Role of GABA<sub>B</sub> Receptor-Mediated Inhibition in Reciprocal Interareal Pathways of Rat Visual Cortex

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INTRODUCTION

Neurons in mammalian primary visual cortex respond with increased firing when a stimulus falls into the receptive field. Although retinal inputs provide the primary excitatory drive, responses can be modulated by the context in which a stimulus appears, by attention, and by signals that are unrelated to the current sensory stimulus (Lamme 1995; Motter 1993; Press et al. 1997; Zipser et al. 1996). Unlike neuronal responses to stimuli in the classical receptive field that encode orientation, spatial position, stereoscopic depth, illusory contours, and faces which all show selective tuning a few milliseconds after onset of firing (Cleeremans et al. 1993; Oram and Perrett 1992; Rolls et al. 1991; Thorpe et al.; von der Heydt and Peterhans 1989), modulatory responses evoked from outside the classical receptive field and by attention are delayed by tens to hundreds of milliseconds (Knierim and Van Essen 1992; Motter 1993; Roelfsema et al. 1998; Zipser et al. 1996). To account for this delay it was suggested that visual inputs are first sent to the top of the cortical hierarchy and after some latency return to V1 via interareal feedback connections (Hupé et al. 1998; Zipser et al. 1996). However, latency measurements in different cortical areas have shown that many neurons at hierarchically different levels can be activated simultaneously (Nowak et al. 1995; Raiguel et al. 1989). This argues against large differences in the arrival of forward and feedback activity in different areas. An alternative to this “delay-line” hypothesis is that responses to forward and feedback inputs are simultaneous but that each pathway elicits a different temporal pattern of polysynaptic excitation.

In the rat, forward and feedback projections between primary and secondary visual cortex (Coogan and Burkhalter 1993) arise from pyramidal cells (Jiang et al. 1993) that provide monosynaptic excitation to postsynaptic neurons (Domenici et al. 1995; Shao and Burkhalter 1996). Most of these neurons are pyramidal cells, and only ~10% of axons form synapses with GABAergic nonpyramidal cells (Johnson and Burkhalter 1996, 1997) which in turn innervate pyramidal neurons that receive input from interareal connections (Shao and Burkhalter 1996). In the forward pathway this disynaptic inhibition produces powerful hyperpolarizing fast inhibitory postsynaptic potentials (IPSPs) (Shao and Burkhalter 1996). By contrast, in the feedback pathway hyperpolarizing fast IPSPs are rare (Shao and Burkhalter 1996).

Inhibition in the cortex is mediated by γ-aminobutyric acid-A (GABA<sub>A</sub>) and GABA<sub>B</sub> receptors (Connors et al. 1988). Together these receptor mechanisms contribute to the balance of excitation and inhibition that influences the magnitude and temporal pattern of spike discharge (Berman et al. 1992; Connors et al. 1988; McCormick 1989). Excitatory responses consist of monosynaptic and polysynaptic excitatory postsynaptic potentials (EPSPs). Although monosynaptic EPSPs result from direct connections between pairs of pyramidal cells, polysynaptic EPSPs arise in chains of pyramidal cells that are linked by local axon collaterals (Douglas et al. 1995). The recurrent excitatory interactions produced in this network are important for the amplification of afferent inputs (Douglas and Martin 1991; Douglas et al. 1995). It is known from developmental studies in rat somatosensory cortex that polysynaptic excitation is regulated by slow inhibition (Fukuda et al. 1993; Luhmann and Prince 1990a,b). This suggests that GABA<sub>B</sub> receptor-

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mediated IPSPs play a role in the modulation of signal amplification. Excitation from feedback connections that is unopposed by slow inhibition might be important in this process. We therefore studied the effects of GABA<sub>B</sub> receptor-mediated inhibition on polysynaptic excitation in reciprocal interareal circuits of rat primary and secondary visual cortex.

**METHODS**

**Slice preparation**

Slices were prepared from 3- to 6-wk-old male Long-Evans rats. For this purpose animals were anesthetized with pentobarbital (40 mg/kg body weight ip) and decapitated. The left occipital pole of the forebrain was removed and placed in ice-cold, oxygenated artificial cerebrospinal fluid (ACSF) in which NaCl was substituted by sucrose (Aghajanian and Rasmussen 1989) [sucrose ACSF (in mM): 252 sucrose, 3.3 KCl, 2.5 CaCl<sub>2</sub>, 1.2 MgSO<sub>4</sub>, 25.5 NaHCO<sub>3</sub>, 1.2 KHPO<sub>4</sub>, 15 d-glucose, pH 7.4]. Tissue blocks were glued with the cut anterior surface down onto the stage of a Vibratome, submersed in sucrose ACSF and sectioned at 400 μm in the coronal plane. To ensure the integrity of reciprocal connections between primary visual cortex (area 17) and the secondary visual area latero-medial visual area (LM) we collected slices at 1.6–2 mm in front of the posterior pole (Domenici et al. 1995). The area 17/LM border was identified as a sharp transition between the two areas. Stimulation electrodes were positioned in the slice under a dissecting microscope. To study synaptic potentials/currents elicited by forward connections stimulating electrodes/glutamate puffer pipettes were placed in layer 2/3 of area LM. For studying synaptic inputs of feedback connections intracellular injection of biocytin. To allow subsequent identification of the cells recorded with patch pipettes, in selected experiments 0.2–0.5% biocytin was added to the intracellular solution containing (in mM) 120 K-gluconate, 5 NaCl, 1 CaCl<sub>2</sub>, 1 MgCl<sub>2</sub>, 5 EGTA, 10 HEPES, 0.2 GTP, 2 ATP, pH 7.4; 285 mosmol/l, adjusted with sucrose). For recording of inhibitory post-synaptic currents (IPSCs) pipettes were filled with a solution in which K<sup>+</sup> was replaced by Cs<sup>+</sup> and 5 mM QX-314 was added to block GABA<sub>B</sub>-receptor activated K<sup>+</sup> currents and delayed Na<sup>+</sup> currents (Gähwiler and Brown 1985; Nathan et al. 1990; Otis et al. 1993). Field potentials were recorded with extracellular electrodes pulled from glass capillaries filled with 3 M NaCl (impedance: 2–5 MΩ).

