Glutamatergic Inhibition in Sensory Neocortex

In the mammalian brain, glutamate and γ-aminobutyric acid are considered major excitatory and inhibitory neurotransmitters, respectively. However, we have found evidence that glutamate can also act as a postsynaptic inhibitory neurotransmitter in layer 4 of the neocortex. Using whole-cell recordings from layer 4 neurons in slice preparations from the mouse visual, auditory, and somatosensory cortices, we found that metabotropic glutamate receptor (mGluR) agonists (ACPD, APDC, and DCG IV) elicit a robust, long-lasting hyperpolarization that is abolished by the group II mGluR antagonist, MCCG. This response largely involves a K⁺ conductance mediated by G-protein activity and GIRK channels. Furthermore, electrical and photostimulation of the intracortical inputs to layer 4 elicits a similar hyperpolarization that is blocked by group II mGluR antagonists. This novel inhibition mediated by group II mGluRs may be an unappreciated mechanism for refining cortical receptive fields in layer 4 and may enable synaptic gain control during periods of high activity.

Keywords: auditory, cortex, layer 4, layer 6, metabotropic glutamate receptors, somatosensory, visual

Introduction

In the mammalian brain, sensory information is transmitted from peripheral receptors, such as in the retina, through the thalamus en route to primary sensory areas in the neocortex, such as the primary visual cortex (V1). Layer 4 of the neocortex is the main target of the ascending excitatory thalamocortical input (Sherman and Guillery 2002) as well as the target of convergent excitatory inputs from multiple intracortical (Stratford et al. 1996; Hirsch and Martinez 2006) and corticocortical (Rockland and Pandya 1979; Zeki and Shipp 1988) sources. For instance, neurons in layers 4 and 6 project intracortically to layer 4 (Stratford et al. 1996) and supragranular layers 2/3 project to layer 4 of higher cortical areas (Rockland and Pandya 1979). Cortical interneurons strongly inhibit layer 4 neurons to attenuate these multiple excitatory influences (Hirsch et al. 2003). Thus, cortical layer 4 represents a major site for integrating ascending thalamocortical input with information processed within the cortical network (Sherman and Guillery 2002; Hirsch and Martinez 2006). How these multiple excitatory and inhibitory influences are integrated at both the network and cellular levels to construct cortical receptive fields is a fundamentally unresolved question in the visual system (Priebe and Ferster 2005; Hirsch and Martinez 2006) as well as in other sensory modalities (Miller et al. 2002; Alonoso and Swadlow 2005).

In this regard, one canonical principle of neurotransmission at layer 4 synapses and forebrain synapses, in general, states that the excitatory responses in these forebrain pathways are largely evoked at glutamatergic synapses (Cartmell and Schoepp 2000; Nakanishi 2004), whereas the inhibitory responses are elicited mainly at γ-aminobutyric acidergic (GABAergic) synapses (Farrant and Nusser 2006) but with some cholinergic inhibition in layer 5 (Gulledge and Stuart 2005). Although generally true, this principle is confounded by metabotropic postsynaptic receptors, whose downstream effects on membrane potential can differ dramatically from those of ionotropic receptors (Collingridge and Lester 1989; Cartmell and Schoepp 2000). For example, group I metabotropic glutamate receptors (mGluR) mediate a biphasic activation in layer 5 of the prefrontal cortex (Hagenston et al. 2008). However, of particular interest is the group II mGluR, which has been surprisingly shown to have inhibitory postsynaptic effects in some brain regions (Cox and Sherman 1999; Dutar et al. 2000), although this is the exception rather than the rule. Indeed, such postsynaptic inhibitory effects at group II mGluRs have not been previously demonstrated in layer 4 of the sensory neocortex, although previous anatomical staining suggests that the receptors are indeed present (Ohishi et al. 1998).

