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1 **Voltage tunes mGlu5 receptor function, impacting synaptic transmission**

2 Marin Boutonnet¹, Camille Carpena¹, Nathalie Bouquier¹, Yan Chastagnier¹, Joan Font-
3 Ingles^{1,2}, Enora Moutin¹, Ludovic Tricoire³, Jean Chemin¹, Julie Perroy^{1*}

4

5 ¹IGF, University of Montpellier, CNRS, INSERM, Montpellier, France.

6 ²Present address: SpliceBio, Barcelona, Spain.

7 ³Neuroscience Paris Seine, Institut de biologie Paris Seine, Sorbonne universite, CNRS,
8 INSERM, Paris, France

9

10 *Corresponding author: julie.perroy@igf.cnrs.fr

11

12 **Short title:** Voltage-dependent control of synaptic transmission by GPCRs

13 **Key words:** G-Protein Coupled Receptors (GPCR), metabotropic glutamate receptor (mGlu5),
14 voltage sensitivity, signaling, biosensors, synaptic transmission, neuronal plasticity

15 **Data Availability Statement** - The data that support the findings of this study are available
16 from the corresponding author upon reasonable request. Some data may not be made
17 available because of privacy or ethical restrictions.

18

19 **Bulleted point summary**

20 *What is already known:*

21 - Some of the GPCRs are voltage-sensitive.

22 *What this study adds:*

23 - mGlu5 activation and signaling are optimal at resting membrane potential of neurons.

24 - mGlu5 primes NMDA receptor activation at the resting potential of neurons

25 *Clinical significance:*

26 - mGlu5 and/or NMDA receptors involvement in inducing plasticity may depend on recent
27 neuronal activity

28 - mGlu5 voltage-sensitivity may play a critical role in pathologies associated with variations
29 in intrinsic excitability

30

31

32 **Abstract**

33 Background and Purpose

34 Voltage sensitivity is a common feature of many membrane proteins, including some G-
35 protein coupled receptors (GPCRs). However, the functional consequences of voltage
36 sensitivity in GPCRs are not well understood.

37 Experimental approach

38 In this study, we investigated the voltage sensitivity of the post-synaptic metabotropic
39 glutamate receptor mGlu5 and its impact on synaptic transmission. Using biosensors and
40 electrophysiological recordings in non-excitabile HEK293T cells or neurons.

41 Key Results

42 We found that mGlu5 receptor function is optimal at resting membrane potentials. We
43 observed that membrane depolarization significantly reduced mGlu5 receptor activation, Gq-
44 PLC/PKC stimulation, Ca²⁺ release, and mGlu5 receptor-gated currents through TRPC6
45 channels or NMDA receptors. Notably, we report a previously unknown activity of the NMDA
46 receptor at the resting potential of neurons, enabled by mGlu5.

47 Conclusions & Implications

48 Our findings suggest that mGlu5 receptor activity is directly regulated by membrane voltage
49 which may have a significant impact on synaptic processes and pathophysiological functions.

50 **Introduction**

51 G-protein coupled receptors (GPCRs) are 7-transmembrane receptors involved in numerous
52 physiological processes and remain the most extensively targeted protein family by approved
53 drugs (Lagerström and Schiöth, 2008). Recent research has unveiled that the activity of certain
54 GPCRs can be modulated by the membrane voltage (V_m), about thirty out of the thousand
55 members of this big family, including acetylcholine (Ben-Chaim et al., 2003, 2006; Navarro-
56 Polanco et al., 2011; Rinne et al., 2015), purine (Martinez-Pinna et al., 2005), opioid (Ruland
57 et al., 2020), dopamine (Sahlholm et al., 2008), and prostanoid receptors (Kurz et al., 2020).
58 In neurons, where V_m undergoes permanent changes, this emerging property could
59 significantly impact the functioning of neurotransmitter-activated GPCRs and their role as
60 modulators of synaptic transmission. Although the structural mechanisms that underlie the
61 sensitivity of GPCRs to V_m are still being elucidated (Ohana et al., 2006; Rinne et al., 2015;
62 Barchad-Avitzur et al., 2016; Hoppe et al., 2018; López-Serrano et al., 2020), functional studies
63 using site-directed mutagenesis of the V_m sensor suggest that GPCR activity can be affected,
64 leading to impaired neurotransmitter release (Kupchik et al., 2011; Zhang et al., 2020) or
65 synaptic plasticity and behavior (Rozenfeld et al., 2021).

66 Glutamate is the primary excitatory neurotransmitter in the brain that binds to both
67 ionotropic (AMPA, NMDA, Kainate receptors) and metabotropic glutamate (mGlu) receptors.
68 The mGlu receptor family, comprising eight G protein-coupled receptors (mGlu1-8), has been
69 extensively studied for their modulatory role in synaptic transmission and plasticity
70 (Scheefhals and MacGillavry, 2018). Preclinical and clinical studies have targeted these
71 receptors in various neurological disorders, such as Autism, Fragile X syndrom, Schizophrenia,
72 Parkinson's, and Alzheimer's disease (Niswender and Conn, 2010). So far, only one study, in

73 the *Xenopus* oocytes expression system, suggests a direct sensitivity of some mGlu receptors
74 to V_m (mGlu1 and 3, (Ohana et al., 2006)). Yet, due to their synaptic location, these receptors
75 are permanently exposed to membrane voltage fluctuations. Therefore, demonstrating the
76 sensitivity of mGlu receptors to V_m could provide insights into their role depending on the
77 state of neuronal activity. In this study, we have selected the postsynaptic mGlu5 receptor as
78 a model receptor, known for its role as neuromodulator of synaptic transmission. mGlu5 is
79 also a key trigger in the induction of synaptic plasticity, working in concert with ionotropic
80 NMDA receptors through physical and functional crosstalk (Reiner and Levitz, 2018).
81 Interestingly, NMDA receptors are a prototype of receptors whose activity is regulated by the
82 membrane potential. As a detector of neuronal activity coincidence, NMDA activation is
83 limited to synapses whose pre- and postsynaptic elements are activated simultaneously
84 (Seeburg et al., 1995; Kwag and Paulsen, 2012). Thus, a sensitivity of the mGlu5 receptor to
85 V_m could similarly specify the identity of synapses on which mGlu5 exerts its functional effects
86 depending on the neuron's activity history.

87 In this report, using a variety of biosensors and patch clamp recordings, we explore and
88 investigate the impact of V_m on the intricate signaling pathways associated with the
89 conformational change of postsynaptic mGlu5 receptor induced by glutamate binding. This
90 conformational change leads to the activation of $G_{q/11}$ -type proteins, the activation of the PLC-
91 PKC pathway, and the subsequent release of Ca^{2+} from internal stores through IP3 receptors
92 (Niswender and Conn, 2010), ultimately fine-tuning synaptic transmission by gating channels
93 and ionotropic receptors, including NMDA (Aniksztejn et al., 1992; Mannaioni et al., 2001;
94 O'Neill et al., 2018), AMPA (Hu et al., 2010; Holz et al., 2019), and transient receptor potential
95 canonical (TRPC) channels (Lepannetier et al., 2018). Our data reveals that the mGlu5 receptor
96 functions optimally at voltage close to the resting potential of the cells.

97

98

99 **Results**

100

101 *V_m regulates mGlu5-induced Ca²⁺ release*

102 To investigate the effect of V_m on mGlu5 function and determine the optimal experimental
103 conditions, we examined mGlu5-induced Ca²⁺ release from intracellular stores. mGlu5
104 stimulation is known to induce a transient increase of Ca²⁺ oscillations in various cell types,
105 including cultured astrocytes (Nakahara et al., 1997), hippocampal neurons (Rae et al., 2000),
106 midbrain neurons (O'Malley et al., 2003), as well as in heterologous expression systems such
107 as Chinese Hamster Ovary cells (Nash et al., 2002) and Human Embryonic Kidney 293T
108 (HEK293T) cells (Gómez-Santacana et al., 2017). In line with these findings, in HEK293T cells
109 expressing the GCaMP6s fluorescent Ca²⁺ sensor (**Figure 1A**), mGlu5 agonist (RS)-3,5-
110 dihydroxyphenylglycine (DHPG, 100 μM) induced a Ca²⁺ increase exclusively in cells expressing
111 mGlu5, as evidenced by GCaMP6s fluorescence fluctuations measured in the cell population
112 using a fluorimeter (**Figure 1B**). The mGlu5 agonist-induced Ca²⁺ increase in mGlu5-expressing
113 cells was inhibited by application of MPEP (10 μM), a mGlu5-specific negative allosteric
114 modulator (NAM), confirming the requirement of mGlu5 receptor activation (**Figure 1B**).

115

116 In order to determine the effect of V_m on mGlu5-induced Ca²⁺ increase, we treated cells with
117 equimolar solutions containing increasing concentrations of KCl (from 3 mM, a physiological
118 concentration referred to as "V_{rest}", up to 100 mM) to induce a systematic, well-controlled,

119 and long-lasting membrane depolarization (as previously demonstrated in neurons (Moutin
120 et al., 2021)). Interestingly, KCl 100 mM had no effect on basal intracellular Ca^{2+}
121 concentrations (**Figure Supp. 1A**), but significantly reduced mGlu5 agonist-induced Ca^{2+}
122 increase by $22.3 \pm 7.1\%$ compared to KCl 3 mM (**Figure 1C**). We measured that KCl 100 mM
123 application increased the membrane potential by 23.6 ± 0.9 mV (**Figure 1D**), referred to as
124 “ V_{depol} ”. Whole cell patch clamp recordings indeed reported a reversible switch from $V_{\text{rest}} = -$
125 28.4 ± 3.5 mV at physiological KCl concentration (3 mM) to $V_{\text{depol}} = -4.8 \pm 0.9$ mV with KCl 100
126 mM (**Figure 1D**). The dose response curve of DHPG-induced Ca^{2+} release further revealed a
127 reduction of the agonist efficacy at V_{depol} compared to V_{rest} (**Figure 1E**). Additionally, saturating
128 concentrations of another mGlu5 agonist, quisqualate (10 μ M), triggered a Ca^{2+} increase at
129 V_{rest} , which was reduced by $45 \pm 9.5\%$ at V_{depol} (**Figure 1F**). These initial findings indicate that
130 the ability of the mGlu5 receptor to increase intracellular Ca^{2+} is dependent on V_m . To
131 investigate whether the effect of V_m was specific to the mGlu5 receptor, we examined another
132 G_q -coupled receptor, the angiotensin II type 1 receptor (AT1), and did not observe any
133 influence of V_m on AT1 receptor-mediated Ca^{2+} release, thereby supporting a specific effect of
134 V_{depol} on mGlu5 signaling in this context (**Figure 1G**).