Voltage signals from intracellular recordings were amplified with an Axoprobe-1 amplifier (Axon Instruments, Foster City, CA). An active bridge balance allowed simultaneous passage of current and measurements of voltage with a single electrode. Signals were displayed simultaneously on an oscilloscope and after digitization (at 25 kHz) with a 1401 plus interface (Cambridge Electronic Design, Science Park, Cambridge, UK) and Spike-2 software (Cambridge Electronic Design) on-line on a monitor. Data were stored on optical disk or digital tape for off-line analysis. Only cells with resting membrane potentials of less than or equal to −68 mV and overshooting action potentials were used for analyses. Peak amplitudes of EPSPs and IPSPs/IPSCs (average of 3–6 sweeps) were measured as maximal positive or negative deflections from baseline. The amplitude of glutamate stimulated IPSCs was measured as peak within 150 ms of response onset. Peak latencies reflect the time elapsed between stimulus and the maximal amplitude of the response. Mean PSPs of a population of cells were obtained by averaging responses of different neurons recorded under comparable conditions and pooling three sweeps per cell.

**Voltage-clamp recordings** were made with standard patch-clamp methods (Blanton et al. 1989). The liquid junction potential (≤5 mV) was measured immediately after making contact between electrode and slice and was offset in the amplifier output so that the voltage and current measurements remained unaffected. Recordings were filtered at 10 kHz and digitized at 25–50 kHz with an Axoclamp-2B amplifier (Axon Instruments) in continuous mode and a Digidata 1200 A/D converter (Axon Instruments) with p-Clamp 6.0 software (Axon Instruments). Only cells with access resistance of <15 MΩ were used for analyses.

**Pharmacology**

The effects of GABA<sub>B</sub> receptor blockade on PSPs were studied by bath application of 200 μM 2-OH-saclofen (Tocris Cookson, Ballwin, MO) or CGP55845 (1–4 μM, gift from Drs. Bittinger and Olpe, Novartis, Switzerland).

**INTRACELLULAR INJECTION OF BIOCYTIN.** To allow subsequent identification of the cells recorded with patch pipettes, in selected experiments 0.2–0.5% biocytin was added to the intracellular solution. Slices with injected cells were fixed for 1–3 days in a mixture of 4% paraformaldehyde and 0.5% glutaraldehyde. The tissue was cryoprotected in 30% sucrose and sectioned at 80 μm. Sections were incubated with 10% methanol and 1% H<sub>2</sub>O<sub>2</sub> for 10 min, washed in 0.1 M 2-OH-saclofen (Tocris Cookson, Ballwin, MO) or CGP55845 (1–4 μM, gift from Drs. Bittinger and Olpe, Novartis, Switzerland).
M phosphate buffer, and processed with the avidin–biotin–peroxidase method, and staining was intensified with AgNO₃ and HAuCl₄ as described by Jiang et al. (1993). Stained sections were mounted on slides, treated with ethanol followed by xylene, and coverslipped with DPX mountant. Biocytin-filled cells were analyzed under the microscope with a ×60 oil immersion lens with high numerical aperture.

RESULTS

Cell types recorded

Intracellular recordings with sharp electrodes were performed from 92 randomly selected layer 2/3 neurons in areas 17 and LM. In the majority of cells (83/92) injection of depolarizing current pulses showed relatively broad action potentials (half-width at half-maximal amplitude: 0.8 ± 0.1 ms; n = 83) and marked accommodation of firing (Fig. 7). These neurons were classified as regular spiking and presumed to represent pyramidal cells (Huettnet and Baughman 1988; Kawaguchi 1995; McCormick et al. 1985). Resting membrane potential (V_m) and input resistance (R_m) of cells recorded in areas 17 (V_m: −75 ± 5 mV; R_m: 35 ± 6 MΩ; n = 66) and LM (V_m: −76 ± 4 mV; R_m: 35 ± 5; n = 17) were similar. In a small group of nine cells action potentials were narrow (half width at half maximal amplitude: 0.4 ± 0.05 ms), and firing frequency remained constant during the injection of a 200-ms pulse of positive current. These cells were classified as fast spiking (Huettnet and Baughman 1988; Kawaguchi 1995; McCormick et al. 1985) and were not included in this study.