Given the importance of layer 4 in sensory cortical processing, the importance of inhibition in forming cortical receptive fields (Priebe and Ferster 2005; Hirsch and Martinez 2006) and the putative roles that group II mGluRs may have in such varied processes as gain control (McLean and Palmer 1996) and synaptic plasticity (Renger et al. 2002), we investigated pharmacologically whether layer 4 neurons in the sensory neocortex are inhibited postsynaptically by group II mGluRs. Furthermore, we sought to identify potential forebrain pathways that could synaptically elicit an inhibitory group II mGluR response. Surprisingly, we have found that such glutamatergic inhibition can be elicited postsynaptically in layer 4 from the activation of intracortical circuitry, and these novel findings are described below.

Materials and Methods

Slice Preparation and Recording

Slices were prepared from BALB/c mice (ages 10-16 days), which were anesthetized with isoflurane and decapitated. All procedures were approved by the Institutional Animal Care and Use Committee of the University of Chicago. Whole brains were quickly submerged in cool, oxygenated, artificial cerebral spinal fluid (ACSF; in millimoles: 125 NaCl, 25 NaHCO₃, 3 KCl, 1.25 NaH₂PO₄, 1 MgCl₂, 2 CaCl₂, and 25 glucose). Brains were blocked coronally, vibratome sectioned (Campden Instruments, Lafayette, IN), then recovered in physiological ACSF for 1 h at 32 °C. The slices were then placed in a submersion-type recording chamber on a modified microscope stage and maintained at 32 °C with constant perfusion of ACSF.

Glass pipettes containing intracellular solution (135 potassium gluconate, 7 NaCl, 10 (2-hydroxyethyl)-1-piperazineethanesulfonic acid, 1.2 Na₂ATP, 0.5 guanosine triphosphate (GTP), and 2 MgCl₂ at a pH of 7.3, obtained with KOH and osmolality of 290 mOsm obtained...
with distilled water) were used for whole-cell patch recordings. Differential interference contrast (DIC) optics were used to identify cortical layers for recording and stimulation. Current or voltage clamp recordings were made using the Axoclamp 2A amplifier and pCLAMP software (Axon Instruments, Union City, CA), which were uncorrected for liquid junction potentials (~10 nV). Spiking characteristics of layer 4 neurons were determined with depolarizing current injections, and neurons were classified as regular spiking (RS) if they fired at slow adapting frequencies (~50 Hz) with small and slow afterhyperpolarizations (AHPs; 5–10 mV) (Lee and Sherman 2008). The acquired data were digitized using a Digidata 1200 board and then stored in a computer for later analysis.

**Pharmacology**

Agonist and antagonist stock solutions were prepared in distilled water and diluted to their final concentration just before use. Pharmacological agents were bath applied by injecting a constant bolus (30–60 s) into the chamber flow line, which was fed by a motorized stage pump. Based on the rate of injection and chamber perfusion, the final bath concentration was generally estimated to be one-fourth of the initial concentration.

Agonists for mGluRs (TOCRIS, Ellisville, MO) were applied at the following concentrations: ACPD (100 μM), APDC (100 μM), and DCG IV (10 μM). To block the group II mGluRs, MCCG (50 μM) was applied to the bath. To block the group I mGluRs, LY367385 (50 μM) for mGluR1 and MPEP (30 μM) for mGluR5 were added together to the bath. To block GABA receptors (GABARs), SR 95531 (20 μM) for GABAA and CGP 46381 (40 μM) for GABAB were used. To block ionotropic glutamate receptors (iGluRs) during high-frequency stimulation, DNQX (50 μM) for AMPA and MK-801 (40 μM) for N-methyl-D-aspartate (NMDA) were used. To reduce synaptic activity and block action potentials, a low-Ca2+ (0.2 mM)/high-Mg2+ (6 mM) ACSF solution and TTX (1 μM) were used.

