behavior that requires the optic tectum. Increasing and decreasing I/E in tectal neurons blocked the tectally-mediated visual avoidance behavior. Because ICL expression, \( \gamma_2 \)-subunit knockdown and diazepam did not directly affect excitatory synaptic transmission, we interpret the results of our study as evidence that partially decreasing or increasing the ratio of inhibition to excitation disrupts several measures of visual system information processing and visually-guided behavior in an intact vertebrate.
Introduction

The CNS is composed of networks of excitatory and inhibitory neurons that process information and control behavior. The strengths of excitatory and inhibitory synaptic inputs to neurons are balanced, such that the distribution and strengths of excitatory inputs appear to be matched by inhibitory inputs (Ascoli et al. 2008; Burkhalter 2008). A balance of excitatory and inhibitory inputs is postulated to allow neurons to operate over greater dynamic range in response to inputs (Tiesinga et al. 2008), and imbalances in excitatory and inhibitory inputs are thought to disrupt information flow within and between brain networks (Rubenstein and Merzenich 2003).

We were interested in testing the consequences of manipulating the ratio of inhibitory to excitatory inputs (I/E) on visual response properties and behavior. We used the visual system of Xenopus tadpoles to test this idea. The visual system is particularly useful for probing the postulated function of a balance of inhibition to excitation on circuit function. Neuronal responses to visual inputs, visual information processing and visually-controlled behaviors have been well characterized in a variety of experimental systems, and perturbations in these response properties can be readily recognized. For instance, information about the sensory world is represented in the timing of stimulus-induced spikes (Daw et al. 2006; Haider et al. 2006; Hasenstaub et al. 2005; Priebe and Ferster 2008; Tiesinga et al. 2008), spatial and temporal receptive fields of neurons (Liu et al. 2009; Nowak et al. 2009; Richards et al. 2010; Tao and Poo 2005) and input/output relations (Carvalho and Buonomano 2009; Mittmann et al. 2005; Pouille and Scanziani 2001; Tiesinga et al. 2008). In addition, visually-guided behaviors provide a readout of visual system function (Dong et al. 2009; Gahtan et al. 2005; Orger et al. 2008; Prusky et al. 2004).

In the visual system of Xenopus tadpoles excitatory inputs from retinal ganglion cells activate a highly-interconnected circuit of inhibitory and excitatory tectal neurons (Akerman and Cline 2006; Dong et al. 2009; Pratt et al. 2008; Tao and Poo 2005), some of which project to the hindbrain and spinal cord to control swimming behavior (Gahtan et al. 2005; Orger et al. 2008). The majority of synaptic GABA<sub>A</sub>R in the CNS contain the γ<sub>2</sub> subunit (Fritschy et al. 2003; Sieghart and Sperk 2002), which is required for anchoring GABA<sub>A</sub>R at postsynaptic sites (Alldred et al. 2005; Essrich et al. 1998; Meier and Grantyn 2004; Schweizer et al. 2003). Furthermore a peptide, called ICL, that corresponds to 95 amino acids of the intracellular loop between transmembrane domains 3 and 4 of the γ<sub>2</sub> subunit prevents anchoring of γ<sub>2</sub> subunit-containing GABA<sub>A</sub>R to synaptic sites (Alldred et al. 2005; Essrich et al. 1998; Meier and Grantyn 2004;
Switzerland et al. 2003). Our strategy was to selectively decrease GABA_A-R-receptor mediated synaptic transmission by expression of ICL or knocking down the γ2 subunit with antisense morpholino oligonucleotides. We increased GABA_A-R-mediated synaptic transmission by exposure to the GABA_A-R positive allosteric modulator, diazepam (DZ). Because these manipulations did not directly affect excitatory synaptic transmission, they provided us with the opportunity to assess the effect of decreased and increased ratios of inhibitory to excitatory synaptic input on visual response properties of tectal neurons and visually-guided behavior. The data indicate that modulating I/E in tectal neurons interferes with visual circuit function and visually-guided behavior.

**Materials and Methods**

All experimental protocols were approved by the Institutional Animal Care and Use Committee.

**Animals and Transfection**

*Xenopus* laevis tadpoles were generated from the lab colony or purchased from Nasco (Fort Atkinson, WI) and reared from stage 23 in an incubator at 16°C with a 12 hr dark/12 hr light cycle. Albino tadpoles of either sex were used for experiments. For tectal neuron transfections, stage 47/48 tadpoles were anesthetized in 0.02% MS-222 (Tricane methanesulfonate; Sigma) and electroporated with DNA plasmids (~0.6 µM) or morpholinos (200 µM) (Bestman et al. 2006). Bulk electroporation with CMV promotor constructs and morpholinos transfects both inhibitory and excitatory neurons in the optic tectum. Electroporation of the left or right half of the brain is accomplished by using unipolar current pulses, whereas bilateral electroporation is accomplished by switching the polarity of current pulses, as described (Haas et al. 2002).

**Plasmids and Morpholinos**

A dual CMV promoter plasmid expressing EGFP and ICL, EGFP and mICL (mutant ICL) or EGFP alone was used for transfections. ICL was modified from the murine sequence of ICL (kindly provided by Dr. Simon Rumpel) (Shen et al. 2009). The mICL is generated by exchanging Ser (S) to Ala (A) of ICL (HYFVSNRKPSKDKKKNPLLRMFŠFKAPTIDIPRSATIQMNTHLQERDEEYGY ECLDGKDCASFFCCFEDCRTGAWRHGRIHIIRIKMS) (Shen et al. 2009). The *Xenopus laevis* cDNA of the GABA_A-R γ2 subunit was cloned from a cDNA library made with the SMART RACE cDNA Amplification kit (Clontech Laboratories, Mountain View,
CA) according to the manufacturer’s protocol and sequenced. To knock down the
GABA<sub>AR</sub> γ2 subunit, we transfected neurons with a translation-blocking morpholino
against Xenopus γ2 subunit (γ2-MO; GATCGCATCTCTCGCGTGTTCGA) or a five
pair nucleotide mismatch control morpholino (Ctrl-MO; GATgGCATgTCTCcTCGCcTTTgGA) from Gene Tools (Philomath, OR), both of which
were tagged with carboxyfluorescein.

**Western Blots**
Stage 47 tadpole midbrains were dissected and immediately homogenized in ice-cold
radioimmunoprecipitation assay (RIPA) buffer containing a protease inhibitor cocktail
(Roche Applied Science) and 1 mM sodium orthovanadate. Equal amounts of protein
homogenates for each experimental group were separated by SDS-polyacrylamide gel
electrophoresis (PAGE, 12%) and transferred to nitrocellulose membranes. Blots were
blocked in 5% non-fat milk with 0.1% TBS and TritonX-100 (Sigma), and incubated with
rabbit polyclonal GABA<sub>AR</sub> γ2 antibody (1:200, ab4073, Abcam, Cambridge, MA) diluted
in blocking solution. Blots were rinsed and incubated with HRP-conjugated secondary
antibodies (Amersham Biosciences, Piscataway, NJ). Bands were visualized using ECL
reagent (Amersham Biosciences). Blots were stripped and reprobed with β-tubulin
antibody (1:200, SC-9104, Santa Cruz Biotechnology, Santa Cruz, CA).

**Immunofluorescence**
Anesthetized stage 47 tadpoles were dissected to remove the skin and meninges over
the brain. Animals were transferred to 4% paraformaldehyde (Electron Microscopy
Sciences, Fort Washington, PA) in phosphate buffer (PB, pH 7.4), exposed to a brief
microwave pulse and left to fix for an additional 2 hrs at room temperature. Animals
were cryoprotected overnight in 30% sucrose (Sigma) and brains were removed and
cut into 20 µm cryostat sections. Sections were permeabilized with 1% TritonX-100 in
PB, and blocked with 5% goat serum (Gibco, Grand Island, NY) in 1% TritonX-100 and
incubated in rabbit polyclonal GABA<sub>AR</sub> γ2 antibody (diluted 1:200 in 2% goat serum
with 0.1% TritonX-100 in PB) overnight at 4°C. Sections were rinsed with PB and
incubated in secondary antibodies (Alexa Fluor 568 goat anti rabbit, Invitrogen, Eugene,
OR). To quantify the immunoreactivity, background subtracted fluorescence intensity
was measured per unit area in the neuropil with ImageJ 1.36 (W. Rasband, NIH,
Bethesda, MD). We compared the ratio of intensity between the MO-transfected and
contralateral control sides of tectum, by measuring intensity in the same size and
location of neuropil area. For each tectum, the immunofluorescence intensity from three
different locations was averaged.

Electrophysiology

Experiments were performed at room temperature (20 - 22°C). For all recordings, the
brains were perfused with extracellular saline containing (in mM: 115 NaCl, 2 KCl, 3
CaCl₂, 1.5 MgCl₂, 5 HEPES, 10 glucose, and 0.01 glycine, pH 7.2, osmolality 255
mOsm) throughout the recording. For recordings from neurons in isolated brains,
tadpoles were anesthetized with 0.02% MS-222 (Sigma), brains were dissected and
neurons were recorded in whole cell mode as described (Shen et al. 2009). When
required, TTX (1 µM, Sigma), diazepam (10 µM, Sigma), NBQX (20 µM, Sigma) and dl-
APV (50 µM, Sigma) were included in the extracellular saline. Miniature synaptic
currents (mIPSCs and mEPSCs) were recorded from neurons in isolated brains using a
Cs⁺-based pipette solution (in mM: 114 Cs-methane sulfonate, 1.5 MgCl₂, 3 TEA-Cl, 20
HEPES, 10 EGTA, 2 ATP, and 0.3 GTP, pH 7.2, osmolality 255 mOsm) in the presence
of 1 µM TTX. The first 30 miniature events per neuron were used for analysis to ensure
similar sampling from each cell. For electrical stimulation experiments, a bipolar
stimulating electrode (Frederick Haer Company, Bowdoinham, ME) was placed in the
optic chiasm or tectum to stimulate axons with a Master-8 stimulator (AMPI, Israel).