Whole cell patch-clamp recordings were made from 16 randomly selected layer 2/3 neurons in areas 17 and LM. Blockade of K⁺ channels by intracellular Cs⁺ precluded the physiological determination of cell types. However all of the eight biocytin-filled neurons (5 in area 17, 3 in LM) were pyramidal cells, suggesting that this type of neuron represents the majority of cells tested. The average resting membrane potential and input resistance of cells in areas 17 (V_m: −63.6 ± 6 mV; R_m: 114.6 ± 44 MΩ) and LM (V_m: −64.1 ± 5 mV; R_m: 120 ± 38 MΩ) were similar.

Synaptic responses to forward and feedback inputs

We compared intracellularly recorded PSPs evoked by forward and feedback inputs of different strengths. The stimuli used ranged from threshold intensity for EPSP activation to more than double the intensity necessary to elicit the maximal EPSP amplitude. Forward and feedback responses of layer 2/3 neurons showed very different waveforms, especially when recorded at membrane potentials 10–15 mV depolarized from rest (Fig. 1, A and B). Threshold stimulation (1T) of forward inputs evoked small EPSPs. Stronger stimuli (1.2T) elicited larger responses, which reached the peak more rapidly. Over 90% of responses had mono- and early polysynaptic components (Fig. 1C), which resembled those recorded in the frontal cortex (Sutor and Hablitz 1989a). The different EPSPs were easily distinguished by stimulating at different frequencies. Early polysynaptic components had variable amplitudes and decayed to baseline within <100 ms. Raising the stimulus intensity to 1.4T revealed effects of fast IPSPs that led to an accelerated decay of monosynaptic EPSPs and abolished early polysynaptic components. In addition, in >90% of trials 1.4T stimuli evoked late polysynaptic EPSPs with variable peaks at latencies of ~100–400 ms. Early and late polysynaptic EPSPs were confined to a narrow window of stimulation intensities and were not seen with stronger stimuli (>1.5T). The disappearance of polysynaptic EPSPs correlated with the appearance of hyperpolarizing fast and slow IPSPs whose amplitudes grew larger with increasing stimulus strength (Fig. 1A). Slow IPSPs were always preceded by a small depolarizing wave that temporally overlapped with the decay of fast IPSPs. Because polysynaptic responses were variable we averaged recordings from seven cells, six trials each, to obtain the mean forward response ± SD (Fig. 1D). This average response showed the same dependence on stimulus intensity as the individual re-
responses (Fig. 1A). However, the late depolarizing peak of the average response was smaller than the individual response caused by trial-by-trial variability of polysynaptic EPSPs.

Unlike polysynaptic EPSPs in the forward pathway, which were seen only after weak stimulation, polysynaptic EPSPs in the feedback pathway were elicited over a broad range of stimulus intensities (Fig. 1B). Most (>90%) responses showed multiple peaks whose amplitude and time course varied from trial to trial but often lasted >500 ms (Figs. 1B and 5). Fast IPSPs were weak, and their presence was only evident at higher stimulus strengths by an accelerated decay of the early depolarization. In the majority of cases fast IPSPs were too weak to hyperpolarize the membrane. The most interesting difference, however, was that at all stimulus intensities tested feedback inputs failed to evoke slow hyperpolarizing IPSPs (Figs. 1B and 5).

Although the responses shown in Fig. 1 were seen in the majority of neurons, recordings with sharp electrodes revealed cells with a range of different properties. Of 17 cells that gave responses to activation (1.8T) of forward inputs, 13 (76%) responded with hyperpolarizing fast and slow IPSPs. As shown in Table 1 the distribution of responses evoked by stimulation of the L6/WM border and horizontal connections was similar to that seen in the forward pathway, and 78–86% of the cells tested showed both early and late IPSPs. In marked contrast, in the feedback pathway hyperpolarizing fast and slow IPSPs were rare, and most responses (18/27, 67%; Fig. 2Bd) resembled that shown in Fig. 2Bc. Few cells (5/27, 18%) showed fast IPSPs only (Fig. 2, Ac and Ad). In every case slow IPSPs, if present, were preceded by fast IPSPs. As shown in Table 1 the proportion of feedback PSPs with fast and slow hyperpolarizing IPSPs filled columns: IPSPfast + IPSPslow. Hatched columns: IPSPfast. Open columns: IPSPslow.

**TABLE 1. Proportion of hyperpolarizing IPSPs**

<table>
<thead>
<tr>
<th></th>
<th>FW</th>
<th>HC</th>
<th>WM</th>
<th>FB</th>
</tr>
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<tbody>
<tr>
<td>No IPSP</td>
<td>17</td>
<td>18</td>
<td>21</td>
<td>27</td>
</tr>
<tr>
<td>IPSPfast</td>
<td>3 (18)</td>
<td>1 (5)</td>
<td>1 (5)</td>
<td>5 (18)</td>
</tr>
<tr>
<td>IPSPslow</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>IPSPfast + slow</td>
<td>13 (76)</td>
<td>14 (78)</td>
<td>18 (86)</td>
<td>4 (15)</td>
</tr>
</tbody>
</table>

Values in parentheses are percentages; n is number of cells. IPSP, inhibitory postsynaptic potential; FW, forward; HC, horizontal; WM, white matter; FB, feedback.

