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Control of Dendritic Outputs of Inhibitory Interneurons in the Lateral Geniculate Nucleus

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Summary

The thalamic relay to neocortex is dynamically gated. The inhibitory interneuron, which we have studied in the lateral geniculate nucleus, is important to this process. In addition to axonal outputs, these cells have dendritic terminals that are both presynaptic and postsynaptic. Even with action potentials blocked, activation of ionotropic and metabotropic glutamate receptors on these terminals increases their output, whereas activation of metabotropic (M_2 muscarinic) but not nicotinic cholinergic receptors decreases their output. These actions can strongly affect retinogeniculate transmission.

Introduction

The thalamic relay nuclei are strategically situated to convey information from the periphery to neocortex. A traditional and stubborn view is that these nuclei serve as a passive, trivial relay to the neocortex. However, there is growing evidence indicating that the thalamus plays more of a dynamic and consequential role in the information transfer to neocortex (for review, see Sherman and Guillery, 1996). This dynamic role of the thalamus is strongly supported by anatomical evidence that the primary information-bearing input (i.e., the driver input; Sherman and Guillery, 1998), which are retinal axons for the lateral geniculate nucleus, contributes only 5%–10% of the total synapses contacting relay neurons (Van Horn et al., 2000). That is, the predominant number of synapses onto relay cells are nonretinal and originate from other sources such as pyramidal cells in layer 6 of visual cortex, various brainstem afferents, and local inhibitory cells. These nonretinal afferents are called modulators (Sherman and Guillery, 1998) because they appear mainly to modulate retinogeniculate transmission. In this study, we focus on the function of GABAergic thalamic interneurons within the lateral geniculate nucleus; these provide a major source of inhibitory innervation onto many relay neurons. While it is commonly assumed that the inhibitory influence of the interneurons would certainly modify relay cell output and thereby the relay of information through the thalamus, the more specific inhibitory role of these interneurons is not well understood.

These interneurons are also of interest because unlike conventional neurons, they give rise to two distinct types of inhibitory output: axonal and dendritic. The axonal terminals, also known as F1 terminals, serve only a presynaptic role and form conventional axodendritic/axosomatic inhibitory synapses onto relay neurons. The second output is via GABA-containing presynaptic dendrites that form dendrodendritic synapses onto relay cell dendrites (Guillery, 1969; Ralston, 1971; Famiglietti and Peters, 1972; Hamos et al., 1985; Montero, 1986). These are known as F2 terminals, and unlike F1 terminals, they are both pre- and postsynaptic. These F2 terminals also comprise a part of triadic synaptic arrangements that are found throughout the thalamus in complex synaptic zones known as glomeruli (for review, see Sherman and Guillery, 1996). Two types of triad have been described (Figure 1A): (1) a single retinal terminal contacts an F2 terminal and relay cell dendrite, and the F2 terminal contacts the same relay cell dendrite, or (2) a cholinergic axon terminal emanating from the brainstem parabrachial region contacts an F2 terminal, which contacts a relay cell dendrite, and another nearby terminal of the same parabrachial axon contacts the same relay cell dendrite. Furthermore, studies of the cat's lateral geniculate nucleus indicate that triadic circuitry and glomeruli are associated with relay X cells, whereas the relay Y cells receive simpler inputs mostly devoid of triads and glomeruli (Friedlander et al., 1981; Wilson et al., 1984). While these triadic arrangements have been anatomically identified, their functional significance remains elusive.

Recent studies have provided evidence that both metabotropic glutamate receptors (mGluRs) and muscarinic cholinergic receptors, which are also metabotropic, are localized on F2 terminals (Godwin et al., 1996; Carden and Bickford, 1999; Plummer et al., 1999). The relevant glutamatergic input arises from axons of retinal ganglion cells, and the cholinergic innervation arises from parabrachial axons (Erisir et al., 1997). We have recently made use of this anatomical information to show pharmacologically that activation of mGluRs on the F2 terminals increases their inhibitory output (Cox et al., 1998). In our present study, we have extended our initial findings to characterize whether a specific mGluR subtype was responsible for activation of F2 receptors and whether this action is limited to mGluRs or may also be mediated by ionotropic glutamate receptors (iGluRs). Furthermore, we have investigated the role of the cholinergic innervation of F2 terminals by axons emanating from the parabrachial region.

Results

Our results derive from whole-cell recordings from a total of 136 thalamic relay neurons, including 88 from the cat's lateral geniculate nucleus and 48 from the rat's thalamus (23 from the lateral geniculate nucleus and 25 from the ventrobasal complex). All recordings described here were made in voltage clamp mode with the membrane voltage held at depolarized holding potentials to maximize spontaneous inhibitory postsynaptic currents (sIPSCs). These outward currents were completely attenuated by the selective GABA antagonists

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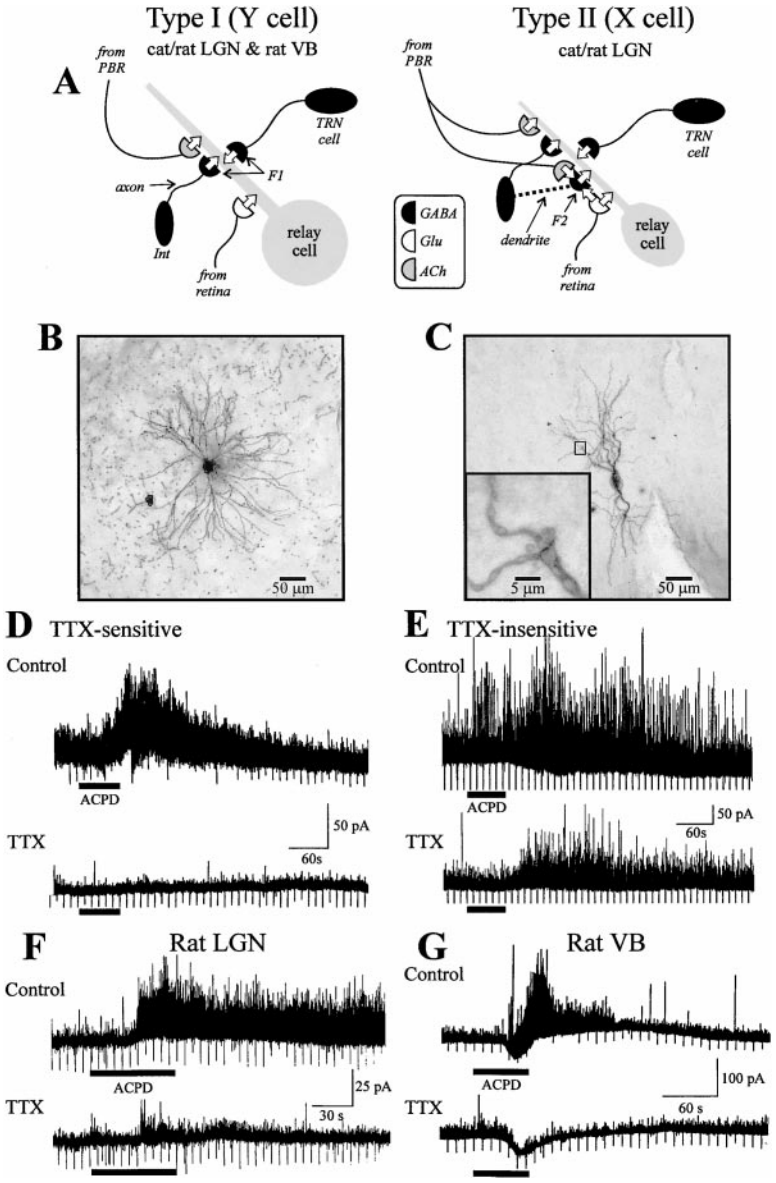

Figure 1. Activation of mGluRs Produces TTX-Insensitive Increase of sIPSC Activity in Geniculate Neurons

(A) Schematic of cholinergic and glutamatergic input onto Type I (Y cell) and Type II (X cell) neurons. Both cell types are found in the cat's and rat's lateral geniculate nucleus, but only Type I cells are found in the rat's ventrobasal complex. Both cell types receive GABAergic input from axonal terminals (F1) of cells of the thalamic reticular nucleus (TRN) and geniculate interneurons (*Int*). For Type I cells, retinal inputs form simple monosynaptic excitatory contacts onto dendrites; in the rat's ventrobasal complex, medial lemniscal input replaces retinal input. Also, cholinergic terminals from the parabrachial region contact the dendrite. For Type II cells, retinal terminals form synaptic triads in which a retinal terminal contacts both the relay cell and a F2 terminal, and the F2 terminal contacts the same relay cell. There is also cholinergic input from the parabrachial region in which a single axon has terminals that contact the dendrite and F2 terminals, forming a sort of functional triad.

(B) Photomicrograph of a biocytin-filled Type I neuron. This cell shows radial dendritic morphology that is consistent with Type I morphology. In this cell, ACPD produced a TTX-sensitive response to ACPD, similar to that shown in (D).

(C) Photomicrograph of a biocytin-filled Type II neuron. This cell has typical bipolar dendritic morphology consistent with Type II neurons, and swellings along dendrites and near branch points consistent with Type II neurons (inset). In this cell, ACPD produced a TTS-insensitive increase in sIPSC activity similar to that shown in (E).

(D and E) TTX differentiates two distinct types of mGluR-mediated facilitation of sIPSCs in the cat's geniculate neurons.

(D) TTX-sensitive alteration of sIPSC activity by ACPD. ACPD (125 μM) produces robust increase in sIPSC frequency (top trace). In TTX (1 μM), the baseline activity is reduced and ACPD no longer alters sIPSC activity. The downward deflections in this and Figures 2–8 are membrane responses to voltage steps used to monitor access resistance but have been truncated in the figures.

