Differential actions of group I mGluRs on human pyramidal neurons and fast-spiking interneurons


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Abstract

Metabotropic glutamate receptors (mGLuRs) are involved in remodeling of neuronal connectivity underlying memory and cognition in the brain. While their physiology has been carefully described in different brain areas of rodents, no data is available on mGLuR physiology in human neurons. Additionally, most rodent studies were performed in principal neurons. Here, we investigated the physiological effects of the group I subtypes of the mGLuR family that have been described to induce long-term depression in rodents when activated. The effect of group I mGLuR activation was investigated in human temporal cortex pyramidal neurons and fast-spiking interneurons. Both cell types showed an acute increase of excitatory postsynaptic currents during agonist application. After washout, spontaneous excitatory currents were only mildly modulated in pyramidal neurons while fast-spiking interneuron currents were markedly depressed. However, evoked currents in pyramidal neurons were significantly depressed for a prolonged period after application of a group I mGLuR agonist, indicating that activation of these receptors mediates significant plasticity changes in human neurons akin to what has been described in rodent models. This study offers the first physiological data-set on mGLuR activity in human temporal cortex pyramidal and fast-spiking interneurons showing that group I mGLuR activation leads to modulation of synaptic strength and long-term depression, similar to that observed in the rodent model.
Introduction

The metabotropic glutamate receptor family

The brain is by no means a rigid structure but is continuously remodeled to store new memories and to help adapt to internal and environmental changes. Multiple different signaling cascades have been described that mediate plasticity changes, amongst which G-protein coupled metabotropic glutamate receptors (mGluRs). At the cellular level, mGluRs are a large family of transmembrane proteins that respond to extracellular glutamatergic signals and transmit these via intracellular signaling pathways to alter cell activity (Willard and Koochekpour, 2013). While mGluR signaling studied in rodent brain circuits and in cell lines showed that a substantial subset of fundamental cellular processes are modulated by activation of mGluRs (see Niswender and Conn, 2010 for review), their effect on adult human circuits remains unknown. Therefore, in this study we set out to investigate plasticity of glutamatergic synapses on human pyramidal (Pyr) cells and fast-spiking interneurons (FS-INs) induced by mGluR activation. Specifically mGluR1 and mGluR5 that are combined to group I mGluRs are investigated, whose activation has been shown to cause synaptic depression in glutamatergic synapses in rodents (Fitzjohn et al., 1999; Huber et al., 2000; Mannaioni et al., 2001; Palmer et al., 1997).

Group I mGluRs are one of the three groups (I-III), the eight mGluR subtypes (mGluR1-8) have been assigned to, based on homology, G-protein subtype and sensitivity to neurotransmitters (Conn and Pin, 1997; Niswender and Conn, 2010). In rodent models, activation of group I mGluRs leads to Ca\(^{2+}\) influx and protein kinase C (PKC) activation through induction of hydrolysis of inositol-1,4,5-triphosphate (IP\(_3\)) and diacylglycerol (DAG) by phospholipase C\(_{\beta}\) (PLC) (Abe et al., 1992; Houamed et al., 1991; Masu et al., 1991; Niswender and Conn, 2010). Other downstream targets include the mitogen-activated protein kinase/extracellular receptor kinase (MAP/ERK) and the mammalian target of rapamycin (mTOR) pathways, known to regulate many essential cellular processes including cell growth and cell cycle (Hou and Klann, 2004; Niswender and Conn, 2010; Page et al., 2006). Thus, in rodent models, activation of the mGluR family triggers intracellular signaling cascades that modulate synapses and neuronal activity.

Distinct spatio-temporal expression pattern of mGluR subtypes

Neuronal expression of group I receptors is widespread for both mGluR1 (Baude et al., 1994; Petralia et al., 1997; Shigemoto et al., 1992) and mGluR5 (Romano et al., 1995; Romano et al., 1996; Shigemoto et al., 1993), but their exact spatiotemporal expression patterns differ. Whereas mGluR5 is detected embryonically in rodents and peaks early in life before it reaches adult levels, mGluR1 only increases expression levels postnatally and steadily increases to finally reach adult levels (Catania et al., 1994; Di Giorgi Gerevini et al., 1994; Petralia et al., 1997; Shigemoto et al., 1992).
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2004; Lopez-Bendito et al., 2002; Minakami et al., 1992; Romano et al., 1996; Ryo et al., 1993; Scheiwe et al., 2002; Shigemoto et al., 1992). Similarly, in humans mGluR5 but not mGluR1a immunoreactivity has been observed at 9 gestational weeks. At 13 gestational weeks mGluR1a immunoreactivity emerges while mGluR5 immunoreactivity persists (Boer et al., 2010). In rodent hippocampus and neocortex distinct spatial expression pattern can be seen for mGluR1 and mGluR5 (Catania et al., 1994; Fotuhi et al., 1994; Lein et al., 2007; Minakami et al., 1995; Shigemoto et al., 1997). While mGluR5 and the mGluR1b splice variant are mainly localized postsynaptically or extrasynaptically in principal neurons (Ferraguti et al., 1998; Lopez-Bendito et al., 2002; Lujan et al., 1996; Lujan et al., 1997) the mGluR1a variant has mainly been found in interneurons (Baude et al., 1994; Ferraguti et al., 1998; Lopez-Bendito et al., 2002; Martin et al., 1992; Petralia et al., 1997; Shigemoto et al., 1997; Stinehelfer et al., 2000). Similar findings have been described in human tissue but more data on the exact spatial distribution are needed especially in neocortical tissue to verify these expression patterns (Blumcke et al., 2000; Blumcke et al., 1996; Ong et al., 1998; Oka and Takashima, 1999; Aronica et al., 2003; Geurts et al., 2003; Boer et al., 2010 and in primates Muly et al., 2003).