**FIG. 2.** The proportion of layer 2/3 neurons responding with hyperpolarizing fast and slow IPSPs is greater in the forward (FW) pathway (area 17 → area LM) than in the feedback (FB) pathway (LM → 17). Aa–Ac: intracellular recordings of PSPs in 3 different layer 2/3 neurons of area LM elicited by strong (1.8T) stimulation of forward inputs. Aa: PSP shows powerful fast and slow IPSPs. Ab: PSP showing fast but no slow IPSP. Ac: depolarizing response without hyperpolarizing IPSPs. Ad: proportion of forward PSPs showing fast and slow hyperpolarizing IPSPs. Ba–Bc: intracellular recordings of PSPs in 3 different layer 2/3 neurons in area 17 elicited by stimulation (1.8T) of feedback inputs. Ba: response showing fast and slow hyperpolarizing IPSPs. Bb: PSP lacking slow hyperpolarizing IPSP. Bc: PSP lacking hyperpolarizing IPSPs. Bd: proportion of feedback PSPs with fast and slow hyperpolarizing IPSPs. Filled columns: IPSPfast + IPSPslow. Hatched columns: IPSPfast. Open columns: IPSPslow.

**Pathway-specificity of PSPs**

**ISOLATION OF PATHWAYS.** To assess whether the responses shown in Fig. 1 are pathway specific it was necessary to control for stimulation of fibers of passage. For this purpose we voltage clamped layer 2/3 neurons in areas 17 and LM at the reversal potential of EPSCs (~0 mV) and compared IPSCs elicited by electrical stimulation or drop application of glutamate at the same site. On the basis of previous observations that disynaptic inhibition in forward pathways is stronger than in feedback pathways (Shao and Burkhalter 1996), the expectation was that IPSC amplitudes show similar pathway differences and that these differences can also be demonstrated after stimulation with glutamate. As expected, in the forward pathway IPSCs elicited by medium- to high-intensity (1.5–1.8T) electrical shocks were larger (538 ± 90 pA) and decayed more slowly than in the feedback pathway (263 ± 50 pA; Fig. 3, A and B). For eight cells tested in each pathway the average IPSC
amplitude in the feedback pathway was ~49% smaller \((P < 0.01)\). Stimulation with glutamate evoked smaller and longer-lasting responses than electrical stimulation. However the relative difference \((40\%, P < 0.05)\) in IPSC amplitude between forward \((120 \pm 31\) pA) and feedback pathways \((48 \pm 22\) pA) was similar to that in tests with electric shocks (Fig. 3, C and D).

These results show that activating forward or feedback projecting neurons with glutamate evokes disynaptic inhibition that is qualitatively similar to that seen with electrical stimuli in the intensity range used in this study. This suggests that under these conditions activation of fibers of passage is not a serious problem.

**PATHWAY-SPECIFIC ACTIVATION OF SLOW IPSPS.** To directly test whether activation of slow IPSPs is pathway specific we compared responses of individual area 17 neurons with converging inputs from feedback (FB), intralaminar (WM), and horizontal (HC) pathways. The results show that the incidence of undershooting fast and slow IPSPs evoked by the different inputs are similar to those of experiments in which neurons were activated from a single pathway (Table 1).

After stimulating the L6/WM border with intensities of 1.5–1.8T most cells \((13/15, 86\%)\) responded with short-latency EPSPs followed by fast and slow IPSPs (Fig. 4, A and C). Slow IPSPs peaked at 146 \pm 13\ ms and had a mean amplitude of 4.7 \pm 1.5\ mV. Similar responses were seen in the majority of cells \((11/15, 74\%)\) stimulated by inputs from horizontal connections (Fig. 4, A and C), although slow IPSPs were slightly smaller \((3.5 \pm 1.2\ mV)\). Responses to feedback inputs differed in that repolarization of the early depolarizing wave was slower than after stimulation of both the L6/WM border or forward inputs. This indicates that early IPSPs in the feedback pathway were weak. Interestingly, in the feedback pathway only 20\% \((3/15)\) of the cells that after WM and HC stimulation showed the usual fast and slow IPSPs responded with hyperpolarizing slow IPSPs (Fig. 4, B and C). The presence of cells with slow IPSPs, similar to those seen after HC stimulation (peak amplitude: 3.1 \pm 1.4\ mV; peak latency 134 \pm 14\ ms), resulted in a small, slow hyperpolarization of the average feedback response at high stimulation intensities (Fig. 5). In spite of this, the mean slow IPSP evoked by high-intensity feedback input was much smaller than the response elicited by high-intensity stimulation of the L6/WM border or horizontal inputs (Fig. 5). In fact the amplitude time integral of the average slow hyperpolarization after strong stimulation of the feedback pathway was a mere 3\% of that evoked by L6/WM stimulation and was only 7\% of that seen after HC stimulation. This indicates that the net hyperpolarizing effect of the slow IPSP is largely independent of stimulus strength and that its weakness is a characteristic feature of the feedback circuit.