(E) TTX-insensitive alteration in sIPSC activity by ACPD. In a different neuron, ACPD (125 μM) evokes a large increase in sIPSC activity. This agonist induced increase in sIPSCs persists in TTX (1 μM), although the overall effect is partially attenuated.

(F) Recording from rat geniculate relay neuron. ACPD (125 μM) produces robust increase in sIPSC amplitude and frequency similar to that observed in the cat's relay neurons. In TTX (1 μM), the ACPD alteration of sIPSCs is only partially attenuated.

(G) Recording from rat ventrobasal relay neuron. In this cell, ACPD produces a robust but short-lived increase in sIPSCs. In TTX (1 μM), ACPD no longer alters the sIPSC activity.

bicuculline methiodide (BMI; 30 μM) or 2-(3-carboxypropyl)-3-amino-6-(4-methoxyphenyl)pyridazinium bromide (S-95331; 10–20 μM), indicating that these events were IPSCs mediated by $GABA_A$ receptors (see below).

Figure 1A schematically illustrates the known circuits for providing GABAergic synaptic inputs to thalamic relay cells. These synapses can arise from two types of terminals: one is the F1 terminal, which is a conventional, strictly presynaptic terminal that emanates from *axons* of either local inhibitory interneurons or thalamic reticular cells; the other is the F2 terminal, which serves both a presynaptic and postsynaptic role, and derives from peripheral *dendrites* of local interneurons. There are two patterns of innervation by these terminal types that relate to two distinct relay cell classes (Figure 1A). One, which we refer here to as Type I, receives GABAergic innervation only or nearly so via F1 terminals. In the lateral geniculate nucleus of the cat, this is characteristic of relay Y cells. The other, Type II, receives GABAergic innervation from the same pattern of F1 terminals, but in addition receives inputs from F2 terminals. This is the pattern characteristic of relay X cells in the cat's lateral

geniculate nucleus. While F1 terminals form simple axodendritic synapses onto relay cells, the F2 terminals participate in more complex triadic arrangements, and these again have been best characterized in the cat's lateral geniculate nucleus (Guillery, 1969; Ralston, 1971; Famiglietti and Peters, 1972; Hamos et al., 1985; Montero, 1986). Here, a particular presynaptic element, usually from either a glutamatergic retinal axon or a cholinergic axon from the parabrachial region of the brainstem, synapses onto both the F2 terminal and a relay cell dendrite, while the F2 terminal contacts the same relay cell dendrite (Figure 1A). Our chief goal in this study was to characterize the nature of the glutamatergic and cholinergic inputs onto the F2 terminals.

The morphology of X (Type I) and Y (Type II) geniculate cells have been described for the cat (Friedlander et al., 1981), and we observed equivalent morphological features in geniculate cells we labeled with biocytin. Figure 1B shows the Type I morphology, suggesting that this was a Y cell, and Figure 1C shows the Type II morphology, suggesting that this was an X cell.

Glutamatergic Influence on F2 Terminals

Metabotropic Glutamate Receptors

Effects of the General Agonist, ACPD. Our initial experiments confirmed and extended our previous finding that activation of metabotropic glutamate receptors by the general agonist ACPD increases the output of inhibitory, dendritic F2 terminals (Cox et al., 1998). This was evident in increases of both frequency and amplitude of sIPSCs. In most cases, both effects (frequency and amplitude) were clearly detected simply by inspecting the traces as illustrated previously (Figure 1 of Cox et al., 1998). In the present study, we quantitatively measured frequency and amplitude of sIPSCs when we applied various receptor antagonists or applied low Ca^{2+}/high Mg^{2+} conditions (see below). It is worth noting that a change in frequency of sIPSCs alone strongly implicates a presynaptic site of action, but a change in amplitude could occur pre- or postsynaptically.

In 59 of 64 (92%) relay cells recorded in the cat's lateral geniculate nucleus, ACPD (125–250 μM) produced a robust, long-lasting increase in both frequency and amplitude of sIPSCs (Figures 1D and 1E, Control). There are four obvious possible sources for this increase (Figure 1A): (1) ACPD could excite cells of the thalamic reticular nucleus, and elevated activity in reticulothalamic axons would increase sIPSCs via the axonal F1 terminals; (2) similarly, ACPD could act by exciting interneurons, thereby increasing activity in their axons and F1 outputs onto the relay cell; (3) ACPD could directly stimulate F1 terminals to increase their output onto the relay cell; and (4) ACPD could similarly act directly on the F2 terminals. To explore the first two possibilities, tetrodotoxin (TTX, 1 μM) was bath applied to attenuate action potential propagation down the axons of reticular cells and interneurons. In 24 of the 59 (41%) neurons, TTX completely abolished the ACPD-mediated increase in sIPSC activity that was observed in control conditions (Figure 1D: TTX-sensitive). For these cells, we conclude that their only source of GABAergic input was via axonal activation of F1 terminals, and furthermore, there was no direct effect of ACPD on these F1 terminals. Since earlier findings indicate that ACPD has no detectable effect on the firing of interneurons (Pape and McCormick, 1995; Cox et al., 1998), we conclude that the ACPD-mediated increase in sIPSCS seen in these cells before TTX application was carried via reticulothalamic axons.

More interesting was the effect on the remaining 35 (59%) relay cells in the presence of TTX. In these neurons, the ACPD-mediated increase in sIPSC activity persisted in TTX despite some attenuation (Figure 1E; TTX-insensitive). The partial attenuation suggests that a component of the response is similar to that described above (TTX-sensitive). More important to the present study is the persistence of a robust TTX-insensitive increase in sIPSC activity by ACPD. We have previously shown that this TTX-insensitive effect of ACPD is blocked by BMI and is Ca^{2+} dependent, both of which indicate that it involves synaptic release of GABA (Figure 2 of Cox et al., 1998). Since the TTX blocks any effects due to changes in axonal outputs of interneurons or reticular cells, the TTX-insensitive increase in sIPSCs in response to ACPD could result from a direct, probably depolarizing, action on F1 and/or F2 terminals.

As noted above, ACPD has no discernable direct effect on F1 terminals in the TTX-sensitive cells, and thus, the likely route for ACPD action in the TTX-insensitive cells is the F2 terminal. To explore this further, we took advantage of the singular distribution of GABAergic interneurons in the rat's thalamus. Although the rat lateral geniculate nucleus includes many interneurons, the ventrobasal complex effectively lacks interneurons and, thus, F2 terminals (Ottersen and Storm-Mathisen, 1984; Arcelli et al., 1997). Thus, GABAergic innervation of rat ventrobasal relay cells follows the Type I pattern shown in Figure 1A. In order to verify that the TTX-insensitive action of ACPD was not restricted to the cat's lateral geniculate nucleus, we initially tested ACPD on rat geniculate relay neurons. As in the cat, ACPD produced a robust increase in the frequency and amplitude of sIPSCs in 21 of 23 (91%) cells tested (Figure 1F). In 12 of the 21 (57%) cells showing an ACPD effect, we further tested the effects of adding TTX and found that the ACPD effect was completely attenuated in 4 (33%; a TTX-sensitive effect; data not shown) but was only partially attenuated in the remaining 8 (67%) cells (Figure 1F). Similarly, in rat ventrobasal relay cells, ACPD here produced a robust increase in sIPSC activity in 14 of 23 (61%) relay cells (Figure 1G). However, in 13 of the 14 cells showing this increase, we further tested for effects of TTX and found, in contrast to rat geniculate neurons, that the ACPD-mediated increase in sIPSCs was completely attenuated by TTX in all 13 cells (Figure 1G). This complete TTX sensitivity of the ACPD effect suggests that the increased sIPSC activity in VB neurons during control conditions results solely from depolarization of thalamic reticular neurons. More importantly, these results suggest that ACPD does not have any detectable direct action on F1 terminals, and the TTX-insensitive increase in sIPSCs by ACPD recorded in geniculate relay cells is due to an action at F2 terminals.

Morphological Correlates. Our results indicate two distinct populations of relay cells that can be distinguished by the TTX sensitivity of the ACPD-mediated alteration in sIPSC activity. As illustrated in Figure 1A,

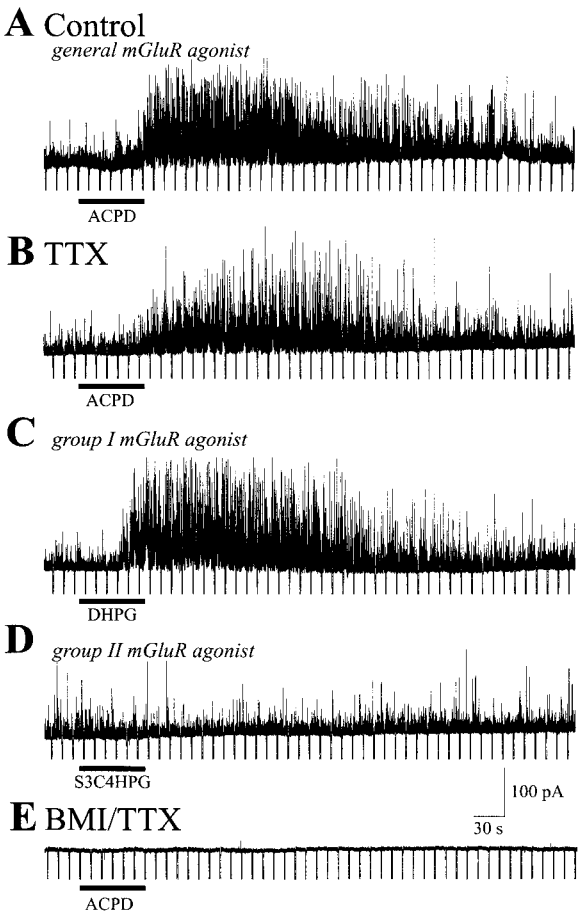

Figure 2. The Increase in sIPSC Activity Is Mediated by Group I mGluRs in a Geniculate Cell from a Cat

(A) In control conditions, ACPD (125 μM) produces a robust increase in sIPSC activity.