For in vivo recordings, we used an regenerating ATP internal solution (Wang et al.
2003): (in mM) K-Gluconate 95, MgCl₂-6H₂O 1.5, Na₂-phosphocreatine 10, KCl 13,
HEPES 20, EGTA 10, ATP 4, GTP 0.3, and creatine phosphokinase 10 units/ml. Cell-
attached recording was started at least 5 minutes after gigaseal was obtained. After the
cell-attached recording, the membrane in the patch was ruptured and membrane
potential was voltage clamped at - 60 mV to record AMPA receptor (AMPAR)-mediated
currents or at 0 mV to record GABA₉R-mediated currents.

Recording micropipettes were pulled from borosilicate glass capillaries (Warner
Instruments, Hamden, CT) with a pipette puller (P-97, Sutter Instrument Company,
Novato, CA) and had resistances in the range of 7 - 9 MΩ when filled with Cs⁺-based
pipette solution. All cells were recorded from the middle of the rostrocaudal axis of the
tectum. Liquid junction potential was adjusted during recording. Signals were filtered at
2 kHz with a Multiclamp 700B amplifier (Molecular Devices, Palo Alto, CA) and a
Digidata 1440A Data Acquisition System (Molecular Devices). Data were sampled at
10 kHz with Clampex 10 (Molecular Devices) and analyzed using a ClampFit 10
(Molecular Devices), Mini Analysis Program (Synaptosoft) or Matlab (The Mathworks,
Natick, MA). IPSCs decay kinetics were measured by a weighted decay time constant ($\tau_{\text{decay}}$) by fitting with an exponential function with ClampFit 10.

The full field visual stimuli with three luminance steps (10, 20 and 250 cd/m$^2$) were generated by Matlab and presented from lowest to highest luminance from a projector (Samsung, sp-p310ME LED projector). Luminance values were measured by a photometer (Model: IL1400BL, International Light, MA). Each stimulus was presented 10 times at a frequency of 0.1 Hz and with an interval of 0.05 Hz. Because the majority of tectal neurons at these stages show predominant light off responses, we analyzed the responses, including spikes, excitatory compound synaptic currents (eCSCs) and inhibitory compound synaptic currents (iCSCs) to light off stimuli. As controls, eCSCs and iCSCs were recorded from cells transfected with control morpholinos and mICL. The ratios of iCSCs to eCSCs were not significantly different for Ctrl-MO and mICL neurons and were therefore combined. The iCSC/eCSC ratios for the 3 luminance values tested for the Ctrl-MO and mICL neurons were Luminance 10 cd/m$^2$: 1.89±0.71 (Ctrl-MO) 2.06±1.32 (mICL); Luminance 20 cd/m$^2$: 1.57±0.41(Ctrl-MO) 2.04±1.01 (mICL); Luminance 250 cd/m$^2$: 1.32±0.47(Ctrl-MO), 0.97±0.28 (mICL); N=4/3; p>0.05 for all comparisons.

**Receptive Field Mapping**

For temporal receptive field mapping, full-field Gaussian white noise flicker stimuli at 50 Hz were presented to the eye from a fiber optic and neurons from the contralateral tectum were recorded. The stimulus luminance was gamma corrected. Spikes were recorded from tectal neurons in cell-attached mode for 20 minutes. Temporal receptive fields were measured by spike-triggered average for cell-attached recording or reverse correlation for whole-cell recording (Vislay-Meltzer et al. 2006). Many tectal cells in tadpoles have monophasic temporal receptive fields with negative deflections, consistent with observations that responses to light-off stimuli are stronger than light-on stimuli in the majority of tectal cells. The temporal receptive fields were normalized to the peak response over a window of 600 ms, when the average response returns to baseline. The full width at half maximum (FWHM) of the normalized receptive field was used as the measure for the length of the temporal receptive fields (Butts et al. 2007; Vislay-Meltzer et al. 2006).

Spatial receptive fields were mapped using methods modified from previous studies (Butts et al. 2007; Dong et al. 2009; Engert et al. 2002; Vislay-Meltzer et al. 2006). A projector (Samsung, sp-p310ME LED projector) was used to project computer-
generated stimuli to the retina through a back-projector screen (Figure 5A). Tadpoles were anesthetized and the skin peeled back to expose the tectum. Tadpoles were stabilized on a sylgard cushion with pins. The sylgard cushion was attached to a bar in the recording chamber which can be rotated so that tadpole was mounted with one eye facing the projector screen. Brightness was controlled by neutral density filters (Kodak) (luminance range: 3 - 55 cd/m²) and image size from the projector was controlled by a convex lens. The receptive field was measured with an 8×8 grid of 0.5×0.5 cm² non-overlapping squares covering a 4.5×4.5 cm² area in the visual field. White squares on a dark background or dark squares on a white background were presented for 1.5 s in random order 64 times with 5 s intervals until the entire visual field is mapped. The mapping sequence was repeated two or three times to eliminate possible contamination by spontaneous activity. The response at each grid position was determined as the average number of total spikes per stimulus and presented as a grey scale map. After cell-attached recording, negative pressure was applied to the electrode to break through for whole-cell voltage clamp recording. The same stimuli were used to measure inhibitory spatial receptive fields and excitatory spatial receptive fields by holding membrane potential at 0 mV and -60 mV respectively. Total synaptic charge transfer over 0.6 s from the onset of stimulus was normalized and computed by Matlab to show spatial receptive field size. All values that are larger than 3 times the standard deviation of spontaneous activity were included in the measurements of spatial receptive fields. Only neurons from which both excitatory receptive fields (eRFs) and inhibitory receptive fields (iRFs) were successfully recorded were included in the correlation analysis. For cell-attached recording, the value at each grid position was normalized to the total number of evoked spikes within 0.6 s after the onset of the off stimulus. Spatial receptive fields for synaptic currents are represented on a map with each square of the grid coded in a grey scale according to the normalized total charge transfer in response to the visual stimulus. Spontaneous activity was measured during last 1 s after each off stimulus.

Visual Avoidance Assay

Animals were screened using an optomotor response, which does not require the optic tectum (Dong et al. 2009; Pronych et al. 1996) and serves as an indicator of non-tectal visual function and animal health. Animals were placed in a 16 X10 cm tank filled with ~1 cm rearing solution and exposed to a stimulus of alternating black and white bars drifting in one direction. After 30 seconds we counted the number of animals located in the half of the chamber toward which the gratings drifted, as previously described (Dong et al.)
Animals that do not respond to the optomotor stimulus are not used for further behavior tests. We then screened animals a second time with a visual stimulus of randomized moving spots. Only tadpoles that show an avoidance response to the moving spots stimulus with a diameter of 0.4 cm are used for further analysis. Animals in which the optic tectum was electroporated with EGFP, mICL, ICL, Ctrl-MO, or γ2-MO exhibit a normal optomotor response, indicating that electroporation itself or the specific reagents do not affect the ability of tadpoles to swim or respond to visual stimuli. Animals treated with diazepam (10 µM or 30 µM) show normal swimming behavior (Figure 9F). In some experiments we injected 1~2 µl of diazepam (1 mM) or vehicle (1% DMSO in 0.9% NaCl) into the tectal ventricle with a micropipette and let the tadpoles recover for 2 hours before testing behavior.

For the visual avoidance assay, single tadpoles are placed in a 16 X10 cm tank filled ~1 cm with rearing solution. The custom-made tank with a back-projection screen is mounted on top of a clear Plexiglas. Each animal is given 2 minutes to distribute within the tank and then visual stimuli are presented for 30 seconds/stimulus using a microprojector (3M, MPro110, luminance range: 3-90 cd/m²) positioned below the tank. The positions of the tadpoles are illuminated by four arrays of IR LEDs. Videos of tadpoles and the stimuli captured on the screen are recorded with a Hamamatsu ORCA-ER digital camera at 15 frames/sec. The entire system is enclosed in light-tight compartment. Visual stimuli are generated and presented by MATLAB 2009b (The MathWorks, Psychophysics Toolbox extensions). Tadpoles are tested once for a series of different stimuli in which spot size, luminance, velocity and contrast are varied and presented in random order. An avoidance event is counted when a tadpole displays a sharp turn as a spot approaches the head at an angle between 75 and 105°. Avoidance behavior is counted from the first ten encounters with moving spots and plotted as % avoidance. Wild type and albino tadpoles show similar avoidance behavior when either of them had prescreening with optomotor test and spot test (data not shown). The Wilcoxon signed-rank test is used to test whether behavior was significantly different from random. A Mann–Whitney test is used to test for significant differences between experimental groups.

To estimate the size (S) of the spots used as visual stimuli, we used the equation

\[ V = 2 \arctan \left( \frac{S}{2D} \right) \]

to calculate the visual angle (V) corresponding to each spot size. D represents the distance from tadpole’s eye to the spot. Because the height of the solution in the tank is ~1 cm, we estimate the distance D from the eye to the spot is at
most 1 cm. Visual angles of 75°, 55°, 30°, 16°, and 6° correspond to spots with estimated diameters of 0.6, 0.4, 0.2, 0.2 and 0.04 cm.

**Statistical Analysis**

Student’s t-tests were used for paired data. An ANOVA test followed by post hoc Tukey’s test was used for multiple group data. Where noted Wilcoxon signed-rank tests or Mann-Whitney tests were used. Data are presented as individual data points or as mean ± SEM unless otherwise noted. Correlations were evaluated by Pearson’s correlation coefficient. Experiments and analysis were performed blind to the experimental condition, unless noted.