135 To investigate the effect of V_m on mGlu5-induced Ca^{2+} increase at a cellular level, we
136 performed live cell imaging of GCaMP6s fluorescence (**Figure 2A** and **supplemental Video1**).
137 Initially, large recordings in the entire field of view showed a global increase in Ca^{2+} induced
138 by the mGlu5 agonist, quisqualate (10 μ M), at V_{rest} . This increase was significantly reduced at
139 V_{depol} (30 \pm 10 % reduction compared to V_{rest} at the peak of fluorescence, **Figure 2B** and
140 **supplemental Video2**). The global Ca^{2+} increase was an average of the single cell Ca^{2+}
141 fluctuations, which were classified into 3 distinct populations of cells depending on the type
142 of responses: silent cells, cells displaying 1 single Ca^{2+} spike, and Ca^{2+} oscillating cells (**Figure**

143 **2C**), as reported previously (Bradley et al., 2009, 2011). V_{depol} modified the distribution of cells
144 in each of these categories, with a global shift towards less oscillating populations (**Figure 2C**),
145 and a significant reduction in the proportion of cells with an oscillating Ca^{2+} pattern (10.6%
146 reduction of the proportion of oscillating cells compared to V_{rest} , **Figure 2D**). Subsequently, for
147 each oscillating cell, we studied the global frequency of Ca^{2+} oscillations induced by mGlu5
148 agonist. Quisqualate induced a mean of 0.49 ± 0.02 oscillations/min per cell at V_{rest} , but only
149 0.39 ± 0.02 oscillations per cell at V_{depol} . Thus, V_{depol} induced a $20.3 \pm 4.8\%$ reduction in the
150 frequency of Ca^{2+} oscillations triggered by mGlu5 stimulation (**Figure 2E**).

151 Given that depolarization in neurons can also occur following receptor stimulation, we
152 investigated the impact of V_{depol} subsequent to mGlu5 activation. Specifically, we measured
153 the effect of V_{depol} compared to V_{rest} on oscillating cells that had undergone Ca^{2+} oscillations at
154 resting membrane potential as a result of mGlu5 stimulation. To do so, we determined the
155 instantaneous frequency, which represents the average inter-oscillation frequency between
156 the first and last oscillations. Our findings consistently revealed a significant reduction in the
157 instantaneous frequency, from 1.18 ± 0.11 at V_{rest} to 0.85 ± 0.04 oscillation/min at V_{depol} ,
158 representing a $28 \pm 4.7\%$ decrease (**Figure 2F**). These results suggest that a membrane
159 depolarization of approximately 20-25mV, whether it occurs before or after mGlu5 activation,
160 can diminish the receptor's ability to induce Ca^{2+} release from intracellular stores, in few
161 seconds. Specifically, V_{depol} reduced the number of agonist-responsive cells and the efficiency
162 of the responding cells, resembling the characteristics of a NAM (Bradley et al., 2009, 2011).

163

164 *V_m tunes mGlu5 receptor probability of activation*

165 The reduced ability of mGlu5 receptor to induce Ca^{2+} release from intracellular stores at V_{depol}
166 may be attributed to a decrease in the expression of receptors at the plasma membrane
167 and/or a diminished activation of these receptors under depolarized conditions. To investigate
168 the first hypothesis, we measured the receptor cell surface expression using an enhance
169 bystander Bioluminescence Resonance Energy Transfer (ebBRET) biosensor. ebBRET is based
170 on naturally interacting chromophores luciferase (RLuc) and green fluorescent protein (rGFP)
171 from *Renilla reniformis*, enabling to quantify the cell surface expression of an RLuc-tagged
172 protein expressed with the rGFP anchored at the plasma membrane by a CAAX sequence
173 ((Namkung et al., 2016) **Figure Supp. 2A**). In mGlu5-RLucII and rGFP-CAAX co-transfected cells,
174 incubation of MPEP for 5 to 30 minutes resulted in an expected increase in receptor
175 expression at the cell surface, as reported by an increase in ebBRET (**Figure Supp. 2B**). This
176 control experiment validated the use of the ebBRET biosensor. However, we did not observe
177 any ebBRET variation induced by shorter (2 min) applications of MPEP or quisqualate, nor by
178 V_{depol} alone or combined with these ligands (**Figure Supp. 2C**). Therefore, this experiment ruled
179 out any potential effect of V_{depol} on the receptor cell surface expression, in these experimental
180 conditions.

181 To investigate the proportion of active and inactive mGlu5 receptors at the cell surface, we
182 then used a Time-Resolved Fluorescence Resonance Energy Transfer (TR-FRET) biosensor,
183 reporting structural dynamics and drug action at the mGlu5 receptor (Doumazane et al., 2013;
184 Scholler et al., 2017). The conformational changes of the extracellular ligand-binding domains
185 (ECDs) of mGlu5 dimers are associated with receptor activation: SNAP-mGlu5 (ST-mGlu5)
186 labeled with the Lumi4-Tb donor and the fluorescein acceptor, display a high FRET signal in
187 the absence of ligand or in presence of the antagonist and a low FRET signal in the presence
188 of glutamate or other orthosteric agonists (**Figure 3A**). Any approach expected to stabilize the

189 active conformation of the effector domain increases the agonist potency in stabilizing the
190 active ECDs conformation (Doumazane et al., 2013). Hence, TR-FRET measurements allowed
191 to determine the proportion of active and inactive ST-mGlu5 receptors, depending on V_m .
192 Importantly, labelling mGlu5 receptors separately on distinct cell populations, we first
193 controlled that neither the fluorescein nor the Lumi4-Tb emissions were affected by V_{depol}
194 (**Figure Supp 1B**), ruling out any effect of V_{depol} on the performance of the biosensor. In ST-
195 mGlu5 expressing cells co-labeled with Lumi4-Tb and fluorescein (**Figure 3B**), quisqualate
196 (10 μ M) induced a decrease in TR-FRET at V_{rest} ($-17.2 \pm 4.7\%$), reporting an agonist-induced
197 increase of mGlu5 receptors in an active-like conformation. However, TR-FRET intensities
198 measured in presence of quisqualate were significantly increased at V_{depol} ($+7.0 \pm 2.6\%$)
199 compared to V_{rest} , indicating that V_{depol} reduced the ability of the agonist to trigger
200 conformational activation of the receptor (**Figure 3B**). This lower efficiency of quisqualate at
201 V_{depol} to decrease the TR-FRET reports a higher proportion of mGlu5 receptors in an inactive-
202 like conformation at V_{depol} compared to V_{rest} . Consistently, we noticed a shift in IC_{50} of the
203 LY341495 mGlu competitive antagonist, from 31.28 μ M at V_{rest} to 6.75 μ M at V_{depol} (**Figure 3C**),
204 demonstrating a V_m effect on ligand affinity for the mGlu5 receptor orthosteric pocket. These
205 results demonstrate the influence of V_m on the conformation of the receptor. V_{depol} stabilizes
206 the inactive-like conformation of the receptor, increasing the antagonist potency.

207

208 *V_m modulates mGlu5-mediated $G_{q/11}$ activation*

209 We then assessed the influence of V_m on the activation by mGlu5 receptor of canonical
210 effectors, starting with the $G_{q/11}$ activation. We monitored $G_{q/11}$ activation by mGlu5 using
211 Effector Membrane Translocation Assay (EMTA) ebBRET (Avet et al., 2022) in single-cell BRET

212 imaging ((Goyet et al., 2016), **Figure 4**) and cell population (**Figure Supp. 3**). HEK293T cells
213 were transfected with mGlu5, $G_{q/11}$, p63hRhoGEF-RlucII and rGFP-CAAX coding plasmids. $G_{q/11}$
214 activation triggers the recruitment of its cytosolic effector, p63RhoGEF-RlucII, to the plasma
215 membrane, which is reported by an increase in ebBRET with the plasma membrane-anchored
216 rGFP-CAAX. The ebBRET signal is therefore a measure of $G_{q/11}$ activation ((Avet et al., 2022),
217 **Figure 4A**). Of note, the ebBRET signal was not affected by V_{depol} compared to V_{rest} , validating
218 the use of this biosensor to assess the influence of V_m on mGlu5-induced $G_{q/11}$ activation
219 (**Figure Supp 1C**). Application of DHPG (100 μ M) induced a slight but significant increase in
220 ebBRET at V_{rest} and V_{depol} (**Figure 4B and 4C**). Paired measurement of the DHPG net effect from
221 individual cell ebBRET values revealed a mean DHPG-induced increase of 97 ± 9.9 milliBRET
222 units at V_{rest} , and only 22.8 ± 6.2 at V_{depol} (**Figure 4D**). These findings indicate a significant
223 reduction of mGlu5 receptor-induced $G_{q/11}$ activation at V_{depol} . Similar results were obtained
224 with ebBRET measurement in cell populations, where the mGlu5 agonist-induced increase of
225 ebBRET was significantly reduced by V_{depol} (agonist-induced ebBRET increase was reduced by
226 24.8 ± 7.2 % at V_{depol} compared to V_{rest} , **Figure Supp 3A**). The same protocol applied to AT1
227 receptor showed no influence of V_m on AT1 receptor agonist-induced $G_{q/11}$ -coupling (**Figure**
228 **Supp 3B**), suggesting once again that V_{depol} specifically affects mGlu5 receptor signaling.

229

230 *V_m affects mGlu5 gating of TRPC6 channels*

231 Transient Receptor Potential Channels (TRPC) are well known effectors of GPCRs intracellular
232 secondary messengers. Typically, TRPC6 opening is triggered by the PLC signaling cascade that
233 triggers formation of diacylglycerol (DAG) and inositol 1,4,5-trisphosphate (IP3, (Hof et al.,
234 2019)). In particular, mGlu5 receptor induces TRPC6-dependent Ca^{2+} influx (Nagy et al., 2013;

235 Wang et al., 2019). We then further tested the influence of V_m on the ability of mGlu5 to gate
236 TRPC6 channels. For this purpose, we co-transfected mGlu5-Venus and TRPC6-tomato in
237 HEK293T cells and recorded whole-cell currents induced by DHPG (100 μ M), in voltage-clamp
238 experiments (**Figure 5**). In cells co-expressing mGlu5 and TRPC6 (**Figure 5B**), but not in cells
239 expressing mGlu5 alone (**Figure 5A**), DHPG applied at a holding potential of -80mV triggered
240 a large inward current with similar kinetics and amplitude than previously reported in
241 HEK293T cells (Itsuki et al., 2014). The current-voltage relationship (I/V curve, **Figure 5C**) also
242 displayed typical TRPC6 permeation and rectification properties (Dryer and Kim, 2018). Given
243 that the ions flow through open channels is governed by the membrane potential, we could
244 not simply compare the current density generated and recorded by activation of mGlu5 at
245 different potentials. We therefore perfused the mGlu5 agonist at different holding potentials
246 (either -80 mV or -20 mV) and then rapidly recorded the currents generated over the full range
247 of V_m ramping (from -80mV to +60mV in 100ms, **Figure 5C**). When DHPG was applied at a
248 holding potential of -20mV instead of -80 mV, the current amplitude recorded with the same
249 voltage ramp protocol was strongly reduced. For example, we recorded a mean inward
250 current density of 46.65 ± 10.32 pA/pF at -80 mV triggered by DHPG application at -80mV,
251 versus 17.69 ± 6.36 pA/pF at -80 mV when DHPG was applied at -20mV (**top insert, Figure 5C**).
252 Importantly, these two protocols triggered the same I/V curve in non-stimulated cells,
253 excluding a holding potential-dependent artefact during the recordings (**Figure Supp 4**). These
254 results confirm that mGlu5 is more active at resting potentials compared to depolarized
255 potentials, which impacts TRPC6 gating by mGlu5 downstream effectors.