(B) In TTX (1 μM), the ACPD-mediated increase in sIPSC activity persists.

(C) The selective Group I mGluR agonist DHPG (250 μM) also produces a robust increase in sIPSC activity.

(D) In contrast, the selective Group II agonist S3-C4HPG (500 μM) produces no apparent change in sIPSC activity.

(E) Following application of BMI (30 μM), the spontaneous baseline activity is attenuated and ACPD no longer produces any further change in the baseline.

the F2 innervation only occurs on Type II and not Type I neurons (for review, see Sherman and Guillery, 1996). From our recordings, we have recovered 13 neurons with adequate labeling of the dendritic morphology to permit identification of neuronal cell type (Figures 1B and 1C). The 13 cells of the present study were analyzed anatomically in a "blind" manner—meaning that the anatomical analysis was done with no knowledge of the physiological data from the same cells. In every case, the morphological identification matched the physiological data. That is, 11 of 13 cells displayed Type II morphology (Figure 1C), and in each of these neurons, there was a TTX-insensitive action of mGluR agonists. In the remaining two neurons with Type I morphology (Figure 1B), the effect of mGluR agonists on sIPSC activity was entirely TTX sensitive.

Participation of Group I versus Group II Metabotropic Glutamate Receptors. Three major groups of metabotropic glutamate receptor have been identified based on pharmacology, structural homology, and signal transduction mechanisms (Nakanishi, 1992; Conn and Pin, 1997). Two of these—Group I and Group II metabotropic glutamate receptors—have been localized within the cat's lateral geniculate nucleus and adjacent thalamic reticular nucleus (Van Horn et al., 1995; Godwin et al., 1996). Group I receptors are found at postsynaptic dendritic sites on relay cells and neurons of the thalamic reticular nucleus and on F2 terminals in the geniculate neuropil. Group II receptors are primarily localized within the thalamic reticular nucleus, staining somata and dendrites, with little staining within the lateral geniculate nucleus. This suggests that the TTX-insensitive increase in sIPSCs mediated by ACPD involves Group I and not Group II metabotropic glutamate receptors, and we sought to test this hypothesis.

Figure 2 illustrates responses of a relay cell from the cat's lateral geniculate nucleus with a TTX-insensitive increase in sIPSC activity in response to ACPD (Figures 2A and 2B). The specific Group I agonist (RS)-3,5-dihydroxyphenylglycine (DHPG; 250 μM) was tested on a total of 12 geniculate neurons from the cat (6 control and TTX; 5 TTX only; 1 control only). As with ACPD, DHPG produced a robust increase in sIPSC activity in all 12 cells tested in control conditions. The DHPG-mediated increase in sIPSC activity also persisted in TTX in 8 of the 11 (73%) neurons so tested (Figures 2C and 3B). In 6 cells in which DHPG was tested in both control and TTX conditions, the DHPG increase persisted in 3 and was attenuated in the remaining 3 cells. These results indicate that the DHPG-mediated effects were similar to that observed with the general mGluR agonist ACPD, producing TTX-sensitive increases in sIPSCs in some cells and TTX-insensitive increases in the others.

In contrast, the specific Group II agonist S-3-carboxy-4-hydroxyphenylglycine (S3C4HPG; 500 μM) produced no apparent change in sIPSC activity (Figure 2D). That is, the Group II agonist did not alter sIPSC activity regardless of the presence or absence of TTX in any of the 4 geniculate cells tested in the cat. The application of the GABA antagonist BMI (30 μM) completely attenuated the spontaneous outward currents, indicating that the events were $GABA_A$-mediated sIPSCs (Figures 2E, 3, and 5–7). To further support the conclusion that the effect on sIPSC activity was a result of Group I metabotropic glutamate receptors, we used various, specific antagonists to attenuate the agonist actions. In 6 relay cells from the cat's lateral geniculate nucleus, the ACPD- or DHPG-mediated actions were attenuated by the general mGluR antagonist (RS)-α-methyl-4-carboxyphenylglycine (MCPG; Figure 3A; $n = 3$) or by the specific Group I antagonist (S)-4-carboxyphenylglycine (4CPG; Figure 3B; $n = 3$). In these 6 neurons, the mGluR agonist produced an average increase in sIPSC frequency by 205% ± 79%, which was significantly different from control levels ($p < 0.02$). In the presence of the antagonist, the increase in sIPSC frequency was attenuated to an average of 143% ± 55%, significantly different from agonist-mediated increase in sIPSC frequency in control conditions ($p < 0.02$). Taken together, these data suggest that the effect of ACPD on F2 terminals is conveyed

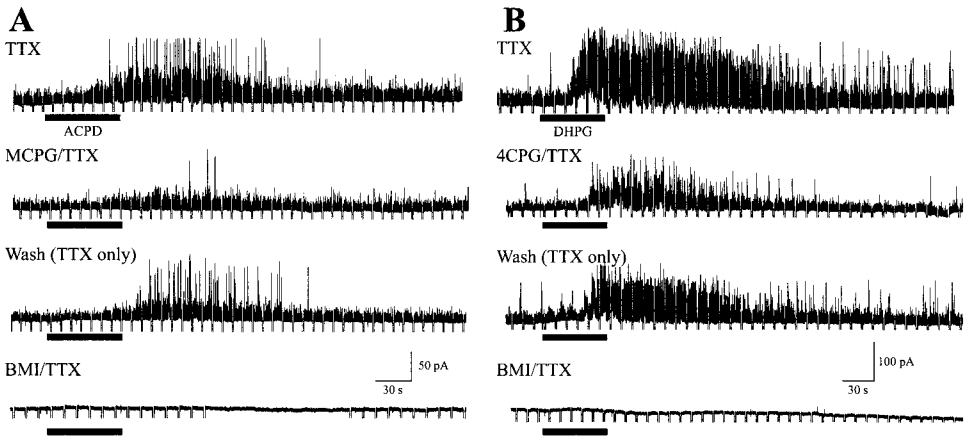

Figure 3. The Increased sIPSC Activity Is Attenuated by mGluR Antagonists in Geniculate Cells from a Cat

(A) TTX: ACPD (125 μM) produces a large increase in sIPSC activity in TTX (1 μM). MCPG/TTX: In presence of the general mGluR antagonist MCPG (500 μM), the ACPD-mediated increase in sIPSC activity is significantly attenuated. Wash: The ACPD-mediated effect partially recovers following 23 min wash in TTX-only solution. BMI/TTX: The baseline activity and ACPD-mediated effect are completely suppressed by the $GABA_A$ antagonist BMI (30 μM).

(B) TTX: In a different neuron, the Group I agonist DHPG (250 μM) produces a large increase in sIPSC activity in the presence of TTX (1 μM). 4CPG/TTX: Following addition of the selective Group I antagonist 4CPG (500 μM), the DHPG-mediated effect is significantly reduced. Wash: The DHPG-mediated response recovers following 33 min washout of the antagonist. BMI/TTX: The baseline activity and DHPG response is suppressed by BMI (30 μM).

via Group I rather than Group II metabotropic glutamate receptors.

Ionotropic Glutamate Receptors

Anatomical data regarding the localization of ionotropic glutamate receptors on F1 and/or F2 terminals is currently lacking, but we sought to test the potential role of these receptors in regulating the sIPSC activity described above. The ionotropic glutamate receptor agonist (RS)-α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) was tested on a total of 34 relay cells from the cat's lateral geniculate nucleus. This included 18 cells studied with AMPA applied to the control condition (normal ACSF) and again in the presence of TTX; 14 cells in which AMPA was applied only in the presence of TTX-containing ACSF; and 2 cells studied only in the presence of normal ACSF. In control conditions, AMPA produced a robust increase in sIPSC activity in all cells tested (Figure 4A). With the addition of TTX (1 μM), the increase in sIPSC activity was still observed in 26 of 32 (81%) cells so tested. In addition to the robust increase in sIPSC activity, there was usually an underlying inward current (Figure 4) that was independent of the alteration in sIPSCs (e.g., Figure 4C). This current likely results from a difference between the holding potential and reversal potential produced by the agonist or may be due to a space clamp problem. Nonetheless, this did not appear to influence the agonist effect on sIPSC activity. In a subset of cells in which AMPA was applied in both control and TTX conditions, the robust increase in sIPSC activity persisted in 15 of 18 (83%) neurons (Figures 4A and 4B). In 2 cells further tested, the AMPA-mediated increase in sISPC activity was attenuated by the ionotropic glutamate receptor antagonist 6,7-dintroquinoxaline-2,3-dione (DNQX; 30 μM; Figure 4B). That is, the TTX-insensitive increase in sIPSCs by AMPA, like ACPD, may have a direct depolarizing effect on F1 and/or F2 terminals.

