INTRODUCTION

The perception of a visual stimulus is strongly influenced by the spatial and temporal context in which the stimulus is presented (Albright and Stoner 2002; Felsen and Dan 2005; Rust and Movshon 2005). The search for a mechanistic understanding of contextual influences in the CNS presents a major challenge in neuroscience (Fitzpatrick 2000; Gilbert 1998).

We studied the neural mechanisms of contextual influences in the retinotectal pathway of birds (Luksch 2003). The avian tectal stratum griseum centrale (SGC) contains wide-field neurons with strong contextual influence on their response to moving stimuli; they respond preferentially to small moving objects, but do not respond to whole-field motion (Frost 1993). The morphologically and physiologically identified SGC-I neurons, one type of wide-field neurons, have somata in tectal layer 13, have large circular dendritic fields, extend their dendrites radially, and terminate with specialized dendritic endings in layer 5 (Karten et al. 1997; Luksch et al. 1998; Mahani et al. 2006) (Fig. 1A). There they make monosynaptic contact with axon terminals from retinal ganglion cells (RGCs) (Tömöl and Németh 1999). The RGC axons form a topographic map on the tectal surface and penetrate the outer tectal layers radially. The arrival of a RGC spike causes a probabilistic all-or-none SGC-I spiking response that is generated remotely from the soma, presumably at the dendritic ending (Luksch et al. 2001). The response probability to subsequent RGC spikes is reduced, an effect that is mediated at the dendritic spines (Luksch and Golz 2003). The morphological and physiological properties of SGC-I neurons were studied using in vitro electrophysiological recordings (Lundström et al. 2001, 2004), except that 20-µm-diameter tungsten electrodes were carried out as described previously (Luksch et al. 2001, 2004), except that 50-µm-diameter tungsten wire was used in this study. We obtained stable whole cell patch recordings from a total of 225 SGC-I neurons. The series resistance of the recordings was 30 ± 16 MΩ (mean ± SD) and was routinely compensated. The cells had a stable resting potential of −60 ± 5 mV (mean ± SD). SGC-I neurons usually have their somata in the outer aspects of the SGC layer and were further classified as SGC-I neurons by their characteristic physiological features. SGC-I neurons respond with rhythmic bursting to somatic current injection, respond with a sharp-onset response to synaptic stimulation in layers 2–4, and fail to respond to a second synaptic stimulation 30 ms after the first (Luksch et al. 2001, 2004).

 METHODS

Experiments

Three hundred White Leghorn chick hatchlings (Gallus gallus; <5 days old) were used in this study. All procedures used in this study were approved by the local authorities and conform to the guidelines of the National Institutes of Health on the Care and Use of Laboratory Animals. Tectal slice preparation, SGC-I soma whole cell recording, and electrostimulation with bipolar tungsten electrodes were carried out as described previously (Luksch et al. 2001, 2004), except that 50-µm-diameter tungsten wire was used in this study. We obtained stable whole cell patch recordings from a total of 225 SGC-I neurons. The series resistance of the recordings was 30 ± 16 MΩ (mean ± SD) and was routinely compensated. The cells had a stable resting potential of −60 ± 5 mV (mean ± SD). SGC-I neurons usually have their somata in the outer aspects of the SGC layer and were further classified as SGC-I neurons by their characteristic physiological features. SGC-I neurons respond with rhythmic bursting to somatic current injection, respond with a sharp-onset response to synaptic stimulation in layers 2–4, and fail to respond to a second synaptic stimulation 30 ms after the first (Luksch et al. 2001, 2004). Pulse train stimuli were typically repeated ten times for each stimulation interval in a pseudorandom sequence of stimulation intervals with a waiting time of 5 min between pulse train stimuli. The response probability for a sharp-onset response for each stimulus pulse within the pulse train was derived from the number of responses divided by the number of trials. The mean steady-state response probability is the mean of response probabilities of pulse numbers two to ten (P2–P10). To quantify whether the SGC-I responses were stationary over such a long recording time, we also separately calculated the mean steady-state response probabilities for the first five trials and second five trials. For all cells considered, the mean steady-state response probabilities were on average 0.26 ± 0.02 for the first five trials and 0.23 ± 0.02 for the second five trials. In conclusion, on average SGC-I responses were stable throughout the recording time of 50 min.
of bipolar stimulus electrodes with biphasic current pulses (20–200 µA, 500 µs) delivered from a stimulus isolator ensures a spatially restricted stimulation. For each stimulation site, the current amplitude was increased until reliable responses to isolated pulse stimulations were generated. The following observations provide evidence that the effective stimulation radius is <100 μm. First, electrical stimulation is not successful at all locations. In some of these cases, however, repositioning the stimulation electrode resulted in successful stimulation. Second, when postsynaptic inhibition was blocked with intracellular picrotoxin, the stimulation of one electrode before stimulation of a second electrode did not reduce the response probability to the second stimulation (Fig. 4C), thus suggesting that one stimulus electrode did not stimulate synapses at the other electrode.