Functional role of mGluRs in health and disease

Group I mGluRs have been shown to mediate synaptic plasticity (Stanton et al., 1991). In rodent CA1, concurrent activation of mGluR1 and mGluR5 with the selective group I agonist (S)3,5Dihydroxyphenylglycine (DHPG) induces long-term depression (LTD, Huber et al., 2001). mGluR-LTD is distinct from the earlier described N-methyl-D-aspartate (NMDA) receptor-mediated LTD and has been found in Pyr cells (Fitzjohn et al., 1999; Huber et al., 2000; Mannaioni et al., 2001; Palmer et al., 1997) as well as in interneurons (Le Duigou et al., 2011). The underlying group I mGluR-LTD mechanism involves α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPA) receptor internalization in addition to ERK and/or mTOR mediated local synaptic protein translation (Bellone et al., 2008; Hou and Klann, 2004; Huber et al., 2000; Page et al., 2006; Snyder et al., 2001; Volk et al., 2006). There are no data available describing the physiology of mGluR group I activation in human neurons.

The early expression onset, brain-wide distribution, and influence upon cell signaling and synaptic plasticity suggests that group I mGluR signaling could be implicated in the mechanisms underlying neurodevelopmental disorders (NDDs). Indeed, impaired cognitive function has been linked to group I mGluR signaling in the intellectual disability and autistic spectrum disorder fragile X syndrome (FXS, Bear et al., 2004; Meredith et al., 2012; Zoghbi and Bear, 2012). In FXS an expanded CGG repeat upstream of the FMR1 gene causes silencing of the fragile X mental retardation protein (FMRP) expression (Verkerk et al., 1991).
FMRF is a negative regulator of mGluR induced local translation (Bassell and Warren, 2008; Darnell et al., 2011; Weiler et al., 1997). One function of those locally translated proteins is to stabilize the mGluR induced AMPA and NMDA receptor internalization underlying LTD. In the FXS mouse model, group I-mediated DHPG induced hippocampal LTD is severely exaggerated – likely by uninhibited protein translation and therefore exaggerated stabilization of internalized AMPA receptors due to FMRP absence (Huber et al., 2002). Attenuation of mGluR5 expression or signaling, in both rodent and fly models of FXS, has shown to rescue several behavioral deficits and aberrant physiological phenotypes (Dolen et al 2007, Aschrafi 2005, Meredith 2011, Michalon et al., 2012, Gatto & Broadie, 2009, Gatto & Broadie, 2008, DeVrij et al., 2008). On the basis of these preclinical findings, clinical trials were set up to evaluate the efficacy of mGluR5-signaling attenuation in alleviating symptoms of patients suffering from FXS. Initially, promising results were obtained by inhibiting group I mGluRs directly or their downstream targets (Berry-Kravis et al., 2009; Berry-Kravis et al., 2008; Jacquemont et al., 2014; Jacquemont et al., 2011). However, recently clinical trials from different pharmaceutical companies ceased due to lack of significance upon their clinically defined endpoints for FXS phenotypes (www.clinicaltrials.gov; NOVARTIS NCT01348087 for AFQ056in adults and NCT01433354 for AFQ056 in adolescents; HOFFMAN-LAROCHE NCT01517698 for RO4917523 in adults and adolescents and NCT01750957 for RO4917523 in 5-13 year old children, Mullard 2015). To-date it is not clear why these trials failed.

mGluRs in human temporal cortex

Current literature lacks data about the physiology of group I mGluRs in humans not only on excitatory Pyr synapses but also on excitatory synapses on FS-INs. Understanding of mGluR physiology in the non-pathological human neocortex is essential to understand pathology in FXS. We propose that data on group I mGluR physiology in human pyramidal cells and interneurons can refine the working mGluR model for FXS pathophysiology that until now, is purely based on rodent glutamatergic synapses on principal neurons. This may alter our understanding of how mGluR signaling is affected in integrated neuronal circuits containing pyramidal and interneurons in human brain of FXS patients. On this basis, we addressed two fundamental questions regarding DHPG-mediated synaptic plasticity and cell type specific signaling in non-FXS human brain slices. Firstly, we tested whether in adult human temporal cortex DHPG-induced LTD of synaptic plasticity occurs at excitatory synapses upon pyramidal neurons, akin to that observed on pyramidal neurons in rodent brain slices. Secondly, we investigated how group I mGluR activation modulated excitatory synaptic function and plasticity upon identified interneuron subtypes in adult human temporal cortex. The novel data generated herein will enhance our understanding of group I modulation of excitatory synapses on Pyr cells and FS-INs in mature neurotypical human
neuronal networks. Additionally, these data will aid in expanding the working model for FXS pathophysiology by integrating group I mGluR modulation of neuronal activity upon both excitatory and inhibitory signaling cascades.

