Modulatory effects of activation of metabotropic glutamate receptors on GABAergic circuits in the mouse thalamus

Tingting Liu, Iraklis Petrof, and S. Murray Sherman

Department of Neurobiology, University of Chicago, Chicago, Illinois

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Liu T, Petrof I, Sherman SM. Modulatory effects of activation of metabotropic glutamate receptors on GABAergic circuits in the mouse thalamus. J Neurophysiol 113: 2646-2652, 2015. First published February 4, 2015; doi:10.1152/jn.01014.2014.--Metabotropic glutamate receptors (mGluRs) are widely distributed in the central nervous system and modulate the release of neurotransmitters in different ways. We have previously shown that activation of presynaptic group II mGluRs reduces the gain of GABAergic inputs in both primary visual and auditory cortices (V1 and A1). In the present study, we sought to determine whether activation of mGluRs can also affect the inhibitory inputs in thalamus. Using whole cell recordings in a mouse slice preparation, we studied two GABAergic inputs to thalamic relay cells: that of the thalamic reticular nucleus (TRN) to cells of the ventral posteromedial nucleus (VPM) and that of interneurons to cells of the lateral geniculate nucleus (LGN). We found that activation of mGluRs significantly reduced the amplitudes of inhibitory postsynaptic currents (IPSCs) evoked from TRN inputs to VPM cells, and further experiments indicated that this was due to activation of presynaptic group I and group II mGluRs. Similar results were found in the interneuronal inputs to LGN cells. Activation of presynaptic group I (type 1 but not type 5) and group II mGluRs significantly reduced the amplitudes of evoked IPSCs of the axonal inputs to relay cells, and additional experiments were consistent with previous observations that activation of type 5 mGluRs on the dendritic terminals of interneurons enhanced postsynaptic IPSCs. We concluded that group I and II mGluRs may generally reduce the amplitude of evoked GABAergic IPSCs of axonal inputs to thalamic relay cells, operating through presynaptic mechanisms, and this extends our previous findings in cortex.

metabotropic glutamate receptors; thalamic interneurons; thalamic reticular nucleus; thalamus

METABOTROPIC GLUTAMATE RECEPTORS (mGluRs) are a family of G protein-coupled receptors that are ubiquitously distributed throughout the mammalian brain (Hollmann and Heinemann 1994). Evidence from previous studies has indicated the presence of mGluRs on GABAergic terminals (Cartmell and Schoepp 2000; Pinheiro and Mulle 2008; Schoepp 2011) and a variety of effects on GABA release induced by activation of mGluRs in different brain regions, such as hippocampus (Jouvenceau et al. 1995; Liu et al. 1993), thalamus (Salt and Eaton 1995; Salt and Turner 1998b), olfactory bulb (Hayashi et al. 1993), tectum (Farazifard and Wu 2010; Neale and Salt 2006), striatum (Hanania and Johnson 1999; Wang et al. 1999), cerebellum (Mitchell and Silver 2000), and parts of brain stem (Chen and Bonham 2005; Jones et al. 1998). Group I mGluRs have been shown to reduce excitatory synaptic transmission in the retinogeniculate pathway (Govindaiah et al. 2012) as well

as in thalamocortical and corticocortical pathways (De Pasquale and Sherman 2013). Similarly, group II mGluRs have been observed to reduce the amplitude of evoked responses following stimulation of thalamocortical (Lee and Sherman 2012) and intracortical inputs (De Pasquale and Sherman 2012).

Related to this, we recently reported that in primary visual and auditory cortex activation of mGluRs by agonist application reduces the gain of GABAergic inputs (Liu et al. 2014). Furthermore, we showed that this effect was due to the presynaptic activation of group II mGluRs. In the present study, we wanted to examine the possible generality of this phenomenon by studying the effects of mGluR activation on GABAergic inputs in thalamus. We thus looked at the GABAergic inputs in mice from the thalamic reticular nucleus (TRN) to relay cells of the ventral posteromedial nucleus (VPM) and the GABAergic input of local interneurons to relay cells of the lateral geniculate nucleus (LGN). Similar to our observations in cortex, we found that the gain of GABAergic input in these pathways was reduced and that this was due to the activation of both group I and group II mGluRs located presynaptically.

MATERIALS AND METHODS

We adopted our previously described methods (De Pasquale and Sherman 2011, 2012, 2013; Liu et al. 2014; Theyel et al. 2010); these are briefly summarized below. All procedures were approved by the Institutional Animal Care and Use Committee of the University of Chicago.

Slice preparation. Slices were prepared from BALB/c mice (age 9-25 days; Harlan). Animals were deeply anesthetized by isoflurane inhalation (AErrane; Baxter Pharmaceuticals) and were then decapitated. Brains were removed and placed in cold $(0-4^{\circ}C)$, oxygenated (95% O₂-5% CO₂) slicing solution containing (in mM) 2.5 KCl, 1.25 NaH₂PO₄, 10 MgSO₄, 0.5 CaCl₂, 25 NaHCO₃, 11 glucose, and 206 sucrose. For the TRN-to-VPM pathway, a dorsal-ventral cut 45° from the midline (i.e., at 10:30 or 1:30 on a watch face) was made first. Then 400- μ m slices were collected with the cut surface facing the platform. For the LGN recording, coronal slices were used. Slices (400 μ m thick) were prepared as described previously (Lam and Sherman 2013) and kept in artificial cerebrospinal fluid (ACSF) containing (in mM) 125 NaCl, 25 NaHCO₃, 3 KCl, 1.25 NaH₂PO₄, 1 MgCl₂, 2 CaCl₂, and 25 glucose at room temperature. In some experiments designed to block synaptic transmission, we used ACSF with low Ca^{2+} and high Mg^{2+} concentrations, containing (in mM) 125 NaCl, 25 NaHCO₃, 3 KCl, 1.25 NaH₂PO₄, 1 CaCl₂, 3 MgCl₂, and 25 glucose.