To manipulate the inhibitory system in the slice, we added either picrotoxin (1 mM) or bicuculline (100 μM) to the bath. To manipulate specifically the inhibition onto the recorded SGC-I cell, we added 50 μM picrotoxin, which also blocks chloride channels when applied intracellulary (Akaite et al. 1985; Cupello et al. 1991; Inomata et al. 1988), to the patch electrode recording solution. Small molecules diffuse about 1 μm/ms and distance increases with the square root of time, so 1 mm (approximate distance to dendritic tips) is 1,000 s, or 15 min. Because larger molecules like picrotoxin should be slower, this number provides an estimated lower bound for the time it takes for picrotoxin to diffuse from the pipette to the dendritic endings. For the sequential stimulation experiments (Fig. 4C), we observed physiological changes about 20 min after the start of whole cell recording. For trials during the first 15 min the response probability to a pulse delivered to the second electrode was 0.49 ± 0.09 (n = 6 cells), a value that is not significantly different from the sequential stimulation response probability, 0.39 ± 0.07 (n = 31 cells) (Fig. 1D), measured under control conditions. In contrast, for intracellular picrotoxin, the response probability for subsequent trials (20 min after the start of whole cell recording) was 0.93 ± 0.07 (n = 6 cells). Previous studies with sharp electrodes filled with drugs reported that it took between 20 and 40 min for the drug to take effect (Dudek and Friedlander 1996; Hefti and Smith 2000). It is important to note that, because of the physics of diffusion (Berg 1993), the effect of a drug is not turned on at a discrete point in time. Rather the concentration of the drug gradually increases at the synapse from the moment whole cell recording has started and until a steady state is reached. Application of the physics of diffusion involves the assumption that the drug diffuses passively. This is not necessarily a given.

The ensemble average was derived from those cells for which the mean steady-state response probabilities was measured for the same stimulation intervals and the same number of stimulation electrodes. Typically, each ensemble average (i.e., each data point) was based on ten cells for Fig. 1C, six cells for Fig. 2, and seven cells for Fig. 5. For the response probability versus pulse number (Fig. 3, A and B), data from different numbers of stimulus electrodes were pooled and, typically, each ensemble average (i.e., each data point) was based on 49 cells for Fig. 3A and 24 cells for Fig. 3B. All data are presented as means ± SE, unless noted otherwise.

The RGC axons form a topographic map on the tectal surface and radially penetrate the outer tectal layers. An important aspect of the synaptic stimulation experiments is the ability to stimulate independently separate groups of RGC axons, which in turn synapse onto separate dendritic endings of the same SGC-I neuron under investigation (Fig. 1A). This experimental paradigm was physically possible because of the large spatial extent of a single SGC-I neuron dendritic field, which spans over a diameter of 4,000 μm (Mahani et al. 2006), thus allowing the spacing of multiple stimulus electrodes within the same dendritic field at a distance of >200 μm in layers 2 to 4. The use of picrotoxin increased the response probability to the second stimulation (Fig. 4C), thus suggesting that one stimulus electrode did not stimulate synapses at the other electrode.

![Image](http://www.jn.org/assets/1/12/104279/709218/f1.png)  
**FIG. 1.** Tectal interaction of multiple retinal inputs. A: schematic of the tectum slice and the characteristic features of the stratum griseum centrale type I (SGC-I) neuron (one shown in yellow) with soma in layer 13 and dendritic endings in layer 5 (gray). Other SGC-I somata (black dots) are shown in layer 13. Retinal ganglion cell (RGC) axons (not shown) enter from the tectal surface and project topographically to tectal layer 5. Stimulation electrodes (red), positioned above layer 5, locally stimulate small groups of RGC axons. Scale bar = 1,000 μm. B: response of one SGC-I neuron to pulse train synaptic stimulation at three locations. Bottom trace: pulse trains of 10 stimulus pulses simultaneously delivered to 3 locations above layer 5. Stimulation interval, Δt, was 2,000 ms. Center trace: typical SGC-I response to one trial of 10 pulses. Resting membrane potential was ~59 mV. Top traces: response raster plots of the same neuron for 10 trials. Each tick mark indicates the occurrence of a sharp-onset response. C: steady-state SGC-I response probability as a function of the stimulation interval, Δt, between synaptic stimulation for the measured responses (red dots) to m = 1 synaptic stimulation site, the exponential fit (red curve), the calculated independence-based steady-state model SGC-I response probabilities to simultaneous synaptic stimulation at multiple locations, m = 2–4 (black curves), and the measured SGC-I response probabilities to simultaneous synaptic stimulation at multiple locations (black symbols). D: response probabilities for isolated stimulations at one site and for sequential two-site stimulation with a delay of D = 100 ms.

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![Image](http://www.jn.org/assets/1/12/104279/709218/f2.png)  
**FIG. 2.** Steady-state response probabilities in the presence of picrotoxin. Measured SGC-I steady-state response probabilities (n = 20 cells) in the presence of 1 mM picrotoxin as a function of the stimulation interval for m = 1 (blue dots) and m = 2–4 (black symbols) stimulus electrodes. Based on the m = 1 data, the independence-based model SGC-I response probabilities to simultaneous synaptic stimulation at multiple locations, m = 2–4, (thin black curves) are calculated and shown for comparison. Exponential fit to the m = 1 control data (Fig. 1C) is shown for comparison (red curve).
Models

Independence-based prediction of response probability

In the independence-based prediction of the response probability \( P(\Delta t, m) \), we assumed that all dendritic endings have the same value for the response probability \( P(\Delta t, m = 1) \). In reality, however, the response probabilities vary between dendritic endings. The simplifying assumption provides a useful independence-based prediction of the response probability \( P(\Delta t, m) \) as long as the deviation of individual response probabilities from the dendritic ending population average is small, as indicated by the error bars in Fig. 1C. In general, the equation \( P(\Delta t, m) = 1 - [1 - P(\Delta t, m = 1)]^m \) is valid for average values of \( P(\Delta t, m) \) and \( P(\Delta t, m = 1) \).