**Results**

**Knockdown of γ2-subunit of GABA<sub>A</sub> Receptors**

Our previous studies showed that expression of ICL decreases the frequency of GABAergic mIPSCs without affecting mEPSCs, and decreases the inhibition/excitation ratio of optic nerve-evoked retinotectal synaptic responses in *Xenopus* tectal neurons (Shen et al. 2009). To establish an independent method to decrease inhibitory synaptic transmission, we transfected tectal neurons with antisense morpholinos directed against the γ2-subunit of GABA<sub>A</sub>R (γ2-MO) to knock down endogenous γ2 subunit expression. γ2-MO transfection decreased γ2 subunit immunolabeling in tissue sections of the optic tectum (Figure 1A-C) and in Western blots by about 36% 2 days after transfection (Figure 1D), suggesting that γ2-MO-transfected cells have lower expression of γ2 subunit than controls. The partial loss of the γ2-subunit from transfected tecta could arise from partial transfection of the population of tectal cells or partial knockdown of protein in MO-transfected neurons.

**γ2-subunit Knockdown Selectively Decreases GABAergic Synaptic Transmission**

To assess the effect of γ2 subunit knockdown on GABAergic and glutamatergic synaptic transmission, we transfected neurons with γ2-MO or control morpholinos (hereafter referred to as γ2-MO neurons and Ctrl-MO neurons, respectively). We recorded GABA<sub>A</sub>R-mediated mIPSCs and glutamate-mediated mEPSCs 2 days later. The inter-event interval of mIPSCs in γ2-MO neurons is significantly greater than in Ctrl-MO neurons (Figure 2A, C), whereas the amplitudes of mIPSCs were not statistically different (Figure 2C). In contrast, the inter-event interval and the amplitudes of mEPSCs
in γ2-MO neurons are not significantly different from Ctrl-MO neurons (Figure 2D).

These data indicate that γ2-MO transfection selectively decreases GABAergic synaptic inputs, similar to the effect of ICL expression (Shen et al. 2009).

The γ2 subunit is required for benzodiazepine binding to GABA$_A$R (Sigel 2002). We therefore used the response to diazepam to test whether γ2-MOs decrease γ2 subunit-containing GABA$_A$Rs at postsynaptic sites. We used a stimulating and recording configuration that allowed us to record direct inhibitory synaptic activity from optic tectal neurons (Figure 2E): we placed a stimulating electrode in the tectum to excite local axons and collected whole cell patch clamp recordings at 0 mV holding potential from nearby neurons in the presence of NBQX to block excitatory transmission. Current injection through the stimulating electrode evoked direct inhibitory synaptic inputs in the recorded neurons (Figure 2F). To test whether inhibitory synaptic transmission onto γ2-MO neurons can be enhanced with diazepam, we recorded evoked inhibitory synaptic currents from γ2-MO neurons or control neurons before and after perfusion of diazepam (10 µM). Diazepam doubled the amplitude of evoked GABA$_A$R-mediated currents in Ctrl-MO neurons and lengthened the decay time of evoked inhibitory responses by ~50%, consistent with the presence of γ2-subunit containing synaptic GABA$_A$R, but diazepam did not affect the amplitude or time-course of evoked GABA$_A$R-mediated currents in γ2-MO cells (Figure 2F-H). Together these results indicate that γ2-MO selectively decreases inhibitory synaptic inputs without affecting AMPAR-mediated mEPSCs, and that this results from a selective loss of γ2 subunit-containing synaptic GABA$_A$R. The experiments with diazepam also show that diazepam significantly enhances monosynaptic evoked GABAergic responses in control tectal neurons, but that the inhibitory synaptic responses remaining in γ2-MO cells are mediated by GABA$_A$R that lack γ2 subunits. We reported a similar specificity of ICL expression in decreasing synaptic γ2-subunit containing synaptic GABA$_A$R responses (Shen et al. 2009).

γ2-MO Decreases and Diazepam Increases Inhibition/Excitation Ratio

Glutamatergic retinal inputs synapse on both excitatory and inhibitory tectal neurons and the inhibitory neurons form feedforward synapses on tectal neurons which are driven by visual input (Akerman and Cline 2006). Consequently, optic nerve stimulation evokes both excitatory and inhibitory synaptic inputs in the majority of tectal neurons. We tested whether γ2-MOs can be used to decrease the ratio of evoked retinotectal inhibitory to excitatory synaptic inputs in tectal neurons, and whether diazepam can be used to increase the ratio of evoked retinotectal inhibitory to excitatory synaptic inputs. We
placed a stimulation electrode in the optic chiasm of the isolated brain preparation and recorded optic nerve stimulation-evoked synaptic responses sequentially at holding potentials of 0 mV and -60 mV from the same neurons (Figure 3A). Since the amplitudes of the evoked synaptic responses depend on the numbers of retinal axons stimulated, we normalized the peak amplitudes of evoked IPSCs to the peak amplitudes of evoked EPSCs for each neuron. The ratio of the amplitudes of evoked IPSCs to EPSCs (or the I/E ratio) in γ2-MO cells is less than half the I/E ratio in Ctrl-MO cells, while the ratio of the amplitudes of evoked retinotectal IPSCs to EPSCs was significantly increased in diazepam (Figure 3B,C). These results indicate that γ2-MO-transfection can be used to decrease the ratio of evoked inhibitory to excitatory synaptic responses in tectal neurons, similar to our previous finding with ICL expression (Shen et al. 2009), and that diazepam can be used to increase the ratio of evoked inhibitory to excitatory synaptic responses.

**Extrasynaptic GABAergic Responses are Unaffected by ICL or γ2-subunit Knockdown**

Activation of extrasynaptic GABAₐR has been shown to affect neuronal excitability, sensory gating and responses to synaptic inputs (Farrant and Nusser 2005; Mody and Pearce 2004; Tang et al. 2011). Most extrasynaptic receptors are comprised of α, β and δ subunits, and lack γ2-subunits (Jacob et al. 2008; Mody and Pearce 2004). Extrasynaptic GABAₐR have approximately 10 fold higher affinity for GABA than synaptic GABAₐR, consistent with their activation by spillover of GABA from synaptic sites (Farrant and Nusser 2005; Mody and Pearce 2004; Sanna et al. 2009). Although tectal neurons have extrasynaptic GABAₐR (Akerman and Cline 2006), we predicted that neither ICL nor γ2-MO would affect responses of extrasynaptic GABAergic responses, because they lack γ2-subunits. To test this directly, we recorded tonic GABAergic currents in whole cell mode (holding at -60 mV) from control, γ2-MO and ICL-expressing tectal neurons from isolated brains, while blocking glutamatergic currents with kynurenic acid (2 mM) (Figure 4). Traces from representative cells are shown in Figure 4A,C and E. We collected a 5-minute period of baseline recording, during which spontaneous inhibitory postsynaptic currents (sIPSCs) can be seen in control neurons (Figure 4A). We applied 1 µM muscimol to activate high affinity extrasynaptic GABAₐR, as described (Sanna et al. 2009). Application of muscimol induced a hyperpolarizing shift in the holding current and increased the noise variance in all neurons recorded, consistent with the presence of extrasynaptic GABAergic receptors (Farrant and Nusser 2005; Mody and Pearce 2004; Sanna et al. 2009). Application of the GABAₐR antagonist, bicuculine
(100 μM) blocked the tonic current. Note that exposure to bicuculine does not result in a significant shift from the baseline (Figure 4A,C, E), suggesting that there is little ambient GABA available to activate extrasynaptic receptors in the isolated brain preparation used for these experiments. Although γ2-MO and ICL-expressing neurons have few sIPSCs during the baseline period, their tonic response to muscimol is comparable to control cells (Figure 4C,E). We generated all-point histograms of the current amplitudes for 2 minutes each during the baseline recording period, in the presence of muscimol, and in the presence of bicuculine for control cells, γ2-MO transfected neurons and ICL-expressing neurons (Figure 4 B,D,F). Muscimol application produced a comparable increase in tonic current in all groups of cells, which was blocked by bicuculine. There is no significant difference in tonic responses between control and γ2-MO transfected neurons or ICL-expressing neurons (Figure 4G). These data indicate that ICL expression and γ2 subunit knockdown do not affect extrasynaptic GABAAR.

**Evoked Visual Responses Show Shifts in Inhibition/Excitation**

Results from experiments in Figures 2 and 3 indicate the ICL and γ2-MO decrease the ratio of optic nerve stimulation evoked inhibitory to excitatory synaptic inputs to tectal neurons. Here we determined the effect of ICL- and γ2-MO transfection and of diazepam exposure on compound synaptic currents recorded from tectal neurons following full field visual stimulation. Visual stimulation evokes compound synaptic currents (CSCs) in tectal neurons that are the result of direct excitatory retinal input and polysynaptic excitatory and inhibitory tectal activity (Dong et al. 2009; Pratt et al. 2008; Zhang et al. 2000). To determine whether manipulation of inhibitory synaptic inputs affects visual responses in intact animals, we recorded CSCs from tectal neurons following whole-field visual stimulation of increasing luminance steps (10, 20 and 250 cd/m²). We recorded the evoked excitatory CSCs (eCSCs) at a holding membrane potential of -60 mV, and evoked inhibitory CSCs (iCSCs) at a holding potential of 0 mV. Tectal neurons were transfected with γ2-MO or Ctrl-MO and recorded 2 days later, or they were transfected with a dual promoter plasmid expressing EGFP and either ICL or miCL, and EGFP-expressing neurons were recorded 2 days later. In addition, we recorded CSCs in the presence of diazepam (10 μM) to test the effect of increasing inhibitory synaptic transmission on visually-evoked responses (Figure 5A, B). Data from Ctrl-MO and miCL-expressing neurons were not significantly different and were combined as controls (see methods).
Visual stimuli of increasing luminance values from 10 to 250 cd/m² evoked iCSCs and eCSCs with corresponding increases in integrated current in control neurons (Figure 5B). Visually-evoked eCSCS have an initial component which is the response to direct retinal inputs and a later component of prolonged synaptic currents from recurrent inputs. Visually-evoked iCSCs in control neurons are larger and longer-lasting than eCSCs, as previously reported (Akerman and Cline 2006; Richards et al. 2010; Tao and Poo 2005; Zhang et al. 2003). Visually-evoked CSCs recorded from ICL- and γ2-MO neurons, and in the presence of diazepam are different from controls (Figure 5B, Table 1). To test whether ICL expression, γ2-MO or diazepam change the ratio of inhibition to excitation in evoked visual responses, we determined iCSC/eCSC ratios of total integrated charge transfer (Figure 5C). For control neurons, the average iCSC/eCSC ratio was greater for lower luminance values and responses were more variable between cells than for responses to higher luminance stimuli. For ICL- and γ2-MO neurons, the iCSC/eCSC ratios were significantly lower than control neurons for stimuli of 10 and 20 cd/m² luminance but the iCSC/eCSC ratios were not different between control and ICL-expressing or γ2-MO-transfected neurons for stimuli with stronger luminance intensity values (250 cd/m²). iCSC/eCSC ratios were significantly greater in diazepam-treated neurons compared to controls for stimuli of 20, 250 cd/m² luminance (Figure 5C). These data suggest that luminance intensity of the stimulus differentially recruits excitatory and inhibitory inputs to tectal neurons in the control optic tectum, as seen in visual cortex, hippocampus and pyriform cortex (Carvalho and Buonomano 2009; Stokes and Isaacson 2010; Tucker and Fitzpatrick 2006). It is interesting to note that, despite the variability in individual CSCs, the ratios of inhibitory to excitatory CSCs are less variable, consistent with the idea that inhibition and excitation are coordinately recruited within the circuit. The data also indicate that increasing or decreasing the ratio of inhibitory to excitatory synaptic transmission onto individual tectal neurons within the tectal circuit has ramifying effects on circuit responses to visual input.