256

257 *V_m regulates mGlu5-induced Ca^{2+} release in hippocampal neurons*

258 We further investigated whether mGlu5 voltage-sensitivity also controls its function in
259 neurons. We therefore first studied the ability of mGlu5 receptors to induce calcium release
260 from intracellular stores in hippocampal neurons depending on the membrane potential. To
261 address this, we systematically inhibited other sources of calcium fluctuations in neurons,
262 including voltage-dependent calcium channels (100 μ M Nickel and 100 μ M Cadmium),
263 ionotropic glutamate receptors of the AMPA (10 μ M NBQX) and NMDA (50 μ M AP5) types,
264 and metabotropic receptors of the mGlu1 type (100 μ M CPCCOEt). Neurons were incubated
265 in a medium containing 3 mM (V_{rest}) or 55 mM KCl (V_{depol} , which depolarizes neurons from -70
266 mV to -30 mV (Moutin et al., 2021)) and GCaMP6s fluorescence fluctuations were recorded
267 before and after mGlu5 receptor stimulation with DHPG (50 μ M). Activation of mGlu5
268 receptors resulted in calcium transients that diffused throughout the neuron and recurred at
269 a regular frequency (**Figure 6 and Supplementary Videos 3 and 4**). The number of events
270 induced by DHPG was reduced at V_{depol} compared to V_{rest} (**Figure 6A and C**), along with a
271 decrease in the number of oscillations per neuron (**Figure 6A and B**). These neuronal findings
272 closely parallel those obtained in HEK cells (described in **Figures 1 and 2**), confirming the
273 responsiveness of mGlu5 receptors to membrane potential and the optimal release of calcium
274 from intracellular stores at the resting potential of neurons.

275

276 *V_m regulates mGlu5-NMDA receptors crosstalk in hippocampal neurons*

277 The NMDA receptor conductance is well established to be regulated by type I mGlu receptors
278 (Lutzu and Castillo, 2021). Specifically, NMDA receptor potentiation by mGlu5 receptors
279 involves the G_q -protein-coupled receptor (GPCR) pathway, which includes protein kinase C
280 (PKC) and Src signaling, in various neuronal contexts (Aniksztejn et al., 1992; Pisani et al., 1997;

281 Mannaioni et al., 2001; Benquet et al., 2002; Heidinger et al., 2002; O’neill et al., 2018). We
282 investigated whether V_m could regulate the crosstalk between mGlu5 and NMDA receptors in
283 primary cell cultures of hippocampal neurons (**Figure 7**). To avoid stimulation of mGlu1
284 receptors, we used the mGlu1-specific negative allosteric modulator (NAM), CPCCOEt (100
285 μ M), while stimulating mGlu5 receptors with DHPG. We recorded NMDA-induced currents in
286 the absence of magnesium (Mg^{2+}) to prevent voltage-dependent blockade of NMDA
287 receptors. When applied alone at a holding potential of -80 mV, DHPG (50 μ M) had a negligible
288 effect (1.046 ± 0.45 pA/pF). In contrast, NMDA (30 μ M) induced an inward current of $23.4 \pm$
289 3.7 pA/pF (**Figure 7A**), which was potentiated by $39.5 \pm 6.5\%$ by DHPG when applied at -80 mV
290 ($I_{NMDA + DHPG}/I_{NMDA}$ ratio measured just before and after DHPG application, **Figure 7B, 7C left**).
291 However, at a holding potential of -40 mV, DHPG only induced a $25.4 \pm 5.2\%$ potentiation of
292 NMDA current density (**Figure 7B, 7C right**). Paired measurements of $I_{NMDA + DHPG}/I_{NMDA}$
293 performed subsequently at -80 mV or -40 mV on the same neuron in a random order
294 confirmed a reduction of DHPG-induced NMDA receptor potentiation at -40 mV ($+25 \pm 4.8\%$)
295 compared to -80 mV ($+38.11 \pm 8.2\%$) (**Figure 7D**). These results demonstrate the crucial role
296 of V_m in the control of mGlu5-NMDA crosstalk in neurons, and they corroborate our previous
297 findings on the global voltage-dependence of mGlu5 receptor activity, which is inhibited by
298 depolarized membrane potentials.

299 Therefore, the optimal functioning of the mGlu5 receptor, which enhances NMDA receptor
300 activity in neurons, occurs at the resting potential of neurons. This finding appears to conflict
301 with the fact that NMDA receptors are typically blocked by Mg^{2+} at resting membrane
302 potential. Activation of the NMDA receptor indeed requires depolarization of the postsynaptic
303 element to release Mg^{2+} from the channel pore, and simultaneous binding of glutamate
304 released from the depolarized axon terminal. However, recent studies have shown that the

305 physiological concentration of Mg^{2+} in the interstitial medium (0.7 mM, (Ding et al., 2016)) is
306 lower than what is typically used experimentally in the ACSF composition to record ex vivo
307 neuronal activity, suggesting that the NMDA receptor activity at resting potential in
308 physiological concentrations of Mg^{2+} may have been underestimated (Chiu and Carter, 2022).
309 Furthermore, GluN2D-containing receptors may exhibit reduced Mg^{2+} sensitivity compared to
310 GluN2A- or GluN2B-containing receptors (Sobczyk et al., 2005). Therefore, to resolve the
311 physiological relevance of optimal potentiation of NMDA receptors by mGlu5 at a potential at
312 which NMDA receptors are predominantly inactive, we investigated the mGlu5-induced
313 potentiation of NMDA receptors at resting potential in the presence of 0.7 mM Mg^{2+} , by
314 recording currents and calcium transients (**Figure 8**). Whole-cell current recordings revealed
315 a residual NMDA-induced current of 1.80 ± 0.30 pA/pF density, which was potentiated to 2.40
316 ± 0.40 pA/pF by DHPG co-application (**Figure 8A**). The full current potential relationships are
317 displayed in **Figure 8B**. In a second set of experiments, we measured calcium fluctuations, still
318 in presence of physiological concentrations of Mg^{2+} at resting potential, before and after DHPG
319 application, followed by application of AP5 at the end of the experiment to identify NMDA-
320 dependent events (**Figure 8C and Suppl Video 5**). GCaMP6s fluorescence fluctuations
321 highlighted basal spontaneous Ca^{2+} transients of various shape, size, and kinetics, as illustrated
322 by a projection of all spontaneous Ca^{2+} transients recorded during 2 min and 15 sec (**Figure**
323 **8C₁**). The variety of these Ca^{2+} events certainly relies on the nature of the receptors, channels
324 and intracellular stores involved in their triggering. DHPG increased the number of calcium
325 transients (**Figure 8D**) and their area under curve (AUC) significantly increased from $0.102 \pm$
326 0.001 to 0.154 ± 0.002 following DHPG application (**Figure 8E**). The majority of events were
327 blocked by AP5 (50 μ M) at the end of the experiment, revealing NMDA-dependent events
328 (**Figure 8C₄ and 8D**). To further focus on the effect of DHPG on NMDA-dependent events, we

329 first filtered and selected small and non-propagating calcium transients (which are properties
330 expected from NMDA-dependent synaptic events in a spine-confined environment, **Figure**
331 **8C₂₋₄**), and further identified *a posteriori* regions of interest (ROIs) that were extinguished by
332 AP5 on **Figure 8C₄** because the events previously recorded in these areas (**Figure 8C₂₋₃**)
333 depended on NMDA receptors. Interestingly, DHPG changed the shape of the NMDA-
334 dependent events, inducing a more sustained calcium inflow over time (**Figure 8F**). Taken
335 together, our data show that the mGlu5 receptor potentiates NMDA receptor activity at
336 resting membrane potential, increasing currents and particularly calcium influx, thus
337 expanding the functional importance of NMDA receptors to resting neurons.

338

339

340 **Discussion**

341

342 *The mGlu5 receptor functions optimally at the resting potential of cells.*

343 In this study, we demonstrate that the activity and signaling of the mGlu5 receptor is
344 influenced by the membrane potential of the cell, highlighting an aspect of the receptor that
345 has previously been overlooked. Specifically, a depolarization of the membrane promotes an
346 inactive-like conformation of the receptor, a reduction in mGlu5 coupling to G_{q/11} and
347 subsequent release of Ca²⁺ from intracellular stores. This results in a reduced activation of
348 downstream effectors, including TRPC6 channels and ionotropic NMDA receptors. In the exact
349 same experimental conditions, no effect of V_m was observed on activation of the AT1 receptor,
350 which however activates the same pathway as the mGlu5 receptor (Rössig et al., 1996; García-

351 Sáinz et al., 1997; Inuzuka et al., 2016), highlighting the specificity of V_m action on mGlu5
352 receptor signaling. The mGlu5 receptor is a critical component in the modulation of synaptic
353 transmission, and it plays a role in both inducing and regulating Hebbian and homeostatic
354 synaptic plasticity processes. As such, the sensitivity of the mGlu5 receptor to membrane
355 potential will impact its functions, and its role may vary depending on the context of neuronal
356 activity.

357

358 *The mGlu5 receptor senses moderate V_m variations, similar to natural fluctuations of*
359 *membrane potential occurring at synapses.*

360 A modest increase in membrane potential by approximately 20-25 mV is sufficient to reduce
361 mGlu5 receptor activity by half. Our findings reveal that the mGlu5 receptor is highly sensitive
362 to changes in membrane potential compared to other GPCRs which require stronger
363 depolarizations, at least twice the level used in our study, and sometimes up to 140 mV, to
364 induce a similar shift in receptor efficacy (Navarro-Polanco et al., 2013; Birk et al., 2015; Rinne
365 et al., 2015). Both patch clamp and KCl depolarization methods resulted in the same polarity
366 of membrane potential effect on mGlu5 receptor function. Moreover, the effect of
367 depolarization was comparable when applied before or during agonist perfusion, suggesting
368 that membrane potential can either prevent optimal activation or rapidly reverse ongoing
369 activation of the mGlu5 receptor. Notably, changes in dendritic spine membrane potential
370 during post-synaptic potentials have been reported to be in the range of 20-25 mV, a
371 magnitude similar to that observed in our study (Acker et al., 2016; Jayant et al., 2017; Cornejo
372 et al., 2022). Thus, local synaptic potentials may mediate rapid modulation of mGlu5 function
373 in neurons. Although membrane potential changes were applied tonically in our study, similar

374 results have been obtained by others using physiological frequency ranges, mimicking trains
375 of action potentials (Hoppe et al., 2018; Zhang et al., 2020). A more resolute investigation
376 would provide better insights into the intensity and duration of membrane potential
377 variations required to modulate mGlu5 receptor activity and refine our understanding of the
378 type of neuronal activity of similar magnitude and temporal order that could affect mGlu5
379 function.

380

381 *Membrane depolarization controls mGlu5 receptor activity, as a NAM.*

382 The scope of this study did not include the identification of the voltage sensor within the
383 mGlu5 receptor. However, previous studies on other GPCRs have identified molecular
384 determinants of V_m sensitivity, such as the binding sites of orthosteric or allosteric ligands, G
385 protein binding domains, or Na^+ ions within the 7TM (David et al., 2022). These studies mainly
386 concern class A GPCRs, where the molecular sites mentioned above are located in the 7TM.
387 Unfortunately, very little is known about the voltage-sensing domains of class C GPCRs, to
388 which the mGlu5 receptor belongs. Nevertheless, it is reasonable to assume that the voltage
389 sensor is also located in the 7TM, directly exposed to the magnetic field. This hypothesis is
390 supported by structural studies showing that activation of the 7TM stabilize the active
391 conformation of the extracellular domains of the mGlu5 receptor (Nasrallah et al., 2021). In
392 the atypical structure of class C GPCRs, the extracellular domains of the receptor (and the
393 orthosteric binding site) are approximately 10 nm distant from the membrane (Nasrallah et
394 al., 2021), out of the membrane electric field, which excludes their involvement as a voltage
395 sensor. In contrast, allosteric modulators bind to the 7TM of the receptor. Interestingly, many
396 studies show that V_m affects the efficiency or agonist potency of class A GPCRs in a manner

397 similar to allosteric modulators (Navarro-Polanco et al., 2013; Birk et al., 2015; Hoppe et al.,
398 2018). Specifically, tyrosine residues located in the allosteric site are involved in V_m detection
399 by GPCRs (Barchad-Avitzur et al., 2016). Of note, the allosteric binding site of the mGlu5
400 receptor also contains a tyrosine residue (Tyr6593.44) that enables NAM inhibitory effects
401 (Mølck et al., 2012; Nasrallah et al., 2021).