We also assumed that a single electrode in our experiments stimulates only one SGC-I synapse. Here we derive that the analysis is independent of this assumption. Consider that, on average, each electrode stimulates \( n \) different synapses. Using the same argument as above, \( P_p = 1 - (1 - P_s)^n \), where \( P_p \) and \( P_s \) describe the average probabilities of synaptic transmission and electrode response, respectively. If we use \( m \) electrodes, we are stimulating \( m \times n \) synapses, on average. Therefore \( P_{mn} = 1 - (1 - P_s)^m \), where \( P_{mn} \) is the probability of response to \( m \) electrodes. Eliminating \( P_p \) from the two preceding equations results in \( P_{mn} = 1 - (1 - P_s)^m \), connecting the single-electrode and multiplet electrode response probabilities together as before.

Temporal aspects of probability functions

We developed a phenomenological model to reproduce the temporal properties of the SGC-I response (Fig. 3). The model assumes that the overall response probability for a certain pulse number has two time-dependent components, the multiplication of which determines the final response probability function, up to a constant factor. One component probability, \( P_1(n) \), decreases every time an RGC spike arrives and then recovers exponentially over time. The other component probability, \( P_2(n) \), dips after the first spike and then recovers in a sigmoidal fashion. The recovery occurs despite the continuing arrival of new RGC spikes. The choice of a “sigmoidal” recovery function despite repeated inputs was entirely motivated by the picrotoxin results (Fig. 3B), without any reference to biophysical mechanisms. However, it is tempting to speculate that a recovery despite continued inputs suggests that synaptic depression has a significant postsynaptic component (e.g., receptor desensitization), which may recover while transmitter release is reduced (e.g., vesicle depletion). The dynamics of presynaptic transmitter release, in turn, may be captured by the sum of decaying exponentials. The average response probability for the first pulse is averaged across different interspike intervals and used as a constant factor that multiplies the time-dependent factors to yield the final response probability. The first component probability is given by the exponentially decaying function of the sum of decaying exponentials

\[
P_1(n) = \exp(-1/A) \times \sum_{j=1}^{n-1} \exp(-\Delta t/T_j)
\]

Here, \( \exp(-\Delta t/T_j) \) is the decaying exponential in response to pulse number \( j \), \( n \) is the number of pulses received so far, \( \Delta t \) is the interpulse interval, and the time constant \( T_j \) and amplitude \( A \) are model parameters to be fit to the experimental data. The sigmoidal function of time is inspired by the time course of response in the presence of picrotoxin (Fig. 3B) and is given by

\[
P_2(n) = 0.5 \times \left(1 + \tanh\left(\frac{n - 1}{\Delta t/T} - \frac{T}{S}\right)\right)
\]

Here, the parameter \( T_2 \) determines the center of the sigmoid where \( P_2(n) = 0.5 \) and \( S \) determines the slope of the curve. The overall response probability is \( P(n) = P_{max} \times P_1(n) \times P_2(n) \), with \( P_{max} \) being the average response probability for the first pulse. A direct search method was used to find the set of four parameters (\( T_1; T_2; A; S \)) that provide the best fit to the experimental data. Two sets of parameters were found: one for the control condition (9,600 ms; 4.0; 3,700 ms; 8,600 ms), and one for the bath-applied picrotoxin condition (42,500 ms; 6.5; 10,000 ms; 5,000 ms). We used the same set of parameters to fit all interpulse interval data under each of the two above-cited conditions. The resolution of our search grid for the time parameters was 100 ms and the resolution for the amplitude \( A \) was 0.1. Each curve fit was based on 10 data points. To evaluate the goodness of the fit, we used a chi-square definition in which chi-square equals the sum of the squared differences between the observed mean and the model prediction, with each term divided by the squared measured SD. The chi-square values are then interpreted as follows. If chi-square equals zero, the agreement is perfect. In general, the individual terms in the
sum are expected to be of the order of one, and there are 10 terms in the sum. Thus if chi-square is $< 10$, the fit is acceptable. The thus defined chi-square evaluation of the best fits to the experimental data provided chi-square values of $< 0.5$ (range 0.02–0.41) for the four curves in the control condition (Fig. 3A) and $< 0.7$ (range 0.38–0.64) for the four curves in the picrotoxin condition (Fig. 3B).

**Poisson pulse train stimulation**

In the steady-state regime, the SGC-I response is quantified by the SGC-I firing rate $r_{SGC}$, which is determined from the number of spikes within time bins after the fifth bin. Within the independence assumption, for uncorrelated RGC spiking, and for low SGC-I firing rates, the SGC-I firing rate is, approximately, $r_{SGC} = mr_{RGC}P(\Delta t, m = 1)$ (Fig. 5C), i.e., the product of the number of $m$ of stimulated dendritic endings, the RGC rate $r_{RGC}$ and the response probability $P(\Delta t, m = 1) = P_{\max}(1 - e^{-\Delta t/t_0})$, for one dendritic ending. In this estimate, $\Delta t = 1/r_{RGC}$ represents the average RGC interspike interval. When the ratio $(\Delta t/t_0)$ is $<<1$, we can replace the exponential by its first-order approximation, $e^{-\Delta t/t_0} = 1 - (\Delta t/t_0)$. Because $t_0$ is $> 2$ s, this approximation is valid even at RGC rates as low as 1 Hz and we get $P(\Delta t) = P_{\max}(\Delta t/t_0)$. Thus the steady-state SGC-I firing rate is $r_{SGC} = mr_{RGC}P(\Delta t) = (mP_{\max}(t_0))$, which increases linearly with $m$.