Methods

Patients and tissue preparation
All procedures carried out involving patient tissue were approved by the VU University Medical Center Medical Ethical Committee and in accordance with the Dutch law and the declaration of Helsinki. Written informed consent was provided by the patients before surgery. For this study tissue from 12 patients (6 male, 6 female) was used (Supplementary Table 6.1). Half of the patients suffered from mesial temporal sclerosis, the others from various other abnormalities causing drug resistant epilepsy. Anterior temporal lobe tissue was obtained from surgeries of these drug resistant epileptic patients. Non-pathological tissue was resected, in order to reach the epileptic foci in deeper brain structures. Resected neocortical tissue that showed no structural abnormalities in pre-surgery MRI scans and that was judged non-pathological by the resident pathologist of the VU University Medical Center was used for electrophysiological recordings in human tissue.

Immediately after dissection the tissue was stored in ice cold slicing solution containing (in mM): 110 Choline chloride, 26 NaHCO₃, 10 D-glucose, 11.6 sodium ascorbate, 7 MgCl₂, 3.1 sodium pyruvate, 2.5 KCl, 1.25 NaH₂PO₄ (Merck), and 0.5 CaCl₂ and transported into the laboratory within 15 minutes. The pia was removed from the tissue block in the same carbogenated solution and slices (350-450 µm) perpendicular to the gyrus and the white matter were obtained containing gray and white matter. Slices were left to recover for approximately 15 minutes in warmed 35°C carbogenated artificial cerebrospinal fluid (aCSF) containing (in mM): 125 NaCl, 26 NaHCO₃, 10 D-glucose, 3 KCl, 2 CaCl₂, 1 MgCl₂, and 1.25 NaH₂PO₄ and for minimally 90 more minutes at room temperature.

Electrophysiology
For electrophysiological recordings slices were placed under a microscope (LUMPlan FI/IR 60x objective, Olympus), held in place with a platinum harp and continuously perfused with warmed (~33 °C) and carbogenated aCSF. Borosilicate glass pipettes filled with intracellular solution containing (in mM): 148 K-gluconate, 1 KCl, 10 Hepes, 4 Mg-ATP, 4 K₂-phosphocreatine, 0.4 GTP and 0.2-0.5 % biocytin, adjusted with KOH to pH 7.3 and a resistance between 3 and 5.5 MΩ were used to patch cells. Pipettes were positioned using micro-manipulators (Luigs-Neumann). Recordings were obtained using a Multiclamp 700B amplifier (Axon CNS) and digitized by a Digidata 1440A (Axon CNS). Clampex 10 software (Molecular Devices) was used to record post synaptic currents and spike profiles.
Cell type identification and anatomical reconstruction

FS-IN and Pyr were selected in superficial layers based on cell body size and shape. After obtaining gigaseal configuration, neurons were opened in order to obtain whole cell recordings. A step profile (from -200 to 300 pA in 50 pA steps, Figure 6.1A) was recorded and the distinct short action potential halfwidth and high firing frequency of FS-INs compared to Pyr cells was used to distinguish Pyr cells and FS-INs (see results section for quantifications). Classical post-hoc DAB biocytin staining was used to anatomically confirm the cell type (Horikawa and Armstrong, 1988). A subset of cells was anatomically reconstructed following Streptavidin-Alexa Fluor 488 staining and confocal imaging (Figure 6.1B). Before imaging slices containing neurons for reconstruction were fixed in 4 % paraformaldehyde (PFA) at 4 °C for two days. After that, slices were washed (three times, ten minutes each) with phosphate buffered saline (PBS, containing in mM: 137 NaCl, 2.7 KCl, 10 Na₂HPO₄, pH 7.4). The slices were processed with a PBS + 0.5 % triton solution for one hour, and left to incubate with Streptavidin-Alexa Fluor 488 in 1:500 solution with PBS and 0.1 % Triton at room temperature. After two days slices were washed using PBS (four times, 10 minutes each) and dried and imbedded with Mowiol Polyvinyl Alcohol.

A laser-scanning confocal microscope (Nikon Instruments, Melville, NY, USA) was used, to acquire z-stacks of the neurons (10x magnification, NA 0.5, pinhole at 16.6 µm, pixel-resolution (x,y,z): 1.25x1.25x1 µm).

For reconstruction the freeware tool Neuromatic V1.7.5 and Neuroexplorer (MicroBrightfield Bioscience, Colchester, USA) was used. Reconstructed trees were exported to Neuroexplorer for measurement and analysis (Myatt et al., 2012).
Spontaneous EPSC recordings

Recordings of spontaneous excitatory post-synaptic currents (sEPSCs) were performed in the presence of 10 µM Gabazine in order to isolate glutamatergic currents on Pyr cells and FS-INs. Gabazine was washed in for at least 10 minutes before the first recording to ensure full blockade of γ-Aminobutyric acid (GABA)a currents. Before application of DHPG a 5 minute gap-free baseline was recorded in voltage clamp mode. After a 5 minute 25 µM DHPG wash-in, a 5 minute wash-out was allowed before a 5 minute recording was acquired (Figure 6.2A). 25 µM DHPG has been shown to reliably evoke LTD in CA3 (Huber et al., 2001)1. sEPSCs were analyzed using Minianalysis (Synaptosoft Inc., USA).