Monosynaptic responses evoked in the same neuron by stimulating the L6/WM border or horizontal connections were on average larger than early and late EPSPs, except after weak stimulation (Fig. 5). The same was true for interareal forward connections (Fig. 1C). Feedback connections differed in that late polysynaptic EPSPs elicited by weak and medium-strength

**FIG. 3.** Comparison of fast IPSCs elicited by electrical or chemical stimulation of interareal forward (FW) and feedback (FB) pathways between area 17 and LM of rat visual cortex. Whole cell patch-clamp recordings of layer 2/3 neurons. Membrane voltage is clamped at 0 mV. Recording pipettes contain Cs\(^+\) and QX314. A: IPSPs after electrical stimulation \((1.8\)T) of FW and FB pathways. B: average \((\pm SD)\) peak amplitudes of fast IPSC recorded in layer 2/3 neurons in areas LM and 17 after electrical activation of FW and FB inputs, respectively. C: IPSCs of layer 2/3 neurons after puffer application of glutamate \((1\) mM) at the site from which electrical stimulation of FW and FB pathways was performed \((A)\). The arrow indicates the onset of the mechanical artifact produced by the delivery of a droplet of glutamate. D: average peak amplitudes of IPSC recorded in layer 2/3 neurons in areas LM and 17 after glutamate stimulation of FW and FB projecting neurons, respectively.
stimuli were larger than monosynaptic responses (Fig. 5). More importantly, unlike the monophasic responses elicited by inputs from the L6/WM border, by horizontal and interareal forward connections, depolarizing responses evoked by feedback connections showed distinct early and late components of polysynaptic EPSPs over the entire range of stimulus intensities tested. By contrast in “forward-type” pathways polysynaptic EPSPs were attenuated by powerful fast and slow IPSPs whose thresholds of activation were substantially lower than in the feedback pathway.

Identification of slow IPSP

Because slow IPSPs were rare in the feedback pathway detailed analyses of slow hyperpolarizing responses were focused on the forward pathway.

VOLTAGE DEPENDENCE. To test the voltage dependence of slow IPSPs current injection was used to alter the membrane potential. During the 500 ms of current injection, forward inputs were activated by stimulating at 1.8T in upper layers of area 17. In all 13 cells tested monosynaptic EPSPs were followed by fast and slow IPSPs. Membrane depolarization away from the equilibrium potential of Cl\(^{-}\) and K\(^{+}\) progressively increased amplitudes of fast and slow IPSPs. On average, fast IPSPs reversed polarity at approximately \(-68\) mV, whereas slow IPSPs reversed at approximately \(-95\) mV. Similar reversal potentials were obtained in two cells tested in the feedback pathway. Although because of the temporal overlap of EPSPs and IPSPs these measurements represent approximations and suggest that fast and slow IPSPs reverse polarity near the equilibrium potential for Cl\(^{-}\) and K\(^{+}\), respectively.

BLOCKADE OF GABA\(_{B}\) RECEPTORS. The apparent reversal of polarity at membrane potentials below \(-90\) mV strongly suggested the involvement of a GABA\(_{B}\) receptor-mediated K\(^{+}\)...
Effects of slow IPSP on neuronal firing

To study whether late IPSPs can influence neuronal firing we compared the effects of feedback inputs with responses to stimulation of the L6/WM border. Recordings were performed in area 17 neurons that received convergent input from both the feedback pathway and connections from the L6/WM border. The magnitude of neuronal discharge was varied by injecting 500-ms pulses of positive current of different amplitudes. Synaptic responses were evoked by delivering stimuli of constant intensity (100 µs, 1.8T) 200 ms after the onset of intracellular current injection to either area LM or the L6/WM border. Each of five neurons tested behaved similarly to the cell shown in Fig. 7 and exhibited radically different responses to inputs from each stimulation site. At the resting membrane potential (no current injection) feedback input evoked a series of EPSPs (Fig. 7&Aa). When the membrane depolarization reached firing threshold but was still too weak to sustain firing, feedback input triggered a train of action potentials that was maintained for the duration of the DC pulse and showed pronounced spike frequency adaptation (Fig. 7&Ad). As the firing rate increased with stronger depolarization, feedback input produced a further increase in firing, but the relative change in rate imposed by the synaptic inputs was less than at firing threshold (Fig. 7&Ae). At the highest firing frequency tested feedback input had no detectable effect on discharge rate (Fig. 7&Af).

Unlike feedback inputs, subthreshold activation of inputs from the L6/WM border evoked a sequence of EPSPs followed by fast and slow IPSPs (Fig. 7, Ba–Be). Near spike threshold synaptic inputs produced a transient increase in firing by evoking a single additional spike. This was followed by powerful fast and slow IPSPs that inhibited firing for most of the duration of the depolarizing current injection. Additional depolarization slightly increased the firing rate and made the transient suppression of repetitive firing by synaptic input less effective than at more negative potentials (Fig. 7&Fb). This shortening of the delay of firing was accompanied by a reduction in the amplitude of the slow IPSP.