We investigated a phenomenological model with spatial interaction and temporal dynamics to determine model SGC-I responses to multiple uncorrelated Poisson pulse trains. In this model, an RGC spike arriving at a synapse has three effects. First, it generates an SGC-I dendritic response with probability $P_{\max}$, which has a value of $\sim 1$. Second, it determines the response probability for subsequent RGC spikes arriving at this synapse. Third, it determines the response probability for subsequent RGC spikes arriving at other synapses. This latter step implements the tectal interaction of retinal inputs. Before each trial we use the probability $P_{\text{int}}$ to decide whether spikes in RGC axon $\alpha$ will interact with retinotectal synapse $\beta$. We do this for all pairs ($\alpha, \beta$) and thus find the subset of RGC axons $\alpha'$ whose spikes will determine the response probability for subsequent RGC spikes arriving at this synapse $\beta$. This interaction of retinal inputs is not necessarily symmetric. Once we have determined the subset of RGC axons $\alpha'$, we combine their spike trains together with the spike train from $\beta$ for the purpose of calculating the component probabilities $P(n) = P_{\max} \times P_{2}(n) = P_{\alpha}(n)$ (Fig. 3C) for synapse $\beta$. One component probability decreases every time an RGC spike affects its synapse, then recovers exponentially. The other component probability recovers in a sigmoidal fashion after a strong initial dip after the first spike affecting its synapse. The recovery occurs despite the continuing arrival of new RGC spikes. We observed that the parameters fitted to regular pulse train experiments (Fig. 3A) did not exactly reproduce the steady-state Poisson results. We suspected that this could be the result of an overestimation of the response probability immediately after an RGC spike. We therefore added a linear term that varied between 10 and zero as time goes from 30 to 500 ms after a pulse. Other than this adjustment, other parameters of the model were the same as those fitted to the control experiments in the regular pulse train section (Fig. 3A). The probability of interaction parameter ($P_{\text{int}} = 0.7$) was chosen to simultaneously fit the experimental data for average Poisson pulse rates of 1, 5, and 10 Hz.

**Processing visual context**

Visual stimuli and retinal representations were simulated as described elsewhere (Mahan et al. 2006). The small moving object had a size of $1 \times 1^\circ$ and moved with a speed of 50°/s. For whole-field motion, we used a $50 \times 50^\circ$ object covering the dendritic field of the SGC-I cell, which has an average diameter of $40^\circ$. Within our simplifying assumption (Lukshch et al. 2004; Mahani et al. 2006) that an RGC responds with a Poisson spike train of a certain mean rate as long as a stimulus is within its receptive field, the retinal representations of whole-field motion and a stationary stimulus covering the whole field are indistinguishable. For computational convenience we therefore used the latter stimulus. In response to a visual stimulus within an RGC receptive field, the RGC cell responded with an initial response of one spike followed by a Poisson spike train with a mean rate of 1 Hz (Fig. 6, B and C) or 50 Hz. The SGC-I dendritic responses were determined as described above for the Poisson pulse train stimulation. The only difference was that the spatial range of the synaptic interaction was taken into account; i.e., for the interaction probability $P_{\text{int}}$ we used the hyperbolic tangent function

$$P_{\text{int}} = 0.5 \times \left(1 - \tanh \left(\frac{x}{a} - b\right)\right)$$

with slope $a = 200 \mu m$ and shift $b = 2$. The average SGC-I firing rate was calculated for the time during which the object traveled the $40^\circ$ distance across this dendritic field. At 50°/s, the time it takes the object to cross the receptive field of the cell is 0.8 s. Therefore we divided the number of spikes elicited from the cell in each trial by 0.8 to find an average firing rate for each set of conditions. For the whole-field simulations, we waited until the response reached a steady state, counted the spikes for 4 s, and calculated an average firing rate. A total of 1,000 trials were used for simulations involving small objects and 100 trials were used for the whole-field motion simulations.

**RESULTS**

Tectal interaction of multiple retinal inputs

Contextual influence requires the interaction of visual inputs. To test the hypothesis that multiple retinal inputs interact

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**FIG. 5.** Synaptic stimulation with multiple uncorrelated Poisson RGC pulses. A: representative SGC-I response to stimulation with $m = 3$ uncorrelated RGC Poisson pulse trains each with a mean rate of 5 Hz. B: average number of SGC-I spikes per 200-ms bins and trial when stimulated with $m = 1$–4 RGC pulse trains each with a mean rate of 5 Hz. Measurements are plotted as symbols and model results are plotted as thin curves of same color. C: comparison of the (measured) steady-state SGC-I rate as a function of RGC rate for $m = 1$–4 RGC pulse trains with the independence-based (dashed curve) and interaction (solid curve) model predictions. D: comparison of the measured (black dots) average number of SGC-I spikes per first bin and trial as a function of the number $m$ of RGC pulse trains for mean RGC rates of 1, 5, and 10 Hz with the independence-based (dashed curve) and interaction (solid curve) model predictions. First bin widths were 1,000, 500, and 100 ms for mean RGC rates of 1, 5, and 10 Hz, respectively.
in the tectum, we compared stimulated SGC-I responses in vitro with an independence-based prediction. In a tectal slice preparation, we stimulated separate groups of RGC axons, which in turn synapse onto separate dendritic endings of the same SGC-I neuron under investigation (Fig. 1A). We delivered synchronous regular trains of 10 short current pulses with either one or multiple \((m = 1–4)\) stimulus electrodes in tectal layers 2 to 4 and recorded the probabilistic binary responses in the SGC-I soma (Fig. 1B). Because of retinotectal synaptic dynamics, the response probability is reduced after the first pulse (Lukesch et al. 2004). We derived the measured SGC-I steady-state response probability when stimulated with \(m = 1\) stimulus electrode from repeated trials with the same stimulation interval \(\Delta t\) and averaged over pulse numbers 2 to 10 (Fig. 1C). The measured steady-state response probabilities were fitted by an exponential function, \(P(\Delta t, m = 1) = P_{\text{max}}(1 - e^{-\Delta t/\tau_0})\), with \(P_{\text{max}} = 0.87\) and \(\tau_0 = 2.820\) ms.