Evoked EPSC recordings

Evoked EPSC (eEPSC) recordings were only performed on Pyr neurons. To measure eEPSCs a stimulation pipette filled with aCSF and containing a unipolar stimulation electrode was positioned proximal to the patched neuron (within 100 µm). Current pulses ranging between 16 and 35 µV were applied (driven by an ISO-Flex stim box (A.M.P.I) and timed by an Master 8/9 (A.M.P.I)) and the stimulation pipette was moved to a position that elicited a distinctive response. Criteria for a distinctive response were a clear separation from the stimulation artifact, a single response current and a clear deviation upon stimulation. Pulses were applied every 15 seconds and a 10 minutes baseline was recorded when the amplitude became stable. To track the access resistance 100 ms square pulses of 10 mV were applied during each sweep. Access resistance was required to be below 15 MΩ at the onset of the recording and traces deviating more than 30 % from initial values during the course of the whole experiment were discarded. After recording the baseline, 25 µM DHPG was perfused for 5 minutes. After wash-in, eEPSCs were recorded for 60 minutes. Custom MATLAB (Mathworks) scripts were used to quantify the access resistance and the amplitude of the evoked currents over time. eEPSC amplitudes were normalized to the baseline amplitude and statistics were performed on 10 minute bins. In a subset of cells, recordings of eEPSCs were preceded by a gap-free recording in the presence of Gabazine, in order to obtain a baseline measure of sEPSC frequency for the acute sEPSC responses (n=3).

Acute DHPG responses

During 25 µM DHPG application gap-free recordings were performed for both spontaneous and evoked EPSC experiments. In a number of Pyr cells, DHPG application was followed by the occurrence of one or multiple ‘network events’ (see results). These events were defined as having an amplitude exceeding the regular sEPSC amplitude by more than 10 times and being prolonged in time lasting several hundred ms. Amplitudes could not be quantified, since events were usually truncated with the gain settings needed to quantify the sEPSCs.

1 Note that Huber et al., 2001 use 50 µM of a racemic mix of DHPG while we use an enantiopure version and therefore 25 µM.
To determine whether there are additional acute effects of DHPG application in the periods between network events as well as in traces without network events, sEPSC frequency and amplitude in periods lacking network events were analyzed using Minianalysis (Synapto-soft Inc., USA) and compared to baseline. There were not enough FS-INs to characterize network events at a statistically relevant level, but sEPSCs in periods without network events were quantified accordingly.

Statistics
Differences were accepted as significant for p-values smaller than p=0.05. SPSS (IBM) and Prism (Graphpad software) were used to perform statistical analysis.

For comparison of the spiking profiles and baseline sEPSCs, first data were tested for normality and sets of measurements that were significant for both the Kolmogorov-Smirnov and the Shapiro-Wilk test were analyzed using the non-parametric independent samples Mann-Whitney U test. All other measurements were analyzed using a two-sided unpaired student t-test. Where applicable, values adjusted for the differences in variances (tested with Levene’s test for similarity of variances) were used.

For spontaneous events (pre vs. post and pre vs. during DHPG) the same statistical analysis procedure was used except that a paired-t test and a related-samples Wilcoxon signed rank non-parametric test was used since the data were acquired in a repeated design.

Amplitudes of post DHPG eEPSCs were binned into 10 minute bins and normalized to baseline. One sample t-tests were performed to determine whether evoked responses deviated significantly from baseline after DHPG application.

Data presented as mean± standard error mean (SEM) throughout this chapter.

Results

Human pyramidal cells and fast-spiking interneurons of superficial temporal cortex show distinct spiking profiles

Neurons were recorded from superficial layers of human temporal lobe cortex. Two types of neurons were analyzed: Pyrs and FS-INs. Neuronal types were first identified electrophysiologically (Table 6.1, Figure 6.1A). Pyr cells were characterized by their slower action potential halfwidth (1.14±0.05 ms, mean ± SEM) compared to fast FS-IN (0.50±0.04 ms, p<0.01) and relatively greater interspike-interval (17.1±2.6 ms) compared to FS-IN (8.2±0.8 ms, p<0.01). Additional analysis of active and passive properties showed that neurons exhibited comparable resting membrane potential (Pyr: 71.3±4.7 vs. FS-IN: 71.2±8 mV, p=0.96) and AP threshold (Pyr: 41.7±1.0 vs. FS-IN: 41.8±1.8 mV, p=0.97). FS-INs exhibited significantly
smaller action potential peaks than Pyrs (73.0±1.6 vs. 83.2±2.1 mV, p<0.01). These data reflect differences seen in multiple neocortical brain regions of other species (McCormick et al., 1985, Chen et al., 1996) and allowed for reliable identification of cell type. Additionally, biocytin staining was used to post-hoc confirm anatomy of neuronal types in most cases (Figure 6.1B, reconstruction of a Pyr left and a FS-IN right based on Streptavidin-Alexa Fluor 488 stain).

Table 6.1: Analysis of active and passive properties of human temporal lobe Pyr cells and FS-INs.