To test the effects of intracellular calcium, BAPTA (10 mM) was added to the intracellular solution. To examine the role of adenylate cyclase, SQ 22536 (400 μM), an inhibitor of adenylate cyclase activity, was added to the bath solution. In order to block G protein-coupled activity, the nonhydrolyzable GTP analog, GDPβS (10 mM) was added to the intracellular solution. The involvement of GIRK channels was tested with the inclusion of QX-314 (10 mM) to the intracellular solution. Following concentrations: ACPD (100 μM), APDC (100 μM), and DCG IV (10 μM). To block the group II mGluRs, MCCG (50 μM) was applied to the bath. To block the group I mGluRs, LY367385 (50 μM) for mGluR1 and MPEP (30 μM) for mGluR5 were added together to the bath. To block GABA receptors (GABARs), SR 95531 (20 μM) for GABAA and CGP 46381 (40 μM) for GABAB were used. To block ionotropic glutamate receptors (iGluRs) during high-frequency stimulation, DNQX (50 μM) for AMPA and MK-801 (40 μM) for N-methyl-D-aspartate (NMDA) were used. To reduce synaptic activity and block action potentials, a low-Ca2+ (0.2 mM)/high-Mg2+ (6 mM) ACSF solution and TTX (1 μM) were used.

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Hyperpolarizing current pulses (10 μA, 200 ms, 0 Hz) were applied to the neurons to test the effect of agonists on neuronal input resistance. Changes in membrane conductance in response to agonists were quantified by applying slow ramped voltage commands (resistance. Changes in membrane conductance in response to agonists (Andrade 1991; Dutar et al. 2000).

**Results**

To investigate initially whether postsynaptic metabotropic glutamatergic receptors are present in the sensory neocortex, we recorded from neurons in layer 4 (Regular spiking or RS: n = 33) of the primary visual (V1: n = 7), auditory (A1: n = 8), and somatosensory (S1: n = 18) cortices of the mouse. These RS cells had an average resting potential of ~61.2 ± 4.6 mV, input resistance of 263.8 ± 118.3 MΩ, and fired at slow-adapting frequencies (~22.1 ± 6.2 Hz) with small AHPs (5.9 ± 1.1 mV).

Current clamp recordings of layer 4 neurons in each area revealed a robust hyperpolarization (12.1 ± 3.5 mV) in response to bath application of the general mGluR agonist, ACPD (100 μM), as demonstrated for a neuron in S1 (Fig. 1A). This response was not due to an action-potential-elicited synaptic release of other inhibitory neurotransmitters, such as GABA, because the response could be elicited in the presence of TTX (1 μM), low-Ca2+ (0.2 mM)/high-Mg2+ (6 mM) ACSF, and GABA receptor antagonists (SR 95531: 20 μM; CGP 46381: 40 μM) (see Materials and Methods) with no significant decrement in the amplitude of the observed response (11.5 ± 2.7 mV, P > 0.05, t-test) (Fig. 1B). In contrast, this effect was abolished in the presence of the group II mGluR antagonist MCCG (50 μM) (Fig. 1C), which often resulted in a slightly depolarizing response (0.33 ± 3.2 mV; Fig. 1D).

Because the above results suggest the possibility that the hyperpolarizing actions of ACPD are due to the direct activation of group II mGluRs on layer 4 neurons, we tested this specifically by further recording from layer 4 neurons followed by bath application of group II specific agonists, APDC (n = 10) (100 μM) (Fig. 1D) and DCG IV (n = 6) (10 μM) (Fig. 1F). As above, this was performed in the presence of TTX, low-Ca2+/high-Mg2+ ACSF, and GABA receptor antagonists to eliminate any indirect synaptic effects. In the presence of both agonists, layer 4 neurons robustly hyperpolarized (APDC: 8.6 ± 1.4 mV; DCG IV: 10.3 ± 4.6 mV) with an amplitude that did not statistically differ from each other or ACPD (P > 0.05, analysis of variance [ANOVA]; Fig. 1H). In addition, the group II mGluR-specific antagonist, MCCG, significantly reduced the response to APDC (n = 3; 1.8 ± 0.9 mV; P < 0.05, t-test) (Fig. 1G). Furthermore, we tested the response to APDC from neurons in lower layer 6 of S1 (n = 3) but did not observe a hyperpolarizing response. Thus, direct activation of group II mGluRs hyperpolarizes neurons in layer 4 of the sensory neocortex.