Table 1. Charge transfer of visual stimulation evoked eCSCs and eCSCs at different luminance intensities

<table>
<thead>
<tr>
<th></th>
<th>Lum (cd/m²)</th>
<th>10</th>
<th>20</th>
<th>250</th>
</tr>
</thead>
<tbody>
<tr>
<td>eCSCs</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>4408±578</td>
<td>8966±2349</td>
<td>18557±4551</td>
<td></td>
</tr>
<tr>
<td>ICL</td>
<td>2446±955</td>
<td>3928±1363</td>
<td>6100±1616*</td>
<td></td>
</tr>
<tr>
<td>γ2-MO</td>
<td>4132±1345</td>
<td>8486±2489</td>
<td>13345±2925</td>
<td></td>
</tr>
<tr>
<td>DZ</td>
<td>6357±3164</td>
<td>12056±4181</td>
<td>21579±6077</td>
<td></td>
</tr>
<tr>
<td>iCSCs</td>
<td>11570±4077</td>
<td>13432±3521</td>
<td>26431±11422</td>
<td></td>
</tr>
</tbody>
</table>

*significant difference compared to control
Asterisks represent statistical differences between controls and ICL, γ2-MO, or DZ groups.

**Regulation of Spontaneous Activity by I/E**

We analyzed spontaneous spiking in cell-attached recordings and spontaneous synaptic currents with whole-cell recordings from control EGFP- or ICL-expressing tectal neurons and diazepam-treated neurons in intact animals. TTX was not present during these recordings so the synaptic currents are a report of spiking activity in tectal neurons presynaptic to the recorded neuron. The frequency of spontaneous action potential spiking in ICL-expressing neurons is significantly greater than control neurons (Figure 6A). These data indicate that ICL increases spontaneous spiking activity in ICL-expressing neurons, which could have an effect on neurons throughout the tectal circuit. Furthermore, the frequency of spontaneous inhibitory synaptic currents is lower in ICL-expressing neurons compared to controls, while the frequency of excitatory spontaneous synaptic currents is not significantly different (Figure 6B,C). Although diazepam did not affect spontaneous action potential activity in tectal neurons, it increased the frequency of spontaneous inhibitory currents and decreased the frequency of spontaneous excitatory synaptic currents (Figure 6B,C).

**Temporal Fidelity of Visual Responses**

The precision of spike timing is important for coding information and for accurate information transfer between neurons (Tiesinga et al. 2008). We tested whether increasing or decreasing I/E affects the temporal fidelity of first spike latency of visual responses in tectal neurons. We recorded in cell-attached mode from neurons transfected with γ2-MO or ICL, with Ctrl-MO or mICL as controls, and from neurons exposed to diazepam. Individual responses and superimposed traces show that the first spike latency is more variable in neurons expressing γ2-MO or ICL and less variable in neurons exposed to diazepam compared to controls (Figure 7A). A plot of the variation of first-spike latencies relative to the average latency per cell shows that the temporal fidelity of the first-spike latency is lower in ICL and γ2-MO neurons and higher in diazepam-treated neurons (Figure 7B).

We analyzed the effect of luminance on the temporal fidelity of the first-spike latency, by determining the coefficient of variance (CV) of the first-spike latencies
relative to the median latency of each cell at each luminance. First-spike latency is consistently reliable in control neurons for visual stimuli across luminance values from 10-250 cd/m². When inhibition is decreased with ICL or γ2-MO, the variation of first-spike latency is significantly greater for stimuli of 10 cd/m² luminance, but is comparable to controls for stimuli of 250 cd/m² (Figure 7C, Table 2). Diazepam decreases the CV for responses to 20 cd/m² luminance compared to controls, but not for other luminances tested, suggesting increased I/E can improve temporal fidelity of responses for some stimuli.

The synaptic inhibition/excitation ratio has been shown to affect the latency to fire action potentials (Carvalho and Buonomano 2009; Marder and Buonomano 2004; Tiesinga et al. 2008). First-spike latencies are significantly longer in response to a stimulus of 10 cd/m² than for a stimulus of 250 cd/m² for all cell groups tested. Furthermore, first spike latencies in response to stimuli of 10 cd/m² are significantly longer in ICL, γ2-MO and diazepam-treated neurons compared to controls (Figure 7D, Table 2). Analysis of inter-spike intervals over the first 50 ms of the visually-evoked response shows that inter-spike intervals decrease with increasing luminance values in control neurons, but not in ICL, γ2-MO or diazepam-treated neurons (Figure 7E, Table 2), consistent with a previous study indicating that I/E affects inter-spike intervals (ISI) within a burst (Berg et al. 2007).

### Table 2. Coefficient variation of first spike latency, first spike latency and inter-spike-interval of visual stimulation evoked spikes at different luminance intensities

<table>
<thead>
<tr>
<th>Lum (cd/m²)</th>
<th>10</th>
<th>20</th>
<th>250</th>
</tr>
</thead>
<tbody>
<tr>
<td>CV of First Spike Latency (ms)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0.05±0.01</td>
<td>0.06±0.01</td>
<td>0.04±0.01</td>
</tr>
<tr>
<td>ICL</td>
<td>0.17±0.03*</td>
<td>0.16±0.05*</td>
<td>0.07±0.03</td>
</tr>
<tr>
<td>γ2-MO</td>
<td>0.11±0.02*</td>
<td>0.12±0.02</td>
<td>0.04±0.01</td>
</tr>
<tr>
<td>DZ</td>
<td>0.07±0.01</td>
<td>0.03±0.00*</td>
<td>0.03±0.00</td>
</tr>
<tr>
<td>First Spike Latency (ms)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>110.2±9.4</td>
<td>86.7±5.6</td>
<td>71.2±4.0</td>
</tr>
<tr>
<td>ICL</td>
<td>191.9±30.3*</td>
<td>104.1±13.1</td>
<td>91.7±14.1</td>
</tr>
<tr>
<td>γ2-MO</td>
<td>181.7±23.0*</td>
<td>104.5±20.3</td>
<td>71.8±6.0</td>
</tr>
<tr>
<td>DZ</td>
<td>188.0±24.1*</td>
<td>130.0±8.5*</td>
<td>128.7±8.3*</td>
</tr>
<tr>
<td>Inter-spike-interval (ms)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>17.5±2.3</td>
<td>11.9±0.6</td>
<td>9.8±0.6</td>
</tr>
<tr>
<td>ICL</td>
<td>18.7±1.8</td>
<td>19.9±4.1*</td>
<td>18.2±2.3*</td>
</tr>
<tr>
<td>γ2-MO</td>
<td>16.7±3.8</td>
<td>15.1±3.4</td>
<td>16.3±2.3*</td>
</tr>
<tr>
<td>DZ</td>
<td>21.5±0.9</td>
<td>17.7±1.2*</td>
<td>17.7±1.5*</td>
</tr>
</tbody>
</table>

Asterisks represent statistic difference between control groups and ICL, γ2-MO, or DZ groups.
These data indicate that decreasing inhibitory synaptic inputs with ICL expression or γ2 subunit knockdown increases the variance in first-spike latency of visual responses and can increase the latency of visual responses, while increasing inhibitory synaptic transmission with diazepam increases the fidelity of visual responses. The data suggest that increasing or decreasing the I/E ratio of synaptic inputs to tectal neurons significantly affects spike timing in response to visual inputs. In the following experiments we tested the effect of decreasing or increasing I/E on visual information processing in the optic tectum and visually-guided behavioral output, using ICL expression, γ2 subunit knockdown or diazepam.