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<tr>
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<th>Pyramidal cells</th>
<th>Fast-spiking Interneuron</th>
<th>Statistics</th>
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<tbody>
<tr>
<td>Resting membrane potential (mV)</td>
<td>-71.3±4.7</td>
<td>-71.2±8.0</td>
<td>t(14.1)=-0.06, p=0.96, ~</td>
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<tr>
<td>Input Resistance (MΩ)</td>
<td>86±10</td>
<td>220±24</td>
<td>t(28)=-5.97, p&lt;0.01</td>
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<tr>
<td>Tau (ms)</td>
<td>12.5±0.5</td>
<td>10.9±1.2</td>
<td>t(13.7)=1.18, p=0.26, ~</td>
</tr>
<tr>
<td>AP threshold (mV)</td>
<td>-41.7±1.0</td>
<td>-41.8±1.8</td>
<td>t(28)=0.94, p=0.97</td>
</tr>
<tr>
<td>Mean ISI (ms)</td>
<td>17.1±2.6</td>
<td>8.2±0.8</td>
<td>U(28)=39.50, p&lt;0.01, #^</td>
</tr>
<tr>
<td>Mean HW (ms)</td>
<td>1.14±0.05</td>
<td>0.50±0.04</td>
<td>t(27.7)=9.91, p&lt;0.01, ~</td>
</tr>
<tr>
<td>Mean AP peak (mV)</td>
<td>83.2±2.1</td>
<td>73.0±1.6</td>
<td>t(28.0)=3.88, p&lt;0.01, ~</td>
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~corrected t and df used due to inequality of variances tested with Levene’s test for equality of variances
^one outlier (>3*SD; removed from statistics)
#independent sample Mann-Whitney U test was used for non-normal distribution of values

AP peak: action potential height from threshold to peak, ISI: inter spike interval, HW: halfwidth
n=19 pyramidal cells and 11 fast-spiking interneurons

Activation of group I mGluRs
causes differential acute effects in Pyramidal cells and FS-INs

To determine the effect of mGluR activation upon human neurons, we set out to describe group I mGluR physiology in adult human pyramidal neurons and fast-spiking interneurons. First, the acute effects of group I mGluR activation was investigated using the selective group I mGluR agonist DHPG to verify that superficial layers of adult human temporal lobe cortex are affected by group I mGluR activation. During a five minute wash in period with 25 µM DHPG (Figure 6.2A), pyramidal neurons saw an increase in the amount of spontaneous sEPSCs (Figure 6.2B, inset) compared to activity in the same pyramidal neurons before and after DHPG application (Figure 6.2B top and bottom). In half of the pyramidal neurons recorded (9 of 19), acute DHPG application resulted in one or more periods of large synaptic bursts (Figure 6.2B, middle, 6.2D, see methods). Simultaneous recording of multiple pyramidal neurons (n=3 pairs/triplets) showed that these large bursts of sEPSCs always occurred
Figure 6.2: mGluR activation elicits comparable acute effects in human pyramidal cells and fast-spiking interneurons. (A) Experimental protocol for spontaneous EPSC recordings before, during and after DHPG application. (B) Typical voltage recordings of two synchronously recorded pyramidal neurons before (top), during (middle) and after (bottom) DHPG application. Huge synchronous network events are visible during DHPG exposure while spontaneous EPSCs (inset) remain uncorrelated. (C) Quantification of sEPSC frequency excluding periods with network activity demonstrates a significant increase of sEPSC frequency in pyramidal neurons. Data acquired during gap-free recordings before and during DHPG application of both spontaneous (solid lines) and evoked experiments (dashed lines). (D) Occurrence of network events in all pyramidal neurons recorded. (E) Quantification of sEPSC frequency of fast-spiking interneurons resembles the increase in frequency during DHPG exposure found in pyramidal neurons. n=19 pyramidal neurons, 7 fast-spiking interneurons.

synchronously in all neurons recorded (Figure 6.2B, middle) suggesting that they reflect a widespread response across the network. Therefore, these events are referred to as ‘network events’. In contrast, sEPSCs were uncorrelated (Figure 6.2B, inset). Quantification of sEPSCs
Differential actions of group I mGluRs on human neurons during DHPG application in Pyr cells, excluding those periods with network events, show no change in sEPSC amplitude (32±13 vs. 28±8 pA, p=0.41, data not shown). However, sEPSC frequency increased significantly upon DHPG wash-in (2.55±0.31 to 3.80±0.66 Hz, p=0.02, Figure 6.2C), confirming the acute effect of mGluRs on human Pyr cells.

Similar analysis was performed for FS-INs. Like Pyr cells, the frequency of excitatory input to FS-INs increased significantly upon DHPG application (from 0.7±0.11 to 1.5±0.5 Hz, Z(7)=2.028, p=0.04, Figure 6.2E) while all other readouts did not change significantly. There were not enough human FS-INs to quantify network events at a statistically significant level similar to those recorded in Pyr cells. Together these data demonstrate that acute group I mGluR activation causes a transient increase in synaptic excitation of both Pyr neurons and FS-INs in human temporal cortex and, for a subset of Pyr neurons in the cortical network, can lead to synchronous excitatory bursting.

**Lasting effects of group I mGluR activation on sEPSCs in Pyr cells and FS-INs**

Acute application of a group I mGluR agonist causes immediate increases in synaptic excitation in human Pyr cells and FS-InNs neurons. However, the function of group I mGluRs described in animal models in literature involves lasting changes of synaptic strength on medium and longer timescales after cessation of mGluR stimulation (Huber et al., 2001). Therefore, spontaneous and evoked glutamatergic currents were recorded before and after exposure to DHPG (Figure 6.3A,B).