Because activation of group II mGluRs has previously been shown to result in the opening of downstream K+ channels in other brain regions (Cox and Sherman 1999; Dutar et al. 2000), we investigated whether similar mechanisms underlie this response in layer 4 cortical neurons. Hyperpolarizing current pulses were injected to measure changes in input resistance during the agonist-mediated hyperpolarization (see Materials and Methods; Fig. 2A). During the response to group II agonists,
input resistance decreased (Fig. 2A(ii)) relative to predrug (Fig. 2A(i)) and postdrug (Fig. 2A(iii)) conditions. This was observed for each agonist in all tested areas, thus indicating that group II mGluR activation results in the opening of ion channels.

To better assess the ionic basis for the response, ramped voltage commands (-55 to -115 mV) were used to examine changes in input conductance during voltage clamp recordings (Fig. 2B). In all experiments (n = 8), during bath application of agonists in the presence of TTX, low-Ca²⁺/high-Mg²⁺ ACSF, and GABA receptor antagonists, the slope of the current response to the voltage commands increased (Fig. 2B(ii)), indicating an increase in the input conductance. The agonist-altered current was linear over the range of voltages tested, and the reversal potential (-83.4 ± 4.8 mV; n = 8) was near but somewhat depolarized with respect to the predicted Nernst reversal potential for K⁺ (-97 mV) (Fig. 2B(iii)), which may in part be accounted for by the uncorrected junction potential (-10 mV). This pattern was found to be the case in all areas and for each agonist and is consistent with group II mGluR activation resulting in the downstream opening of linear K⁺ channels. Furthermore, when the concentration of K⁺ in the extracellular solution was increased (6 mM), the reversal potential (-68.8 ± 2.7 mV; n = 3), uncorrected for junction potential (-10 mV), shifted to a more depolarized level as predicted by the Nernst reversal potential for K⁺ (-80 mV). We conclude that K⁺ is likely the major ion involved in the observed hyperpolarizing response, but it is also plausible that other ionic conductances, such as Iₚ, may also be involved.

To further characterize the mechanisms underlying this hyperpolarizing conductance, BAPTA (10 mM), SQ 22536 (400 μM), GDPβS (10 mM), or QX-314 (10 mM) were added to the intracellular or extracellular solutions, respectively. Addition of the calcium chelator, BAPTA, to the intracellular solution (n = 7) resulted in no significant decrease (P > 0.05, t-test) of the hyperpolarizing response to ACPD (9.5 ± 2.8 mV) (Fig. 3A,E). Similarly, bath application of the adenylyl cyclase inhibitor, SQ 22536, in the extracellular solution (n = 5) resulted in no significant decrease (P > 0.05, t-test) in the hyperpolarizing response to ACPD (10.6 ± 2.5 mV) (Fig. 3B,E). However, inclusion of either a blocker of G-protein activity (GDPβS) (n = 7) or a GIRK channel blocker (QX-314) (Andrade 1991; Dutar et al. 2000) (n = 6) resulted in a significant reduction (P < 0.05, t-test) of the hyperpolarizing response to ACPD (GDPβS: 2.2 ± 0.8 mV; QX-314: 0.97 ± 0.41 mV) (Fig. 3C–E).

Thus, the group II mGluR-mediated hyperpolarization is dependent on G-protein activity and GIRK channels but is independent of intracellular calcium and adenylyl cyclase inhibition.