402 Functional data collected in this study suggests that membrane depolarization causes a NAM
403 effect on the mGlu5 receptor. Indeed, NAMs cause a decrease in agonist efficacy with virtually
404 no changes on agonist potency (Niswender and Conn, 2010), similar to the effect of
405 depolarization on the dose-response curve (**Figure 1E**). Furthermore, mGlu5 receptor-
406 mediated Ca^{2+} oscillations are known to undergo frequency reduction by NAMs, such as MPEP,
407 supported by a decrease in the proportion of oscillating cells (Bradley et al., 2009, 2011).
408 Interestingly, a similar process is induced by membrane depolarization, namely, a reduction
409 in frequency when depolarization is applied before or after the initiation of oscillations,
410 correlated with a decrease in the proportion of oscillating cells (**Figure 2 and 6**).

411

412 *Reconsideration of mGlu5 and NMDA functions*

413 Our study suggests that the optimal functioning of mGlu5 receptors at resting membrane
414 potential may potentiate NMDA receptors too. Traditionally, NMDA receptors are known to
415 be “coincidence detectors” of pre- and post-synaptic activation because they are blocked by
416 the Mg^{2+} ion at resting potential (SEEBURG et al., 1995; Kwag and Paulsen, 2012). However,
417 our results indicate that they may also play an unexpected role at resting potential, facilitated
418 by the permissive effect of mGlu5 receptors. Our findings add complexity to the functional
419 cross-talk between mGlu5 and NMDA receptors, with mGlu5 priming NMDA receptor

420 activation on recently resting neurons. Once the membrane potential is depolarized enough
421 to unblock NMDA receptors, they can function autonomously, while negative feedback from
422 V_m on mGlu5 receptors limits NMDA facilitation and synapse overexcitability. This finding is
423 likely to have significant implications for the nature of induced neuronal plasticity, which relies
424 on the concerted activities of mGlu5 and NMDA receptors (Francesconi et al., 2004; Neyman
425 and Manahan-Vaughan, 2008). Different cellular mechanisms for inducing plasticity will come
426 into play depending on the type of activatable receptor following network activity.

427 The acute effect of depolarization on the activity and signaling of mGlu5 receptors adds to the
428 extensive regulation of protein complexes around these receptors and their functional
429 interactions that are regulated by neuronal activity. The mGlu5 receptor has been proposed
430 as a homeostatic regulator of synaptic transmission, a role that requires activity-induced
431 monomeric Homer1a expression (Hu et al., 2010; Diering et al., 2017; Bockaert et al., 2021).
432 Within the neuronal environment, the function of mGlu5 receptors is dynamically regulated
433 by interactions with multimeric Homer proteins, which is dampened by the monomeric
434 Homer1a protein during sustained synaptic activity (Ango et al., 2001; Moutin et al., 2012).
435 Our team recently demonstrated an instantaneous disruption of the mGlu5-Homer
436 interaction following membrane depolarization in hippocampal neurons (Moutin et al., 2021),
437 which cannot be explained by the induction of monomeric Homer1a synthesis, a process
438 taking place several minutes later. This depolarization-dependent disruption occurs even if
439 NMDA receptors are blocked (Moutin et al., 2021) and may be attributed to the direct
440 sensitivity of the mGlu5 receptor to membrane potential, as demonstrated in this study.
441 However, testing this hypothesis would require knowledge of and mutations to the voltage
442 sensor of the mGlu5 receptor without affecting the receptor's response to its ligand.

443 Following the induction of a first plasticity event, the expression of Homer1a blocks the activity
444 of the mGlu5 and NMDA receptors to prevent the induction of subsequent plasticity, allowing
445 cellular signaling required for the expression of the engaged plasticity and the maintenance
446 of the neuron in the functional network in which it has been committed (Moutin et al., 2021).
447 It is worth noting that the direct effect of V_{depol} on mGlu5 receptor functioning described in
448 this study is similar to the effect of Homer1a, which inhibits canonical receptor signaling. The
449 acute effect of depolarization is likely to control the function of the mGlu5 receptor on a
450 shorter time scale. The decreased affinity of the receptor for multimeric Homer, first by
451 membrane depolarization (seconds) and then by CamKII-dependent Homer phosphorylation
452 (minutes, (Guo et al., 2015)), would promote decreased competition and enable the
453 interaction with monomeric Homer1a once this protein is expressed (20-30 minutes, (Moutin
454 et al., 2012)), enabling long-lasting plasticity.

455

456 In conclusion, this study has identified mGlu5 receptor as a member of the small yet expanding
457 group of voltage-sensitive GPCRs. The high sensitivity of mGlu5 receptor to changes in
458 membrane potential at synapses suggests that its effects extend beyond canonical signaling
459 and may even impact its cognate NMDA receptor. Optimal functioning of mGlu5 receptor at
460 hyperpolarized potentials could have far-reaching consequences, such as restricting its
461 activation to specific locations by integrating glutamate spill-over and electrical propagation
462 at synapses. Additionally, mGlu5 receptor may exert cell-type-dependent actions, conditioned
463 by the resting potential of cells. Finally, given the crucial role of this receptor in neuronal
464 physiology, it is likely that its voltage sensitivity plays a critical role in pathological conditions
465 associated with variations in intrinsic excitability.

466

467

468 **Material and methods**

469

470 *Plasmids*

471 Plasmids were amplified using E.Coli DH5 α strain (Life Technologies). DNA was then purified
472 using the NucleoBond[®] Xtra Plus Midi-Prep kit (Machery-Nagel[®]). The plasmids used were:
473 pcDNA3.1-rGFP-CAAX (Namkung et al., 2016), pcDNA3.1-p63RhoGEF-RlucII (Avet et al., 2022),
474 pcDNA3.1-G α q (gift from Jean-Philippe Pin), pRK5-SNAPtag-AT1 (CIS bio international), pRK5-
475 Myc-mGlu5a and pcDNA3.1-Myc-mGlu5a-Venus (Perroy et al., 2008). pCAGGS-TRPC6-ires2-
476 mbtdtomato was obtained by cloning mouse TRPC6 sequence in pCAGGS plasmid (Niwa et al.,
477 1991) upstream the sequences of IRES2 and membrane-anchored tdtomato.

478

479 *Reagents*

480 (S)-3,5- Dihydroxyphenylglycine ([DHPG](#), HelloBio[®]); [Quisqualate](#) (Tocris); [LY341495](#) (Tocris[®]);
481 2-methyl-6-(phenylethynyl)-pyridine hydrochloride ([MPEP](#), HelloBio[®]); human angiotensin II
482 (HelloBio[®]); N-Methyl-D-Aspartate ([NMDA](#), Tocris[®]). 7-(Hydroxyimino)cyclopropa[*b*]chromen-
483 1a-carboxylate ethyl ester ([CPCCOEt](#), Tocris[®]); [Nickel](#) (Merck[®]); [Cadmium](#) (Merck[®]); 2,3-Dioxo-
484 6-nitro-1,2,3,4-tetrahydrobenzo[*f*]quinoxaline-7-sulfonamide disodium salt ([NBQX](#),
485 HelloBio[®]); [Tetrodotoxin](#) (TTX) Citrate (Latoxan[®]), [Bicuculline](#) (HelloBio[®]). Except for dose
486 response curves, all the mGlu5, AT1 and mGlu1 ligands are applied at saturating
487 concentrations.

488

489 *Cell cultures and transfection*

490 HEK293T cell lines (RRID:CVCL_0063) grown in DMEM GlutaMAX[®] medium (Gibco[™])
491 supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin were maintained
492 at 37°C in a humidity-controlled atmosphere with 5% CO₂. The ST-hmGlu5 HEK293T cell line
493 enables inducible expression of the hmGlu5 receptor containing a SNAP-tagged extracellular
494 VFT domain, as previously described (Ricart-Ortega et al., 2020). Briefly, the release of the
495 constitutive Tet repression of the ST-hmGlu5 gene is induced by doxycycline (Dox), allowing a
496 reproducible and controlled expression, proportional to Dox concentration and incubation
497 time. Cells were maintained in ST-hmGlu5 transgene selection medium in the presence of 100
498 µg/ml hygromycin B and 15 µg/ml blasticidin. Dox (1µg/ml) was added 6h (Ca²⁺ experiments)
499 or 10 to 24h (TR-FRET measurement) before experiments. Cell lines transduction with the
500 pWPT-EF1a-GcaMP6s-P2A-Scarlet lentivirus (gift from Vincent Compan) enabled stable
501 expression of GcaMP6s fluorescent Ca²⁺ sensor. Plasmids transfection was performed on
502 adherent cells the day after the seeding in antibiotic-free culture medium using Lipofectamine
503 2000[™] (Life Technologies) diluted in OptiMEM GlutaMAX[®] (Life Technologies) or jetPEI[®]
504 (Polyplus, only for patch clamp experiments), 24 to 48h before experiments.

505 Primary cultures of hippocampal neurons were prepared from C57BL/6 mice P0-2 pups as
506 previously described (Moutin et al., 2020). Neurons were seeded onto plastic dishes coated
507 with Poly-L-ornithine (0.03 mg/ml) and laminin (1µg/ml) in Neurobasal, B27, Glutamax,
508 Glutamine, serum and 1% penicillin/streptomycin medium. Neurons were then maintained at
509 37°C with 5% CO₂ and incubated from DIV2 to DIV3 with AraC (1 µM) to inhibit glial cell

510 proliferation. The medium was then progressively replaced by a serum-free medium
511 (BrainPhys, B27, Glutamax).

512

513 *Recording Solutions*

514 In all experiments, the extracellular solution contained (in mM): NaCl 140, CaCl₂ 2, MgCl₂ 0 to
515 2, KCl 3, HEPES 10, D-glucose 10 (pH = 7.4, 330 mOsm). For neurons, Glycine 0.01, bicuculline
516 0.01, and tetrodotoxin 0.0003 were added the day of the experiment. For whole-cell voltage
517 clamp recordings, the pipette was filled with an intracellular solution (in mM): CsCl 140, EGTA
518 5, CaCl₂ 0.5, HEPES 10, ATP-Na₂ 2 and D-glucose 10 (pH = 7.2, 300 mOsm). Equimolar solutions
519 containing increased KCl concentrations (from 3mM to 100 mM) were obtained by equal
520 decrease of NaCl concentration.