We also tested the Ca^{2+} dependence of the TTX-insensitive AMPA-mediated response in 9 cells. Our earlier findings indicated that the increase in sIPSC frequency by ACPD was Ca^{2+} dependent (Cox et al., 1998). In the presence of TTX, ACPD produces a robust increase in sIPSC frequency (Figure 5A; a 308% increase above pre-ACPD application) that is effectively completely attenuated (90% of the pre-ACPD level) in the presence of a low Ca^{2+} (0.2 mM)/high Mg^{2+} (6.0 mM)-containing extracellular solution. The amplitude of sIPSCs was significantly increased following application of ACPD ($p < 0.001$; Figure 5A, top cumulative probability distribution). However, the amplitudes of the remaining IPSCs in the low Ca^{2+}/high Mg^{2+} solution were unchanged in ACPD (Figure 5A, bottom cumulative probability distribution), suggesting that ACPD is not producing a direct postsynaptic action on the relay cell that increases IPSC amplitude. As illustrated in Figure 5B, AMPA produced a robust TTX-insensitive increase in sIPSC frequency (165% above pre-AMPA application) in a different relay neuron. In all 9 neurons tested, the AMPA-mediated increase in sIPSC frequency was significantly attenuated in the low Ca^{2+}/high Mg^{2+} solution. Quantitatively, AMPA produced a 356% ± 168% increase in sIPSC frequency in TTX, which is significantly higher than before AMPA application ($p < 0.005$). In the low Ca^{2+} solution, the AMPA-mediated increase was significantly reduced relative to that observed in normal Ca^{2+} solution (152% ± 111%, $p < 0.005$). Furthermore, the remaining increase in sIPSC frequency by AMPA in the low Ca^{2+} solution was not statistically significant from pre-AMPA values ($p > 0.1$). In 5 of these neurons, the AMPA effect was completely attenuated (Figure 5B). As with ACPD, the TTX-insensitive increase in sIPSC frequency elicited by AMPA was associated with a significant increase in IPSC amplitude ($p < 0.01$; Figure 5B, top cumulative probability distribution). In these

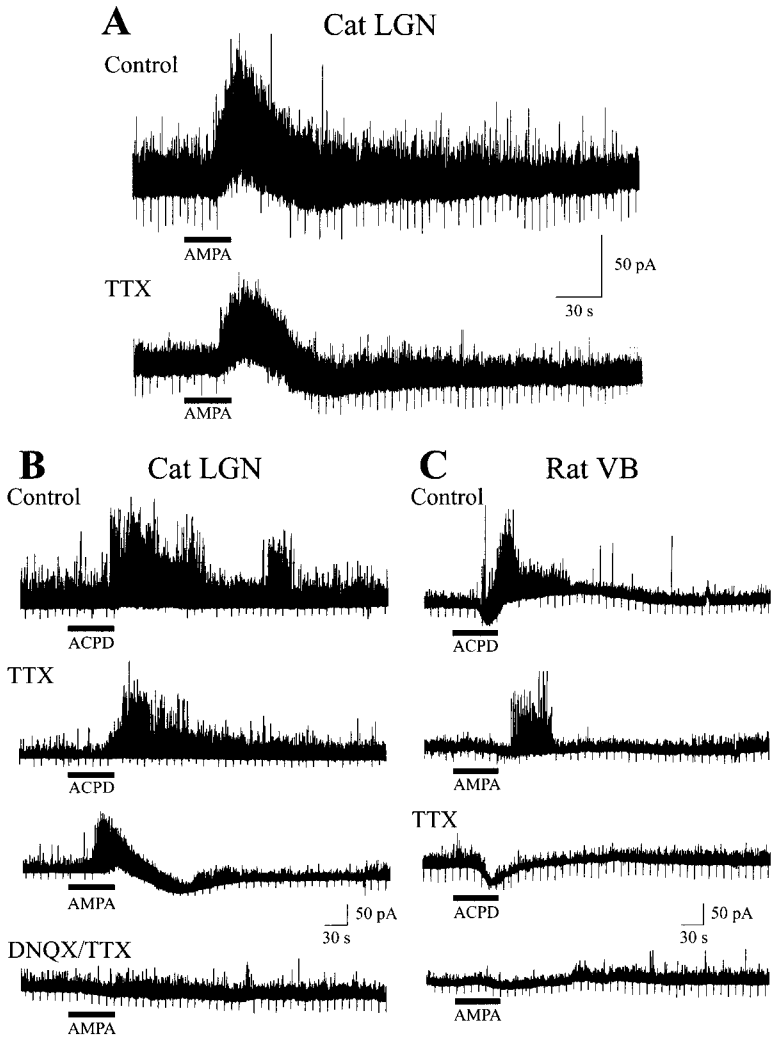

Figure 4. Agonists to iGluRs Alter sIPSC Activity in Cat and Rat Lateral Geniculate Nucleus but Not in Rat Ventrobasal Complex

(A) Control: In a recording from a cat's geniculate relay neuron, AMPA (5 μM) produces a robust increase in sIPSC activity. TTX: Following application of TTX (1 μM), the AMPA-mediated increase in sIPSC activity persists. (B) Control: In a recording from a different cat's geniculate relay neuron, ACPD (250 μM) produces a robust increase in sIPSC activity. TTX: Following application of TTX (1 μM), ACPD still produces a large increase in sIPSC activity although it is partially reduced. AMPA (5 μM) also produces a large increase in sIPSC activity, although this response is shorter in duration than that observed with ACPD. DNQX/TTX: In the presence of DNQX (30 μM), subsequent application of AMPA no longer alters the sIPSC activity.

(C) Control: In a recording from a rat VB neuron, both ACPD (250 μM) and AMPA (5 μM) produce robust increases in sIPSC activity in control conditions. TTX: In TTX (1 μM), neither agonist, ACPD or AMPA, produces an increase in sIPSC activity.

cells, the amplitudes of the IPSCs remaining in the low Ca^{2+}/high Mg^{2+} solution were not significantly altered by AMPA (Figure 5B, bottom cumulative probability distribution). While our data indicate that there may be a Ca^{2+}-independent component of the AMPA response in some neurons, these data nonetheless indicate a strong Ca^{2+}-dependent component that is presumed to be presynaptic because of the lack of alteration in IPSC amplitude in the low Ca^{2+} condition. Thus, similar to the ACPD effect (Figure 5A and Cox et al., 1998), the AMPA-mediated response seems due to the presynaptic effect on F2 terminals.

Because these observations suggested that AMPA could be acting on F1 or F2 terminals, or both, we once again took advantage of the lack of interneurons and F2 terminals in the rat's ventrobasal complex. As illustrated in Figure 4C, AMPA in control conditions produced a robust increase in sIPSC activity in 4 of 5 (80%) ventrobasal neurons so tested, and this increase in sIPSC activity was completely abolished in TTX in all 4 neurons. This suggests that the AMPA does not act directly on the F1 terminals. By applying the logic used above with ACPD-mediated effects, we conclude that the TTX-insensitive increase in sIPSCs mediated by AMPA in relay cells of the cat's lateral geniculate nucleus may be operating via ionotropic glutamate receptors on F2 terminals.

Effects of application of both AMPA and ACPD were tested together in a total of 16 relay cells from the cat's lateral geniculate nucleus. In 7 cells, both the ACPD- and AMPA-mediated increase in sIPSC activity was TTX insensitive (Figure 4B). In 5 other neurons, the increase in sIPSC activity in response to ACPD and AMPA was TTX sensitive. Thus, in these 12 neurons, the data are consistent with two different neuronal populations (e.g., Type I and Type II; see Figure 1A). However the remaining 4 neurons responded in an manner inconsistent with this conclusion. In 3 of these neurons, the response to ACPD was TTX sensitive and the AMPA-mediated response was TTX insensitive. In the remaining neuron, the ACPD-mediated action was TTX insensitive, whereas the AMPA-mediated effect was TTX sensitive. It is possible that in a minority of Type II cells with inputs from F2 terminals, glutamatergic transmission onto the afferent F2 terminals is dominated by iGluRs or mGluRs.

Cholinergic Influence on F2 Terminals

Morphological data have indicated that F2 terminals are often postsynaptic to terminals from axons of cholinergic cells of the brainstem parabrachial region (Figure

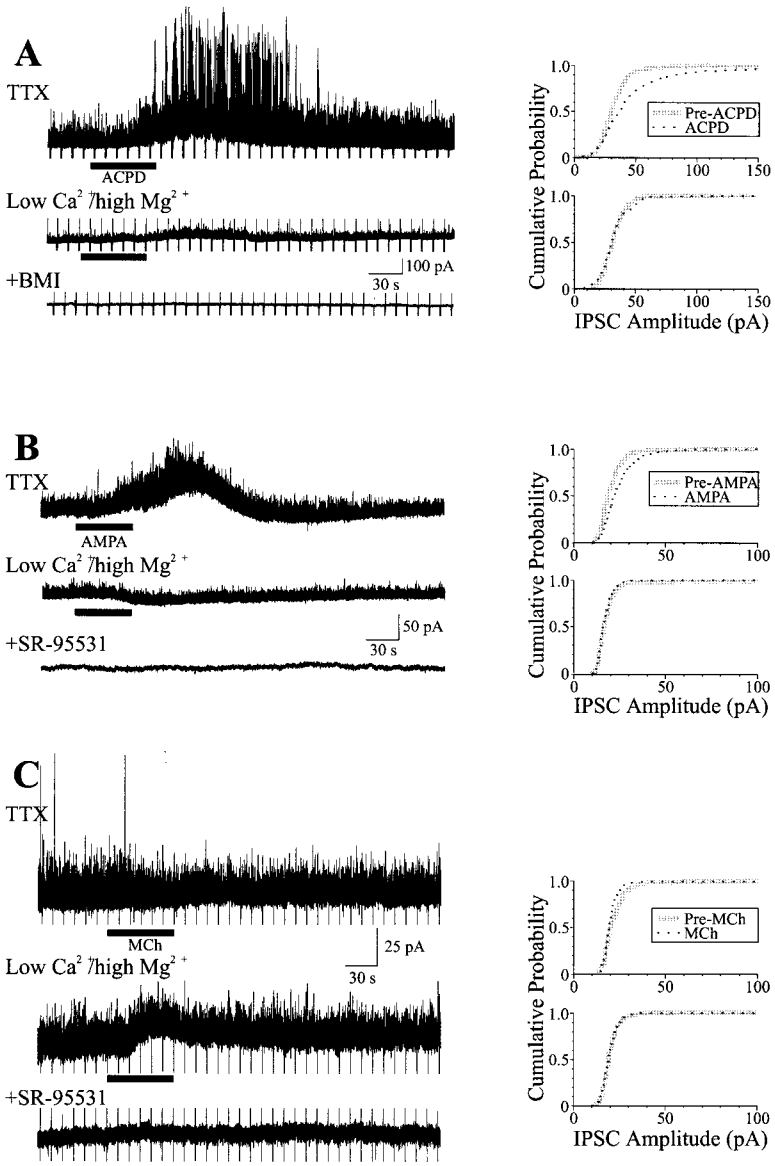

Figure 5. TTX-Insenstive Alteration in sIPSC Activity Is Ca^{2+} Dependent in Geniculate Neurons from the Cat