Electrophysiology. Whole cell recordings were obtained from VPM and LGN. Recording pipettes were filled with intracellular solution containing (in mM) 117 K-gluconate, 13 KCl, 1 MgCl₂, 0.07 CaCl₂, 10 HEPES, 0.1 EGTA, 2 Na₂-ATP, and 0.4 Na-GTP, with 0.1–0.5% biocytin; pH 7.3, 290 mosM. In experiments aimed at interfering with

Address for reprint requests and other correspondence: S. M. Sherman, Dept. of Neurobiology, Univ. of Chicago, 947 E. 58th St., MC 0926, 316 Abbott, Chicago, IL 60637 (e-mail: msherman@bsd.uchicago.edu).

the postsynaptic action of mGluRs, the nonhydrolyzable GTP analog guanosine 5'-[β -thio]diphosphate (GDP β S, 1 mM; Sigma-Aldrich) was added to the intracellular solution in order to block postsynaptic G protein-coupled activity; we have previously found that this concentration of the drug is effective in blocking postsynaptic mGluR responses (DePasquale and Sherman 2012, 2013).

All experiments were performed on a visualized slice setup under a differential interference contrast-equipped Axioskop 2FS microscope (Carl Zeiss Instruments). Current- and voltage-clamp signals were collected and amplified with pCLAMP software and a Multiclamp 700B amplifier (Axon Instruments). Only cells with a stable access resistance between 12 and 20 M Ω were recorded. Hyperpolarizing currents were injected to identify I_h , while depolarizing currents were injected to identify regular, tonic, or bursting spike patterns. We recorded postsynaptic responses mostly in voltage clamp.

In some experiments in the TRN-to-VPM pathway, we stimulated inputs to the recorded cells by placing a bipolar concentric electrode (FHC) in a location guided by glutamate photostimulation (i.e., uncaging) maps. Electrical stimulation consisted of 4 pulses at 25 Hz. The duration of each pulse was 0.1 or 0.2 ms. We used a stimulation intensity defined as follows: for any given recorded cell, we identified the stimulation intensity that produced inhibitory postsynaptic currents (IPSCs) \sim 50% of the time; we then increased stimulation by 5- μ A increments until we observed IPSCs every time, and we used this as our stimulation intensity.

Laser photostimulation of glutamate and GABA. For photostimulation to uncage glutamate or GABA, we used our previously described methods (Liu et al. 2014).

Pharmacology. In some experiments, we bath-applied various mGluR agonists and antagonists in addition to AMPA and NMDA antagonists as described previously (De Pasquale and Sherman 2011, 2012, 2013; Liu et al. 2014; Theyel et al. 2010). These agents were delivered to the recording chamber by injecting a bolus into the flow line of the recirculating ACSF. A minimum of 5 min had to elapse after the administration of the agonists and antagonists before any stimulation protocols could commence.

AMPA and NMDA antagonists were used at the following concentrations: the AMPA receptor antagonist DNQX (R&D Tocris), 50 μ M; the NMDA receptor antagonist MK-801 (Sigma-Aldrich), 40 μ M. The concentration of mGluR agonists and antagonists used was as follows: the general mGluR agonist ACPD (R&D Tocris), 100 μ M; the group I mGluR agonist DHPG (R&D Tocris), 100 μ M; the group II mGluR agonist APDC (R&D Tocris), 100 μ M; the type 5 mGluR agonist CHPG (R&D Tocris), 100 μ M; the type 5 mGluR antagonist MPEP (R&D Tocris), 100 μ M. In some experiments on photostimulation of glutamate in the LGN, tetrodotoxin (TTX, 1 μ m; R&D Tocris) was used to block the sodium channels in the axonal pathways.

Normalization and statistical procedures. In our analyses, IPSC values represented the average of 10 trials. For each cell, IPSC values of the repeated stimulation (IPSC_x) were normalized to the value of the corresponding IPSC in the control condition (IPSC_{control}) according to the following procedure: IPSC_{normalized} = (IPSC_x/IPSC_{control}), expressed as a percentage. The effect of the application of an agonist or antagonist on each IPSC was evaluated with a Wilcoxon signed-rank test. Where multiple comparisons were involved, critical values were adjusted with the Bonferroni correction.

RESULTS

We recorded from a total of 79 cells: 55 in VPM and 24 in LGN. The neurons in VPM had an average resting membrane potential of -55.7 ± 5.2 mV (mean \pm SD) and an average input resistance of 221.8 \pm 85.7 M Ω . The respective values of LGN relay cells were -57.7 ± 5.2 mV and 255.4 \pm 98.4 M Ω .