Spontaneous events, reflecting AMPAR currents (see material & methods), were recorded during baseline conditions from Pyr cells (n=14) and FS-interneurons (n=7). The frequency of sEPSCs was significantly higher in Pyr cells (2.63±0.31 Hz) than in FS-Ins (0.72±0.12 Hz, p<0.001), while FS-INs had a higher amplitude (32.26±5.53 pA) compared to Pyr cells (19.06±1.25 pA, p<0.001). Both rise and decay times of sEPSCs were significantly larger in Pyr cells (rise: 2.07±0.12 ms, decay: 4.99±0.32 ms) than in the FS-INS (rise: 0.71±0.10 ms, p<0.0001, decay: 2.81±0.33 ms, p<0.001).

The lasting effects of DHPG exposure were assessed after a 5 minute washout period (Figure 6.2, top). In Pyr cells, the frequency (2.62±0.30 Hz, p=0.99), the amplitude (18±1.6 pA, p=0.59) and the rise time (2.13±0.12 ms, p=0.10) of spontaneous EPSCs were not different from before activation of the group I mGluRs. However, a small but consistent increase in decay time (5.37±0.41 ms, p=0.04) was observed (Figure 6.3A).

In contrast to the weak lasting effects of transient group I mGluR activation in human pyramidal neurons, distinct effects were observed in FS-INs. While the frequency of sEPSCs did not change (0.72±0.12 vs. 0.91±0.27 Hz, p=0.43), a clear decrease of sEPSC amplitude was observed (from 37±5.5 to 24±2.8 pA, p=0.03; Figure 6.3B). At the same time the rise and
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Figure 6.3: sEPSCs of human pyramidal cells and fast-spiking interneurons are differentially affected after DHPG application. (A) Typical examples of sEPSCs in human pyramidal neurons before and after DHPG application (top). A slight increase in decay time was observed (right bottom) while frequency, amplitude and rise-time remained unaffected by DHPG. (B) Representative traces of fast-spiking interneurons before and after activation of group I mGluRs. DHPG application induced a significant decrease of sEPSC amplitude, accompanied by a significant increase in rise and decay time. No change of frequency was observed. n=14 pyramidal neurons, 7 fast-spiking interneurons. Scalebars: 10 pA, 100 ms

decay time of the currents significantly increased (from 0.72±0.10 to 0.96±0.15 ms, p=0.01; from 2.81±0.33 to 3.88±0.62 ms, p<0.05; Figure 6.3B). Interestingly the area of the sEPSCs did not change (73±14.6 vs. 77±18.3 pA*ms, p=0.95; data not shown) indicating that the same amount of charge is transferred per synaptic event but that the duration of charge transfer is broadened.

In summary, sEPSC properties recorded in Pyr cells and FS-INs are markedly different at baseline. After group I mGluR activation sEPSCs of pyramidal neurons are at most weakly affected, while sEPSCs of interneurons show a clear depression of sEPSC amplitude and a slowdown of sEPSC kinetics.
Prominent and sustained effect of DHPG on evoked responses in pyramidal cells

The long lasting changes on sEPSCs in human Pyr neurons induced by DHPG application were minimal despite clear acute effects of group I mGluR activation. This could be due to differential effects of DHPG on the different combination of inputs e.g. both proximal and distal synaptic synapses that are measured in sEPSCs. To isolate the effect of DHPG on proximal synaptic inputs, we studied local evoked responses in Pyr neurons before and up to 60 min after DHPG application (Figure 6.4A) to determine whether human neurons exhibited mGluR-induced LTD.

Activation of group I mGluRs induced a long-lasting synaptic depression in proximal synaptic inputs to pyramidal neurons (n=6, Figure 6.4Bi, Bii 0-10 min: 58±14 % of original EPSC size, 50-60 min: 70±22 %). The initial change in synaptic response was clear in all neurons recorded but variability in plasticity was observed after 20 minutes, which suggested that there might be subgroups of neurons behaving differently (Figure 6.4Bii). Closer inspection of individual neurons revealed four cells with a clear sustained LTD (Supplementary Figure 6.1A) and two Pyrs with an initial depression followed by a slight potentiation or no change of the evoked currents (supplementary Figure 6.1B).

There are two effects of DHPG that could explain the LTD observed. First the activation of group I mGluRs could have increased the number of failures to evoke an eEPSC or it could directly affect the amplitude. Detailed analysis revealed that of the neurons exhibiting LTD, three out of the four showed a clear increase in the number of failures, defined as evoked currents with an absolute amplitude smaller than 10 pA (between 3.00 and 8.44 fold). Additionally, the magnitude of the amplitudes of the evoked responses not classified as failures consistently decreased to on average 49 % of the original magnitude during the first 10 min

Figure 6.4: Human pyramidal neurons show marked LTD of evoked responses upon DHPG application. (A) i Experimental protocol and ii setup of evoked EPSC recordings. (B) Typical course of amplitude development of a pyramidal neuron during before and after DHPG application. Average responses before (blue), right after (0-10 min, dotted line) and at the end of the experiment (black, 50-60 min) are indicated. Scalebars: 100pA, 5ms. (C) Normalized amplitude of evoked responses in % of baseline show an average decrease of evoked EPSC amplitude compared to baseline (blue dotted line) after DHPG application. n=6 pyramidal neurons.
Differential actions of group I mGluRs on human neurons after DHPG (Figure 6.4B, inset). Thus for those neurons showing sustained LTD the depression seems to result from both a marked increase in failures and a clear decrease in the amplitude of the remaining responses. In summary, in contrast to a weak effect of DHPG application of sEPSCs in pyramidal neurons, eEPSCs were markedly and persistently decreased.