To search for potential synaptic sources that activate group II mGluRs in layer 4, we examined the intracortical pathways in each area (V1: n = 3; A1: n = 5; and S1: n = 9) because previous work in our lab has demonstrated that the thalamocortical projections to layer 4 in both the auditory and somatosensory pathways do not elicit a metabotropic glutamate response (Lee and Sherman 2008). Photostimulation with caged glutamate was used to identify the intracortical (Fig. 4A) and thalamic regions that projected to the recorded layer 4 neurons (Papageorgiou et al. 1999; Shepherd et al. 2003; Lam and Sherman 2007). For the intracortical input, a concentric bipolar stimulating electrode was placed in the cortical regions (in subjacent layer 6 and

**Figure 1.** Direct activation of group II mGluRs in layer 4 neurons of sensory neocortex results in a large hyperpolarizing response. (A) Bath application of the general mGluR agonist ACPD under control conditions in normal ACSF hyperpolarizes a layer 4 neuron in S1. (D) ACPD-mediated hyperpolarization persists under conditions where GABAergic and presynaptic activity are blocked with TTX and GABAR antagonists in a low-Ca²⁺/high-Mg²⁺ ACSF (cocktail). (C) However, application of the group II mGluR antagonist, MCCG, abolishes the hyperpolarization. (D) The average amplitude of the hyperpolarization is not affected by the cocktail but is significantly reduced by MCCG (P < 0.05, t-test). (E-F) Bath application of the specific group II mGluR agonists, ACPD (E) and DCG IV (F), with TTX and GABAR antagonists in a low-Ca²⁺/high-Mg²⁺ ACSF, result in similar hyperpolarizing responses. (G) The ACPD-induced hyperpolarization is largely eliminated in the presence of MCCG. (H) Group II mGluR-specific agonists result in similar hyperpolarizations compared with ACPD.
nearby layer 4) that elicited robust responses to photostimulation. To isolate the group II mGluR-mediated response, antagonists to ionotropic glutamate receptors (DNQX: 50 μM; MK-801: 40 μM), group I mGluRs (LY367385: 50 μM; MPEP: 30 μM), and GABARs (SR 95531: 20 μM; CGP 46381: 40 μM) were applied. Subsequent electrical stimulation with a high-frequency tetanus (125 Hz for 800 ms; see Materials and Methods) (Reichova and Sherman 2004) resulted in a prolonged and robust (3.9 ± 1.2 mV; n = 17) hyperpolarizing response (Fig. 4C), which reversed near −80 mV (Fig. 7A). This hyperpolarizing response was largely abolished (0.8 ± 0.5 mV; n = 17) following the subsequent addition of the group II antagonist, MCCG (50 μM), and was recovered after a wash of the antagonist (2.61 ± 0.9 mV; n = 17) (Fig. 4C,D).

Because electrical stimulation may activate fibers of passage, we further utilized photostimulation with caged glutamate to selectively elicit and identify the intracortical sources that evoke group II mGluR responses. Stimulation via photorelease of glutamate only results in activation at the cell body or proximal dendrites and thus avoids the fiber-of-passage problem (Shepherd et al. 2003). In each area (V1: n = 3; A1: n = 3; S1: n = 5), antagonists to NMDA glutamate receptors (MK-801: 40 μM), group I mGluRs (LY367385: 50 μM; MPEP: 30 μM), and GABARs (SR 95531: 20 μM; CGP 46381: 40 μM) were applied to isolate the group II mGluR response. Photostimulation in subjacent layer 6 and nearby layer 4 resulted in a large depolarizing AMPA-mediated response that was followed by a prolonged hyperpolarization (4.2 ± 0.8 mV; n = 11) (Fig. 5C), which reversed near −80 mV (Fig. 7D). Photostimulation outside of layers 4 and 6 failed to elicit such a response, demonstrating the specificity of the input. This hyperpolarization was largely eliminated (0.7 ± 0.6 mV; n = 11)
following the application of MCCG (50 μM), a group II antagonist, but was recovered after a wash of the antagonist (3.1 ± 0.7 mV; n = 11). Furthermore, the hyperpolarization was not attributed to direct activation of the recorded cell because switching to low-Ca²⁺/high-Mg²⁺ ACSF abolished the response. Interestingly, testing direct photostimulation of the recorded cell in layer 4 neurons showed that photostimulation of the intracortical pathways, in the presence of iGluR, GABAR, and group I mGluR antagonists, resulted in a large hyperpolarizing response (top trace) that was abolished with the addition of antagonist to group II mGluRs (MCCG) (middle trace) and recovered following a wash (bottom trace). The group II mGluR antagonist, MCCG, decreased the average amplitude of the response but is recovered following a wash.
neuron in this condition also failed to elicit a hyperpolarizing response, even when the concentration of caged glutamate was doubled, suggesting that the local release of glutamate by photostimulation is insufficient to directly activate group II mGluRs.