GABAergic inputs in VPM activated from TRN. Figure 1A illustrates the stimulation and recording arrangement for all the



Fig. 1. Effects of the general metabotropic glutamate receptor (mGluR) agonist ACPD on the inhibitory inputs from thalamic reticular nucleus (TRN) to ventral posteromedial nucleus (VPM). Significance of comparisons: *P <0.05, ***P < 0.001. A: schematic showing the placement of a bipolar concentric electrode in TRN (red) and the recorded cell in VPM (yellow star). VPL, ventral posterolateral nucleus; D, dorsal; L, lateral; M, medial; V, ventral. B: effects of ACPD on inhibitory postsynaptic currents (IPSCs) evoked by 4 pulses delivered at 25 Hz. Bi: traces showing an example experiment. Bii: graph showing the normalized amplitudes of the 4 IPSCs before (Control) and after ACPD application (ACPD) as well as those after the recovery by washout (Washout). Each of the 4 IPSCs was decreased significantly by ACPD application and was partly recovered after washout. Biii: graph showing that ACPD application increased the ratio of the 4th IPSC to the 1st IPSC of the recorded cells and washout mostly reversed the effect. C: effects of ACPD on the rise times (20-80%) and the decay times (80-20%) of the 4 evoked IPSCs in VPM. Ci: rise times of the 4 IPSCs before (Control) and after ACPD application (ACPD) using normal intracellular solution. ACPD application had no effect on the rise times of any of the 4 IPSCs. Cii: decay times of the 4 IPSCs before (Control) and after ACPD application (ACPD). ACPD application had no significant effect on the decay times of any of the 4 IPSCs.

cells recorded in VPM, with electrical stimulation (4 pulses at 25 Hz) applied in TRN with a concentric bipolar electrode. We used photostimulation (glutamate uncaging) to locate the "hot spot" in TRN as a source of GABAergic inputs to a recorded cell in VPM and then placed the stimulating electrode over that hot spot for electrical stimulation. To help isolate and identify IPSCs, we maintained each cell membrane potential at 0 mV and added AMPA and NMDA antagonists (DNQX 50 μ M and MK-801 40 μ M, respectively) to the bath. Electrical stimulation of TRN evoked IPSCs in every recorded cell in VPM.

Figure 1*B* shows the effect of application of the general mGluR agonist ACPD on the IPSCs in 13 VPM neurons evoked in TRN. A train of four IPSCs was recorded before and during the application of ACPD. We found that the amplitudes of all four IPSCs were significantly decreased during the application of ACPD and that this decrease was partially reversed by washing out the ACPD (Fig. 1, *Bi* and *Bii*). This

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	ACPD, % of corresponding control IPSC			GDPβS in Intracellular Solution, % of corresponding control IPSC		DHPG, % of corresponding control IPSC			APDC, % of corresponding control IPSC			
	Control	ACPD	Washout	Control	ACPD	Washout	Control	DHPG	Washout	Control	APDC	Washout
IPSC1 IPSC2 IPSC3 IPSC4	100 100 100 100	$7.7 \pm 1.8^{***}$ $14.4 \pm 3.0^{***}$ $19.3 \pm 4.0^{***}$ $20.5 \pm 5.0^{***}$	$51.9 \pm 14.4 \\58.3 \pm 10.5 \\54.3 \pm 5.5 \\49.9 \pm 7.6$	100 100 100 100	$\begin{array}{c} 15.2 \pm 2.6^{**} \\ 22.4 \pm 4.1^{**} \\ 22.0 \pm 6.1^{**} \\ 38.4 \pm 13.6^{*} \end{array}$	83.1 ± 13.7 67.9 ± 8.8 83.1 ± 12.9 111.8 ± 34.0	100 100 100 100	$\begin{array}{c} 42.1 \pm 4.8^{***} \\ 55.2 \pm 5.8^{***} \\ 57.4 \pm 8.6^{**} \\ 58.6 \pm 5.2^{***} \end{array}$	$\begin{array}{l} 82.2 \pm 19.4 \\ 69.8 \pm 14.1 \\ 97.1 \pm 26.8 \\ 72.5 \pm 14.9 \end{array}$	100 100 100 100	$\begin{array}{c} 10.1 \pm 2.6^{***} \\ 17.1 \pm 3.2^{***} \\ 18.6 \pm 2.4^{***} \\ 23.7 \pm 4.9^{***} \end{array}$	$\begin{array}{c} 39.2 \pm 5.5 \\ 68.3 \pm 16.9 \\ 64.9 \pm 16.0 \\ 68.4 \pm 13.9 \end{array}$

Values are average \pm SE effect of agonists (ACPD, DHPG, and APDC) on the amplitudes of inhibitory postsynaptic currents (IPSCs) (% of the 1st IPSC in control group) evoked from thalamic reticular nucleus (TRN) inputs to cells of the ventral posteromedial nucleus (VPM). GDP β S, guanosine 5'-[β -thio]diphosphate. *P < 0.05, **P < 0.01, ***P < 0.001.

