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Activation requirements for metabotropic glutamate receptors

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HIGHLIGHTS

- We examined Group I and II mGluR responses to variable stimulation parameters.
- The activation patterns of Group I and II mGluRs were very similar in nature.
- High-frequency/intensity stimulation is not necessary to activate these receptors.
- mGluRs can often be activated by only 2 stimulation pulses but never by one.

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ABSTRACT

It has been common experimentally to use high frequency, tetanic, stimulation to activate metabotropic glutamate receptors (mGluRs) in cortex and thalamus. To determine what type of stimulation is actually necessary to activate mGluRs we examined the effects of varying stimulation duration and intensity on activating mGluR responses. We used a thalamocortical and an intracortical slice preparation from mice and performed whole cell recordings from neurons in the ventral posterior medial nucleus or in layer 4 of primary somatosensory cortex (S1) while electrically stimulating in layer 6 of S1. Extracellular ionotropic glutamate receptor antagonists and $GABA_A$ receptor antagonists were used to isolate Group I or Group II mGluR responses. We observed that high frequency stimulation is not necessary for the activation of either Group I or Group II mGluRs. Either could be activated with as few as 2–3 pulses at stimulation frequencies around 15–20 Hz. Additionally, increasing the number of pulses, intensity of stimulation, or stimulation frequency increased amplitude and duration of the mGluR response.

1. Introduction

Metabotropic glutamate receptors (mGluRs) are G-protein-coupled receptors that can be found in many parts of the mammalian brain, including the thalamus and cortex [16]. Unlike the fast action of ionotropic glutamate receptors (iGluRs), mGluRs are slow to respond, and the effects of their activation can last for several hundreds of milliseconds, or even seconds [6,18,40].

Due to their distribution, Group I and Group II mGluRs are of particular interest with regards to cortical and thalamic function [10,15,17,26,27,29,34]. A major difference between these two receptor groups is that while activation of Group I mGluRs results in postsynaptic depolarization of the cell, activation of Group II mGluRs has hyperpolarizing postsynaptic effects [8,10,13,19,23].

In thalamus and cortex, mGluRs can be activated by inputs that exhibit a modulatory (or Class 2) synaptic profile such as the projection from layer 6 to layer 4 in several cortical areas [10,23,24], from cortical layer 6 to thalamus [30,33] and some intracortical pathways [7,9]. On the other hand, mGluRs do not become activated by glutamatergic inputs with driver (or Class 1) synaptic characteristics, such as the retinogeniculate pathway [33], the mammilothalamic pathway [32] and some thalamocortical [22,37,38] and corticothalamic [33] projections.

Experiments making use of *in vitro* slice preparations have typically used high-frequency (>50 Hz) and often high intensity (>150 pA) stimulation of an afferent pathway to activate mGluRs [2,4,20], especially in cases where stimulation of axons was involved [32]. This raises questions regarding how commonly mGluRs are activated under more physiological conditions. For instance, some studies have suggested that much less activity is required among glutamatergic afferents to activate Group I mGluR responses in thalamus [30] and cerebellum [14].

To help clarify this issue, we chose to characterize the stimulation parameters required to activate Group I and Group II mGluRs

Abbreviations: EPSP, excitatory post synaptic potential; iGluR, ionotropic glutamate receptor; mGluR, metabotropic glutamate receptor; MGNv, ventral portion of the medial geniculate nucleus; S1, primary somatosensory cortex; VPM, ventral posterior medial thalamic nucleus.

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in two modulatory pathways: the projection from layer 6 to layer 4 in the primary somatosensory cortex (S1) and the feedback projection from layer 6 of S1 to the ventral posterior medial nucleus (VPM) of the thalamus.

2. Methods

2.1. Slice preparation

All procedures were approved by the Institutional Animal Care and Use Committee of the University of Chicago. BALB/c mice (Harlan) of either sex (age 7–16 days postnatal) were anaesthetized with isoflurane and decapitated. For studying corticothalamic projections, thalamocortical slices (500 μm thick) were prepared by blocking the brain at a 55° angle from the midsagittal plane and then gluing the blocked side onto a vibratome platform (Leica, Germany) for slicing [1]. For studying intracortical projections, we prepared 400 μm-thick coronal slices. Following sectioning, the brain slices were placed in oxygenated artificial cerebrospinal fluid containing (in mM) 125 NaCl, 3 KCl, 1.25 NaH_2PO_4, 1 $MgCl_2$, 2 $CaCl_2$, 25 $NaHCO_3$ and 25 glucose.

2.2. Electrophysiology

Whole-cell recordings in current clamp mode were performed as described before [37]. Recording glass pipettes (input resistances 3-8 MΩ) were filled with intracellular solution containing (in mM) 117 K-gluconate, 13 KCl, 1 $MgCl_2$, 0.07 $CaCl_2$, 10 HEPES, 0.1 EGTA, 2 Na_2-ATP, and 0.4 Na-GTP; pH 7.3, 290 mOsm. For both corticothalamic and intracortical projections, electrical stimulation of layer 6 was delivered by a concentric bipolar electrode (FHC, Bowdoinham, ME). For studying the corticothalamic pathway, recordings were performed in VPM, and for studying intracortical projections, recordings were performed in layer 4 barrels of primary somatosensory cortex.