(A) TTX: In TTX (1 μM), ACPD (125 μM) produces a robust increase in frequency and amplitude of sIPSCs. The top cumulative probability distribution illustrates the increase in IPSC amplitudes by ACPD (thin dotted black line) relative to pre-ACPD application (thick gray line). Low Ca^{2+}/high Mg^{2+}: In a low Ca^{2+} (0.2 mM)/high Mg^{2+} (6.0 mM), TTX-containing ACSF, the ACPD effect is attenuated. The bottom cumulative probability distribution illustrates that the IPSC amplitudes are unchanged by ACPD. +BMI: The addition of BMI (30 μM) attenuates the sIPSCs.

(B) Ca^{2+} dependence of AMPA effect on sIPSCs in a different neuron. TTX: In TTX (1 μM), AMPA (5 μM) produces a large increase in sIPSC activity. The increase in sIPSC amplitude is illustrated by the top cumulative probability distribution. Low Ca^{2+}/high Mg^{2+}: In low Ca^{2+}/high Mg^{2+} solution, AMPA no longer alters the frequency of sIPSCs. In addition, the amplitude of the sIPSCs is also unchanged by AMPA (bottom cumulative probability distribution). +SR95531: The addition of SR95531 (20 μM) attenuates the sIPSCs.

(C) Ca^{2+} dependence of MCh effect on sIPSCs in a different neuron. TTX: In TTX (1 μM), MCh (250 μM) suppresses the frequency of sIPSCs. As shown in the top cumulative probability distribution, sIPSC amplitudes are reduced in MCh. Low Ca^{2+}/high Mg^{2+}: In low Ca^{2+}/high Mg^{2+} solution, the frequency of sIPSCs is partially attenuated. As illustrated in the bottom cumulative probability distribution, the amplitude of sIPSCs is unchanged in MCh. +SR95531: The baseline sIPSCs are attenuated by SR95531 (30 μM).

1A; Erisir et al., 1997). We thus used an experimental design similar to that described above for glutamate pharmacology to test for the effects of cholinergic transmission in the inhibitory circuits depicted in Figure 1A. Because the TTX-insensitive actions of mGluR activation are likely to be associated with Type II circuitry involving relay X cells, and these were the cells of interest, we altered our strategy somewhat. Relay X cells are smaller than are relay Y cells (Friedlander et al., 1981). Instead of recording from cells regardless of size, we took advantage of the optics of our recording set-up to target smaller neurons selectively, and as noted below, this strategy seemed to work in the sense that the great majority of recorded neurons behaved as if they were associated with Type II circuitry. In these experiments, we used agonists for both muscarinic (i.e., metabotropic) and nicotinic (i.e., ionotropic) receptors.

Muscarinic Receptors

The general muscarinic agonist acetyl-β-methylcholine chloride (MCh, 250–500 μM) produced an initial transient increase in sIPSC activity followed by a long-lasting suppression of sIPSCs in 17 of 18 (94%) relay cells of the cat's lateral geniculate nucleus (Figure 6A). In the remaining cell, MCh did not alter the sIPSC activity. In TTX (1 μM), the MCh-mediated transient increase in sIPSCs was attenuated in all 17 cells, indicating that this is mediated via increased activity in axons of interneurons and/or reticular cells and does not involve F2 terminals. However, the long-lasting suppression of sIPSC activity persisted in 16 of the 18 neurons (i.e., TTX insensitive). In a subset of 12 neurons that were tested in control and in TTX, the MCh-mediated suppression of sIPSC activity persisted in both conditions in 11 of these (92%) cells. Note that the TTX-insensitive effects of MCh—suppression of sIPSCs—are opposite in sign to those of ACPD, which involves an increase in sIPSC activity (see also below). The presence of strong TTX-insensitive effects implies that muscarinic receptors are activated directly on F1 and/or F2 terminals. Furthermore, this activation results in suppression of transmitter release, which in turn suggests that MCh may hyperpolarize these terminals.

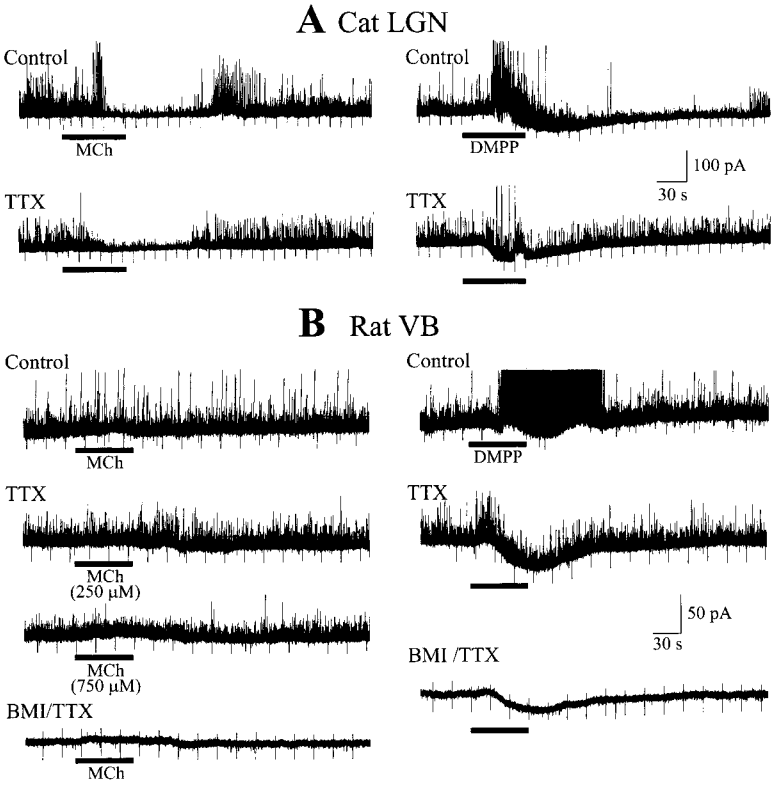

Figure 6. Cholinergic Agonists Modify Inhibitory Response in Thalamic Relay Neurons

(A) Left panel, Control: In a geniculate relay neuron from a cat, MCh (250 μM) produces a transient increase, followed by a long-lasting suppression in sIPSC activity. TTX: In TTX (1 μM), MCh still produces a strong suppression of sIPSC activity. Note that the short-lasting increase in sIPSC activity by MCh is attenuated. Right panel, Control: In the same cell, the nicotinic agonist DMPP (250 μM) produces a robust increase in sIPSC activity superimposed on a small inward current. TTX: In TTX (1 μM), DMPP no longer alters sIPSC activity, although the small inward current still persists.

(B) Recording from a rat ventrobasal neuron. Left panel, Control: MCh (250 μM) produces no apparent change in sIPSC activity in control conditions. TTX: In TTX (1 μM), MCh still produces no obvious change in sIPSC activity at original (250 μM) and at a higher concentration (750 μM). BMI/TTX: Following the addition of BMI (30 μM), the baseline sISPC activity is completely suppressed and MCh does not alter the baseline. Right panel, Control: In the same cell, DMPP (250 μM) produces a robust increase in sIPSC activity. TTX: In TTX (1 μM), DMPP no longer alters sIPSC activity, although there is an obvious inward current. BMI/TTX: The baseline is completely suppressed in BMI (30 μM), and DMPP only produces a small inward current.

In order to test whether the TTX-insensitive suppression of sIPSC activity by muscarinic agonists acts on F1 and/or F2 terminals, we again turned to ventrobasal relay cells in the rat. From recordings of 4 such neurons, we failed to observe any MCh-mediated reduction in sIPSC activity with or without the addition of TTX (Figure 6B). These data indicate that the TTX-insensitive decrease in sIPSCs mediated by MCh in the cat's lateral geniculate nucleus is not due to a direct action on F1 terminals and thus involves only F2 terminals. This also suggests that MCh has no detectable effect on reticulothalamic axons innervating these relay neurons, perhaps because these axons have been severed from their parent somata in the process of preparing the slices for recording or as a result of low spontaneous activity of these cells in the slice preparation.