effect was particularly dramatic for the first IPSC, which was decreased by 92% compared with its control (i.e., here and below we compare each IPSC to its control, meaning 1st to 1st, 2nd to 2nd, etc.; P < 0.001), but also very strong for the second (decreased by 86%; P < 0.001), third (decreased by 81%; P <0.001), and fourth (decreased by 80%; P < 0.001) IPSCs (Fig. 1Bii, Table 1; Bonferroni-adjusted Wilcoxon signed-rank test). After the washout, the amplitudes of all four IPSCs were partially recovered (1st IPSC back to 52% of control, 2nd to 58%, 3rd to 54%, and 4th to 50%; see also Table 1). Of the 13 cells studied, 6 went through all three test conditions (control, ACPD application, and washout), while the other 7 cells only went through the first two (control and ACPD application). Among the six cells with washout data, five showed a depressing pattern of IPSCs (i.e., each IPSC had an amplitude that was smaller than the previous) under the control condition (i.e., before ACPD application), while one cell responded with facilitation (i.e., each IPSC had a greater amplitude than the previous) (Fig. 1Biii). Application of ACPD shifted this pattern: all six cells showed a significantly less depressing pattern of responses (Fig. 1Biii; P < 0.05, Bonferroni-adjusted Wilcoxon signed-rank test). The washout reversed this effect back to baseline levels (washout compared with control: P > 0.05, washout compared with the ACPD group: P < 0.05; Bonferroni-adjusted Wilcoxon signed-rank test). The general effects of ACPD on paired-pulse dynamics suggest a presynaptic site for this change in evoked IPSCs. Experiments described below support this conclusion.

We measured the effects of ACPD on the rise time of the evoked IPSCs, which was defined as the time elapsed between 20% and 80% of the evoked IPSC peak value. Figure 1*Ci* shows that application of ACPD had no significant effect on this parameter for the evoked IPSCs (P > 0.6 for all comparisons on Mann-Whitney *U*-tests). To check whether the reduction of evoked IPSCs might be associated with postsynaptic GABA receptor desensitization, we also measured the effects of ACPD on the decay time (between 80% and 20% of the peak value of the evoked IPSCs after baseline adjustment). As shown in Fig. 1*Cii*, the application of ACPD had no significant effect on the decay time of the four IPSCs (P > 0.2 for all comparisons on Mann-Whitney *U*-tests). The lack of effect of ACPD on temporal dynamics of the evoked IPSCs is consistent with the effects of ACPD being mainly presynaptic.

In a complementary set of experiments, we added GDP β S to the intracellular solution for eight additional cells recorded in VPM. The goal here was to interfere with the effects of postsynaptic metabotropic receptors (see MATERIALS AND METH-ODS). For seven of the eight cells, we obtained data from all three conditions, control, ACPD application, and washout, whereas for the remaining cell we obtained data for only the first two conditions. For these cells, GDP β S failed to prevent the effects of ACPD on evoked IPSCs (Fig. 2, *Ai* and *Aii*): the first IPSC was decreased by 85% (*P* < 0.01; Bonferroniadjusted Wilcoxon signed-rank test; see Table 1), the second by 78% (*P* < 0.01), the third by 78% (*P* < 0.01), and the fourth



Fig. 2. Pre- and postsynaptic effects of the general mGluR agonist ACPD on the inhibitory inputs from TRN to VPM. Significance of comparisons: *P <0.05. A: effects of ACPD and washout on the evoked IPSCs while blocking the postsynaptic G protein-coupled pathway with guanosine 5'-[\beta-thio]diphosphate (GDPBS) in the intracellular solution. Ai: traces showing an example experiment. Aii: graph showing the normalized amplitudes of 4 IPSCs before (Control), during ACPD application (ACPD), and after washout (Washout). B: effects of ACPD on rise and decay times of IPSCs recorded in VPM cells while stimulating in TRN, with GDPBS in the intracellular solution. ACPD application produced no significant change in the rise or decay times of the IPSCs. C: effect of ACPD on direct applications of GABA to recorded cells achieved by GABA photostimulation in a low-Ca2+, high-Mg2+ ACSF. Ci: outward currents before (blue) and after the ACPD application (red) evoked by GABA photostimulation; arrow shows the time of photostimulation, 100 ms after the recording starts. Cii: no significant change of outward currents after ACPD application.

by 62% (P < 0.05). After washout, the amplitudes of all four IPSCs were mostly recovered (1st IPSC back to 83% of control, 2nd back to 68%, 3rd back to 83%, and 4th back to 112%; see also Table 1). Furthermore, application of ACPD had no significant effect on IPSC rise or decay time (Fig. 2, Bi and Bii; P > 0.05 for all 4 IPSCs on Mann-Whitney U-tests) in these experiments. These observations further support a presynaptic site for the effects of ACPD on the evoked IPSCs.

Although we believe our use of GDP β S in these experiments strongly supports the conclusion of a presynaptic site as just mentioned, we considered the question of effects of GDP β S alone on evoked IPSCs. Because GDPBS is used in the intracellular recording solution, we could not in practice compare IPSCs evoked before and after its application on individual cells. However, we could indirectly address this by comparing the evoked IPSCs between cells recorded with and without GDP β S in the electrode (i.e., by comparing the data in Fig. 1B with those in Fig. 2A). This comparison revealed no significant differences between the normalized second, third, and fourth IPSCs of the two groups (for 2nd IPSCs, P = 0.5382; for 3rd IPSCs, P = 0.1803; for 4th IPSCs, P = 0.4470; Mann-Whitney U-tests). This analysis suggests that GDP β S did not have any significant effect on the paired-pulse pattern for these IPSCs.