Assuming that individual dendritic endings respond independently, the predicted SGC-I steady-state response probability \(P(\Delta t, m)\), when stimulated with \(m\) stimulus electrodes, is equal to the probability that any one or more of its \(m\) stimulated dendritic endings spike. The probability of not having a spike in a dendritic ending is \(1 - P(\Delta t, m = 1)\). The probability of not generating a spike in any of the \(m\) stimulated dendritic endings is \(\left[1 - P(\Delta t, m = 1)\right]^m\). The sum of the probabilities of all possible events of dendritic spiking must be 1. Therefore the probability that any one or more of its \(m\) stimulated dendritic endings spike is \(P(\Delta t, m) = 1 - \left[1 - P(\Delta t, m = 1)\right]^m\).

This relation predicts that the SGC-I steady-state response probability increases with increasing number of activated stimulus electrodes (Fig. 1C).

In contrast, the measured steady-state response probabilities, when stimulated simultaneously with \(m = 2–4\) stimulus electrodes, clearly did not increase with increasing \(m\) (Fig. 1C). There is no significant difference in the steady-state response probabilities for different number of stimulus electrodes in the range from \(m = 1\) to \(m = 4\) [ANOVA, \(F = 1.3\), degrees of freedom (df) = 3, \(P = 0.33\)]. Thus we conclude that the independence assumption is not valid. Rather, the data suggest that spatially separate retinal inputs interact in the tectum. The interaction is such that the steady-state response probability at individual dendritic endings decreases with increasing number of stimulus electrodes.

To elucidate the temporal aspects of interaction, we conducted sequential two-site stimulation experiments. We placed two stimulus electrodes in layers 2 to 4 for synaptic stimulation. The two electrodes were typically 300 \(\mu m\) apart from each other. A stimulus pulse was delivered to one electrode and 100 ms later to the second electrode. The response probability to the second stimulation was derived from the responses to repeated trials. Alternating with responses to sequential two-site stimulation trials, responses to isolated stimulations were recorded and yielded a response probability of \(0.95 \pm 0.02\).

The stimulation of one electrode before stimulation of the second reduced the response probability to \(0.39 \pm 0.07\) (\(n = 31\) cells) (Fig. 1D). The effect was smaller, but did not vanish, for delays of 1,000 ms, where the response probability to the second stimulation was \(0.51 \pm 0.15\) (\(n = 8\) cells), or when a train of 10 pulses with 100-ms interval (rather than one pulse) was delivered to the first electrode (response probability = \(0.64 \pm 0.19\), \(n = 5\) cells). The effect also decreased with increasing stimulus electrode distances. The response probability to the second stimulation had a value of \(0.66 \pm 0.12\) (\(n = 10\) cells) when stimulus electrodes were spaced \(>600\) \(\mu m\) apart. In conclusion, tectal interaction of retinal inputs is activated within <100 ms and decays gradually over time.

**GABAergic circuitry interacts with synaptic depression at retinotectal synapses**

To test the potential involvement of GABAergic circuitry in the observed tectal interaction of retinal inputs, we repeated the synchronous pulse-train stimulation experiments, but now with bath application of picrotoxin, a chloride channel blocker (Fig. 2, \(n = 20\) cells). Comparing the two groups, we found that for bath-applied picrotoxin the steady-state response probabilities (pulse numbers 2 to 10) were significantly lower than for the control group (ANOVA, \(F = 9.5\), df = 4, \(P < 0.001\)). Specifically, in a pairwise comparison, the measured steady-state response probabilities for most stimulus parameters were significantly lower than the \(m = 1\) controls \((t\text{-test}, P < 0.05)\) and largely vanished for \(\Delta t\) values of 500 and 1,000 ms. For four stimulus parameters \((m = 3\) and \(m = 4\) with \(\Delta t\) values of 2,000 and 4,000 ms), the apparent reduction in the measured steady-state response probabilities compared with control was less conclusive \((t\text{-test}, P < 0.08)\). Similar to the control data (Fig. 1C), with bath-applied picrotoxin there was no significant difference in the steady-state response probabilities for different number of stimulus electrodes in the range from \(m = 1\) to \(m = 4\) (ANOVA, \(F = 2.2\), df = 3, \(P = 0.14\)). Bath-applied
picrotoxin had no significant effect on the first-pulse response probability (control: $P_1 = 0.91 \pm 0.03$; picrotoxin: $P_1 = 0.87 \pm 0.04, n = 20$ cells) and also left the shape of the spiking response unchanged (data not shown). Similar results were obtained with bath application of bicuculline ($n = 17$ cells, $m = 4$ stimulus electrodes, $\Delta t$ values of 500 to 4,000 ms; data not shown).

The reduced steady-state response probability for most stimulus parameters with picrotoxin in the bath suggests that the effect of GABAergic circuitry is not postsynaptic inhibition alone. Rather, the results support the hypothesis that retinotectal signal transmission involves at least two distinct, but possibly interacting, mechanisms: 1) a suppressive mechanism located at the retinotectal synapse, which we refer to as “synaptic depression”; and 2) a mechanism mediated by picrotoxin-sensitive GABAergic circuitry that may be located before the postsynaptic side.