Discussion

Our data demonstrate that synaptic excitation of human Pyr cells and FS-INs is modulated by group I mGluRs. Activation of these receptors leads to acute effects in both cell types, increasing the sEPSC frequency and at least in a subset of Pyr neurons, evoking network events. Following DHPG application, Pyr sEPSCs are largely unaffected with only a slightly slower synaptic decay while a strong decrease in amplitude, decay time and rise time is observed in FS-INs. However, in Pyr neurons, DHPG induces a long lasting decrease in synaptic efficacy, thus a long term depression (LTD), of a proximal synaptic input for up to 60 min after induction. This study demonstrates for the first time the physiological effects of group I mGluR activation by DHPG in human temporal cortex circuits, in both Pyr cells and FS-INs.

Recordings of locally evoked EPSCs show that activation of group I mGluRs has a quick and sustained depressing effect on proximal synaptic inputs to Pyr neurons. Therefore, it can be concluded that, like in the juvenile rodent model (Huber et al., 2001), activation of group I mGluRs can cause LTD in human Pyr neurons from human temporal cortex. Specific forms of synaptic depression, namely spike-timing dependent LTD that relies on NMDA receptors, are demonstrated to be developmentally-regulated in rodent cortex, with reduced tendency or no LTD induction reported after late adolescence and into adulthood (Banerjee et al., 2009 in cortex; Dudek and Bear, 1993 and Bear and Abraham, 1996 in hippocampus; Crair and Malenka, 1995 in thalamocortical synapses). Group I mGluR-induced LTD is expressed in adult stages of rodent development and, as demonstrated here, in adult human cortex as well. Persistence of this form of LTD into adulthood may reflect in part the continued upregulation of the mGluR1 receptor subtype expression postnatally, at least in rodent brain (rodent: Shigemoto et al., 1992; Minakami et al., 1992; Ryo et al., 1993; Catania et al., 1994; Scheiwe et al., 2002; Lopez-Bendito et al., 2002; Di Giorgi Gerevini et al., 2004; human: Boer et al., 2010). Further, it may implicate a role for this form of LTD to regulate synaptic strength in the mature nervous system, perhaps as a homeostatic mechanism to downregulate synaptic excitation in response to strong or excessive glutamatergic network excitation (Hu et al., 2010).

mGluR-LTD in mature human Pyr neurons validates occurrence of this type of plasticity in the non-pathological human brain. This implies that the mechanism underlying the mGluR theory for fragile X syndrome could also apply to mature human neurons. However, it was
not possible with these data to experimentally test and validate this theory in human brain. In this study, the human tissue used was from surgical patients who did not present with a genetic form of neurodevelopmental disorder such as fragile X syndrome. Brain tissue suitable for physiology from fragile X patients is not available. An option to assess group I mGluR physiology would be to use human stem cell derived neurons from fragile X syndrome patients. This would allow the effect of the lack of FMRP in human neurons to be studied. However, a caveat of this study is that neurons cultured in this manner would not be embedded in a normally developed intact brain network and therefore would have missed most of the signals from other brain regions and surrounding non-neuronal cells that are essential during phenotypic development of the brain. Consequently, the effect of the lack of FMRP on mGluR physiology during development in human tissue could not be assessed in such a study.

There is a growing body of evidence suggesting that synaptic plasticity rules can differ markedly between brain regions and mammalian species. For example, spike timing-dependent plasticity in juvenile and adult rodent somatosensory cortex and hippocampus requires a ‘pre-post’ pairing paradigm to induce LTP and a ‘post-pre’ paradigm for inducing LTD (Banerjee et al., 2009; Bi and Poo, 1998; Levy and Steward, 1983; Markram et al., 1997). However, pyramidal neurons in both adult rodent and human temporal cortex have been shown to have more or less reversed spike timing-dependent plasticity rules, with LTP being induced in response to a ‘post-pre’ order of pairing (Verhoog et al., 2013). As yet, no rodent data akin to the human data set in this chapter exists from rodent temporal cortex at comparable ages, and so a direct comparison of human and rodent group I mGluR physiology can presently not be made. Nevertheless, the presented data suggests that for cortical pyramidal neurons, group I mGluRs in human tissue act in a manner comparable to group I mGluRs in hippocampus of rodent animal models (Huber et al., 2000).