During the recording of an additional seven cells in VPM, we attempted to isolate potential postsynaptic effects of ACPD in the following manner: recordings were performed in a low-Ca^{2+} and high-Mg^{2+} ACSF to block synaptic transmission. While holding a cell at 0 mV, we applied RuBi-GABA to the bath and photostimulated around the cell body; such photostimulation produced outward currents (see Fig. 2, Ci and Cii). ACPD application had no appreciable effects on outward currents evoked by this direct GABAergic activation (Fig. 2, Ci and Cii). This indicates no appreciable effect on activation of postsynaptic GABA receptors by coactivating postsynaptic mGluRs.

Using the same experimental arrangement as in Fig. 1A, we applied specific mGluR agonists to 13 additional cells recorded in VPM in order to identify the mGluRs involved in the effects shown in Fig. 1, A and B, and Fig. 2A. Application of the group I mGluR agonist DHPG significantly decreased the amplitudes of all four evoked IPSCs (Fig. 3A): the first IPSC was decreased by 58% of control (P < 0.001; Bonferroni-adjusted Wilcoxon signed-rank test; see Table 1), the second by 45% (P < 0.001), the third by 43% (P < 0.05), and the fourth by 41% (P < 0.001). After washout, the amplitudes of the four IPSCs were largely recovered (1st IPSC back to 82% of the baseline, 2nd back to 70%, 3rd back to 97%, and 4th back to 72%; Fig. 3A, Table 1). Application of the group II mGluR agonist APDC also resulted in a significant decrease in the amplitudes of all four evoked IPSCs (Fig. 3B): the first IPSC was decreased by 90% of control (P < 0.001; Bonferroniadjusted Wilcoxon signed-rank test; see Table 1), the second by 83% (P < 0.001), the third by 81% (P < 0.001), and the fourth by 76% (P < 0.001;). Washout partly reversed these effects (1st IPSC back to 39% of the baseline, 2nd back to 68%, 3rd back to 65%, and 4th back to 68%; see Table 1). We thus conclude that the effects documented in Fig. 1, A and B, and Fig. 2A are due to presynaptic activation of both group I and group II mGluRs.

We compared recovery after washout between the use of DHPG and APDC. This analysis showed no significant differ-

Bii Bi) amplitude control) 40pA Control 25ms Washout PSC 9 APDC % IPSC1 IPSC2 IPSC3 IPSC4 Fig. 3. Effects of the specific mGluR agonists on the inhibitory inputs from TRN to VPM. Significance of comparisons: *P < 0.05, **P < 0.01, ***P < 0.010.001. A: effects of the group I mGluR agonist DHPG on IPSCs evoked by 4 pulses delivered at 25 Hz. Ai: traces showing an example experiment. Aii: graph showing the normalized amplitudes of the 4 IPSCs before (Control) and after DHPG application (DHPG), as well as those after the recovery by washout (Washout). The application of DHPG produced a significant decrease in evoked IPSCs, and the effect could be partly reversed by washout. B: effects of the group II mGluR agonist APDC on evoked IPSCs. Bi: traces showing an example experiment. Bii: graph showing the normalized amplitudes of the 4

ence between the use of the two drugs regarding recovery in two pharmacology experiments (1st IPSCs, P = 0.0593; 2nd IPSCs, P = 0.9497; 3rd IPSCs, P = 0.2824; 4th IPSCs, P =0.7546; Mann-Whitney U-tests).

IPSCs before (Control) and after APDC application (APDC), as well as those

after the recovery by washout (Washout). The application of APDC also significantly decreased the amplitudes of evoked IPSCs, and the effect could be

partly reversed by washout.

GABAergic inputs in LGN activated from local interneurons. To test the generality of mGluR effects on GABAergic transmission in thalamus, we performed similar experiments in another GABAergic pathway within thalamus, namely, the GABAergic input of interneurons to LGN relay cells. We selected LGN because it is the only thalamic nucleus with a significant presence of interneurons in the mouse (Arcelli et al. 1997).

LGN interneurons have two outputs: a conventional one via an axon and another via numerous presynaptic dendrites (reviewed in Sherman 2004). Prior work suggests that the dendritic terminals have type 5 mGluRs that, when exposed to glutamate, increase GABA release (Cox and Sherman 2000), and this indicates that the dendritic terminals of interneurons do not respond to mGluR agonists as do the axonal outputs of TRN cells described here or those of cortical interneurons described previously (Liu et al. 2014). Thus to test the generality of these other findings to LGN interneurons, we sought to isolate effects on their axonal outputs.

The experimental arrangement is schematically illustrated in Fig. 4A. We recorded from eight LGN relay cells held at 20 mV [which is 5 mV above the excitatory postsynaptic potential (EPSP) reversal potential (Blitz and Regehr 2005)] in coronal slices, and we used photostimulation (glutamate uncaging) over an 8×8 matrix (50- μ m spacing between points) over the recorded cells to activate local interneuronal inputs to each recorded cell (see Fig. 4Bi). We interpreted hot spots evoking outward currents in the recorded cells as being due to activation of these interneurons. In these experiments, we separately activated group II and type 1 mGluRs, thereby avoiding acti-



vation of the type 5 mGluRs located on interneuronal dendritic terminals (see above). For type 1 mGluR activation, we applied a combination of the group I mGluR agonist DHPG and the type 5 mGluR antagonist MPEP on four cells. For the remaining four cells, we applied the group II mGluR agonist APDC. In all eight cells, we observed the decrease of outward currents after the activation of type 1 mGluR or group II mGluRs (as shown in Fig. 2, Bii and Biii), and this is statistically significant $(P = 2^{-7} \text{ or } P < 0.01; \text{ binomial test}).$