The time course of the SGC-I response to simultaneous regular pulse trains for $m = 1–4$ stimulus electrodes for control (Fig. 3A) and bath-applied picrotoxin (Fig. 3B) provided additional information about the two mechanisms. Because the measured steady-state response probabilities (Fig. 1C and 2) did not show a significant $m$ dependency we averaged data for $m = 1–4$ and thereby obtained a more transparent graphical display of the time dependency (Fig. 3, A and B). In the case of bath-applied picrotoxin, there was a significant dip in the response probability for the second pulse followed by a partial recovery, i.e., one component of the recovery from synaptic depression takes place despite the continuing pulse-train stimulation. In the control experiments, the two distinct mechanisms, GABAergic circuitry and synaptic depression, together generate a response probability with a weaker time dependency after the second stimulus pulse. This observation suggests that, under control conditions and through an unknown mechanism, GABAergic circuitry interacts with synaptic depression at retinotectal synapses. A phenomenological model consisting of two time-dependent component probabilities (Fig. 3C) qualitatively reproduces the general features of the described time course (Fig. 3, A and B).

**Postsynaptic lateral inhibition**

The evidence for GABAergic circuitry interacting with retinotectal signal transmission (Fig. 3) raised the question whether part of the GABAergic circuitry also acts postsynaptically. To address this question, we attempted to block putative postsynaptic chloride channels. We therefore added picrotoxin to the patch electrode recording solution. Physiological changes (see following text) were observed roughly 20 min after the start of whole cell recording, which is consistent with the estimated time it takes for molecules to diffuse into the cell and to the dendritic endings. It was thus possible to record synaptic responses both before and after the putative chloride channels had been blocked. We limited our experiments to one set of stimulation parameters ($\Delta t = 2,000$ ms, $m = 1$ or 2 stimulus electrodes).

Intracellular picrotoxin did not change the measured steady-state response probability $P(\Delta t, m = 1)$ to stimulation with $m = 1$ stimulus electrode, which had a value of $0.23 \pm 0.07$ and $0.20 \pm 0.07$ for control and intracellular picrotoxin, respectively ($n = 6$ cells) (Fig. 4A). Intracellular picrotoxin had no significant effect on the first-pulse response probability (control: $P_1 = 0.81 \pm 0.09$; picrotoxin: $P_1 = 0.86 \pm 0.09, n = 6$ cells) and also left the shape of the spiking response unchanged (data not shown). Also, for intracellular picrotoxin the response probability did not dip at the second pulse (data not shown). However, intracellular picrotoxin reduced the interaction of retinal inputs. When $m = 2$ stimulus electrodes were activated, the measured steady-state response probability of $0.37 \pm 0.15 (n = 7$ cells) approximately equaled the independence-based prediction $P(\Delta t, m = 2) = 1 - (1 - P(\Delta t, m = 1))^2$, which had a value of $0.41 \pm 0.11$ (Fig. 4B). In contrast, in the control condition the measured steady-state response probability of $0.26 \pm 0.05$ was significantly smaller than the independence-based prediction of $0.69 \pm 0.07$ (Fig. 1C). These observations suggest that a postsynaptic component of inhibition is present and mediates part of the tectal interaction of retinal inputs.

Additional evidence for lateral postsynaptic inhibition comes from sequential two-site stimulation experiments. In control experiments, the stimulation of one electrode before stimulation of the second electrode reduced the response probability to the second stimulation (Fig. 1D). However, when postsynaptic inhibition was blocked with intracellular picrotoxin, the stimulation of one electrode before stimulation of the second electrode did not reduce the response probability to the second stimulation ($n = 5$ cells, Fig. 4C). This observation suggests that postsynaptic inhibition acts laterally.

**Synaptic stimulation with uncorrelated Poisson pulse trains**

The stimulation of retinal afferents with simultaneous regular pulse trains or sequential pulses (Figs. 1–4) is a powerful diagnostic tool. Under natural conditions, however, the tectum receives less correlated retinal spike trains. We therefore measured SGC-I responses to stimulation of retinal afferents with up to four uncorrelated Poisson pulse trains with mean rates of 1, 5, or 10 Hz (Fig. 5A). For such stimuli the response probability is not defined. However, the number of spikes within appropriately chosen time bins and averaged over multiple trials with uncorrelated Poisson pulse trains for each trial quantifies the SGC-I responses. The measured SGC-I responses are largely independent of the number of stimulus electrodes and rapidly decline after the onset of the Poisson pulse train (Fig. 5B).

Assuming independently responding dendritic endings and for low SGC-I firing rates, the steady-state SGC-I firing rate in response to uncorrelated RGC spike trains, is independent of the RGC firing rate at high rates and increases linearly with the number $m$ of stimulated dendritic endings. The latter prediction is inconsistent with the experimental data, which show a significantly reduced steady-state SGC-I response (Fig. 5C), thus once again demonstrating that the independence assumption is not valid. Similarly, the independence-based model falsely predicts an approximately linear increase of the number of spikes in the first time bin with increasing number of stimulus electrodes. Rather, the measured average number of spikes in the first time bin was close to 1, largely independent of the number $m$ of stimulus electrodes or the mean RGC rate (Fig. 5D). We thus investigated a phenomenological model, which included the two component probabilities that represent the synaptic dynamics (Fig. 3C) and which allowed for the effect of an individual RGC spike on multiple synapses (see
METHODS). This tectal interaction model qualitatively reproduces the important aspects of the data in response to uncorrelated RGC spike trains (Fig. 5, B–D).