521 *Ca²⁺ measurement*

522 - Cell population: live Ca²⁺ fluctuations were recorded at 37°C on ST-hmGlu5 GcaMP6s-P2A-
523 Scarlet HEK293T cells, or when specified on GcaMP6s-P2A-Scarlet HEK293T cells transfected
524 with pRK-SNAPtag-AT1 (25 ng/well), seeded at 5 x 10⁴ cells per well in 96-wells plates with
525 transparent bottom, with the FDSS/μCell plate reader (Hamamatsu[®]) – Acquisitions of 1Hz;
526 Exc: 480 nm – Em: 540 nm; high-speed digital EM CCD camera. Each independent experiment
527 was performed in triplicate, which average corresponds to n = 1. Data were normalized to the
528 maximum response at V_{rest} in each independent experiment. Basic calculations from the raw
529 data files were performed on Microsoft Excel (Office 2016), statistical analyses and graphics
530 on GraphPad Prism 8.1.

531 - Single cell imaging: GcaMP6s fluorescence fluctuations were recorded on ST-hmGlu5
532 GcaMP6s-P2A-Scarlet HEK293T cells, using an Axio Observer 7 KMAT fluorescence microscope
533 (Carl Zeiss), equipped with a Plan-Neofluar 40x/1.30 EC oil objective (M27, ZEISS®), Exc 470/40
534 nm – Em 525/50 nm filters and ORCA-Quest qCMOS camera (Hamamatsu); all controlled with
535 Metamorph software. Images on HEK cells were acquired at 3Hz and analysed with Fiji using
536 a custom code computing the average fluorescence intensity of the regions of interest. For
537 the frequency analysis, MATLAB was used to automatically detect the oscillations occurrences
538 and compute the instantaneous and global oscillation frequencies. First, baselines were
539 computed as the 10th percentile of the trace using a sliding window of size 400 frames. Signal
540 was then divided by the baseline. Oscillations were detected using the function findpeaks with
541 a minimal peak prominence of 0.01. Instantaneous frequency was computed as the number
542 of oscillations minus one divided by the time between first and last oscillation. Global
543 frequency was computed as the number of oscillations divided by the total duration.
544 Spontaneous Ca²⁺ transients in neurons infected with the pWPT-CAMKIIa-GcaMP6s-P2A-
545 Scarlet lentivirus were acquired at 10 Hz, for series of 2 min and 15 sec. Image stacks were
546 then analyzed as follow: images were filtered with a 5*5 median kernel. Each pixel was divided
547 by its average value over the whole stack. Each pixel was gaussian filtered along the time axis
548 with a sigma of 2 frames. Time derivative was applied to the stack with a step of 6 frames.
549 Images were thresholded with a value of image mean + 6 * image standard deviation. A filter
550 was then applied to keep only voxels with at least 10 thresholded voxels in their 3*3*3
551 neighborhood, to remove isolated voxels. Thresholded voxels spatially connected were
552 assembled as events. Maximal time projection of those events gave their spatial footprint: the
553 list of pixels in the image. Events with lower than 16 pixels were rejected. Events were
554 classified in different categories based on two criterions: a threshold of 1000 pixels separating

555 big events from small events, a threshold of 0.8 px of propagation distance separating events
556 propagating or not. Propagation distance was measured as the difference of position between
557 the centroid of the pixels thresholded at the beginning and at the end of the onset. Only big
558 events were quantified in **Figure 6**, while both thresholds were applied to focus on “small,
559 non-propagating calcium transients” at synapses in **Figure 8C₂₋₄**. Projection images were
560 obtained by accumulation of the events footprint. Fluorescence time traces were extracted
561 for each event as the average fluorescence over the event pixels and normalized by the
562 fluorescence just before the onset of the event. Area under curve was computed between the
563 onset and 4 seconds later.

564

565 *TR-FRET measurement*

566 TR-FRET measurements were performed as previously described (Scholler et al., 2017). Briefly,
567 ST-hmGlu5 HEK293T seeded at 5×10^4 cells per well in 96-wells black bottom plates were
568 incubated for 1h-1h30 with 100nM of SNAP–Lumi4-Tb and/or 60 nM of SNAP–green.
569 Measurements were performed at room temperature using the Pherastar FS (BMG LabTech).
570 Following SNAP–Lumi4-Tb excitation (337 nm), the decays of SNAP–green or SNAP–Lumi4-Tb
571 emission were measured every $10\mu\text{s}$ at 520 nm and 620 nm respectively. TR-FRET ratios and
572 SNAP–Lumi4-Tb decays were calculated following Scholler et al. recommendation for mGlu
573 receptors with the corresponding time intervals: $\frac{\Sigma [50\mu\text{s}-100\mu\text{s}]}{\Sigma [1200\mu\text{s}-1600\mu\text{s}]}$ (Scholler et al. 2017). For
574 cells incubated only with SNAP–green, the fluorescence was measured at 520 nm with a 485
575 nm excitation. Each independent experiment was performed in triplicate, which average
576 corresponds to $n = 1$ and data were normalized to the maximum response at V_{rest} except for
577 LY341495 dose-response curves which were normalized to the maximum response at V_{depol} .

578 Basic calculations from the raw data files were performed on Microsoft Excel (Office 2016),
579 statistical analyses and graphics on GraphPad Prism 8.1.

580

581 *BRET measurement*

582 After coelenterazine H (Coel H, Promega, 2.5 μ M) addition, ebBRET measurements in cell
583 population were performed using the LB940Mithras plate reader (Berthold) as previously
584 described (Namkung et al., 2016; Avet et al., 2022), on cells seeded at 5×10^4 cells per well in
585 96-wells white bottom plates and transfected with pcDNA3.1-rGFP-CAAX (33,3 ng/well),
586 pcDNA3.1-p63RhoGEF-RlucII (5,1 ng/well), pcDNA3.1-Gαq (6,75 ng/well) combined with the
587 pRK5-Myc-mGlu5a (14,7 ng/well) or pRK-SNAPtag-AT1 (14,8 ng/well). BRET, or the 535
588 nm/485 nm ratio, was assessed by calculating the ratio of the light emitted by the acceptor
589 (510–550 nm band-pass filter, Em535) to the light emitted by the donor (460–500 nm band-
590 pass filter, Em480).

591 Single cell BRET imaging was previously described (Goyet et al., 2016). Acquisitions were
592 performed on cells seeded in 35mm diameter glass dishes (MatTek® for microscopy) coated
593 with poly-ornithine (0.03 mg/ml, for 30min), after Coel H (5 μ M) addition, using the Axio
594 Observer 7 KMAT fluorescence microscope (Carl Zeiss), equipped with a Neofluar 40X/1.3
595 planar EC oil objective (M27, ZEISS) and an ORCA-Quest qCMOS camera (Hamamatsu),
596 controlled by Metamorph software. The emission of RlucII (10s exposure, FF01-450nm/70-25
597 Brightline filter, Semrock) and rGFP (20s exposure, HQ535nm/50M filter, Chroma Technology
598 Corp) were measured successively. Images were analyzed using the BRET Analyzer macro on
599 Fiji (Chastagnier et al., 2018). Overall, this analysis uses an open-source toolkit for Fiji
600 (<https://github.com/yhastagnier/BRET-Analyzer>) performing four key steps in the analysis:

601 (1) background subtraction from the image, (2) image alignment over time, (3) composite
602 thresholding of the image, and (4) pixel-by-pixel division of the image and distribution of the
603 BRET ratio intensity on a pseudo-color scale. The BRET ratio corresponds to the
604 acceptor/donor emission ratio (535/450).

605

606 *Electrophysiology*

607 HEK293T cells, seeded in 35 mm diameter plastic dishes at low confluency (1×10^4 cells) and
608 transfected with pcDNA3.1-Myc-mGlu5-Venus (0.25 μg) and pCAGGS-TRPC6-ires2-
609 mbtdtomato (0.5 μg) plasmids, were targeted by fluorescence microscopy. Measurements
610 were performed in the whole cell configuration, in voltage or current clamp mode, with
611 pipettes of 2-4 M Ω resistance when filled with the appropriate intracellular medium.

612 At 10 to 12 days in vitro, hippocampal neurons were used for endogenous NMDA current
613 measurements. Patch clamp recordings were performed in the whole-cell configuration with
614 pipettes of 3-5 M Ω resistance when filled with the appropriate intracellular medium. NMDA
615 stimulations were separated by 2 min to avoid receptor desensitization. All recordings were
616 performed at room temperature for less than 1 h per dish. Drugs were applied using a gravity
617 perfusion system allowing complete exchange of the cellular environment in less than 30ms.
618 Currents were recorded using an Axopatch 200B amplifier (Axon Instruments), with noise
619 removal at 50-60 Hz (Hum Bug Noise, Quest Scientific) and digitized at 3 kHz before being
620 stored in Clampex software (version 8.1). The data were then analyzed using the Clampfit 11.2
621 software from Axon instruments (Molecular Devices).

622

623 *Data and analysis*

624 The statistical tests are indicated in each of the figure legends. Statistical analysis was
625 undertaken only for studies where each group size was at least n=5. Studies were designed to
626 generate groups of equal size, using randomisation and blinded analysis. Group size is the
627 number of independent values, and statistical analysis was done using these independent
628 values. $p= 0.05$ was the level of probability to constitute the threshold for statistical
629 significance for determining whether groups differ. Outliers were included in data analysis and
630 presentation. The manuscript complies with BJP's recommendations and requirements on
631 experimental design and analysis (Curtis MJ, Alexander S, Cirino G, Docherty JR, George CH,
632 Giembycz MA, Hoyer D, Insel PA, Izzo AA, Ji Y, MacEwan DA, Sobey CG, Stanford SC, Teixeira
633 MM, Wonnacott S, Ahluwalia A. Experimental design and analysis and their reporting II:
634 updated and simplified guidance for authors and peer reviewers. *Br J Pharmacology* 175 987–
635 993, 2018).

636

637 *Nomenclature of Targets and Ligands*

638 Key protein targets and ligands in this article are hyperlinked to corresponding entries in
639 <http://www.guidetopharmacology.org>, and are permanently archived in the Concise Guide to
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641

642

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653

654 **Author contributions**

655 MB and JP conceived research and wrote the manuscript; MB carried out the majority of the
656 experiments, conception, design of the protocols, analysis of the results and production of the
657 figures. CC carried out G protein sensor experiments, including protocol design, experiments
658 and analyses. NB performed theoretical and manual supervision of the molecular biology
659 experiments (electrophoresis, plasmid digestion, transfection) and data interpretation. YC
660 provided general assistance with the acquisition and analysis of results in microscopy, he also
661 developed optimised programmes to facilitate and automate signal analysis. JFI helped to
662 design and perform experiments to detect mGlu5 receptor conformation. EM supervised and
663 implemented neuronal cultures with NB and the development of population BRET protocols.
664 LT cloned the pCAGGS-TRPC6-ires2-mbtdtomato plasmid. JC took an active part in the
665 implementation of the experimental protocol and together with LT provided advices for patch
666 clamp recordings on HEK and analysis of results. JP carried out BRET and calcium microscopy

667 experiments in neurons, and was involved in the analysis of results and theoretical supervision
668 at all levels. JP conceived and supervised the full project. All authors contribute to the
669 preparation of the manuscript and approved it.

670

671 **Competing Interests Statement**

672 None - The authors declare no competing interests.

673

674 **Declaration of transparency and scientific rigour**

675 This Declaration acknowledges that this paper adheres to the principles for transparent
676 reporting and scientific rigour of preclinical research as stated in the BJP guidelines for [Design](#)
677 [and Analysis](#), and as recommended by funding agencies, publishers and other organisations
678 engaged with supporting research.