Our working hypothesis is that activation of muscarinic receptors on F2 terminals hyperpolarizes these F2 terminals and thereby decreases GABA release onto the relay cell innervated by these F2 terminals. Similar to the results with ACPD and AMPA application, we thus predict that this would be a Ca^{2+}-dependent process and have tested this in 7 neurons. As illustrated in Figure 5C, in the presence of TTX, MCh reduced sIPSC frequency to 43% of control (i.e., pre-MCh) in this relay neuron. In an extracellular solution containing low Ca^{2+} (0.2 mM)/high Mg^{2+} (6.0 mM), the MCh-mediated suppression in sIPSC frequency was attenuated to 67% of control, showing that much, but not all, of the MCh effect is Ca^{2+} dependent. Similar observations were made in all 7 cells. That is, in TTX containing normal ACSF, MCh significantly reduced the ISPC frequency to 53% $\pm$ 17% of control ($p < 0.02$; $n = 7$), and in the low Ca^{2+}/high Mg^{2+}/TTX solution, the MCh suppression was attenuated to an average 74% $\pm$ 14% of control, significantly greater than in the normal Ca^{2+} solution ($p < 0.03$). The TTX-insensitive reduction in sIPSC frequency by MCh was associated with a significant reduction in IPSC amplitudes ($p < 0.01$; Figure 5C, top cumulative probability distribution). However, sIPSC amplitudes were not significantly altered in the low Ca^{2+}/high Mg^{2+} solution, although MCh still reduced the frequencies of IPSCs (Figure 5C, bottom cumulative probability distribution). Unlike the ACPD- and AMPA-mediated actions, the MCh-mediated suppression of sIPSC frequency was never completely attenuated in the low Ca^{2+} solution, suggesting that there may be a Ca^{2+}-independent presynaptic action that reduces IPSC frequency but is not likely a postsynaptic action that reduces IPSC amplitude. In any case, our data indicate that most of the reduced sIPSCs due to MCh application are presynaptic and operate via F2 terminals.

As with metabotropic glutamate receptors, many subtypes—at least 5—of muscarinic receptors (M_1 through M_5) have been identified (Kubo et al., 1986; Bonner et al., 1987; Caulfield, 1993). Within the thalamus, activation of muscarinic receptors has been associated with both excitatory and inhibitory actions (McCormick and Prince, 1987; McCormick and Pape, 1988). Thus, we used selective muscarinic receptor antagonists to determine whether the reduction in sIPSC activity is associated with a specific receptor subtype. We initially used the muscarinic antagonist pirenzepine, which has a higher affinity for M_1 receptors than for M_2 receptors (Hammer et al., 1980; Watson et al., 1982, 1983; Luthin and Wolfe, 1984). As illustrated for a cat's geniculate relay cell

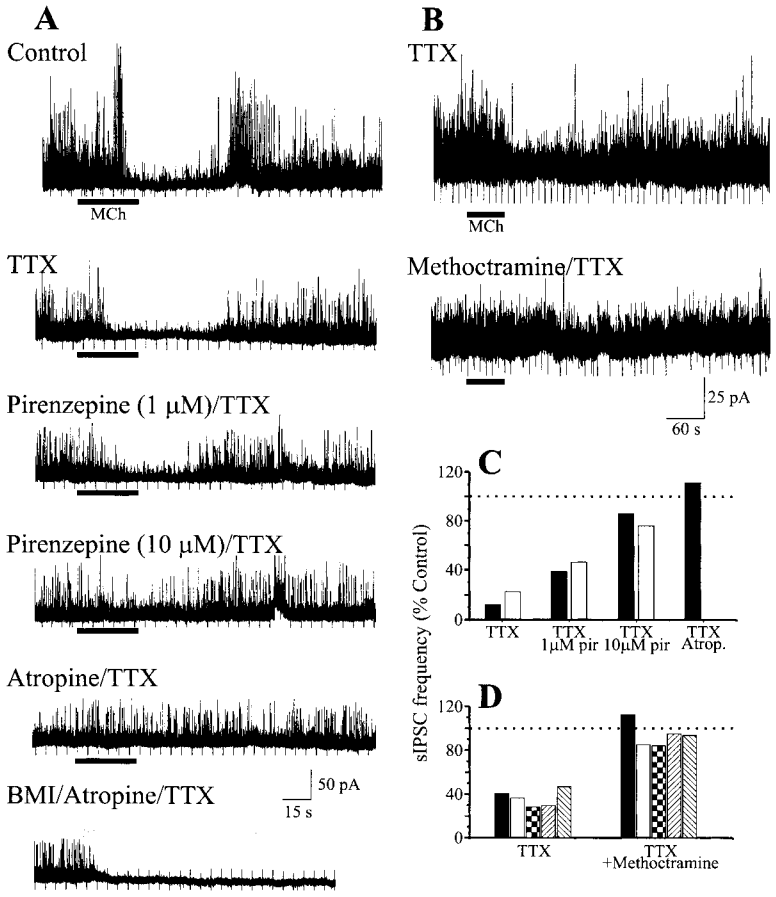

Figure 7. Muscarinic Suppression in sIPSC Activity in Geniculate Neurons from a Cat Is Mediated by M2 Muscarinic Receptor Subtype

(A) Control: MCh (250 μM) produces a short-lasting increase followed by a strong reduction in sIPSC activity. TTX: In TTX (1 μM), the suppression of sIPSC activity by MCh persists. Pirenzepine (1 μM)/TTX: In the presence of low pirenzepine concentration, the MCh-mediated suppression is slightly attenuated. Pirenzepine (10 μM)/TTX: After increasing the pirenzepine concentration to 10 μM, the MCh-mediated suppression of sIPSC activity is clearly attenuated. Atropine/TTX: The MCh suppression of sIPSC activity is completely attenuated in atropine (1 μM). BMI/Atropine/TTX: The addition of the $GABA_A$ antagonist BMI (30 μM) completely suppresses the baseline activity (gray bar).

(B) TTX: In a different relay neuron in the presence of TTX (1 μM), MCh (250 μM) suppresses sIPSC activity. Methoctramine: Following addition of selective M2 antagonist, methoctramine (500 μM), MCh no longer suppresses sIPSC activity.

(C) Summary of the effect of pirenzepine on MCH-mediated changes in sIPSC frequency. The graph depicts the change in sIPSC frequency produced by MCh relative to control in different conditions for 2 neurons. With increasing pirenzepine concentrations, the suppressive action of MCh is reduced.

(D) Summary of methoctramine effect in 5 different neurons. In TTX, MCh significantly suppresses sIPSC frequency. In the presence of methoctramine, the effect of MCh on sIPSC frequency is reduced and is no longer significantly different from control.

by Figure 7A, MCh produces a strong suppression of sIPSC activity in a control condition that persists in the presence of TTX. Following the addition of a relatively low pirenzepine concentration (1 μM), there is little reduction of the TTX-insensitive, MCh-mediated suppression. However, increasing pirenzepine concentration to 10 μM significantly attenuated this suppression of sIPSC activity (Figure 7C). With the addition of the general antagonist atropine (1 μM), the suppressive action of MCh is completely blocked. The effect of pirenzepine on the frequency of sIPSC activity for the two neurons is summarized in Figure 7C. There is clearly a concentration-dependent attenuation of MCh suppression by pirenzepine that suggests the involvement of M_2 muscarinic receptors. To investigate the potential role of this receptor subtype further in 5 neurons, we used the more specific M_2 receptors antagonist methoctramine. As illustrated in Figures 7B and 7D, the MCh-mediated suppression of sIPSCs was clearly attenuated by methoctramine. In the presence of TTX, MCh produced a significant suppression in the frequency of sIPSCs relative to control levels (Figure 7D, black bars; $p < 0.01$). However, in the presence of methoctramine, the effect of MCh on sIPSC frequency was not significantly altered from control ($p > 0.1$). These data further support the role of M_2 muscarinic receptors underlying this suppressive action.

Nicotinic Receptors

In contrast to the muscarinic actions on sIPSC activity, the nicotinic agonist 1,1-dimethyl-4-phenyl-piperazinium iodide (DMPP; 250–500 μM) produced a robust increase in sIPSC activity in all 10 cells tested in the cat's lateral geniculate nucleus (Figure 6A). Also, unlike the effect of MCh, the DMPP-mediated increase was abolished in the presence of TTX in each of the 6 cells further tested (Figure 6A). Like AMPA, DMPP also produced an inward current that appeared independent of the agonist action on sIPSC activity (e.g., Figures 6A and 6B), which again is likely due to a difference in the holding potential and the reversal potential of the DMPP induced current and/or a space clamp problem. These data support the above conclusion that the nicotinic effect seems to be borne by increasing activity in interneuron or reticulothalamic axons and their F1 terminals, and the complete lack of any remaining TTX-insensitive effect further indicates that there is no substantial presence of nicotinic receptors on F2 (or F1) terminals. The TTX-sensitive increase in sIPSC activity by DMPP was observed in both types of MCh-mediated actions: TTX-insensitive suppression in sIPSC activity (Type II, X cells; 6 cells tested) and TTX-sensitive increase in sIPSC (Type I, Y cells; 2 cells tested).

We further tested effects of DMPP in 4 relay cells of the rat's ventrobasal complex. In 2 (50%) of these cells,

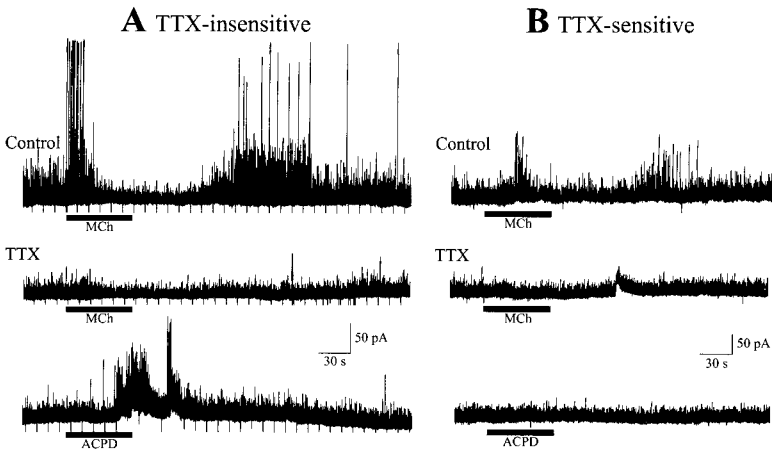

Figure 8. Suppression of sIPSCs by Muscarinic Agonists in Geniculate Relay Cells of the Cat

(A) TTX-insensitive alteration of sIPSC activity. Control: MCh (250 μM) produces transient increase followed by a sustained suppression of sIPSC activity. TTX: In TTX (1 μM), MCh produces a lasting suppression of sIPSC activity. In the same neuron, ACPD (125 μM) produces a robust increase in sIPSC activity (bottom trace).