**Discussion**

Our results show that intracortical feedback inputs from the secondary visual area, LM, rarely evoke slow GABA<sub>B</sub> receptor-mediated hyperpolarizing IPSPs in regular spiking layer 2/3 pyramidal cells (Kawaguchi 1995; McCormick et al. 1985) of area 17. By contrast, forward inputs from area 17 evoke slow IPSPs in most upper layer pyramidal neurons of area LM. A similarly high incidence of GABA<sub>B</sub> responses was found in superficial layers of area 17 after local activation of interlaminar and horizontal inputs. Because in the same neuron feedback inputs evoke weaker slow inhibition than horizontal and
intralaminar inputs, these differences reflect pathway-specific synaptic properties and are not due to recordings from different cell types.

A low incidence of slow IPSPs in the feedback pathway could result from an antagonism by polysynaptic EPSPs (Scharfman 1992). However, we found no evidence that polysynaptic EPSPs are larger in the feedback pathway, suggesting that in feedback-recipient neurons slow IPSPs are less effective.

Unlike in interareal forward, horizontal, and interlaminar circuits in which polysynaptic excitation is curbed by powerful fast and slow IPSPs, in the feedback circuit polysynaptic EPSPs are more common because slow IPSPs are weak. Douglas et al. (1995) have shown that polysynaptic excitation is important for the amplification of afferent visual inputs. Our results suggest that feedback inputs are capable of generating polysynaptic EPSPs that in turn influence the gain of neuronal responses in primary visual cortex.

Source of synaptic potentials

A central assumption of this study is that distinct intracortical pathways were activated and that neurons at the stimulation site were the source of synaptic input to the cells recorded. Although definitive proof is lacking, observations indicate that stimulation at <2T is too weak for activating fibers of passage. Stimuli in this intensity range evoke synaptic activity that corresponds to the laminar distribution of forward and feedback terminals (Domenici et al. 1995). Furthermore, antidromic spikes at these intensities are rare (Asanuma and Sakata 1967; Nowak and Bullier 1996; Shao and Burkhalter 1996; Stoney et al. 1968), which rules out contamination by local axon collaterals. Finally, stimulation of forward and feedback-projecting neurons with electric shocks or glutamate yields a qualitatively similar asymmetry of disynaptic inhibition. From these results we conclude that stimulation of the L6/WM border preferentially activates ascending projections of layer 6 neurons (Burkhalter 1989; Hirsch 1995; Katz 1987) and that activation of thalamocortical (Miller et al. 1993) and cortico-cortical axons (Coogan and Burkhalter 1993; Olavarria and Van Sluyters 1985) was negligible. For similar reasons we believe that stimulation in upper layers of area 17 activates inputs from topographically distant points (Burkhalter and Charles 1990) and that layer 2/3 neurons in areas 17 and LM are the source of forward and feedback inputs, respectively.

Differential expression and effectiveness of slow IPSP

demonstration that distinct intracortical pathways produce different amounts of slow inhibition.

Although the results show that fast and slow hyperpolarizing IPSPs are smaller in the feedback pathway, it is possible that an amount of inhibition equivalent to that seen in the other pathways studied was attenuated by powerful excitation. This seems unlikely, however, because in the feedback pathway the size of polysynaptic EPSPs was similar to that in the forward pathway (Figs. 1C and 5). In addition, polysynaptic EPSPs released by blocking GABA<sub>B</sub> receptors at feedback synapses were often smaller than those released at forward synapses (Fig. 6, A, B, E, and F). This argues against a drug-induced increase in glutamate release from presynaptic terminals (Deisz et al. 1993) and indicates that in the feedback pathway slow inhibition is weak and that strong synaptic activation of GABAergic neurons is necessary to attenuate recurrent polysynaptic EPSPs (Fig. 5). This interpretation is consistent with observations in cat motor cortex where the demonstration of slow IPSPs requires elevation of GABA release from presynaptic terminals (van Bredenrode and Spain 1995). Thus it seems that the small IPSCs evoked by feedback inputs are insufficient to activate GABA<sub>B</sub>-mediated slow IPSPs.

It is important to stress that the same neuron that responds to feedback inputs with small IPSPs gives much larger IPSPs to “forward-type” inputs (Fig. 5). Therefore the weakness of fast and slow IPSPs in the feedback pathway is not due to different intrinsic membrane properties of feedback-and forward-recipient neurons; rather it reflects a distinct synaptic organization of feedback and forward circuits.

Determinants of inhibitory strength

Inhibitory responses in forward and feedback pathways may differ because the density of inhibitory synapses on forward-recipient pyramidal neurons is higher than on feedback-recipient cells. This seems unlikely because White et al. (1994) found no positive correlation between the density of GABAergic inputs and IPSP amplitude. Alternatively, the distribution of GABAergic synapses on target neurons may differ. In both pathways these synapses derive mainly from parvalbumin (PV) immunoreactive neurons (Gonchar and Burkhalter 1999). PV neurons include basket and axo-axonic cells (DeFelipe et al. 1989; Kawaguchi and Kubota 1997), which form synapses with pyramidal cell bodies, dendrites, or axon initial segments, respectively (DeFelipe et al. 1986; Peters et al. 1982). Inhibition by axo-axonic cells is thought to be most effective. Thus the presence of a larger contingent of axo-axonic cells in forward than in feedback circuits may underlie the stronger inhibition in forward pathways. Whether this cellular diversity can account for the pathway difference in GABA<sub>B</sub> responses rests on the demonstration that different GABAergic cells have different affinities for GABA<sub>B</sub> receptors (Benardo 1994; Otis and Mody 1992; Sugita et al. 1992; Thomson and West 1997).