679

680 **References**

- 681 Acker, C. D., Hoyos, E., and Loew, L. M. (2016). EPSPs measured in proximal dendritic spines of
682 cortical pyramidal neurons. *eNeuro* 3. doi: 10.1523/ENEURO.0050-15.2016.
- 683 Ango, F., Prézeau, L., Muller, T., Tu, J. C., Xiao, B., Worley, P. F., et al. (2001). Agonist-independent
684 activation of metabotropic glutamate receptors by the intracellular protein Homer. *Nature* 411.
685 doi: 10.1038/35082096.
- 686 Aniksztejn, L., Otani, S., and Ben-Ari, Y. (1992). Quisqualate Metabotropic Receptors Modulate NMDA
687 Currents and Facilitate Induction of Long-Term Potentiation Through Protein Kinase C.
688 *European Journal of Neuroscience* 4. doi: 10.1111/j.1460-9568.1992.tb00900.x.
- 689 Avet, C., Mancini, A., Breton, B., Gouill, C. Le, Hauser, A. S., Normand, C., et al. (2022). Effector
690 membrane translocation biosensors reveal G protein and Parrestin coupling profiles of 100
691 therapeutically relevant GPCRs. *Elife* 11. doi: 10.7554/eLife.74101.
- 692 Barchad-Avitzur, O., Priest, M. F., Dekel, N., Bezanilla, F., Parnas, H., and Ben-Chaim, Y. (2016). A
693 Novel Voltage Sensor in the Orthosteric Binding Site of the M2 Muscarinic Receptor. *Biophys J*
694 111. doi: 10.1016/j.bpj.2016.08.035.
- 695 Ben-Chaim, Y., Chanda, B., Dascal, N., Bezanilla, F., Parnas, I., and Parnas, H. (2006). Movement of
696 “gating charge” is coupled to ligand binding in a G-protein-coupled receptor. *Nature* 444. doi:
697 10.1038/nature05259.
- 698 Ben-Chaim, Y., Tour, O., Dascal, N., Parnas, I., and Parnas, H. (2003). The M2 muscarinic G-protein-
699 coupled receptor is voltage-sensitive. *Journal of Biological Chemistry* 278. doi:
700 10.1074/jbc.M301146200.
- 701 Benquet, P., Gee, C. E., and Gerber, U. (2002). Two distinct signaling pathways upregulate NMDA
702 receptor responses via two distinct metabotropic glutamate receptor subtypes. *Journal of*
703 *Neuroscience* 22. doi: 10.1523/jneurosci.22-22-09679.2002.
- 704 Birk, A., Rinne, A., and Bünemann, M. (2015). Membrane potential controls the efficacy of
705 catecholamine-induced β 1-Adrenoceptor activity. *Journal of Biological Chemistry* 290, 27311–
706 27320. doi: 10.1074/jbc.M115.665000.
- 707 Bockaert, J., Perroy, J., and Ango, F. (2021). The complex formed by group I metabotropic glutamate
708 receptor (MGLUR) and homer1a plays a central role in metaplasticity and homeostatic synaptic
709 scaling. *Journal of Neuroscience* 41. doi: 10.1523/JNEUROSCI.0026-21.2021.
- 710 Bradley, S. J., Langmead, C. J., Watson, J. M., and Challiss, R. A. J. (2011). Quantitative analysis reveals
711 multiple mechanisms of allosteric modulation of the mGlu5 receptor in rat astroglia. *Mol*
712 *Pharmacol* 79. doi: 10.1124/mol.110.068882.
- 713 Bradley, S. J., Watson, J. M., and Challiss, R. A. J. (2009). Effects of positive allosteric modulators on
714 single-cell oscillatory Ca²⁺ signaling initiated by the type 5 metabotropic glutamate receptor.
715 *Mol Pharmacol* 76. doi: 10.1124/mol.109.059170.
- 716 Chastagnier, Y., Moutin, E., Hemonnot, A. L., and Perroy, J. (2018). Image processing for
717 bioluminescence resonance energy transfer measurement-BRET-Analyzer. *Front Comput*
718 *Neurosci* 11. doi: 10.3389/fncom.2017.00118.

- 719 Chiu, D. N., and Carter, B. C. (2022). Synaptic NMDA receptor activity at resting membrane potentials.
720 *Front Cell Neurosci* 16. doi: 10.3389/fncel.2022.916626.
- 721 Cornejo, V. H., Ofer, N., and Yuste, R. (2022). Voltage compartmentalization in dendritic spines in
722 vivo. *Science (1979)* 375. doi: 10.1126/science.abg0501.
- 723 David, D., Bentulila, Z., Tauber, M., and Ben-Chaim, Y. (2022). G Protein-Coupled Receptors Regulated
724 by Membrane Potential. *Int J Mol Sci* 23. doi: 10.3390/ijms232213988.
- 725 Diering, G. H., Nirujogi, R. S., Roth, R. H., Worley, P. F., Pandey, A., and Huganir, R. L. (2017). Homer1a
726 drives homeostatic scaling-down of excitatory synapses during sleep. *Science (1979)* 355. doi:
727 10.1126/science.aai8355.
- 728 Ding, F., O'donnell, J., Xu, Q., Kang, N., Goldman, N., and Nedergaard, M. (2016). Changes in the
729 composition of brain interstitial ions control the sleep-wake cycle. *Science (1979)* 352. doi:
730 10.1126/science.aad4821.
- 731 Doumazane, E., Scholler, P., Fabre, L., Zwier, J. M., Trinquet, E., Pin, J. P., et al. (2013). Illuminating
732 the activation mechanisms and allosteric properties of metabotropic glutamate receptors. *Proc*
733 *Natl Acad Sci U S A* 110, E1416-25. doi: 10.1073/pnas.1215615110.
- 734 Dryer, S. E., and Kim, E. Y. (2018). Permeation and rectification in canonical transient receptor
735 potential-6 (TRPC6) channels. *Front Physiol* 9. doi: 10.3389/fphys.2018.01055.
- 736 Francesconi, W., Cammalleri, M., and Sanna, P. P. (2004). The metabotropic glutamate receptor 5 is
737 necessary for late-phase long-term potentiation in the hippocampal CA1 region. *Brain Res* 1022.
738 doi: 10.1016/j.brainres.2004.06.060.
- 739 García-Sáinz, J. A., Martínez-Alfaro, M., Romero-Avila, M. T., and González-Espinosa, C. (1997).
740 Characterization of the AT1 angiotensin II receptor expressed in guinea pig liver. *Journal of*
741 *Endocrinology* 154. doi: 10.1677/joe.0.1540133.
- 742 Gómez-Santacana, X., Pittolo, S., Rovira, X., Lopez, M., Zussy, C., Dalton, J. A. R., et al. (2017).
743 Illuminating Phenylazopyridines to Photoswitch Metabotropic Glutamate Receptors: From the
744 Flask to the Animals. *ACS Cent Sci* 3. doi: 10.1021/acscentsci.6b00353.
- 745 Goyet, E., Bouquier, N., Ollendorff, V., and Perroy, J. (2016). Fast and high resolution single-cell BRET
746 imaging. *Sci Rep* 6, 28231. doi: 10.1038/srep28231.
- 747 Guo, W., Ceolin, L., Collins, K. A., Perroy, J., and Huber, K. M. (2015). Elevated CaMKIIalpha and
748 Hyperphosphorylation of Homer Mediate Circuit Dysfunction in a Fragile X Syndrome Mouse
749 Model. *Cell Rep* 13, 2297–2311. doi: 10.1016/j.celrep.2015.11.013.
- 750 Heidinger, V., Manzerra, P., Wang, X. Q., Strasser, U., Yu, S. P., Choi, D. W., et al. (2002).
751 Metabotropic glutamate receptor 1-induced upregulation of NMDA receptor current:
752 Mediation through the Pyk2/Src-family kinase pathway in cortical neurons. *Journal of*
753 *Neuroscience* 22. doi: 10.1523/jneurosci.22-13-05452.2002.
- 754 Hof, T., Chaigne, S., Récalde, A., Sallé, L., Brette, F., and Guinamard, R. (2019). Transient receptor
755 potential channels in cardiac health and disease. *Nat Rev Cardiol* 16. doi: 10.1038/s41569-018-
756 0145-2.

757 Holz, A., Mülsch, F., Schwarz, M. K., Hollmann, M., Döbrössy, M. D., Coenen, V. A., et al. (2019).
758 Enhanced mGlu5 Signaling in Excitatory Neurons Promotes Rapid Antidepressant Effects via
759 AMPA Receptor Activation. *Neuron* 104. doi: 10.1016/j.neuron.2019.07.011.

760 Hoppe, A., Marti-Solano, M., Drabek, M., Bünemann, M., Kolb, P., and Rinne, A. (2018). The allosteric
761 site regulates the voltage sensitivity of muscarinic receptors. *Cell Signal* 42. doi:
762 10.1016/j.cellsig.2017.10.011.

763 Hu, J. H., Park, J. M., Park, S., Xiao, B., Dehoff, M. H., Kim, S., et al. (2010). Homeostatic Scaling
764 Requires Group I mGluR Activation Mediated by Homer1a. *Neuron* 68. doi:
765 10.1016/j.neuron.2010.11.008.

766 Inuzuka, T., Fujioka, Y., Tsuda, M., Fujioka, M., Satoh, A. O., Horiuchi, K., et al. (2016). Attenuation of
767 ligand-induced activation of angiotensin II type 1 receptor signaling by the type 2 receptor via
768 protein kinase C. *Sci Rep* 6. doi: 10.1038/srep21613.

769 Itsuki, K., Imai, Y., Hase, H., Okamura, Y., Inoue, R., and Mori, M. X. (2014). PLC-mediated PI(4,5)P2
770 hydrolysis regulates activation and inactivation of TRPC6/7 channels. *Journal of General*
771 *Physiology* 143. doi: 10.1085/jgp.201311033.

772 Jayant, K., Hirtz, J. J., Plante, I. J. La, Tsai, D. M., De Boer, W. D. A. M., Semonche, A., et al. (2017).
773 Targeted intracellular voltage recordings from dendritic spines using quantum-dot-coated
774 nanopipettes. *Nat Nanotechnol* 12. doi: 10.1038/nnano.2016.268.

775 Kupchik, Y. M., Barchad-Avitzur, O., Wess, J., Ben-Chaim, Y., Parnas, I., and Parnas, H. (2011). A novel
776 fast mechanism for GPCR-mediated signal transduction - Control of neurotransmitter release.
777 *Journal of Cell Biology* 192. doi: 10.1083/jcb.201007053.

778 Kurz, M., Krett, A. L., and Bünemann, M. (2020). Voltage dependence of prostanoid receptors. *Mol*
779 *Pharmacol* 97. doi: 10.1124/mol.119.118372.

780 Kwag, J., and Paulsen, O. (2012). Gating of NMDA receptor-mediated hippocampal spike timing-
781 dependent potentiation by mGluR5. *Neuropharmacology* 63. doi:
782 10.1016/j.neuropharm.2012.05.021.

783 Lagerström, M. C., and Schiöth, H. B. (2008). Erratum: Structural diversity of G protein-coupled
784 receptors and significance for drug discovery. *Nat Rev Drug Discov* 7. doi: 10.1038/nrd2592.

785 Lepannetier, S., Gualdani, R., Tempesta, S., Schakman, O., Seghers, F., Kreis, A., et al. (2018).
786 Activation of TRPC1 Channel by Metabotropic Glutamate Receptor mGluR5 Modulates Synaptic
787 Plasticity and Spatial Working Memory. *Front Cell Neurosci* 12. doi: 10.3389/fncel.2018.00318.