In order to test whether the photostimulation-induced hyperpolarization was occluded by the electrically induced hyperpolarization, antagonists to NMDA glutamate receptors (MK-801: 40 μM), group I mGluRs (LY367385: 50 μM; MPEP: 30 μM), and GABARs (SR 95531: 20 μM; CGP 46381: 40 μM) were added to the bath in order to isolate the group II mGluR response. Both electrical stimulation and photostimulation of the intracortical pathways from subjacent layer 6 or nearby layer 4 resulted in a pronounced hyperpolarizing response (*n = 5; electrical: 3.7 ± 0.7 mV; photostimulation: 4.0 ± 1.5 mV*) (Fig. 6A). The hyperpolarizing response was not significantly changed following simultaneous electrical stimulation and photostimulation (3.9 ± 1.4 mV; *P > 0.05; ANOVA*) (Fig. 6A,B). Because simultaneous stimulation was found to be nonadditive, this suggests that electrical stimulation and photostimulation activate the same afferent pathways and receptor populations.

The postsynaptic inhibition mediated by group II mGluRs is general to all sensory cortical areas investigated (V1, A1, and S1) (Fig. 8). Bath application of mGluR agonists hyperpolarized neurons in each primary sensory cortical area (V1: *n = 7; A1: *n = 8; S1: *n = 18*) (Fig. 8) with no significant difference observed in the amplitude of the hyperpolarizing response among cortical areas (*p > 0.05, ANOVA*; Fig. 8A-C, left panels). In addition, high-frequency electrical stimulation (V1: *n = 3; A1: *n = 5; S1: *n = 9*) and photostimulation (V1: *n = 3; A1: *n = 3; S1: *n = 5*) of the intracortical pathways, in the presence of antagonists to iGluRs, group I mGluRs, and GABARs, resulted in similar hyperpolarizing responses for all areas tested (*p > 0.05, ANOVA*; Fig. 8A-C, right panels).

Finally, to confirm the distribution of group II mGluRs in sensory cortex, we immunostained coronal sections in the mouse with a group II mGluR antibody (Fig. 9). We found significant labeling in layer 4 of all sensory cortical areas, labeling that was not limited to the primary regions (Fig. 9), suggesting that inhibition mediated by group II mGluR is general to the neocortex. This staining pattern appears to coincide with the known distribution of GIRK channels in the neocortex. This staining pattern was also seen in the neuropil (Fig. 9B,DF). A more diffuse distribution of labeling was also seen in the neuropil (Fig. 9B,DF). Layer 5 was also strongly labeled in all cortical areas with lower layer 5b particularly intense (Fig. 9). Layers 2–3 were lightly labeled, whereas layers 1 and 6 were often devoid of labeling (Fig. 9). Other regions of interest that stained darkly included the hippocampus, striatum, and thalamic reticular nucleus (Fig. 9A,CE). This labeling pattern raises the possibility of inhibitory effects of glutamate outside of layer 4 as well.