To isolate possible postsynaptic contributions of mGluRs in reducing IPSC amplitudes in this experiment, we again used photostimulation of GABA in a low- Ca^{2+} and high- Mg^{2+} ACSF in seven additional cells with the same methods as described above for VPM. ACPD application had no appreciable effects on outward currents evoked by the direct GABAergic activation on either the cell bodies or dendrites (Fig. 4, Ci and Cii). This indicates no appreciable effect on activation of postsynaptic GABA receptors by coactivation of postsynaptic mGluRs. We also performed GABA photostimulation over a 4×4 matrix (25-µm spacing between points) on the areas over or adjacent to nine additional recorded cells (Fig. 4, *Ciii-Cv*; for detailed methods refer to Liu et al. 2014). The application of ACPD did not change the outward currents evoked by



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GABAergic activation on the dendrites. On the basis of these observations, we conclude that activation of mGluRs reduces the gain of GABAergic inputs evoked in LGN relay cells by activating presynaptic mGluRs and that both type 1 and group II mGluRs are involved in these effects.

DISCUSSION

We have shown in two examples within thalamic circuitrythe projection of TRN cells to relay cells in VPM and the projection from interneurons to relay cells in LGN-that activation of presynaptic mGluRs on these GABAergic axons leads to a reduction of IPSC amplitudes evoked in the postsynaptic relay cells. In this sense, it is difficult to determine the presynaptic site more precisely; it could involve mGluRs on any cellular component and might even involve glial cell participation (e.g., Fellin et al. 2009; Huang et al. 2004; Neumann et al. 1999; Zhang et al. 2003), but the most parsimonious suggestion for now is that the site of effect is presynaptic axonal terminals. Clearly, this warrants further investigation. In any case, these presynaptic effects do seem to be a property associated with these GABAergic axonal projections, because prior evidence (Cox and Sherman 2000; Govindaiah and Cox 2006; Lam et al. 2005) consistent with limited results in the present study indicates that this is not the case for dendritic outputs of the LGN interneurons: here activation of mGluRs (type 5) increases the amplitudes of postsynaptic IPSCs. Obviously, more examples of GABAergic circuitry in thalamus are needed to determine the extent to which these results can be generalized, but it is interesting that we recently reported very much the same pattern for GABAergic cortical inputs (Liu et al. 2014). That is, we found in a number of examples that activation of mGluRs on intracortical GABAergic inputs reduces postsynaptic IPSC amplitudes.

Downloaded from http://jn.physiology.org/ by 10.220.33.6 on November 9, Fig. 4. Effects of specific mGluR agonists and antagonists on the inhibitory inputs from local interneurons to relay cells in lateral geniculate nucleus (LGN). A: schematic showing the coronal slice, including LGN and the recorded cell in the dorsal division of LGN (yellow star). Shown are the ventral , 2017

and dorsal divisions of LGN (vLGN and dLGN, respectively). B: effects of the activation of type 1 and group II mGluRs on outward currents of the recorded relay cells achieved by glutamate photostimulation. Bi: photomicrograph of recording setup. This shows the recording electrode, which approached from the left, and the blue dot shows the location of the recorded cell, with an 8 \times 8 map for photostimulation (red dots) over it. Bii: a typical example of the effects of the activation of type 1 mGluR on the inhibitory inputs from interneurons to the recorded relay cell. The picture shows the enlarged 8×8 map of glutamate photostimulation. Application of the group I mGluR agonist (DHPG) and the type 5 mGluR antagonist (MPEP) (red traces) decreased the outward currents achieved by glutamate photostimulation compared with the baseline (blue traces). The spots with activated outward currents are marked out by yellow stars. Biii: a typical example of the effects of the activation of group II mGluR on the inhibitory inputs from interneurons to the relay cell. The application of group II mGluR agonist (APDC) (red traces) decreased some of the outward currents achieved by glutamate photostimulation compared with the baseline (blue traces). C: effect of ACPD on direct application of GABA to recorded cells achieved by photostimulation of GABA in a low-Ca²⁺, high-Mg²⁺ ACSF. Ci: evoked outward currents before (blue) and after the ACPD application (red) evoked by photostimulation of GABA; arrow shows the time of photostimulation, 100 ms after the recording starts. Cii: no significant change of ACPD on outward currents. Ciii: recording setup and example of direct GABA responses. The photomicrograph shows the recording electrode, and the blue dot (the yellow dot in the enlarged photo) shows the location of the recorded cell, with examples of outward currents over a 4×4 map (50-µm spacing between points). Civ and Cv: recording setup and example of direct GABA responses over a 4 \times 4 matrix (25- μ m spacing between points) on the areas adjacent (Civ, on the dorsal side of the cell body; Cv, on the ventral side of the cell body) to the recorded cells.

Our finding that the magnitude of TRN inhibition in VPM is reduced by the presynaptic activation of group I and II mGluRs is partially consistent with previous reports. More specifically, both in vivo and in vitro studies have reported that presynaptic group II mGluRs can reduce inhibition in rat VPM (Salt and Eaton 1995; Salt and Turner 1998a; Turner and Salt 2003). However, these earlier studies failed to find a role for presynaptic group I mGluRs, as we did, and except for a possible species difference, we cannot account for this discrepancy.