It is striking that the lasting effects of group I mGluR activation upon spontaneously active synaptic AMPA receptors are very different for the neuronal subtypes in human cortical tissue. In contrast to the weak effect of DHPG on sEPSCs in Pyrs, FS-INs showed a strong depression of sEPSC amplitude accompanied by an increase in rise and decay time. Interestingly, the area of the sEPSCs did not change significantly, meaning that the same amount of current is transmitted during each event but that its duration is prolonged. This increased event duration might allow for a broader time-window during which integration with other inputs can occur in the cell body. Thus, group I mGluRs have a modulatory effect on integration properties of excitatory synapses on FS-INs but this effect is markedly different from group I mGluR induced changes in Pyr cells. Strikingly, there is only a minor change in decay time of sEPSCs in Pyr neurons that persists after DHPG application ended. This
lack of lasting effects of group I mGluR activation on synaptic efficacy in human Pyr cells could be due to differential effects on the mixture of inputs at proximal and distal regions or from axonal connections of different presynaptic partners that are reflected in the overall output of sEPSCs. Another possibility is that there is a delay in effect of the intracellular signaling cascades that cause LTD, although this seems unlikely since DHPG is able to induce decreases in synaptic efficacy within the first 10 minutes following application in classical mGluR-LTD (Huber et al., 2001; Snyder et al., 2001), and as seen in evoked recordings (Figure 6.4, evoked LTD). Given the postsynaptic location of group I mGlu receptors, it is assumed that the locus of change and altered signaling pathways is, at least in part, postsynaptic (Snyder et al., 2001). However, such a clear difference in the ability of DHPG to induce LTD of an evoked input pathway but not of spontaneous synaptic inputs could reflect a change in presynaptic transmission, supported by the increase of failures observed in some evoked LTD experiments (Figure 6.4). In other preparations this has been shown to be the case for DHPG-induced LTD (Fitzjohn et al., 2001; Xu et al., 2013). Multiple different mechanisms can be proposed whereby a selective change in presynaptic transmission in one pathway could occur. One recent study in reduced culture preparations suggests that spontaneous synaptic transmission may utilize different synaptic vesicle release pathways than those recruited during evoked synaptic transmission (Kavalali, 2015). Other studies have shown that the endocannabinoid system can be activated by group I mGluRs (Varma et al., 2001). For example, activation of the cannabinoid receptor 1 reduces the presynaptic vesicle release probability (Gerdeman and Lovinger, 2001). Thus group I mGluR stimulation could selectively modulate one presynaptic pathway on human Pyr neurons in superficial layers of temporal cortex.

In human slice neuronal networks so called sharp waves have been described, waves of prolonged bursting activity throughout the network. It has been shown that these waves are abolished upon GABAa blockade (Kohling et al., 1998). Since experiments have been performed in the presence of the GABAa blocker gabazine, the acute network events observed upon DHPG application in our experiments must be a different type of network activity. The mechanisms underlying DHPG induced network events or long-term epileptogenesis are not clear yet. For both group I mGluR subtypes, their joint activation via G proteins of the Gq type results in activation of phospholipase C, leading to calcium release from intracellular stores. DHPG application in acute brain slices is proposed to mediate network bursting via activation of a voltage-gated cationic current, $I_{\text{mGluR(V)}}$ and via suppression of the afterhyperpolarisation (AHP) phase following cell spiking. $I_{\text{mGluR(V)}}$ is inactive in the hyperpolarized state but is activated during depolarization and is a non-inactivating current. This current may also underlie the suppression of AHPs observed since observations also report prolonged afterdepolarisation (ADP) to effectively broaden the cell spike and prolong
excitation. Baseline sEPSC kinetics were significantly different between human Pyr cells and FS-INs with Pyr neurons receiving more sEPSCs but with a lower amplitude and slower rise and decay times than FS-INs. These results are comparable to data from different brain regions in rodents (Dantzker and Callaway, 2000; Hestrin, 1993). In addition to the differences between neuronal types, evoked recordings suggest that there might be subpopulations within the Pyr cells as well, one type experiences only initial LTD while others sustain prolonged depression of synaptic strength. However, our present sample is not large enough to verify this and to identify distinguishing morphological and/or physiological properties that could explain these subgroups. Future research is needed in order to classify Pyr subtypes in human neocortex.

Finally, the immediate and short-term consequences of group I mGluR activation upon synaptic function and neuronal excitability in human Pyr cells and FS-INs are demonstrated. To determine whether the alterations in synaptic efficacy of both pyramidal and interneurons are due to direct activation by mGluRs, co-localisation immunocytochemical studies are required to correlate receptor expression with particular neuronal types. Further, immune-electron microscopy (EM) would provide a more precise picture of receptor localization in human neurons to determine whether mGluR1 and mGluR5 are also predominantly expressed postsynaptically, as in rodent brain (Shigemoto et al., 1997).

Taken together, this study offers the first dataset on group I physiology in human temporal cortex. Functional group I mGluRs exist in human cortex; their activation leads to LTD similar to data from rodent neocortex and hippocampus, sparking hopes that group I mGluR attenuation can be used in fragile X syndrome patients. However, future studies have to investigate the specific molecular pathways and compare the reported findings to data from rodent temporal cortex at matching ages.
Supplementary material

![Graphs showing LTD course of individual pyramidal neurons]

**Supplementary Figure 6.1**: LTD course of individual pyramidal neurons. (A) Absolute responses of Pyramidal neurons showing a sustained LTD and corresponding spike profiles. (B) Absolute responses of Pyramidal neurons only displaying an initial depression upon DHPG application.

**Supplementary Table 6.1**: Patient data.

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<th>Gender</th>
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Summary: Mean=35.3, 6 female, 6 male
References


Differential actions of group I mGluRs on human neurons


Differential actions of group I mGluRs on human neurons


Ryo, Y., Miyawaki, A., Furuichi, T., and Mikoshiba, K. (1993). Expression of the metabotropic glutamate receptor mGluR1alpha and the ionotropic glutamate receptor GluR1 in the brain during the postnatal development of normal mouse and in the cerebellum from mutant mice. Journal of neuroscience research 36, 19-32.