$GABA_A$ receptors were blocked with SR95531 (20 μM) to prevent inhibitory inputs from the thalamic reticular nucleus in corticothalamic pathway experiments or from cortical interneurons in the intracortical pathway experiments. CGP-46381 (50 nM) was used to block $GABA_B$ receptors. Short term plasticity was assessed as described before [37]. This was done in order to identify the type of input of the stimulated pathway given that only Class 2 inputs are known to activate mGluRs [35]. Isolation of mGluR responses was achieved by blocking ionotropic glutamate receptors with AMPA and NMDA receptor antagonists (DNQX, 50 μM, and AP5, 100 μM respectively). The effects of stimulation intensity, frequency, and number of pulses on mGluR response amplitude and duration were assessed under these conditions. Stimulation intensities ranged from 25 μA to 250 μA for all experiments. The number of pulses was varied from 1 to 60 pulses, and frequencies ranged from 10 Hz to 125 Hz. The duration of each pulse was always 0.1 ms. A response was defined as any depolarization or hyperpolarization exceeding 0.5 mV, lasting at least 450 ms, and occurring within 2 s of stimulation. mGluR response amplitude was measured as the peak amplitude of the response (from baseline), occurring at any time during the response. Group II mGluRs were isolated by blocking type 1 and 5 mGluRs (i.e. Group I mGluR) with LY367385 (40 μM) and MPEP (30 μM), respectively, while Group I mGluRs were isolated by blocking Group II mGluRs with MPPG (300 μM).

mGluR response duration was measured as the time from the initial change in membrane potential to the time the membrane potential returned to baseline. The time to peak mGluR response was measured as the time from the onset of response to when the peak response amplitude occurred. After all measurements were taken, responses were verified as being mediated by Group I or Group II mGluRs by using the relevant antagonists (see above).

3. Results

We performed a series of recordings in 41 excitatory[1] neurons that received direct input from layer 6 (18 in VPM, 23 in layer 4 of S1). In both VPM and layer 4 cells, low frequency stimulation (10 Hz) of layer 6 resulted in EPSPs exhibiting paired-pulse facilitation and an increasing amplitude with increasing stimulation intensity (Supp. Fig. 1), in agreement with previous reports [22,33]. Subsequently, iGluR antagonists were applied to the bath and allowed to wash in for 10 min. Complete block of iGluRs was confirmed by the absence of EPSPs following low frequency (10 Hz), high intensity (200–250 μA) stimulation of layer 6 (Supp. Fig. 1). The subsequent demonstration of mGluR activation (see below) demonstrated that these layer 6 afferents are Class 2 in nature [7,37–39].

3.1. mGluR responses

We were able to elicit mGluR responses in all 41 cells of this study. For cells in thalamus receiving layer 6 input, these responses were always mediated by Group I mGluRs [32,33]. On the other hand, neurons in layer 4 showed responses that were mediated by both Group I and/or Group II mGluRs [23,24], and these responses were isolated using the appropriate antagonists. We studied 14 neurons with Group I mGluR responses and 9 neurons with Group II mGluR responses in layer 4. As noted in Section 4, prior evidence indicates that these responses are due to activation of postsynaptic mGluRs.

Increasing the number of pulses, while keeping stimulation frequency and intensity constant, produced an increase in the peak response amplitude, time to peak response, and response duration. This was true for both Group I and Group II mGluR responses (Figs. 1A, B, and 2a–c). Response amplitude increased in a logarithmic fashion with the greatest increase in amplitude occurring over a range of 2–20 pulses (average increase ± SD over this range: of 2.51 ± 1.0 mV for Group I and 1.8 ± 0.18 mV for Group II) with less significant increases for 20-60 pulses (average increase over this range: 1.21 ± 0.28 mV for Group I and 0.39 ± 0.76 mV for Group II, Fig. 2a, Supp. Table 1). An analysis of the change in response amplitude over number of pulses for these two ranges showed significantly larger increases in response amplitude over the 2–20 pulse range for both Group I and Group II responses (Mann–Whitney, $p < 0.05$ for Group I; $p < 0.01$ for Group II). On the other hand, the time to peak showed a positively monotonic relationship with the number of pulses (Fig. 2b). We observed mGluR responses with as few as 2 pulses, as long as the inter-pulse interval was less than approximately 75 ms, which is consistent with previous findings [30]; however, mGluR responses were never seen following a single pulse, regardless of stimulation intensity (Fig. 1I, J and Supp. Fig. 2).

Next, we assessed the effect of stimulation frequency on the mGluR responses. Increasing the frequency of stimulation once again caused an increase in response amplitude for both Group I and Group II mGluR responses (Figs. 1C, D and 2d). For Group I mGluR responses, response amplitude showed a logarithmic increase as stimulation frequency was increased and response duration showed a similar pattern (Fig. 2 d and f). Group I mGluR response time to peak increased across stimulation frequencies of 10–40 Hz by an average of 0.64 ± 0.9 s over that range (time to peak was significantly larger at 40 Hz than 10 Hz, Mann–Whitney,

[1] In the rodent, VPM is devoid of interneurons, while all cells we recorded from in layer 4 were regular-spiking.