Processing visual context

Encouraged by the successful prediction of the SGC-I responses to multiple uncorrelated Poisson pulse trains we investigated the functional role of the tectal interaction of retinal inputs for visual processing. Avian tectum wide-field neurons demonstrate strong contextual influences on their response to moving stimuli. They respond preferentially to small moving objects, but do not respond to whole-field motion (Frost 1993). With the tectal interaction model, we therefore simulated model SGC-I responses to assumed retinal representations of small moving spots and whole-field motion using realistic spatial distributions of SGC-I dendritic endings (Mahani et al. 2006) (Fig. 6A). The model SGC-I neurons responded (3.3 ± 1.3 Hz, mean ± SD, n = 1,000 trials of 0.8-s duration) to small moving objects (Fig. 6B), but did not respond (0.4 ± 0.4 Hz, mean ± SD, n = 100 trials of 4-s duration) to whole-field motion (Fig. 6C). In contrast, without the interaction, the model SGC-I neurons responded to both object and whole-field motion, 7.0 ± 2.4 and 15.2 ± 1.4 Hz, respectively (Fig. 6, B and C).

The simulation revealed additional insight. To abolish the SGC-I response to whole-field motion, the tectal interaction is crucial when the steady-state RGC activity settles to a low mean firing rate (1 Hz was used in the preceding simulation). In contrast, for large steady-state mean firing rates (e.g., 50 Hz) the component probability with exponential recovery vanishes even without interaction. As a result, the model SGC-I neuron does not respond to whole-field motion. For object motion, however, the RGC response is sufficiently short that changes in the RGC steady-state properties are not relevant and do not affect the SGC-I response.

DISCUSSION

The inflow of sensory information must be filtered and controlled if an animal is to make a contextually appropriate behavioral response to its environment. Our in vitro experiments provided insight into the complex synaptic circuitry that has evolved in the avian tectum to achieve this task. GABAergic circuitry interacts with signal transmission across retinotectal synapses by two distinct pathways (Fig. 7). One pathway mediates postsynaptic lateral inhibition, which acts directly on synaptic layer 5, which receive monosynaptic retinal inputs and long and thick dendrites that apparently are also presynaptic to neural processes (Luksch and Golz 2003).

A tectal system of interacting nonlinear elements

In addition to the experimentally constrained model results described earlier, it is instructive to attempt an intuitive account of the dynamics of this system of interacting nonlinear elements. A single SGC-I neuron has some 160 dendritic endings sparsely distributed in tectal layer 5 over a circular area with a 2-mm radius (Mahani et al. 2006; Fig. 6A). The arrival of an isolated RGC spike has multiple effects in the tectum: 1) it generates a spike in the postsynaptic SGC-I dendrite (Luksch et al. 2001), 2) it depresses the retinotectal synapse, 3) it activates the GABAergic circuitry, which in turn 4) increases the inhibition onto other SGC-I dendritic endings, and 5) interacts, through unknown mechanisms, with the depressed synapse as well as others. As a result of this tectal interaction, on average the response probability to subsequent RGC spikes arriving at any synapse is reduced (Fig. 6B). The average response probability decreases steeply with increasing number of activated RGC axons and, as a result, the SGC-I neuron fails to respond to whole-field motion (Fig. 6C).

GABAergic circuitry in avian tectum

Evidence exists for GABAergic neuronal hardware in avian tectum (Luksch 2003) that could implement the GABAergic circuitry we refer to in this study. The retinorecipient layers of the optic tectum contain several types of horizontal cells that arborize within one layer and have either a local axonal projection or no axons at all. All horizontal cells are likely to be GABAergic and for several types direct retinal input has been shown. For instance, the prime candidate neurons that could mediate the observed lateral postsynaptic inhibition are the GABAergic horizontal cells located in the retinotectal synaptic layer 5, which receive monosynaptic retinal inputs and have long and thick dendrites that apparently are also presynaptic to neural processes (Luksch and Golz 2003).

Two distinct GABAergic pathways

The interpretation that GABAergic circuitry mediates postsynaptic lateral inhibition on SGC-I dendrites relies on the assumption that intracellular picrotoxin in avian SGC-I neu-
rons specifically blocks GABA-mediated current. Previous studies on isolated neurons or membrane patches showed that intracellular picrotoxin blocks GABA-mediated current in frog sensory neurons (Akaike et al. 1985), bullfrog dorsal root ganglion cells (Inomata et al. 1988), and rabbit Deiters’ neurons (Cupello et al. 1991). Subsequently, intracellular picrotoxin has been used to block inhibition in auditory cortex of rat (Metherate and Ashe 1993) and primary visual cortex of cat (Nelson et al. 1994). Together, these studies suggest that the block of GABA-mediated current is a likely effect of intracellular picrotoxin in SGC-I neurons.

Intracellular picrotoxin dramatically increased the response probability in two-site sequential stimulation experiments (Fig. 4C) compared with a control set (Fig. 1D), thus leading to the conclusion that postsynaptic inhibition is lateral. In contrast, when stimulated at one site with one stimulus electrode the steady-state response probability did not change during the time course of intracellular picrotoxin application (Fig. 4A). It is thus tempting to conclude that postsynaptic inhibition is not active locally when stimulated with one stimulus electrode. However, this interpretation is hampered by the fact that the experiments did not provide an independent measure of the time course of the intracellular picrotoxin application at the dendritic endings. The interpretation of the trials during the first 20 min after recording onset as controls is therefore in doubt and no firm conclusion can be drawn about the potential lack of postsynaptic inhibition at the stimulation site.