Recent findings that forward synapses on PV neurons are larger than feedback synapses and are located more proximally on the dendritic tree suggest that the pathway selectivity of inhibitory effects may be linked to the inputs to PV neurons (Gonchar and Burkhalter 1999). This organization may cause stronger activation of inhibitory neurons in the forward pathway (Deuchars et al. 1994; Stuart and Sakmann 1995) and increase the probability for activating GABA<sub>B</sub> receptors (Howe et al. 1987; Kim et al. 1997; Sodickson and Bean 1996).

Polysynaptic circuit

Unlike interareal forward, interlaminar, and horizontal pathways in which polysynaptic EPSPs were confined to weak inputs (Sutor and Hablitz 1989a; this study), strong feedback inputs elicited polysynaptic EPSPs that lasted ≤500 ms. Similar responses were recorded after stimulation of layer 1 (Cauller and Connors 1994), which receives strong feedback input (Coogan and Burkhalter 1993). Because antidromic activity in layers 2/3 of area 17 was rare, it is unlikely that polysynaptic EPSPs arise from reverberations within the reciproc al interareal loop between areas 17 and LM. The two more likely sources of these secondary depolarizations are inputs from feedback-receptor pyramidal cells in the same layer and/or inputs from deep layers of the same column (Burkhalter 1989; Czeiger and White 1993; Elhanany and White 1990; Mason et al. 1991). In layer 2/3 the convergence of inputs to individual pyramidal cells is large (Deuchars et al. 1994; Gabbott et al. 1987; Kivvárday et al. 1986; Markram et al. 1997; Peters 1987). Thus it is thought that summation of unitary EPSPs from a pool of neighboring neurons could easily evoke polysynaptic EPSPs (Douglas and Martin 1991; Douglas et al. 1995). In the feedback pathway this pool consists of interconnected forward projecting neurons with few contacts to inhibitory cells (Johnson and Burkhalter 1997). As a result, recurrent excitation is only weakly opposed by inhibition (Markram et al. 1997; Mason et al. 1991; Thomson and Deuchars 1994). The local connectivity of forward-recipient pyramidal cells is not known. However, the strong inhibitory component in the forward response suggests that they are more intimately connected to inhibitory cells that curb recurrent excitation.

Functional implications

Our study demonstrates that GABA<sub>B</sub> receptor antagonists block slow IPSPs elicited by interareal forward inputs and release polysynaptic EPSPs from inhibition. The role of GABA<sub>B</sub> receptors in the suppression of polysynaptic EPSPs is consistent with the low probability of late EPSPs and an increased spike threshold (Berman et al. 1992; Connors et al. 1988; McCormick 1989) that renders responses to moderately strong interareal forward inputs more transient (Fig. 7). In contrast, in the feedback pathway late IPSPs are weak, and polysynaptic EPSPs are present even at high stimulation strengths and are capable of increasing and prolonging neuronal firing.

Activation of polysynaptic excitation by feedback connections over a broad range of stimulus intensities is advantageous for enhancing striate cortical responses to a variety of afferent inputs. If strong enough, this depolarization can overcome the GABA<sub>B</sub>-mediated slow hyperpolarization generated by forward inputs, increase spike frequency, and make responses more sustained (Fig. 7) (Connors et al. 1988). Therefore the proposed role of the feedback pathway is to modulate late inhibition elicited by forward inputs.

To understand the significance of two types of circuits (i.e., forward type, feedback) with unique balances of excitation and inhibition...
inhibition and partially overlapping but different operating
ranges, consider how convergent inputs are synapticly inte-
grated. Because of the lower activation threshold for inhibition
in forward pathways, two coincident forward-type inputs may
lead to strong amplification of IPSPs and to a complete sup-
pression of recurrent excitation (Hirsch 1995). By contrast,
because inhibition in feedback circuits is weak, coactivation of
forward and feedback inputs may have a much smaller effect
on the summation of inhibition. Indeed, slow inhibition pro-
vided by forward inputs might be suppressed, and late,
poly synaptic, N-methyl-d-aspartate (NMDA) receptor-dependent
excitation (Sutor and Hablitz 1989b) might be enhanced so
that responses to forward inputs are more robust and more
supported. Interestingly, single-unit recordings in cat visual
cortex have shown that the response gain depends on NMDA
receptors that act to amplify small synaptic signals over the
entire range of effective inputs (Fox et al. 1990). Thus the
capacity of feedback inputs to turn on recurrent excitation in
an intensity range in which these circuits are suppressed by for-
ward input-dependent slow IPSPs may provide a mechanism
by which higher cortical areas can amplify responses in lower
areas.

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REFERENCES

AGHAJANIAN, G. K. AND RASMUSSEN, K. Intracellular studies in the facial
nucleus illustrating a simple method for obtaining viable motoneurons in

ASANUMA, S. AND SAKATA, H. Functional organization of a cortical efferent
system examined with focal depth stimulation in cats. J. Neurophysiol.