One limitation of the present account is that it is not clear what sort of specificity exists in these GABAergic circuits as regards the presence of presynaptic mGluRs. That is, if these are found only on a specific subset of terminals or associated with a specific subset of GABAergic TRN cells or interneurons, this would raise the possibility of a means to control GABAergic circuits specified by their postsynaptic targets. Again, more data will be required to test this possibility.

Another question that needs to be answered in future studies will be how the mGluR activation affects the GABAergic responses of the relay cell in thalamus under more physiological conditions. One possible source of the presynaptic glutamate received by the interneurons might be the "spillover" of glutamate released from neighboring excitatory synapses (Mitchell and Silver 2000). In this regard, identifying the source of glutamate that leads to these presynaptic effects remains an important unanswered question. Two obvious candidates are the driver inputs (medial lemniscus for VPM and retina for LGN) and modulatory inputs from layer 6 of cortex.

It might be concluded from these data that greater activity in glutamatergic inputs would reduce inhibition, leading to a positive feedback explosion of activity, but a different conclusion suggests itself. As in the case with cortical circuitry, there is evidence that main glutamatergic inputs to thalamus (such as retinal input to the LGN) are also downregulated by activation of presynaptic mGluRs (Govindaiah et al. 2012; Lam and Sherman 2013). Thus the possibility of balanced downregulation of both excitatory and inhibitory inputs seems a plausible conclusion, and this could act as a sort of homeostatic mechanism for thalamic circuitry. For instance, in the LGN we know that increased firing of retinal or corticogeniculate afferents leads to a reduction of retinogeniculate EPSPs (Govindaiah et al. 2012; Lam and Sherman 2013), and the present account suggests that such increased afferent input might also lead to a balancing reduction of inhibitory inputs to relay cells.

Finally, as noted in the introduction, there are examples in many brain regions of control of inhibitory GABAergic circuitry via activation of presynaptic mGluRs. Our results extend the generality of this finding. Evidence also exists for the control of excitatory glutamatergic circuitry via the activation of mGluRs (reviewed in Sherman 2014). Thus we can suggest a more general function of mGluRs: their activation, among other functions, serves in a sort of homeostatic role to downregulate both excitatory and inhibitory inputs as circuits become more active.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

Author contributions: T.L., I.P., and S.M.S. conception and design of research; T.L. performed experiments; T.L., I.P., and S.M.S. analyzed data; T.L., I.P., and S.M.S. interpreted results of experiments; T.L., I.P., and S.M.S. prepared figures; T.L., I.P., and S.M.S. drafted manuscript; T.L., I.P., and S.M.S. edited and revised manuscript; T.L., I.P., and S.M.S. approved final version of manuscript.

REFERENCES

- Arcelli P, Frassoni C, Regondi MC, De Biasi S, Spreafico R. GABAergic neurons in mammalian thalamus: a marker of thalamic complexity? *Brain Res Bull* 42: 27–37, 1997.
- Blitz DM, Regehr WG. Timing and specificity of feed-forward inhibition within the LGN. *Neuron* 45: 917–928, 2005.
- Cartmell J, Schoepp DD. Regulation of neurotransmitter release by metabotropic glutamate receptors. J Neurochem 75: 889–907, 2000.
- Chen CY, Bonham AC. Glutamate suppresses GABA release via presynaptic metabotropic glutamate receptors at baroreceptor neurones in rats. J Physiol 562: 535–551, 2005.
- **Cox CL, Sherman SM.** Control of dendritic outputs of inhibitory interneurons in the lateral geniculate nucleus. *Neuron* 27: 597–610, 2000.
- **De Pasquale R, Sherman SM.** Synaptic properties of corticocortical connections between the primary and secondary visual cortical areas in the mouse. *J Neurosci* 31: 16494–16506, 2011.
- **De Pasquale R, Sherman SM.** Modulatory effects of metabotropic glutamate receptors on local cortical circuits. *J Neurosci* 32: 7364–7372, 2012.
- **De Pasquale R, Sherman SM.** A modulatory effect of the feedback from higher visual areas to V1 in the mouse. *J Neurophysiol* 109: 2618–2631, 2013.
- Farazifard R, Wu SH. Metabotropic glutamate receptors modulate glutamatergic and GABAergic synaptic transmission in the central nucleus of the inferior colliculus. *Brain Res* 1325: 28–40, 2010.
- Fellin T, Halassa MM, Terunuma M, Succol F, Takano H, Frank M, Moss SJ, Haydon PG. Endogenous nonneuronal modulators of synaptic transmission control cortical slow oscillations in vivo. *Proc Natl Acad Sci USA* 106: 15037–15042, 2009.
- **Govindaiah G, Cox CL.** Metabotropic glutamate receptors differentially regulate GABAergic inhibition in thalamus. *J Neurosci* 26: 13443–13453, 2006.
- **Govindaiah G, Wang T, Gillette MU, Cox CL.** Activity-dependent regulation of retinogeniculate signaling by metabotropic glutamate receptors. *J Neurosci* 32: 12820–12831, 2012.
- Hanania T, Johnson KM. Regulation of NMDA-stimulated [¹⁴C]GABA and [³H]acetylcholine release by striatal glutamate and dopamine receptors. *Brain Res* 844: 106–117, 1999.
- Hayashi Y, Momiyama A, Takahashi T, Ohishi H, Ogawameguro R, Shigemoto R, Mizuno N, Nakanishi S. Role of a metabotropic glutamate receptor in synaptic modulation in the accessory olfactory bulb. *Nature* 366: 687–690, 1993.
- Hollmann M, Heinemann S. Cloned glutamate receptors. *Annu Rev Neurosci* 17: 31–108, 1994.
- Huang YH, Sinha SR, Tanaka K, Rothstein JD, Bergles DE. Astrocyte glutamate transporters regulate metabotropic glutamate receptor-mediated excitation of hippocampal interneurons. J Neurosci 24: 4551–4559, 2004.
- **Jones NM, Monn JA, Beart PM.** Type I and II metabotropic glutamate receptors regulate the outflow of [³H]D-aspartate and [¹⁴C]gamma-aminobutyric acid in rat solitary nucleus. *Eur J Pharmacol* 353: 43–51, 1998.
- Jouvenceau A, Dutar P, Billard JM. Presynaptic depression of inhibitory postsynaptic potentials by metabotropic glutamate receptors in rat hippocampal CA1 pyramidal cells. *Eur J Pharmacol* 281: 131–139, 1995.
- Lam YW, Cox CL, Varela C, Sherman SM. Morphological correlates of triadic circuitry in the lateral geniculate nucleus of cats and rats. J Neurophysiol 93: 748–757, 2005.
- Lam YW, Sherman SM. Activation of both Group I and Group II metabotropic glutamatergic receptors suppress retinogeniculate transmission. *Neuroscience* 242: 78–84, 2013.
- Lee CC, Sherman SM. Intrinsic modulators of auditory thalamocortical transmission. *Hear Res* 287: 43–50, 2012.