788 Lin, M. Z., and Schnitzer, M. J. (2016). Genetically encoded indicators of neuronal activity. *Nat*
789 *Neurosci* 19. doi: 10.1038/nn.4359.

790 López-Serrano, A. L., De Jesús-Pérez, J. J., Zamora-Cárdenas, R., Ferrer, T., Rodríguez-Menchaca, A. A.,
791 Tristani-Firouzi, M., et al. (2020). Voltage-induced structural modifications on M2 muscarinic
792 receptor and their functional implications when interacting with the superagonist iperoxo.
793 *Biochem Pharmacol* 177. doi: 10.1016/j.bcp.2020.113961.

794 Lutz, S., and Castillo, P. E. (2021). Modulation of NMDA Receptors by G-protein-coupled receptors:
795 Role in Synaptic Transmission, Plasticity and Beyond. *Neuroscience* 456. doi:
796 10.1016/j.neuroscience.2020.02.019.

797 Mannaioni, G., Marino, M. J., Valenti, O., Traynelis, S. F., and Conn, P. J. (2001). Metabotropic
798 glutamate receptors 1 and 5 differentially regulate CA1 pyramidal cell function. *Journal of*
799 *Neuroscience* 21. doi: 10.1523/jneurosci.21-16-05925.2001.

800 Martinez-Pinna, J., Gurung, I. S., Vial, C., Leon, C., Gachet, C., Evans, R. J., et al. (2005). Direct voltage
801 control of signaling via P2Y1 and other Gαq-coupled receptors. *Journal of Biological Chemistry*
802 280. doi: 10.1074/jbc.M407783200.

803 Mølck, C., Harpsøe, K., Gloriam, D. E., Clausen, R. P., Madsen, U., Pedersen, L., et al. (2012).
804 Pharmacological characterization and modeling of the binding sites of novel 1,3-
805 Bis(pyridinylethynyl)benzenes as metabotropic glutamate receptor 5-selective negative
806 allosteric modulators. *Mol Pharmacol* 82. doi: 10.1124/mol.112.078808.

807 Moutin, E., Hemonnot, A. L., Seube, V., Linck, N., Rassendren, F., Perroy, J., et al. (2020). Procedures
808 for Culturing and Genetically Manipulating Murine Hippocampal Postnatal Neurons. *Front*
809 *Synaptic Neurosci* 12. doi: 10.3389/fnsyn.2020.00019.

810 Moutin, E., Raynaud, F., Roger, J., Pellegrino, E., Homburger, V., Bertaso, F., et al. (2012). Dynamic
811 remodeling of scaffold interactions in dendritic spines controls synaptic excitability. *Journal of*
812 *Cell Biology* 198, 251–263. doi: 10.1083/jcb.201110101.

813 Moutin, E., Sakkaki, S., Compan, V., Bouquier, N., Giona, F., Areias, J., et al. (2021). Restoring
814 glutamate receptosome dynamics at synapses rescues autism-like deficits in Shank3-deficient
815 mice. *Mol Psychiatry*. doi: 10.1038/s41380-021-01230-x.

816 Nagy, G. A., Botond, G., Borhegyi, Z., Plummer, N. W., Freund, T. F., and Hájos, N. (2013). DAG-
817 sensitive and Ca²⁺ permeable TRPC6 channels are expressed in dentate granule cells and
818 interneurons in the hippocampal formation. *Hippocampus* 23. doi: 10.1002/hipo.22081.

819 Nakahara, K., Okada, M., and Nakanishi, S. (1997). The metabotropic glutamate receptor mGluR5
820 induces calcium oscillations in cultured astrocytes via protein kinase C phosphorylation. *J*
821 *Neurochem* 69. doi: 10.1046/j.1471-4159.1997.69041467.x.

822 Namkung, Y., Le Gouill, C., Lukashova, V., Kobayashi, H., Hogue, M., Khoury, E., et al. (2016).
823 Monitoring G protein-coupled receptor and β-arrestin trafficking in live cells using enhanced
824 bystander BRET. *Nat Commun* 7. doi: 10.1038/ncomms12178.

825 Nash, M. S., Schell, M. J., Atkinson, P. J., Johnston, N. R., Nahorski, S. R., and John Challiss, R. A.
826 (2002). Determinants of metabotropic glutamate receptor-5-mediated Ca²⁺ and inositol 1,4,5-
827 trisphosphate oscillation frequency: Receptor density versus agonist concentration. *Journal of*
828 *Biological Chemistry* 277. doi: 10.1074/jbc.M205622200.

829 Nasrallah, C., Cannone, G., Briot, J., Rottier, K., Berizzi, A. E., Huang, C. Y., et al. (2021). Agonists and
830 allosteric modulators promote signaling from different metabotropic glutamate receptor 5
831 conformations. *Cell Rep* 36. doi: 10.1016/j.celrep.2021.109648.

832 Navarro-Polanco, R. A., Aréchiga-Figueroa, I. A., Salazar-Fajardo, P. D., Benavides-Haro, D. E.,
833 Rodríguez-Elías, J. C., Sachse, F. B., et al. (2013). Voltage sensitivity of M2 muscarinic receptors
834 underlies the delayed rectifier-like activation of ACh-gated K⁺ current by choline in feline atrial
835 myocytes. *Journal of Physiology* 591. doi: 10.1113/jphysiol.2013.255166.

836 Navarro-Polanco, R. A., Galindo, E. G. M., Ferrer-Villada, T., Arias, M., Rigby, J. R., Sánchez-Chapula, J.
837 A., et al. (2011). Conformational changes in the M2 muscarinic receptor induced by membrane
838 voltage and agonist binding. *Journal of Physiology* 589. doi: 10.1113/jphysiol.2010.204107.

839 Neyman, S., and Manahan-Vaughan, D. (2008). Metabotropic glutamate receptor 1 (mGluR1) and 5
840 (mGluR5) regulate late phases of LTP and LTD in the hippocampal CA1 region in vitro. *European*
841 *Journal of Neuroscience* 27. doi: 10.1111/j.1460-9568.2008.06109.x.

842 Niswender, C. M., and Conn, P. J. (2010). Metabotropic glutamate receptors: Physiology,
843 pharmacology, and disease. *Annu Rev Pharmacol Toxicol* 50. doi:
844 10.1146/annurev.pharmtox.011008.145533.

845 Niwa, H., Yamamura, K., and Miyazaki, J. (1991). Efficient selection for high-expression transfectants
846 with a novel eukaryotic vector. *Gene* 108.

847 Ohana, L., Barchad, O., Parnas, I., and Parnas, H. (2006). The metabotropic glutamate G-protein-
848 coupled receptors mGluR3 and mGluR1a are voltage-sensitive. *Journal of Biological Chemistry*
849 281. doi: 10.1074/jbc.M513447200.

850 O'Malley, K. L., Jong, Y. J. I., Gonchar, Y., Burkhalter, A., and Romano, C. (2003). Activation of
851 metabotropic glutamate receptor mGlu5 on nuclear membranes mediates intranuclear Ca²⁺
852 changes in heterologous cell types and neurons. *Journal of Biological Chemistry* 278. doi:
853 10.1074/jbc.M300792200.

854 O'Neill, N., McLaughlin, C., Komiyama, N., and Sylantsev, S. (2018). Biphasic modulation of NMDA
855 receptor function by metabotropic glutamate receptors. *Journal of Neuroscience* 38. doi:
856 10.1523/JNEUROSCI.1000-18.2018.

857 Perroy, J., Raynaud, F., Homburger, V., Rousset, M. C., Tellez, L., Bockaert, J., et al. (2008). Direct
858 interaction enables cross-talk between ionotropic and group I metabotropic glutamate
859 receptors. *Journal of Biological Chemistry*. doi: 10.1074/jbc.M705661200.

860 Pisani, A., Calabresi, P., Centonze, D., and Bernardi, G. (1997). Enhancement of NMDA responses by
861 group I metabotropic glutamate receptor activation in striatal neurones. *Br J Pharmacol* 120.
862 doi: 10.1038/sj.bjp.0700999.

863 Rae, M. G., Martin, D. J., Collingridge, G. L., and Irving, A. J. (2000). Role of Ca²⁺ stores in
864 metabotropic L-glutamate receptor-mediated supralinear Ca²⁺ signaling in rat hippocampal
865 neurons. *Journal of Neuroscience* 20. doi: 10.1523/jneurosci.20-23-08628.2000.

866 Reiner, A., and Levitz, J. (2018). Glutamatergic Signaling in the Central Nervous System: Ionotropic
867 and Metabotropic Receptors in Concert. *Neuron* 98. doi: 10.1016/j.neuron.2018.05.018.

868 Ricart-Ortega, M., Berizzi, A. E., Catena, J., Malhaire, F., Muñoz, L., Serra, C., et al. (2020).
869 Development and validation of a mass spectrometry binding assay for mGlu5 receptor. *Anal*
870 *Bioanal Chem* 412. doi: 10.1007/s00216-020-02772-9.

871 Rinne, A., Mobarec, J. C., Mahaut-Smith, M., Kolb, P., and Bünemann, M. (2015). The mode of agonist
872 binding to a G protein-coupled receptor switches the effect that voltage changes have on
873 signaling. *Sci Signal* 8. doi: 10.1126/scisignal.aac7419.

874 Rössig, L., Zólyomi, A., Catt, K. J., and Balla, T. (1996). Regulation of angiotensin II-stimulated Ca²⁺
875 oscillations by Ca²⁺ influx mechanisms in adrenal glomerulosa cells. *Journal of Biological*
876 *Chemistry* 271. doi: 10.1074/jbc.271.36.22063.

- 877 Rozenfeld, E., Tauber, M., Ben-Chaim, Y., and Parnas, M. (2021). GPCR voltage dependence controls
878 neuronal plasticity and behavior. *Nat Commun* 12. doi: 10.1038/s41467-021-27593-x.
- 879 Ruland, J. G., Kirchhofer, S. B., Klindert, S., Bailey, C. P., and Bünemann, M. (2020). Voltage modulates
880 the effect of μ -receptor activation in a ligand-dependent manner. *Br J Pharmacol* 177. doi:
881 10.1111/bph.15070.
- 882 Sahlholm, K., Nilsson, J., Marcellino, D., Fuxe, K., and Århem, P. (2008). Voltage-dependence of the
883 human dopamine D2 receptor. *Synapse* 62. doi: 10.1002/syn.20509.
- 884 Scheefhals, N., and MacGillavry, H. D. (2018). Functional organization of postsynaptic glutamate
885 receptors. *Molecular and Cellular Neuroscience* 91. doi: 10.1016/j.mcn.2018.05.002.
- 886 Scholler, P., Moreno-Delgado, D., Lecat-Guillet, N., Doumazane, E., Monnier, C., Charrier-Savournin,
887 F., et al. (2017). HTS-compatible FRET-based conformational sensors clarify membrane receptor
888 activation. *Nat Chem Biol* 13. doi: 10.1038/nchembio.2286.
- 889 SEEBURG, P. H., BURNASHEV, N., KÖHR, G., KUNER, T., SPRENGEL, R., and MONYER, H. (1995). "The
890 NMDA Receptor Channel: Molecular Design of a Coincidence Detector," in *Proceedings of the*
891 *1993 Laurentian Hormone Conference* doi: 10.1016/b978-0-12-571150-0.50006-8.
- 892 Sobczyk, A., Scheuss, V., and Svoboda, K. (2005). NMDA receptor subunit-dependent [Ca²⁺] signaling
893 in individual hippocampal dendritic spines. *Journal of Neuroscience* 25. doi:
894 10.1523/JNEUROSCI.1221-05.2005.
- 895 Wang, Q., Wang, D., Shibata, S., Ji, T., Zhang, L., Zhang, R., et al. (2019). Group I metabotropic
896 glutamate receptor activation induces TRPC6-dependent calcium influx and RhoA activation in
897 cultured human kidney podocytes. *Biochem Biophys Res Commun* 511. doi:
898 10.1016/j.bbrc.2019.02.062.
- 899 Zhang, Q., Liu, B., Li, Y., Yin, L., Younus, M., Jiang, X., et al. (2020). Regulating quantal size of
900 neurotransmitter release through a GPCR voltage sensor. *Proc Natl Acad Sci U S A* 117. doi:
901 10.1073/pnas.2005274117.