**Inhibition/Excitation Ratio Shapes Spatial Receptive Fields**

Spatial receptive fields for visual input represent the regions of the visual world from which information is detected and processed in the CNS. Because spatial receptive fields are an emergent property of a circuit generated from organized sensory afferents, local circuit activity and modulatory inputs, they serve as a readout of circuit function in the CNS (Tiesinga et al. 2008). Previous work has shown that inhibitory inputs shape spatial receptive fields (Fagiolini et al. 2004; Gabernet et al. 2005; Hensch 2005; Hicks and Dykes 1983; Liu et al. 2009; Morales et al. 2002; Sillito 1975; Tao and Poo 2005), possibly by affecting a balance of inhibitory to excitatory synaptic inputs (Farrant and Nusser 2005; Hensch 2005; Zhang et al. 2008). Here we tested directly whether increasing or decreasing I/E affects spatial receptive fields. Using a chamber that allows us to map receptive fields without physically removing the lens from the retina, we recorded spatial receptive fields sequentially in cell-attached mode and in whole-cell mode at -60 mV and 0 mV in response to light off visual stimuli presented in a pseudorandom pattern across the 64 positions of an 8X8 grid (Figure 8A-C). Grey scale spatial receptive field maps of the action potential activity and synaptic currents over a 600 ms window following stimulus onset are generated from an average of 2-3 repetitions of the stimuli (Figure 8D). In the example in Figure 8, cell attached recordings reveal a highly focused spiking receptive field (sRF) in which visual stimulation in one square of the visual field produces strong action potential activity in the cell, whereas spiking activity in response to the same stimulus in immediately adjacent areas of the visual field is much lower. By contrast, whole cell recordings taken at -60 mV show that the neuron receives excitatory synaptic inputs in response to light off stimulation over a much larger area of the visual field (Figure 8D, middle). We call
this the excitatory synaptic receptive field (eRF). Similarly, the inhibitory synaptic
receptive field (iRF), recorded in whole cell mode at 0 mV, is larger than the spiking
receptive field (Figure 8D, right). The recordings show that tectal cells receive excitatory
and inhibitory synaptic inputs over a 600 ms window in response to stimuli presented in
every portion of the visual field, but the output of the cell reports visual input over a
subset of the visual field.

To test whether decreasing the ratio of inhibitory to excitatory synaptic
transmission onto tectal neurons affects the cell’s spatial receptive fields, we recorded
spikes first in cell-attached mode followed by whole cell recordings of excitatory and
inhibitory synaptic inputs from neurons transfected with ICL and EGFP or EGFP alone.
We also tested whether increasing ratio of inhibitory to excitatory synaptic transmission
onto tectal neurons affects spatial receptive fields by recording from neurons in animals
exposed to diazepam. Two examples each are shown for EGFP-expressing control
neurons, ICL-expressing neurons and neurons from diazepam-treated animals (Figure
9A). Spiking RFs in ICL-expressing neurons are significantly larger than sRFs in control
neurons, while sRFs are significantly smaller in diazepam-treated neurons compared to
tests (Figure 9A-C). The cumulative distribution of sRF sizes in ICL-expressing
neurons (red line) is shifted significantly toward larger receptive fields compared to
EGFP-expressing neurons (green line, Figure 9B). Conversely, the cumulative
distribution of sRF sizes in diazepam-treated neurons is shifted toward smaller values
(blue line, Figure 9B). Similarly, the mean sRF size of control neurons is greater than
the mean sRF size of diazepam-treated neurons and smaller than the mean sRF size of
ICL-expressing neurons (Figure 9C).

Considering the synaptic inputs, we find that eRFs are significantly larger in ICL-
expressing cells than in controls (Figure 9D). The relative size of the iRF is not different
between ICL-expressing neurons, diazepam-treated neurons and controls (Figure 9E),
even though inhibitory responses are smaller in ICL-expressing neurons and larger in
diazepam-treated neurons, because RF measurements are generated from responses
normalized to the maximum response. Since the iRF size indicates the spatial extent of
feed-forward inhibitory inputs (Akerman and Cline 2006), these data suggest that the
convergence of inhibitory inputs onto the recorded neurons is unaffected by ICL
expression or diazepam, even though the strength of synaptic inputs are affected by the
treatment.

Plots of the sizes of the eRFs versus sRFs show that even though RFs of control
neurons are smaller than ICL-neurons, the sizes of sRFs and eRFs are similarly
correlated in control and ICL-neurons (Figure 9F). Although the sizes of sRFs and iRFs are correlated in control neurons, they are not well correlated in ICL neurons (Figure 9G). Furthermore, the sizes of eRFs and iRFs are correlated in control neurons, but they are not well correlated in either ICL-expressing or diazepam-treated neurons (Figure 9H). These data indicate that partially decreasing or increasing the ratio of inhibitory to excitatory synaptic inputs in tectal neurons is sufficient to disrupt the information processing in the tectal circuit that is represented as spatial receptive fields.

To determine the input-output correlations from the receptive field mapping, we plotted the maximum spikes from the 8X8 grid from each neuron, determined by cell-attached recording and the corresponding eCSCs (Figure 9I) or iCSCs (Figure 9J), determined by whole-cell recordings. ICL-expressing neurons fire more action potentials relative to synaptic inputs compared to control neurons, consistent with data presented in Figure 6A.

To determine the degree to which excitatory and inhibitory inputs are correlated in response to visual stimuli, we plotted the charge transfer of the iCSCs and eCSCs over the 8*8 grid from each neuron (Figures 9K). The currents of iCSCs and eCSCs are well correlated in EGFP-, ICL-expressing and diazepam-treated neurons. The regression line between iCSCs and eCSCs in ICL-expressing neurons (red) are lower than controls (green), consistent with a lower I/E as shown in Figure 5C. In diazepam-treated neurons, the linear trend line (blue) is shifted towards larger values of iCSCs than controls, consistent with a higher I/E in these neurons. This analysis indicates that ICL-expression and diazepam treatment shift the balance of excitation to inhibition in the compound synaptic current inputs recorded with receptive field mapping.

These data demonstrate that spatial receptive fields are sensitive to increases and decreases in inhibition and suggest that both increases and decreases in I/E affect spatial receptive fields. The larger subthreshold inhibitory and excitatory receptive fields relative to the spiking receptive field in control neurons and the change in receptive field size in response to changes in I/E may reflect the capacity for receptive field plasticity seen with sensory training protocols or learning paradigms (Dong et al. 2009; Engert et al. 2002; Gandhi et al. 2008; Moore et al. 1999; Mu and Poo 2006; Richards et al. 2010; Vislay-Meltzer et al. 2006).

**I/E Affects Temporal Response Properties of Spatial Receptive Fields**

We recorded spatial receptive field responses over a 600 ms window after the onset of the stimulus at each location in the visual field. This allowed us to determine the
temporal features of responses at each of the 64 stimulus locations. To test whether ICL expression or diazepam affect temporal parameters of spatial receptive field responses, we generated maps of the spiking receptive fields over intervals of 100 ms for the 600 ms recording period, shown for 3 representative neurons in Figure 10A. Control neurons generate spikes encoding spatial receptive field information within a 100 ms window following stimulus onset while spiking receptive fields in ICL-expressing and DZ-treated neurons can be recorded over larger proportions of the 600 ms period (Figure 10A).

Plotting the temporal distribution of normalized spike numbers for the representative spatial receptive fields in Figure 10A shows that the receptive field in the control is rapid and temporally concise (Figure 10B). The ICL-expressing example fires more spikes than the control neuron. The onset of the spatial receptive field response in the ICL-expressing neuron shown is delayed relative to the control, and persists over 600 ms window. In diazepam, the onset of the response is also delayed, and the cell fires fewer action potentials but they extend over a larger temporal window than controls. We determined the full width at the half maximal response (FWHM) as a measure of response duration (Lesica et al. 2007). The mean FWHM of responses in ICL-expressing neurons is significantly greater than control neurons (Figure 10C), consistent with the idea that the prolongation of spatial receptive field information is due to recurrent excitatory activity in the tectal circuit. The mean FWHM of responses in diazepam was also significantly greater than controls neurons (Figure 10C), suggesting that increased GABAergic transmission and an increased I/E ratio disrupts the temporal response properties of visual information processing. We explored this idea further by plotting mean spike latency for the groups of cells recorded (Figure 10D). Consistent with the spike latencies of the individual representative cells shown in Figure 10A and B, the mean spike latencies of ICL and diazepam cells are greater than control neurons. ICL expression increases the total number of spikes fired over 600ms in response to visual stimulation (Figure 10E). Post-stimulus time histograms show that the temporal distribution of action potentials in ICL-expressing neurons is longer than control neurons, while diazepam-treated neurons fire less than control neurons, but their activity continues longer (Figure 10F). These data indicate that I/E controls spatial and temporal properties of tectal cell visual responses by regulating the recruitment of local tectal circuitry following visual stimulation.

ICL Increases the Synaptic Integration Window
Temporal receptive fields are the period over which synaptic inputs are integrated to
generate neuronal output. Regulation of temporal receptive fields therefore controls
synaptic integration related to information processing and learning rules (Pouille and
Scanziani 2001). As an independent measure of the function of inhibition in controlling
temporal response properties, we calculated temporal receptive fields of visual
responses using a spike-triggered average or reverse correlation to a whole field
Gaussian white noise flicker of luminance steps. The FWHM of temporal receptive fields
of ICL-expressing cells determined from cell-attached or whole cell recording is
significantly longer than control cells (Figure 11A-D). Furthermore, comparing the FWHM
values from cell-attached recordings and whole-cell recordings indicates that the
temporal integration window of synaptic inputs closely matches the temporal receptive
field of the cells output spiking activity. Finally, the decay constant, tau, of the excitatory
synaptic temporal receptive fields of ICL-expressing neurons is significantly greater than
in control neurons (EGFP: 40.8±5.0 ms, n=11; ICL: 66.0±9.6 ms, n=15; p<0.05).
Together, these results indicate that ICL expression lengthens the synaptic integration
window.

**Visually-guided Avoidance Behavior**
The optic tectum receives and processes visual information and transforms sensory
inputs to outputs, some of which in turn regulate behavior mediated by circuits in the
hindbrain and spinal cord (Orger et al. 2008). We have found that the temporal precision
of visually-evoked tectal cell spiking activity (Figure 7), temporal receptive fields (Figure
11), spatial receptive fields (Figure 9 and 10), and tectal cell input/output relations are all
disrupted by experimental manipulations that either increase or decrease inhibition and
alter the ratio of inhibition to excitation by 30-50% of control I/E values. These data
suggest that multiple aspects of visual responses in the optic tectal neurons are
altered disrupted when I/E is modified. We next tested whether a partial decrease or increase in
I/E affects tectally-mediated visually-guided behavior using a visual avoidance behavior
assay modified from Aizenman and colleagues (Dong et al. 2009).