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- Liu T, Petrof I, Sherman SM. Modulatory effects of activation of metabotropic glutamate receptors on GABAergic circuits in the mouse cortex. *J Neurophysiol* 111: 2287–2297, 2014.
- Liu YB, Disterhoft JF, Slater NT. Activation of metabotropic glutamate receptors induces long-term depression of GABAergic inhibition in hippocampus. J Neurophysiol 69: 1000–1004, 1993.
- Mitchell SJ, Silver RA. Glutamate spillover suppresses inhibition by activating presynaptic mGluRs. *Nature* 404: 498–502, 2000.
- Neale SA, Sait TE. Modulation of GABAergic inhibition in the rat superior colliculus by a presynaptic group II metabotropic glutamate receptor. J Physiol 577: 659–669, 2006.
- Neumann H, Pessoa L, Hanse T. Interaction of ON and OFF pathways for visual contrast measurement. *Biol Cybern* 81: 515–532, 1999.
- Pinheiro PS, Mulle C. Presynaptic glutamate receptors: physiological functions and mechanisms of action. *Nat Rev Neurosci* 9: 423–436, 2008.
- Salt TE, Eaton SA. Distinct presynaptic metabotropic receptors for L-AP4 and CCG1 on GABAergic terminals: pharmacological evidence using novel α -methyl derivative mGluR antagonists, MAP4 and MCCG, in the rat thalamus in vivo. *Neuroscience* 65: 5–13, 1995.
- Salt TE, Turner JP. Modulation of sensory inhibition in the ventrobasal thalamus via activation of group II metabotropic glutamate receptors by 2*R*,4*R*-aminopyrrolidine-2,4-dicarboxylate. *Exp Brain Res* 121: 181–185, 1998a.

- Salt TE, Turner JP. Reduction of sensory and metabotropic glutamate receptor responses in the thalamus by the novel metabotropic glutamate receptor-1-selective antagonist *S*-2-methyl-4-carboxy-phenylglycine. *Neuroscience* 85: 655–658, 1998b.
- Schoepp DD. Where will new neuroscience therapies come from? *Nat Rev* Drug Discov 10: 715–716, 2011.
- Sherman SM. Interneurons and triadic circuitry of the thalamus. Trends Neurosci 27: 670–675, 2004.
- Sherman SM. The function of metabotropic glutamate receptors in thalamus and cortex. *Neuroscientist* 20: 136–149, 2014.
- **Theyel BB, Llano DA, Sherman SM.** The corticothalamocortical circuit drives higher-order cortex in the mouse. *Nat Neurosci* 13: 84–88, 2010.
- **Turner JP, Salt TE.** Group II and III metabotropic glutamate receptors and the control of the nucleus reticularis thalami input to rat thalamocortical neurones in vitro. *Neuroscience* 122: 459–469, 2003.
- Wang Y, Qin ZH, Nakai M, Chen RW, Chuang DM, Chase TN. Costimulation of cyclic-AMP-linked metabotropic glutamate receptors in rat striatum attenuates excitotoxin-induced nuclear factor-kappaB activation and apoptosis. *Neuroscience* 94: 1153–1162, 1999.
- Zhang JM, Wang HK, Ye CQ, Ge W, Chen Y, Jiang ZL, Wu CP, Poo MM, Duan S. ATP released by astrocytes mediates glutamatergic activitydependent heterosynaptic suppression. *Neuron* 40: 971–982, 2003.