The GABAergic circuitry could interact with the presynaptic part of retinotectal signal transmission in many ways (Watson 1992). These include control of action potential propagation (Verdier et al. 2003), presynaptic inhibition (or excitation for that matter; Marty and Llano 2005), changes in the conductance of the afferent membrane, and direct effects on transmitter release (Wu and Saggau 1997). Unlike the classic studies of presynaptic inhibition at more accessible synapses (Eccles 1964), the remoteness of the RGC-to–SGC-I synapse from the somatic recording site prohibits an electrophysiological identification of the mechanisms, which was therefore not attempted in this study.

It has long been noted that the net effect of presynaptic inhibition on synaptic transmission is nontrivial (Nicoll and Alger 1979; Segev 1990). Further, the interaction of a GABAergic circuitry with a synapse that itself depresses can lead to nonintuitive filtering properties (Buonomano 2000; Buonomano and Merzenich 1998; Lewis and Maler 2002). For instance, an inhibitory effect of GABA on the RGC axon terminals has two opposing effects on the response probability to the next incoming RGC spike. Inhibition directly decreases the response probability, but inhibition also could reduce depression (Krasne and Bryan 1973; O’Shea and Rowell 1975) and thereby indirectly increase the response probability. The overall effect of inhibition on the response to the next RGC spike would thus depend on the relative strength and time course of the direct decreasing versus indirect increasing influence on the response probability.

The time course of the SGC-I response to simultaneous regular pulse trains (Fig. 3) further illustrates the complexity of GABAergic circuitry interacting with a depressing retinotectal synapse. When the first RGC spike arrives, no GABAergic circuitry has yet been activated. Therefore the amount of synaptic depression caused by the first RGC spike should be the same for control and bath-applied picrotoxin. The subsequently activated inhibition should result in a response probability for the second pulse that is lower for the control than for bath-applied picrotoxin. In contrast, however, there is a significant dip for bath-applied picrotoxin (Fig. 3B) compared with control (Fig. 3A). This apparent conflict is resolved if the effect of GABAergic circuitry is to speed up the recovery from depression. For the control, the arrival of the first RGC spike depresses the synapse and, with a delay, activates the GABAergic circuitry. The GABAergic circuitry in turn speeds up the recovery from depression. This leads to an increased response probability for the second pulse. For bath-applied picrotoxin, on the other hand, because no GABAergic circuitry is available after the first pulse, recovery occurs more slowly, leading to lowered response probability to the second pulse. This speculation hinges on the assumption that there is no spontaneous release of GABA. In the presence of spontaneous GABA release, however, the amount of synaptic depression caused by the first RGC spike could be more for bath-applied picrotoxin than for control. Depending on the relative impact of inhibition versus depression, the response probability for the second pulse could indeed be smaller for bath-applied picrotoxin (Fig. 3B) compared with control (Fig. 3A).

Comparison to other systems

The interaction of GABAergic circuitry with synapses on wide-field neurons may be found in other systems as well. Among vertebrates, for instance, wide-field neurons with the characteristics of avian SGC-I cells also exist in the mammalian superior colliculus (Major et al. 2000). They have large dendritic fields, specialized dendritic endings in upper layers, and receive retinal input. The corresponding layers of the mammalian superior colliculus also contain horizontal cells that are multipolar, have dendritic arborizations parallel to the collicular surface, and are likely to be without axons (Langer and Lund 1974; Mooney et al. 1985; Rhoades et al. 1989). Neurons with this cell morphology were previously shown to be GABAergic (Binns 1999; Mize 1992; Mize and Luo 1992; Mize et al. 1982). Among invertebrates, a striking similarity exists between the avian SGC-I cells and the lobular giant movement detector (LGMD), a wide-field neuron in the locust visual system (Gabbiani et al. 2005). Onto its large dendritic field, the LGMD receives excitatory synaptic inputs sensitive to local motion. It also receives direct feedforward inhibition, however, located on separate dendritic subfields. Further, a lateral inhibitory network between excitatory afferent fibers and presynaptic to the LGMD allows for the interaction of this network with the synapses on this wide-field neuron.

Here we showed that in the avian tectum the combination of the interaction of GABAergic circuitry with synapses on wide-field neurons and the dynamics of synaptic depression together mediate the segregation of object and whole-field motion (Fig. 6C), such as resulting from head and body movement. It is instructive to compare this with retinal mechanisms that apparently evolved to block responses to rapid global shifts of visual scenes (Olveczky et al. 2003; Roska and Werblin 2003). In the retina, fixational eye movements cause jittery whole-field motion. In this case, simultaneous arrival of excitation (from the center) and inhibition (from the surround), both coupled to the jittery whole-field motion trajectory, cancel each
other out on summation in RGCs. In contrast, when motion trajectories for object and background are different, such excitatory and inhibitory pulses are randomly timed relative to each other. The net result is that inhibition does not cancel excitation. This mechanism does not generalize to other types of whole-field motion, such as those resulting from slower and smoother head and body movement. In such cases, there are no distinct transients arising from jitters in motion trajectory to serve as a trigger for excitatory and inhibitory pulses.

The neural processing of context in natural visual scenes

Ever since the discovery of lateral inhibition (Hartline and Ratliff 1974; Ratliff 1965), followed by its functional interpretations (Srinivasan et al. 1982), the neural processing of context in natural visual scenes has grown into a formidable research endeavor in neuroscience (Albright and Stoner 2002; Bar 2004; Felsen and Dan 2005; Fitzpatrick 2000; Gilbert 1998; Kayser et al. 2004; Rust and Movshon 2005; Simoncelli 2003). The contextual interaction of GABAergic circuitry with synaptic depression at retinotectal synapses, presented in this study, adds yet another dimension to be considered in our quest for a combined computational (Carandini et al. 2005) and mechanistic (Hirsch and Martínez 2006) understanding of context processing in the CNS.

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