The behavior assay tests whether animals avoid visual stimuli by determining their
swimming behavior in response to visual stimuli. Animals turn away from moving spots
of certain sizes that approach their eyes at approximately a right angle (Figure 12A).
Data are presented as an avoidance index, or the fraction of times that individual
animals respond to the stimulus out of 10 encounters with the moving spot stimuli.
Control (untransfected) and EGFP-transfected tadpoles selectively avoid moving spots
corresponding to diameters larger than 0.4 cm (about as big as their head) (Figure 12B).

Avoidance of stimuli of 0.4 and 0.04 cm show the maximal and baseline responses in control animals, respectively, which are significantly different from one another (p=0.014; Mann-Whitney test). Animals electroporated with ICL or γ2-MO show deficits in visual avoidance behavior, whereas the animals electroporated with mICL or Ctrl-MO are comparable to controls (Figure 12C, D).

To test whether diazepam affects visual avoidance behavior, 12 tadpoles were tested sequentially in normal Steinberg’s solution, after 1 and 2 hour exposure to 10 µM diazepam, after 1 hour exposure to 30 µM diazepam and finally, after 1 hour in Steinberg’s solution (Figure 12E). Diazepam significantly reduces the avoidance response to stimuli of 0.4 and 0.6 cm. The longer 2h exposure to 10 µM diazepam did not cause a further reduction in the visual avoidance response, however 30 µM diazepam completely blocked the avoidance response. The response recovered after 1 hour in Steinberg’s solution. Diazepam did not affect the time animals spent swimming (Figure 12F) and optomotor responses (data not shown), suggesting it does not affect responses to visual input or locomotor activity. In an effort to limit the potential effect of diazepam on the retina or motor systems, we injected 1 mM diazepam into the tectal ventricle and tested for visual avoidance behavior 2 hour later. Intraventricular diazepam blocked the visual avoidance behavior (Figure 12G), whereas behavior after intraventricular injection of the vehicle, 1% DMSO, was comparable to control (Figure 12H). These data indicate that manipulations that decrease or increase I/E in tectal neurons interfere with visually-guided behavior.

Discussion

Deficits in information processing, cognition and behavior are thought to arise from an imbalance of inhibitory and excitatory synaptic inputs (Berg et al. 2007; Rubenstein and Merzenich 2003). We therefore sought to test the functional significance of modulating I/E on tectal cell visual response properties and visually-guided behavior. We used molecular genetic reagents to decrease inhibitory synaptic transmission and used diazepam, a positive GABA$_{A}$R allosteric modulator, to increase inhibitory synaptic transmission. Because ICL expression, γ2-morpholinos and diazepam do not directly affect excitatory synaptic transmission, we interpret our results as evidence that modulating I/E disrupts several measures of circuit function, notably the precision and latency of visually-evoked spike timing, control of recurrent activity within the tectal
circuit, spatial and temporal receptive fields, input/output relations of tectal cells and visually-guided behavior.

**Spike Timing Precision**

Temporal information coding conveyed by spike timing is largely attributed to the precision of first spike latency (Daw et al. 2006; Johansson and Birznieks 2004; Mainen and Sejnowski 1995; Tiesinga et al. 2008). Temporal precision of spike timing is regulated by feed-forward or feed-back inhibition (Burkhalter 2008; Gabernet et al. 2005; Pouille and Scanziani 2001; Tiesinga et al. 2008), suggesting that inhibition regulates information transfer between neurons. In the tadpole visual system, tectal neurons receive direct excitatory inputs from convergent excitatory retinal ganglion cells and feed-forward inhibition from GABAergic tectal neurons (Akerman and Cline 2006), similar to the configuration of excitatory and inhibitory inputs in other systems (Burkhalter 2008). The onset of feed-forward inhibition curtails excitatory inputs and has three related consequences: 1. the window over which convergent excitatory inputs sum to generate action potentials is narrowed, 2. the window over which action potentials are evoked is shortened, and 3. the temporal precision of spikes in response to repeated stimuli is increased (Akerman and Cline 2006; Kremkow et al.; Mittmann et al. 2005; Pouille and Scanziani 2001; Wehr and Zador 2003).

Our results show that ~50% decrease in the I/E ratio in tectal neurons causes more jitter in the first spike latency, increases the synaptic integration window and increases the length of temporal receptive fields. Conversely, ~30% increase in I/E ratio generates less jitter and increases first spike latency. Together these observations show that spike timing in response to direct sensory input is sensitive to changes in I/E. First-spike latency carries information about stimulus features including intensity and location of sensory inputs (Heil 2004; Petersen et al. 2002; Tiesinga et al. 2008). Interestingly, we found that the effect of modulating I/E on first-spike jitter depends on luminance intensity. The variance in first spike latencies is significantly greater for visual stimuli of low luminance values, while responses to higher luminance stimuli have greater temporal fidelity. Similarly, I/E of visually-evoked synaptic currents is larger and more variable for low luminance stimuli, suggesting that I/E in tectal neurons can convey information about stimulus intensity. Higher luminance stimuli recruit more inhibitory inputs within the tectal circuit, similar to ‘veto’ responses in visual cortex (Tucker and Fitzpatrick 2006), which in turn increases the precision and reliability of spikes in visual cortex (Haider et al. 2010). Evidence for selective recruitment of
inhibitory inputs with stronger stimuli have been reported in slice preparations of the pyriform cortex and hippocampus (Carvalho and Buonomano 2009; Stokes and Isaacson 2010), suggesting that dynamic changes in I/E in response to input strength may be a general property of neuronal circuits. These results demonstrate that the extent to which excitatory and inhibitory components of tectal circuits are engaged in visual responses reflects the strength of the sensory inputs. They further suggest that the dynamic range of changes in I/E may be essential for information processing in vivo and that mechanisms which limit the I/E dynamic range may impair CNS function.

**I/E Ratios Control Input/Output Relations**

A fundamental challenge of neuroscience is to determine the mechanisms that govern the input/output transformation. In many brain regions, including optic tectum, the magnitudes of excitatory and inhibitory synaptic conductance are balanced and temporally offset (Akerman and Cline 2006; Haider et al. 2010; Liu et al. 2007; Nowak et al. 2009; Richards et al. 2010; Tiesinga et al. 2008; Zhang et al. 2003). Modeling studies and experiments in reduced preparations suggest that the ratio of inhibitory and excitatory conductances govern input/output relations of neurons and circuits by affecting, among other parameters, the reliability with which inputs drive spikes in downstream neurons (Carvalho and Buonomano 2009; Kremkow et al.; Marder and Buonomano 2004; Pouille and Scanziani 2001; Tiesinga et al. 2008). Our data provide experimental evidence that modulating I/E disrupts input/output relations of individual neurons and the functional circuit in intact animals. The increased variance in latency to fire spikes and increased recurrent activity when I/E is decreased likely contribute to functional changes in the input/output relation of individual tectal neurons, similar to effects seen with changing the timing of inhibition in cultured hippocampal neurons (Marder and Buonomano 2004; Pouille and Scanziani 2001). It would be interesting to test directly how decreasing or increasing I/E in tectal neurons affects activity in hindbrain and spinal cord neurons that have been shown to trigger swimming in response to visual stimulation (Orger et al. 2008).

**Lack of Homeostatic Control of I/E Ratio**

The balance of inhibition to excitation is actively established and maintained during circuit development by unknown mechanisms (Davis 2006; Farrant and Nusser 2005; Lee et al. 2010; Liu 2004; Sillito 1975). Large-scale modifications in sensory input, such as sensory deprivation, result in homeostatic modifications in both excitatory and
inhibitory synaptic transmission (Farrant and Nusser 2005; Morales et al. 2002; Sillito 1975). Although the strength of excitatory and inhibitory inputs can be modified independently by activity-dependent mechanisms, homeostatic mechanisms may scale the magnitude of changes in synaptic strength to maintain a balance of inhibitory and excitatory inputs (Davis 2006; Liu et al. 2007; Maffei et al. 2006). Despite homeostatic mechanisms that operate to maintain I/E and neuronal firing rate, the relatively moderate and cell autonomous decrease in inhibition accomplished by ICL expression or γ2 subunit knockdown do not trigger homeostatic mechanisms that reset I/E, at least within the time-course of our experiments.

**Tectal I/E Regulates Visually-Guided Swimming Behavior**

Our data indicate that modulating I/E in the tectum blocks visually-guided behavior. They also demonstrate that the tectum is a critical visual processing station for visually-guided behavior. Failure to generate an avoidance response may arise from deficits in information processing and distorted spatiotemporal responses in tectal neurons that interfere with generating output from the tectum to the hindbrain and spinal cord. The deficits observed with ICL expression or γ2-subunit knockdown may be a response to decreased inhibitory input over the 2 days prior to our recordings. Time-lapse imaging showed that the tangential extent of tectal cell dendrites was larger than controls 3 days after transfection with ICL, but were not different from controls 2 days after ICL transfection (Shen et al. 2009). In addition, ICL-expressing neurons fail to show the visual-experience dependent structural plasticity seen in control neurons (Shen et al. 2009). The decreased I/E and resultant increased spiking in transfected cells could affect their development and connections within the visual circuit. Several studies have shown that visual experience and activity-dependent synaptic plasticity refine the developing retinotectal circuit and alter visual response properties, indicating that a variety of mechanisms, including STDP, could translate changes in I/E and activity into changes in circuit connectivity and function (Ruthazer and Aizenman 2010).