BENARDO, J. S. Separate activation of fast and slow inhibitory postsynaptic

BERMAN, N. J., DOUGLAS, R. J., AND MARTIN, K.A.C. GABA-mediated inhi-
bition in the neuronal networks of visual cortex. Prog. Brain Res. 90:

BLANTON, M. G., LOTURCO, J. J., AND KRIEGSTEIN, A. R. Whole cell recording
from neurons in slices of reptilian and mammalian cerebral cortex. J.

BRUGGER, F., WICKL, U., OLPE, H.-R., FRIESTL, W., AND MICKEL, S. The action
of new potent GABA<sub>B</sub> receptor antagonists in the hemisected spinal cord

BURKHALTER, A. Intrinsic connections of rat primary visual cortex: Laminar

BURKHALTER, A. AND CHARLES, V. A. Organization of local axon collateral
of efferent projection neurons in rat neocortex. J. Comp. Neurol. 302:

CAULLER, L. J. AND CONNORS, B. W. Synchronized excitation and inhi-
bition driven by intrinsically bursting neurons in neocortex. J. Comp.

CHAGNAC-AMITAI, Y., LUMANN, H., AND PRINCE, D. A. Burst generating and
regular spiking layer V pyramidal neurons in rat neocortex have different

CHAGNAC-AMITAI, Y., MALENKA, R. C., AND SILVA, L. R. Two inhibitory postsyn-
aptic potentials, an GABA<sub>A</sub> and GABA<sub>B</sub>-mediated responses in

COOGAN, T. A. AND BURKHALTER, A. Hierarchical organization of areas in rat

CZEGER, T. AND WHITE, E. L. Synapses of extrinsic and intrinsic origin made
by callosal projection neurons in mouse visual cortex. J. Comp. Neurol.

DEFEILPE, J., HENDRY, S.H.C., AND JONES, E. G. A correlative electron micro-
scopic study of basket cells and large GABAergic neurons in the monkey

DEFEILPE, J., HENDRY, S.H.C., AND JONES, E. G. Visualization of chandelier
cell axons by parvalbumin immunoreactivity in monkey cerebral cortex.

DEIZ, R. A., BILJARD, M. J., AND ZIEGELGÄNSBERGER, W. Pre- and postsyn-
aptic GABA<sub>B</sub> receptors of rat neocortical neurons differ in their pharmacolo-

DEIZ, R. A. AND PRINCE, D. A. Frequency-dependent depression of inhibition
in guinea-pig neocortex in vitro by GABA<sub>B</sub> receptor feed-back on GABA

DEUCHERS, J., WEST, D. C., AND THOMSON, A. M. Relationships between
morphology and physiology of pyramid-pyramid single axon connections in

DOMENICI, L., HARDING, G. W., AND BURKHALTER, A. Patterns of synaptic
activity in forward and feedback pathways within rat visual cortex. J. Neu-

DOUGLAS, R. J., KOCH, C., MAHOWALD, M., MARTIN, K.A.C., AND SUAREZ,
H. R. Recurrent excitation in neocortical circuits. Science 269: 981–985,
1995.

DOUGLAS, R. J. AND MARTIN, K.A.C. A functional microcircuit for cat visual

ELHANANY, E. AND WHITE, E. L. Intrinsic circuitry: synapses involving the
local axon collaterals of corticocortical projection neurons in the mouse

FOX, K., SATO, H., AND DAW, N. The effect of varying stimulus intensity on
NMDA-receptor activity in cat visual cortex. J. Neurophysiol. 64: 1413–
1428, 1990.

FUKUDA, A., MOODY, L. L., AND PRINCE, D. A. Differential ontogenesis of presyn-
aptic and postsynaptic GABA<sub>B</sub> inhibition in rat somatosensory cortex.

GABBOTT, P.L.A., MARTIN, K.A.C., AND WHITTERIDGE, D. Connections be-
tween pyramidal neurons in layer 5 of cat visual cortex (area 17). J. Comp.

GAWWILER, B. H. AND BROWN, D. A. GABA<sub>B</sub>-receptor-activated K<sup>+</sup>
current in voltage-clamped CA3 pyramidal cells in hippocampal cultures.

GENCHAR, Y. AND BURKHALTER, A. Differential subcellular localization of
forward and feedback interareal inputs to parvalbumin expressing GABA-

HIRSCH, J. A. AND GILBERT, C. D. Synaptic physiology of horizontal connec-

HIRSCH, J. A. Synaptic integration in layer IV of the ferret striate cortex.

HOWE, J. R., SUTOR, B. C., ZIEGELGÄNSBERGER, W. Baclophen reduces
postsynaptic potentials of rat cortical neurons by an action other than its

HUETTNER, J. E. AND BAUGHMAN, R. W. The pharmacology of excitatory
synapses formed by identified corticocortical neurons in primary cultures

HUPÉ, J.-M., JAMES, A. C., PAYNE, B., LOMBER, S. L., GIRARD, P., AND
BULLER, J. Cortical feedback improves discrimination between figure and

JIANG, X.-P., JOHNSON, R. R., AND BURKHALTER, A. Visualization of dendritic
morphology of cortical projection neurons by retrograde axonal tracing.

JOHNSON, R. R. AND BURKHALTER, A. Evidence for excitatory amino acid
neurotransmitters in forward and feedback corticocortical pathways within