(B) TTX-sensitive alteration in sIPSC activity in another neuron. Control: MCh (250 μM) produces a short duration increase followed by a subtle suppression of sIPSC activity. TTX: In the presence of TTX (1 μM), MCh no longer alters sIPSC activity. Subsequent application of ACPD (125 μM) has no effect on sIPSC activity (bottom trace).

DMPP produced a robust increase in sIPSC activity that was completely abolished by TTX (Figure 6B), consistent with an increase in reticular cell activity via activation of nicotinic receptors; in the other 2 relay cells, DMPP had no detectable effect in either condition. These data further indicate that DMPP does not directly affect F1 terminals, supporting the conclusion from our study of the cat's geniculate cells.

Combined Studies of Metabotropic Glutamate and Muscarinic Receptors

In a subset of experiments, agonists to both metabotropic glutamate and muscarinic receptors were applied to the same 11 geniculate relay cells in the cat. As we had observed earlier, two populations of responses could be distinguished by the addition TTX: TTX sensitive and TTX insensitive. In 10 neurons in which the MCh-mediated reduction in sIPSCs persisted in TTX (TTX insensitive), the addition of ACPD also resulted in the TTX insensitive increase in sIPSC activity (Figure 8A). In the remaining neuron, the suppression of sIPSC by MCh was attenuated by TTX (TTX sensitive), and subsequent application of ACPD did not alter sIPSC activity (Figure 8B). These data support two distinct subpopulations: one that is TTX sensitive to both muscarinic and mGluR agonists, consistent with Type I (Y) relay cells (Figures 1A and 1B), whereas the other population has TTX-insensitive responses to the two agonists, consistent with Type II (X) relay cells (Figure 1C).

Discussion

The overwhelming majority of synapses onto individual geniculate relay neurons are nonretinal in origin, and it is likely that these inputs play a potentially major role in controlling the excitability of relay neurons and, hence, the flow of information from retina to neocortex. Our studies have focused on the role of synapses from interneurons via presynaptic dendrites (F2 terminals) that receive both glutamatergic and cholinergic innervation. Our basic results are summarized in Figure 9. Activation of either glutamatergic (iGluRs and mGluRs) or cholinergic (muscarinic only) receptors can modify the release of GABA from F2 terminals. The activation of iGluRs and mGluRs produces a robust increase in sIPSC activity that can occur independent of action potentials, presumably through depolarization of the F2 terminals. This increase in GABA release from F2 terminals increases inhibition in the relay neuron. In contrast, activation of M_2 muscarinic receptors produces a strong suppression of sIPSC activity, which is also independent of action potentials and consequently leads to a disinhibitory action on the relay cell. These opposing actions on the output of the F2 terminals likely have a significant influence on the information relayed through the thalamus.

Furthermore, the alterations in inhibitory activity in the recorded relay cells resulting from activation of the above receptors can be differentiated into two groups based on sensitivity to TTX. This differentiation is consistent with the two morphological classes of relay neurons—Type I (Y) and Type II (X) as shown in Figures 1A and 1B—and these correspond to the classes of cell in the cat's lateral geniculate nucleus identified in vivo (Friedlander et al., 1981). We found without exception that TTX-insensitive effects of the agonists occur in Type II neurons, whereas the TTX-sensitive response was observed in Type I neurons.

F2 and Not F1 Terminals Are Directly Affected by Agonists

We have documented agonist-mediated effects on sIPSCs recorded in relay cells. Because these effects are blocked by $GABA_A$ antagonists, include changes in frequency, and are significantly attenuated by low Ca^{2+}/high Mg^{2+} solution, we conclude that they represent presynaptic effects on GABAergic terminals innervating the recorded cells, although in some cases, additional direct, postsynaptic effects cannot be ruled out. The obvious candidates among terminals to be affected by the agonists are the F1 and F2 terminals that are found throughout much of the thalamic neuropil.

Effects Involving Metabotropic Receptors

The TTX-insensitive response indicates that the agonists to metabotropic receptors for glutamate and acetylcholine act directly on GABAergic terminals, although these results alone cannot distinguish between effects on F1 or F2 terminals, or both. The TTX-sensitive responses to metabotropic agonists observed in the cat's and rat's lateral geniculate nucleus and in the rat's ven-

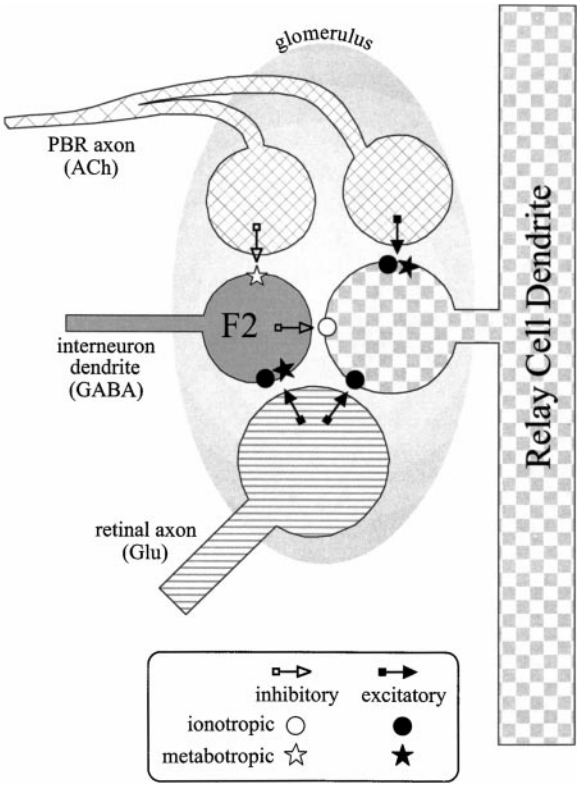

Figure 9. Summary Schematic Illustrating the Innervation of F2 Terminals and Relay Cell Dendrite by Retinal Ganglion Cells and Cholinergic Neurons of the Brainstem Parabrachial Region

The parabrachial input produces direct excitation in the relay neuron via ionotropic (nicotinic) and metabotropic (muscarinic) receptors. In addition, activation of metabotropic muscarinic receptors inhibits the output of F2 terminals, and thus the inhibitory output of these terminals onto the relay cell. The retinal input produces a direct excitatory response in the relay cell via iGluRs. The retinal input also produces an excitatory response via iGluRs and mGluRs in the F2 terminals, thereby increasing the inhibitory output of these terminals and producing an inhibitory action on the relay neuron. These two different triadic arrangements are contained within a glomerulus structure.

trobasal complex suggest that the effect is not mediated through the subset of F1 terminals that derive from cells of the thalamic reticular nucleus (Figure 1A). However, it is possible from just these data that the TTX-insensitive effects could directly alter the F1 and/or F2 terminal outputs of interneurons. At least in terms of activation of metabotropic glutamate and muscarinic receptors, immunohistological studies indicate that these receptors are localized on F2 and not F1 terminals (Godwin et al., 1996; Carden and Bickford, 1999). In further, albeit indirect, support of this conclusion, any effect carried via F1 terminals of interneurons should be seen in both X and Y cells. This is because of physiological evidence that interneurons innervate both X and Y cells (Lindström, 1982), and since F2 terminals are nearly exclusively limited to X cells, this suggests that the interneuronal innervation of Y cells must be via F1 terminals. If this is the case and if F1 terminals were directly activated by the agonists we employed, we should never have observed the TTX-sensitive pattern apparently associated with the larger (Y) cells. Thus, all the data considered together make a fairly strong case that the TTX-insensitive effects we observed in the lateral geniculate nucleus involved direct effects on F2 and not F1 terminals.

Effects Involving Ionotropic Receptors

We found no TTX-insensitive effect in the lateral geniculate nucleus for activation of nicotinic receptors, and thus, there is no evidence that F1 or F2 terminals can be directly affected by nicotinic agonists. However, we did find TTX-independent effects to AMPA that were quite similar to those in response to ACPD. With one exception, we can apply the same logic as applied above to argue that the TTX-sensitive effects of AMPA are likely to be due to direct activation of F2 and not F1 terminals. The exception is that there is at present no immunocytochemical evidence available that can address this issue. We thus conclude that F2 terminals of interneurons have iGluRs and that F1 terminals of either interneurons or cells of the thalamic reticular nucleus lack these receptors but emphasize that this conclusion is weaker than that for metabotropic receptors.