Both increasing and decreasing I/E impair visually guided behavior. These experiments are the first to test experimentally the consequences of opposite changes in I/E on neuronal response properties and behavior. The data indicate that increasing and decreasing I/E do not necessarily have opposing effects on neuronal function and visual response properties. The degraded visually-guided behavior arises because processing of visual information and input/output relations are disrupted in both experimental
conditions, and suggests that diverse deficits in circuit function can disrupt behaviors controlled by the visual circuit.

Our transfections result in ICL expression in 10-20% of tectal neurons and morpholino transfection in approximately 50% of tectal cells, suggesting that decreasing I/E in a subset of tectal cells within the retina-tectum-spinal cord circuit is sufficient to degrade behavioral output. Increases in spontaneous and recurrent activity in transfected neurons indicate that activity in other neurons in the tectal circuit is increased and suggest that the changes in visually-guided behavior may result from these circuit-wide changes in activity. Several studies indicate that altering the function of a minority of cells in the circuit can compromise circuit function. In rat fear conditioning, decreasing glutamatergic transmission in ~ 20% of amygdala neurons blocked the conditioned response (Rumpel et al. 2005), while recent reports suggest that increasing activity in subpopulations of neurons, for instance by expression and activation of channelrhodopsin (ChR2), affects circuit function and behavior (Douglass et al. 2008; Houweling and Brecht 2008; Li et al. 2009; Pagliardini et al. 2011; Tye et al. 2011; Witten et al. 2010).

About 30% of tectal neurons are GABA immunoreactive. We estimate that transfection of 10-20% of tectal neurons results in transfection of a corresponding minority of GABAergic neurons in the tectum. Although decreasing inhibitory input to inhibitory neurons might be expected to increase net inhibition in the tectal circuit, the visually-evoked inhibitory compound synaptic currents in ICL and MO-transfected neurons (Fig 5) indicate that decreasing the ratio of inhibitory to excitatory synaptic inputs to tectal neurons decreases net inhibitory currents following visual stimulation. Therefore, we cannot distinguish a potential contribution of modifying inhibitory input to inhibitory neurons.

I/E is thought to be disrupted in several neurodevelopmental diseases including autism spectrum disorders (ASD) (Rubenstein and Merzenich 2003). Visual processing is also disrupted in some people with ASD (Annaz et al. 2010; Marco et al. 2011). Our study shows that disrupting I/E in a minority of neurons within a circuit is sufficient to cause deficits in information processing and behavior, suggesting that diseases characterized by errors in information processing could arise from deficits in a subset of neurons within a circuit.
Figure Legends

Figure 1. Morpholino-mediated Knockdown of GABA<sub>A</sub>R γ2 Subunit

(A, B) i. images of the distribution of fluorescein-tagged morpholino in optic tectum after unilateral electroporation of Ctrl-MO (A) or γ2-MO (B) into the left tectum only (marked with the star). ii. Immunolabeling for the γ2 subunit in the same section as i. iii: merged image showing morpholino distribution (green) and γ2 subunit immunolabeling (red).

Scale bar: 50 μm. iv, v: higher magnification images of boxed regions in Aii and Bii.

Scale bar: 10 μm. γ2 subunit immunolabeling is lower in the tectal lobe transfected with γ2-MO compared to the contralateral tectum. (C) γ2 subunit immunolabeling is significantly lower in γ2-MO transfected tectum compared to Ctrl-MO transfected tectum (measured as the fluorescent intensity of the entire neuropil per unit area in the MO-transfected side (left) normalized to non-transfected side (right). n=3 animals per condition **p<0.01. (D) Western blots of tectum for control and γ2-MO-transfected brains labeled with anti-γ2 and anti-β-tubulin antibodies, show a 38% decrease in γ2 intensity relative to β-tubulin intensity.

Figure 2. Selective manipulation of GABA<sub>A</sub>R-mediated transmission

(A, B) Representative traces of mIPSCs (A) and mEPSCs (B) in Ctrl-MO- and γ2-MO-transfected neurons. (C) γ2 knockdown increases inter-event-interval of mIPSCs. Cumulative probability plot (left panel) shows a significant shift in the distribution of the inter-event-intervals of mIPSCs in γ2-MO-transfected neurons compared to in Ctrl-MO-transfected cells (Kolmogorov-Smirnov test, **p<0.01). The average inter-event-interval (middle panel) of mIPSCs in γ2-MO-transfected neurons is significantly larger than in Ctrl-MO-transfected cells (Ctrl-MO: 3.72±1.19 sec, n=9 cells; γ2-MO: 23.95±6.09 sec, n=11 cells; Mann-whitney test, **p<0.05). Amplitudes of mIPSCs (right panel, Ctrl-MO: 23.73±4.04 pA; γ2-MO: 20.63±1.70 pA; p>0.05) are not different in Ctrl-MO- and γ2-MO-transfected cells. (D) Amplitudes (Ctrl-MO: 10.46±0.48 pA; γ2-MO: 12.85±0.67 pA; p>0.05) and inter-event-interval (Ctrl-MO: 3.60±1.71 sec; γ2-MO: 2.49±0.68 sec; p>0.05) of mEPSCs are not different in Ctrl-MO- and γ2-MO-transfected cells. (E) Diagram of recording evoked IPSCs in a tectal neuron (green) by stimulation of local inhibitory neurons in the presence of 20 μM NBQX to block excitatory transmission, including retinal inputs (red lines). (F) Representative traces of evoked IPSCs (recorded at 0 mV holding potential) before (black trace) and after perfusion with DZ (10 μM, red trace) in Ctrl-MO and γ2-MO neurons. DZ increases the amplitude of evoked IPSCs in control.
neurons, but does not increase evoked IPSC amplitude in γ2-MO-transfected cells (bottom). (G) Relative change in peak current amplitude after DZ in Ctrl-MO cells: 112.7±29.2%, n=6; γ2-MO cells: 10.7±9.6%, n=5; Mann-Whitney test, *p<0.05. (H) Relative increase in decay time after DZ in Ctrl-MO cells: 46.5±12.8%, n=6; γ2-MO cells: 9.4±7.9%, n=5; Mann-Whitney test, *p<0.05.

Figure 3. Change in I/E ratio of evoked retinotectal synaptic inputs
(A) Diagram of recording evoked IPSCs and EPSCs in a tectal neuron (green) by stimulation of retinal axons in the optic chiasm (red). (B) Representative traces of evoked GABA_A-R-mediated synaptic currents (recorded at 0 mV holding potential) and AMPAR-mediated synaptic currents (recorded at -60 mV holding potential) in response to optic nerve stimulation for control neurons, γ2-MO neurons and diazepam (DZ) treated neurons. (C) The ratio of amplitudes of evoked IPSCs to EPSCs in γ2-MO-transfected cells was significantly lower than in Ctrl-MO-transfected cells (Ctrl-MO: 0.85±0.12, n=5; γ2-MO: 0.28±0.06, n=4; **p<0.01). Diazepam significantly increased the ratio of amplitudes of evoked IPSCs to EPSCs (DZ: 1.28±0.07, n=4; * p<0.05)

Figure 4. ICL and γ2-subunit knockdown do not affect tonic GABAergic currents
(A-F) Representative traces of tonic GABAergic currents in control (A), γ2-MO (C) and ICL (E) neurons, recorded in whole cell mode, at -60 mV from isolated brains. Glutamatergic currents were blocked by kynurenic acid (2 mM). After 5 minutes of baseline recording, application of a GABA agonist muscimol (1 μM) induced a current shift and increased the noise variance in all neurons recorded. All spontaneous inhibitory postsynaptic currents (sIPSCs) were blocked by application of antagonist bicuculine (100 μM). All-point histograms for 2 minutes are shown at the right during baseline recording, muscimol application and bicuculine application in control (B), γ2-MO (D) and ICL (E) neurons. (G) There is no significant difference of current shift between Ctrl and γ2-MO transfected neurons or ICL-expressing neurons. (Ctrl/γ2-MO/ICL neurons, N=10/5/9).

Figure 5. Ratio of inhibition to excitation in visually-evoked responses
(A) Diagram for recording of light OFF evoked inhibitory compound synaptic currents (iCSCs, recorded at 0 mV holding potential) and excitatory compound synaptic currents (eCSCs, recorded at -60 mV holding potential). (B) Representative recordings of iCSCs and eCSCs for Control (top), γ2-MO- (middle, top), ICL-transfected neurons (middle,
bottom) or DZ-treated neurons (bottom) in response to stimuli of increasing luminance (10, 20 and 250 cd/m²). Black traces are the average of three representative traces, shown in grey. (C) The ratio of iCSC/eCSC in Control, ICL-, γ2-MO-, and diazepam (DZ)-treated cells at luminance of 10, 20 and 250 cd/m² (Ctrl: 1.87±0.64, 1.49±0.28, 1.15±0.27, n=7; ICL: 0.37±0.14, 0.58±0.12, 0.79±0.28, n=6; γ2-MO: 0.52±0.12, 0.58±0.15, 0.86±0.17, n=7; DZ: 2.53±1.10, 2.36±0.27, 1.87±0.18, n=8; *p<0.05).

Figure 6. Increasing or decreasing inhibition changes spontaneous activity
(A) Spontaneous spiking activity is greater in ICL-expressing neurons than controls (Ctrl: 0.06±0.02 Hz, n=18; ICL: 0.16±0.04 Hz, n= 15; DZ: 0.03±0.01 Hz, n= 13; Mann-Whitney test, *p<0.05). (B) The frequency of spontaneous inhibitory postsynaptic currents (sIPSCs) is significantly lower in ICL-expressing neurons and significantly higher in DZ-treated neurons compared to controls (Ctrl: 0.42±0.14 Hz, n=14; ICL: 0.09±0.04 Hz, n= 14; DZ: 0.85±0.17 Hz, n= 13; **p<0.01). (C) The frequency of spontaneous excitatory currents is significantly lower in DZ-treated neurons compared to controls (Ctrl: 5.65±1.16 Hz; ICL: 3.69±0.70 Hz; DZ: 1.58±0.27 Hz; **p<0.01).