Discussion

Overall, our results suggest that glutamate inhibits layer 4 neurons in the sensory neocortex via the postsynaptic activation of group II mGluRs, which hyperpolarize the neuron through the downstream opening of a linear K+ conductance. This hyperpolarization is independent of intracellular calcium and inhibition of adenylate cyclase but dependent on G-protein activity and GIRK channels. This glutamatergic inhibition is observed in all primary sensory cortical areas tested, and the presence of the receptor in other cortical areas suggests that it is a general and ubiquitous feature of the neocortex. In addition, we have identified intracortical sources in subjacent layer 6 and nearby layer 4 as potential synaptic sources of this inhibitory glutamatergic input, in addition to its normal excitatory effects (Stratford et al. 1996). These results are surprising given that the normal response to glutamate in the central nervous system is excitatory (Watkins and Evans 1981; Nakanishi 2004), and therefore, these data expand the physiological range and potential roles of glutamatergic neurotransmission in the neocortex.
Furthermore, postsynaptic glutamatergic inhibition via group II mGluRs, although observed in a few other structures (Cox and Sherman 1999; Dutar et al. 2000), is not typical, even at synapses where the receptor is present. Many studies suggest that group II mGluRs are primarily located on the presynaptic terminal (Cartmell and Schoepp 2000; Mateo and Porter 2007), where it reduces neurotransmitter release during periods of high activity. As such, the group II mGluRs usually act as presynaptic autoreceptors (Cartmell and Schoepp 2000) and have been implicated in functions ranging from gain control (McLean and Palmer 1996) to synaptic plasticity (Renger et al. 2002) to early development of the synapse (Beaver et al. 1999; Daw 1999). Interestingly, all of these putative functions ascribed to presynaptic receptors might just...
as easily be served by postsynaptically localized receptors, and thus, it remains an open and intriguing question whether the postsynaptic group II mGluRs on layer 4 neurons are involved in any of these processes.

If so, then our results suggest that the intracortical projection from subjacent layer 6 and nearby layer 4 might be directly involved in some of these functions related to group II mGluRs. This is in contrast to the thalamocortical pathway, which does not postsynaptically activate group II mGluRs (Lee and Sherman 2008), although some evidence suggests that they are activated presynaptically there (Mateo and Porter 2007). Compared with ionotropic GABA\(\text{Rs}\) (Hirsch et al. 2003; Farrant and Nusser 2006), the group II mGluR-mediated inhibition in the intracortical pathways has a slower onset, is more prolonged, lasting several hundred milliseconds, and is elicited during periods of robust activity. This strongly indicates that these intracortical excitatory inputs may become attenuated during periods of high activity as receptors to group II mGluRs are recruited to inhibit the excitatory response. As suggested above, this may be a useful mechanism for synaptic gain control to expand the dynamic range of intracortical synapses. Additionally, should this inhibition overwhelm excitation (Hirsch and Martinez 2006), it might be manifested in the construction of layer 4 receptive field properties, in particular the temporally delayed inhibition seen in the spatiotemporal structure of some cortical receptive fields (Miller et al. 2002;

Figure 9. Group II mGluRs are found in layer 4 of the neocortex in all areas studied as well as in other surrounding cortical areas. (A, B) Primary visual cortex (V1). (C, D) Primary auditory cortex (A1). (E, F) Primary somatosensory cortex (S1). Labeling is primarily found on cell bodies and proximal dendrites (B, D, F), indicative of a postsynaptic location of receptors. Arabic numerals indicate layers.
Shapley et al. 2003; Priebe and Ferster 2005). If so, this would represent a novel and heretofore unappreciated inhibitory mechanism for refining cortical receptive fields in the visual (Priebe and Ferster 2005; Hirsch and Martinez 2006), auditory (Miller et al. 2002), and somatosensory (Alonsoso and Swadlow 2005) systems.

Although the functional importance of group II mGluRs in layer 4 remains to be fully elucidated, our data clearly demonstrate that the distinction between glutamatergic excitation and GABAergic inhibition in the neocortex is not absolute. The perspective provided by our finding of glutamatergic inhibition in layer 4 confounds, refines, and contributes to our ever-evolving understanding of the complexity of sensory neocortical processing.

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