Functional Significance of F2/Triadic Circuitry

Regulation via Glutamate Receptors

Our results, summarized by Figure 9, provide some insight into the function of the F2 terminal and triadic synaptic arrangements associated specifically with relay X cells in the cat's lateral geniculate nucleus and more generally with Type II circuitry in the thalamus. An active retinal terminal will release glutamate, which would activate only iGluRs on the relay cell (McCormick and von Krosigk, 1992) and both mGluR and iGluRs on the F2 terminals. If we assume that activation of iGluRs and mGluRs on F2 terminals has similar properties to such activation elsewhere in the thalamus (e.g., see Scharfman et al., 1990; McCormick and von Krosigk, 1992; von Krosigk et al., 1999), then we can suggest the following scenario. The iGluR-mediated AMPA effect on the F2 terminals would occur reliably at low frequency of afferent retinal input and may simply correlate with the strength of any iGluR-mediated EPSPs activated in the relay cell. In this regard, the relative strength in the relay cell of the monosynaptic EPSP and disynaptic IPSP resulting from iGluR activation would be fairly constant. In contrast, we would predict that the activation of mGluRs would require repetitive, high frequency stimulation, being minimal or nonexistent at lower frequencies (McCormick and von Krosigk, 1992; von Krosigk et al., 1999). Thus, unlike the case with disynaptic inhibition mediated via iGluRs, that mediated via mGluRs relative to the monosynaptic EPSPs would grow dramatically with firing frequency in the retinal input. In addition, activation of iGluRs have a short latency and duration, whereas mGluR activation has a much longer latency and duration, suggesting that mGluR activation produces a lasting inhibitory influence over subsequent excitatory afferent activity. The result of the mGluR-mediated responses would be to dampen the relay cell responses to increasingly active retinal inputs, thereby preventing response saturation and extending the dynamic range of the relay to cortex. This could be regarded as an early form of contrast gain control since

increasing contrast in a visual stimulus results in an increasing response in retinal ganglion cells (Kaplan et al., 1987). If this is correct, then this sort of contrast gain control should be a property associated with relay X and not Y cells, and this is a hypothesis amenable to experimental verification.

Regulation via Cholinergic Receptors

The functional significance of the cholinergic innervation of the F2 terminals is likely related to the behavioral state of the animal. There is strong evidence that the activity of neurons in the parabrachial region is closely related to the arousal state or attentiveness of the animal (for review, see Steckler et al., 1994). We would thus predict that during periods of increased arousal, the release of acetylcholine onto the F2 terminals would result in suppression of inhibition of the relay cell via F2 terminals. This would enhance retinogeniculate transmission through the relay cell. It is also the case that cholinergic inputs to interneurons and cells of the thalamic reticular nucleus tend to inhibit their axonal (F1) outputs (McCormick and Prince, 1986; McCormick and Pape, 1988), so in general, we can conclude that the effect of enhanced cholinergic input is to reduce all inhibition of relay cells via F1 and F2 terminals.

If this muscarinic inhibition of the F2 terminal were sufficiently strong enough to overcome glutamatergic excitation of the F2 terminal, then the F2 terminal ceases to be a factor in controlling responses of geniculate X cells or any thalamic cells with Type II circuitry. Indeed, they would then presumably respond to retinal inputs more like geniculate Y cells or cells with Type I circuitry. In other words, as far as retinogeniculate transmission or its equivalent in other thalamic nuclei, this aspect might be dependent on behaviorally state to the extent that behavioral state dictates activity in parabrachial inputs to thalamus.

Insights into Functioning of Interneurons

Finally, our data may provide further insights regarding the functional role of thalamic interneurons. That is, alterations in the output of distal F2 terminals by mGluR agonists occur without any apparent changes in membrane potential or input resistance at the somatic level (Pape and McCormick, 1995; Cox et al., 1998). These data, in conjunction with computational work, suggest that postsynaptic membrane voltage changes occurring at the F2 terminals are so electrotonically distant from the soma and proximal dendrites that they have negligible influence there (Bloomfield and Sherman, 1989). One may thus view the role of interneurons as a multiplexing integrator with two largely independent input/output paths: one route occurs in the distal dendrites (F2 terminals); the other route involves integration at the soma and proximal dendrites and outputs via the axon (F1 terminals). Thus, a single interneuron could have multiple dendritic regions that may act fairly independently of one another. Of course, this view of interneuronal functioning assumes passive dendrites. Indirect support for this comes from evidence that ACPD, which we conclude depolarizes F2 terminals but has no detectable effect at the soma (Pape and McCormick, 1995; Cox et al., 1998).

Conclusions

Of particular interest in the functioning of F2 terminals in the thalamus is evidence that they possess metabotropic receptors responsive to glutamate and acetylcholine. The former would be activated by retinal inputs but presumably in a manner such that the ratio in the relay cell of the disynaptic IPSP to the monosynaptic EPSP increases with increasing firing frequency of the retinal input. This could prevent response saturation in retinogeniculate transmission and provide a form of contrast gain control. While mGluR activation increases output of the F2 terminal, muscarinic activation decreases it. We suggest that this muscarinic activation depends on the behavioral state of the animal and that the more alert the animal, the more inhibition via the F2 terminal is suppressed. Thus, the involvement of the F2 terminal in Type II circuitry in the thalamus offers different means of controlling transmission of incoming signals through the relay cell.

Experimental Procedures

Thalamic slices were obtained from kittens (postnatal age 4–8 weeks) and young Sprague–Dawly rats (postnatal age 10–21 days). The kittens were deeply anaesthetized with ketamine (25 mg/kg) and xylazine (1 mg/kg), a craniotomy was made, and a block of tissue containing the lateral geniculate nucleus was quickly removed and placed into cold, oxygenated slicing medium (~4°C) containing (in mM): 2.5 KCl, 1.25 NaH_2PO_4, 10 $MgCl_2$, 0.5 $CaCl_2$, 26 $NaHCO_3$, 11 glucose, and 234 sucrose. To obtain rat tissue, the animal was deeply anaesthetized with sodium pentobarbital (50 mg/kg), the brain quickly removed and chilled in the above slicing medium. Tissue slices (300–350 μm) were cut in either a coronal or sagittal plane using a vibrating tissue slicer, transferred to a holding chamber containing oxygenated physiological saline maintained at 30°C, and incubated for at least 2 hr prior to recording. The orientation used for tissue preparation did not influence our results and have therefore been combined. The physiological saline (ACSF) contained (in mM): 126 NaCl, 2.5 KCl, 1.25 NaH_2PO_4, 2 $MgCl_2$, 2 $CaCl_2$, 26 $NaHCO_3$, and 10 glucose. These solutions were gassed with 95% O_2/5% CO_2 to a final pH of 7.4. Individual slices were then transferred to a submersion-type recording chamber on a modified microscope stage and maintained at 30°C ± 1°C. A few experiments with rat tissue were conducted at room temperature (~25°C), but similar findings were observed at 30°C, and thus these data have been combined. The tissue was superfused with oxygenated physiological solution at a rate of 3–4 ml/min.

Whole-cell recordings were obtained using a visualized slice preparation as previously described (Edwards et al., 1989; Stuart et al., 1993; Cox et al., 1998). Recording pipettes were pulled from 1.5 mm OD capillary tubing with tip resistances of 4–6 MΩ when filled with the intracellular solutions listed below. The intracellular solution used in the majority of recordings contained (in mM): 117 Cs-gluconate, 13 CsCl, 1 $MgCl_2$, 0.07 $CaCl_2$, 0.1 ethylene glycol-O,O′-bis(2-aminoethyl)-N,N,N′,N′-tetraacetic acid (EGTA), 10 N-(2-hydroxyethyl)piperazine-N′-(2-ethanesulfonic acid) (HEPES), 2 Na_2-ATP, 0.4 Na-GTP, and 0.5% biocytin. The pH of the solution was adjusted to 7.3 using CsOH, and osmolality was adjusted to 290 mosm with distilled water. The use of this intracellular solution results in ~10 mV junction potential that has been left uncorrected in the voltage measures.

Recordings of spontaneous postsynaptic currents were obtained using continuous single electrode voltage clamp mode of an Axoclamp2A amplifier (Axon Instruments, Foster City, CA). To optimize recording of spontaneous inhibitory postsynaptic currents (sIPSCs), a holding voltage of 0 mV was used to maximize the driving force of these inhibitory currents. In addition, the potassium channel blocker Cs^+ was included in the recording pipette to suppress postsynaptic actions of mGluR or muscarinic receptor activation which has previously been shown to alter K^+ conductances in thalamic relay neu-

rons (McCormick and Prince, 1987; McCormick and von Krosigk, 1992; Cox et al., 1998). Recordings were limited to neurons with a stable access resistance of less than 20 MΩ. Current and voltage protocols were generated using PCLAMP software (Axon Instruments) and data were digitized and stored on an IBM PC-compatible computer and magnetic tape (Neurodata).

The spontaneous postsynaptic currents were detected using commercial minianalysis software program (Synaptosoft, Leonia, NJ) and cumulative probability plots were constructed from the peak measure of each individual detected event. Quantitative analyses of IPSC frequency and amplitude were obtained from 90 s epochs prior to and immediately following agonist application. Such analyses were largely limited to comparisons involving effects of antagonists or of low Ca^{2+}/ high Mg^{2+} solutions. Statistical analyses of the IPSC amplitudes consisted of a Kolmogorov-Smirnov test and for comparison of sIPSC frequencies, Mann-Whitney U test was used. Data are presented as mean ± standard deviation.

Concentrated stock solution of agonists and antagonists were prepared in either 0.1 M NaOH or distilled water and diluted in physiological solution to a final concentration of 100–500 μM. Agonists were applied to the tissue by a bolus injection (20–60 s duration) into the input line of the chamber using a motorized syringe pump or in a few noted cases, bath applied. Based on the rate of agonist injection into the input line and the chamber perfusion rate, the final bath concentration of these agents was estimated to be about one-fourth of the initial concentration in the injection line. All antagonists were diluted to final concentration in physiological solution just prior to use and bath applied. All excitatory amino acid agonists and antagonists were purchased from Tocris Cookson (St. Louis, MO) and remaining chemicals were purchased from Sigma (St. Louis, MO).

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