Figure 7. Increasing or decreasing inhibition alters the temporal precision of visually-evoked spiking
(A) Four representative traces showing Off-response-evoked spikes in Control (left), ICL- (middle left), γ2-MO-transfected neurons (middle right), and diazepam (DZ)-treated neurons (right). Superimposition of all first spikes (in red, bottom) shows more variance in first-spike latency in ICL- and γ2-MO-transfected cells and less variance in DZ-treated cells than control neurons. (B) Variation from the mean of first-spike latency in Ctrl-MO-, ICL-, γ2-MO- and DZ-treated neurons. Decreasing I/E increased the distribution of first-spike latencies for stimuli of 10 cd/m² while increasing I/E narrowed the distribution of first-spike latencies for stimuli of 20 cd/m². Bin width, 5 ms. (C) Coefficient of variation (CV) of first-spike latencies for stimuli of 10, 20, 250 cd/m² shows that CVs are significantly greater for ICL- and γ2-MO- cells compared to controls for stimuli of 10 and 20 cd/m², while CV is significantly lower for DZ-treated cells compared to controls for stimuli of 20 cd/m². n=9/7/7/12; *p<0.05. (D) First-spike latencies in response to visual stimuli of increasing luminance. First-spike latency is significantly greater for stimuli of 10 cd/m² compared to 250 cd/m² in Ctrl-, ICL-, γ2-MO-transfected and DZ-treated cells. First spike latency for control neurons is shorter than ICL-, γ2-MO-transfected and DZ-treated cells at 10 cd/m². n=9/5/7/12; *p<0.05. (E) Inter-spike-interval (ISI) decreases for
stimuli of increasing luminance in control neurons and DZ-treated neurons but not in ICL-, γ2-MO-transfected and DZ-treated neurons. ISI at 10 cd/m² compared to 250 cd/m² is significantly different for controls (*p<0.05) but not for ICL-expressing or γ2-MO or DZ-treated neurons (p=0.70, 0.92 and 0.21).

Figure 8. Mapping spatial receptive fields
(A) Diagram of recording setup for mapping spatial receptive fields in vivo. (B) Traces of cell-attached and whole-cell recordings from a neuron in response to light ON/OFF stimulus (shown on top). Cell attached recordings show the cell fires to the light off stimulus. Whole-cell currents recorded at 0 mV and -60 mV holding potentials show stimulus-evoked inhibited and excitatory synaptic currents, respectively. (C) Maps of evoked spikes (left), eCSCs (middle), and iCSCs (right) from a representative neuron, with responses shown for the stimulus in each of the 64 grid positions. (D) The corresponding receptive field maps are shown in grey scale, with white as the maximal response.

Figure 9. Increasing or decreasing inhibition alters spatial receptive field size
(A) Spatial receptive field maps in two representative EGFP- (left), ICL- (middle), and diazepam (DZ)-treated (right) neurons. (B) Cumulative distribution of sRF sizes in EGFP, ICL and DZ-treated cells. (C) Average spiking receptive field size is larger in ICL-neurons but is smaller in DZ-treated neurons compared to controls (EGFP: 25.6±4.7%, n=17; ICL: 48.9±7.4%, n=13; DZ: 17.6±2.7%, n=13; *p<0.05). (D) Average eRF size of ICL- neurons is significantly larger than controls but not different in DZ-treated neurons (p=0.08) (EGFP: 39.9±5.4%, n=17; ICL: 58.9±3.9%, n=13; DZ: 54.0±5.3%, n=9; **p<0.01). (E) iRFs in EGFP-, ICL- and DZ-treated cells are not different (EGFP: 44.0±4.2%, n=17; ICL: 44.9±5.0%, n=13; DZ: 41.3±5.6%, n=9). (F, G) Relationship between eRF (F) (EGFP/ICL/DZ: r=0.76/0.63; n=11/12) or iRF (G) (EGFP/ICL: r=0.67/0.03) size and spiking RF for EGFP- and ICL-expressing neurons. (H) Relationship of iRF and eRF size for EGFP-, ICL- and DZ-treated neurons (EGFP/ICL/DZ: r=0.75/0.10/0.20; n=11/12/11). (I, J) Relationship between maximum spikes per grid and corresponding excitatory compound currents (I) (EGFP/ICL/DZ: r=0.78/0.56; n=11/12) or inhibitory compound currents (J) (EGFP/ICL: r=0.72/0.26) for EGFP- and ICL-expressing neurons. (K) Relationship between iCSCs and eCSCs for EGFP-, ICL- and DZ-treated neurons respectively (EGFP/ICL/DZ: r=0.69/0.71/0.60; n=11/12/11).
Figure 10. ICL and diazepam lengthen temporal response properties of spatial receptive fields
(A) Temporal extent of spatial receptive field maps from representative EGFP-, ICL- and diazepam (DZ)-treated neurons in 100 ms bins over 600 ms. Responses in the control neuron occur within a 100 ms window (top). Responses in the ICL-expressing neuron occur over a protracted period from 100-600 ms after stimulus onset (middle). Responses in the DZ neuron are delayed by ~200 ms and occur over a protracted period from 200-600 ms after stimulus onset (bottom). (B) Spike numbers for control, ICL- and DZ-treated neurons from (A) over 600 ms in 50 ms bins, normalized to the bin with the most spike counts. (C) The full width at half maxima (FWHM) of the temporal response is larger in ICL- and DZ-neurons compared to controls (EGFP: 112.5±11.5 ms, n=20; ICL: 159.2±10.4 ms, n=26; DZ: 157.4±16.3 ms, n=13; *p<0.05). (D) The latency of total spikes in 50 ms bins is longer in DZ-treated neurons compared to controls. (E) The total number of visual stimulus-evoked spikes is significantly greater in ICL-expressing neurons compared to controls or DZ-treated neurons. (F) Post-stimulus time histogram of visual-stimulus evoked spikes. Mean spike counts in 10 ms bins over the 600 ms recording period. ICL- expressing neurons (n=26) have more recurrent activity than EGFP-expressing cells (n=20). DZ-treated neurons have a lower peak of firing but more recurrent activity than controls (n=13).

Figure 11. Temporal receptive fields are larger in ICL-expressing neurons
Temporal receptive fields determined by cell-attached (A) or whole cell (B) recording using reverse correlation analysis of full field Gaussian white noise flicker stimulus. Data from four representative EGFP (green) - and ICL (red) - cells (left) and averaged temporal receptive fields (right). Data were scaled to the peak response. ICL- expressing neurons have a longer integration window than EGFP- expressing cells. (C) FWHM of spiking temporal receptive fields is significantly greater in ICL- expressing cells then EGFP- expressing cells (EGFP: 57.4±3.7 ms, n=11; ICL: 80.3±10.4 ms, n=15; *p<0.05). (D) FWHM of temporal receptive fields generated from excitatory synaptic currents in ICL- expressing cells is significantly greater than EGFP- expressing cells (EGFP: 53.3±3.7 ms, n=18; ICL: 73.5±7.6 ms, n=15; *p<0.05).

Figure 12. Decreasing or increasing tectal I/E blocks visual avoidance behavior
(A) Images of tadpole visual avoidance behavior in response to moving visual stimuli. Freely moving tadpoles are presented with a field moving spots for 30 seconds. Images taken throughout the 30 seconds are analyzed posthoc to identify encounters of a tadpole with a stimulus. A sequence of three time-lapse images (left to right) show an avoidance behavior in a control tadpole (top panel), a tadpole with ICL-transfected tectal cells (middle panel) and tadpole treated with diazepam (DZ, bottom panel). The upward direction of movement of the spots is shown with the white arrow. The direction of movement of the tadpole is shown with the red arrow. The control tadpole displayed a visual avoidance response characterized as a sharp turn in response to the moving spot, while the ICL-expressing and diazepam-treated tadpole continued to swim in the same direction after the encounter with the moving spot. Panels on the right show swim trajectories of animals, with the start point marked as 0 s and the end point marked as 0.4 or 0.6s. Spot luminance: 90 cd/m². Relative time stamps of image collection are shown in lower left of each image. Scale bar: 0.4 cm. (B) Tuning curves of the avoidance response for stimuli from 0.04 to 0.6cm diameter for control animals (including non-transfected tadpoles (n=7) and EGFP-expressing tadpoles (n=7). Control animals show significantly more avoidance events for stimuli 0.2 cm and larger (avoidance response compared to 0.04 cm: **p<0.01, *p<0.05). (C) Visual avoidance to stimuli of 0.4 cm and 0.6 cm is significantly lower in ICL-transfected tadpoles (n=15) compared to mICL-transfected tadpoles (n=12, *p<0.05). (D) Visual avoidance behavior of γ2-MO-expressing tadpoles (n=17) compared to Ctrl-MO-expressing tadpoles (n=14). Avoidance response to 0.4 cm stimuli is significantly lower in γ2-MO-transfected tadpoles compared to Ctrl-MO-transfected tadpoles (*p<0.05). (E) Visual avoidance to stimuli of 0.4 cm and 0.6 cm is significantly lower in tadpoles after DZ (10 µM for 1h or 2h, n=12, **p<0.01) while avoidance behavior is completely abolished in the same tadpoles after exposure to 30 µM DZ for 1 h (**p<0.01). The behavior recovers after 1 h in Steinberg’s solution. (F) Diazepam-treated tadpoles do not have motor response defects. Graph shows time spent swimming during 50s periods in the absence of moving dot visual stimuli for tadpoles before exposure to diazepam, and after exposures to diazepam or Steinberg’s solution (wash). (G) Injection of 1 mM diazepam into the tectal ventricle decreases avoidance behavior (**p<0.01), while injection of the vehicle, DMSO (1%) did not alter visual avoidance behavior (H).
References


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Figure 7

A

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B

Spike distribution (%)

C

CV of FSL

D

First spike latency (ms)

E

ISI (ms)