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903

904 **Figure titles and legends**

905

906 **Graphical Abstract**

907 mGlu5 receptor function is optimal at resting cell potential. Membrane depolarization
908 stabilizes an inactive-like conformation of mGlu5 receptor and decreases agonist-induced G_q

909 protein activation, Ca^{2+} release from intracellular stores and gating of NMDA and TRPC6
910 channels.

911

912 **Figure 1. Voltage regulates mGlu5 agonist-induced Ca^{2+} release.** HEK cells stably expressing
913 GCaMP6s also transiently expressed mGlu5 (B-F) or AT1 (G) receptors. **A)** Scheme of GCaMP6s
914 fluorescent Ca^{2+} sensor - adapted from (Lin and Schnitzer, 2016). GCaMP6s is composed of a
915 circularly permuted GFP, a calmodulin (CaM) and a peptide from smooth-muscle myosin light-
916 chain kinase (RS20). **B)** DHPG (100 μM)-induced fluorescence fluctuation in cells expressing
917 (black and blue) or not (grey) mGlu5 receptor, in absence (grey and black) or presence (blue)
918 of the mGlu5 NAM, MPEP (10 μM); Representative illustration. **C)** Peak of fluorescence
919 fluctuation induced by quisqualate (10 μM) in isotonic extracellular solutions containing
920 increasing KCl concentrations ([KCl] in mM), normalized to fluorescence fluctuations recorded
921 with the 3mM [KCl] solution. Data are mean \pm SEM of n = 6 triplicates from independent
922 experiments. Statistics: Kruskal-Wallis test, **D)** Membrane potential measured in the current
923 clamp whole-cell configuration with extracellular solutions containing either 3mM (black) or
924 100mM (red) KCl. Data are mean \pm SEM of n = 10 cells from 5 independent experiments.
925 Statistics: Friedman test **E)** Dose-response curves of DHPG-induced fluorescence fluctuation
926 with KCl 3mM (black, V_{rest}) and 100mM (red, V_{depol}), normalized to V_{rest} peak of each
927 experiment. Data are mean \pm SEM of n = 3 independent experiments. **F and G)** Quisqualate
928 (10 μM , F) or Angiotensine II (1 μM , G) -induced fluorescence fluctuation in KCl 3mM (V_{rest}) and
929 100mM (V_{depol}) solutions. Left, representative illustration; Right, mean \pm SEM of n = 7 to 14
930 triplicates from independent experiments; Statistics: one sample Wilcoxon test.

931

932 **Figure 2. Voltage regulates mGlu5 agonist-induced Ca^{2+} oscillations. A)** Representative field
933 of GCaMP6s expressing cells in epifluorescence microscopy - Scale = 10 μm . **B)** Quisqualate
934 (10 μM)-induced fluorescence fluctuation at V_{rest} (left, black) and V_{depol} (middle, red), for
935 individual field of view (thin) and average (thick) aligned to the peak of response, and
936 quantification of the mean \pm SEM of $n = 14$ to 21 independent experiments (right, Statistics:
937 Mann-Whitney test) **C)** Fluorescence fluctuation recorded in representative oscillating, single
938 spike and silent cells at V_{rest} and V_{depol} in response to quisqualate (10 μM) with proportion of
939 cells in each category displayed in a circular diagram, $n = 403$ cells for V_{rest} and 324 cells for
940 V_{depol} **D)** Percentage of oscillating cells (> 2 oscillations) at V_{rest} and V_{depol} . $n = 324$ to 403 cells
941 from 14 to 18 independent experiments. Statistics: N-1 χ^2 test, $p = 0.0001$. **E)** Violin plots with
942 mean indicated by a dotted line of global frequency of fluorescence oscillations induced by
943 quisqualate (10 μM) application at V_{rest} and V_{depol} ($n = 280$ to 408 cells). Statistics: unpaired t-
944 test. **F)** Representative illustrations (left) and violin plots with mean indicated by a dotted line
945 (right) of fluorescence oscillations frequency induced by V_{rest} or V_{depol} imposed after
946 quisqualate (10 μM) application ($n = 21$ to 35 cells - Statistics: unpaired t-test)

947

948 **Figure 3. Voltage tunes mGlu5 receptor activation probability. A)** Scheme of the TR-FRET
949 sensor of mGlu5 conformation (Doumazane et al., 2013) with SNAP-Lumi4-Tb (purple) and
950 SNAP-fluorescein (green) linked on SNAP-tag-mGlu5 VFT domains. The FRET signal is inversely
951 proportional to the number of receptors in an active-like conformation. **B)** TR-FRET intensity
952 at V_{rest} with and without quisqualate 10 μM (left) and quisqualate (10 μM) effect at V_{rest} and
953 V_{depol} (right). Data are mean \pm SEM of $n = 10$ independent experiments. Statistics: Wilcoxon

954 test. **C)** Dose-response curve of LY341495 mGlu antagonist applied at V_{rest} or V_{depol} . Data are
955 mean \pm SEM of $n = 4$ independent experiments normalized to the maximum TR-FRET of V_{depol} .

956

957 **Figure 4. Voltage modulates mGlu5-mediated Gq activation. A)** Scheme of EMTA ebBRET
958 sensor of Gq activation (Avet et al., 2022). When Gq is activated by mGlu5, p63-RhoGEF-Rlucll
959 is recruited to the membrane where it interacts with rGFP-CAAX. **B and C)** Representative
960 illustrations (B) and mean \pm SEM (C) of BRET signals measured before (CT) and after (DHPG,
961 100 μ M) stimulation at V_{rest} or V_{depol} . $N = 86$ to 97 cells from more than 5 independent
962 experiments; statistics: Paired t-test. **D)** Violin plots with mean indicated by a dotted line of
963 single cell BRET measurement of DHPG net effect; statistics: Unpaired t-test.

964

965 **Figure 5. Voltage controls mGlu5 gating of TRPC6 channels. A and B)** - Whole-cell patch clamp
966 current induced by DHPG (100 μ M) on HEK293T cells expressing mGlu5-Venus alone (A) or with
967 TRPC6-tomato (B). **C)** mGlu5-Venus/TRPC6-tomato co-transfected cells were held at -80mV
968 (black) or -20mV (red) during DHPG (100 μ M) application. Current-voltage relationship was
969 then recorded at the maximum response induced by DHPG (100 μ M) for potentials ranging
970 from -80mV to +60mV in 100ms (bottom). Inset: Mean \pm SEM current density at -80mV. Data
971 are mean \pm SEM of $n = 9$ cells per condition from 5 independent experiments, Statistics: Mann-
972 Whitney test.

973

974 **Figure 6. Voltage regulates mGlu5 agonist-induced Ca^{2+} oscillations in hippocampal neurons.**
975 Projection of spontaneous calcium transients, in presence of Nickel, Cadmium, NBQX, AP5 and

976 CPCCOEt, recorded at V_{rest} (black) and V_{depol} (red) during sequential acquisitions (1 min and 30
977 sec) before (faded colors) and after (dark colors) DHPG application. **A)** representative
978 illustration, **B)** measure of fluorescence fluctuation in representative neurons, stimulated
979 (DHPG) or not (CT) just before recording **C)** violin plots with mean indicated by a dotted line
980 of the number of events ($n = 5$ to 7 independent experiments - Statistics: Mann-Whitney test)
981

982 **Figure 7. Voltage regulates mGlu5 gating of NMDA receptor in hippocampal neurons. A)**
983 Representative current and mean density induced by DHPG ($50\mu\text{M}$, left) or NMDA ($30\mu\text{M}$,
984 right) at -80mV . **B - D)** Representative currents (B), mean current density and $I_{\text{NMDA+DHPG}}/I_{\text{NMDA}}$
985 current ratio (C), and percentage of DHPG-induced NMDA current potentiation (D) measured
986 in presence of NMDA ($30\mu\text{M}$) alone or co-applied with DHPG ($50\mu\text{M}$) at -80mV (black) and -
987 40mV (red) holding potential. Measures of I_{NMDA} (blue) and $I_{\text{NMDA+DHPG}}$ (black) used for
988 quantification in C and D are indicated by arrows in B. In D, paired measurement of $I_{\text{NMDA+DHPG}}$
989 / I_{NMDA} were performed subsequently at -80mV or -40mV , in a random order, on the same
990 neuron. C and D data are mean \pm SEM of $n = 10$ to 15 neurons from at least 5 independent
991 experiments; Statistics: Wilcoxon matched-pairs signed rank test.

992

993 **Figure 8. DHPG-induced potentiation of NMDA currents at resting potential, in physiological**
994 **concentration of Mg^{2+} .**

995 **A)** Left - Representative NMDA ($100\mu\text{M}$)-induced current, without (NMDA) or with (Mg^{2+})
996 extracellular Mg^{2+} (0.7 mM), co-applied with DHPG ($50\mu\text{M}$). Right - Mean \pm SEM current
997 density recorded in 0.7 mM Mg^{2+} , from $n = 21$ neurons in 5 independent experiments.
998 Wilcoxon matched-pairs signed rank test. **B)** Current density-voltage relationship ($-80 \rightarrow +60$

999 mV within 100ms every 5s) performed as illustrated in (a) during NMDA application (at the
1000 plateau response) without (blue) or with 0.7 mM Mg^{2+} (black) co-applied with DHPG (red).
1001 Mean from n = 8-10 neurons per condition. **C**) Projection of spontaneous calcium transients,
1002 recorded during 2 min and 15 sec, in Mg^{2+} (0.7mM) at resting potential of neurons in a
1003 representative field of view. **C₁**: all events for the entire field of view. **C₂₋₄**: small non-
1004 propagating calcium transients from the white square in **C₁**, recorded during three consecutive
1005 acquisitions, in control conditions **C₂**, and following application of DHPG (50 μ M, **C₃**) and AP5
1006 (50 μ M, **C₄**). **D**) Number of calcium transients recorded in the whole field of view before (CT)
1007 and after sequential application of DHPG and AP5. **E**) Mean area under curve (AUC) of calcium
1008 transients. Each bar of the histogram is the Mean \pm SEM of all AUC traces recorded in the
1009 whole field of view before and after DHPG application for 2 min and 15 seconds in both
1010 conditions. Statistics: Unpaired t test. **F**) GCaMP6s fluorescence fluctuation during small non-
1011 propagating calcium transients; Mean \pm SEM of 91 and 94 traces, before (black) and after (red)
1012 DHPG application, measured on 10, identical, AP5-sensitive ROI;

Figure 1

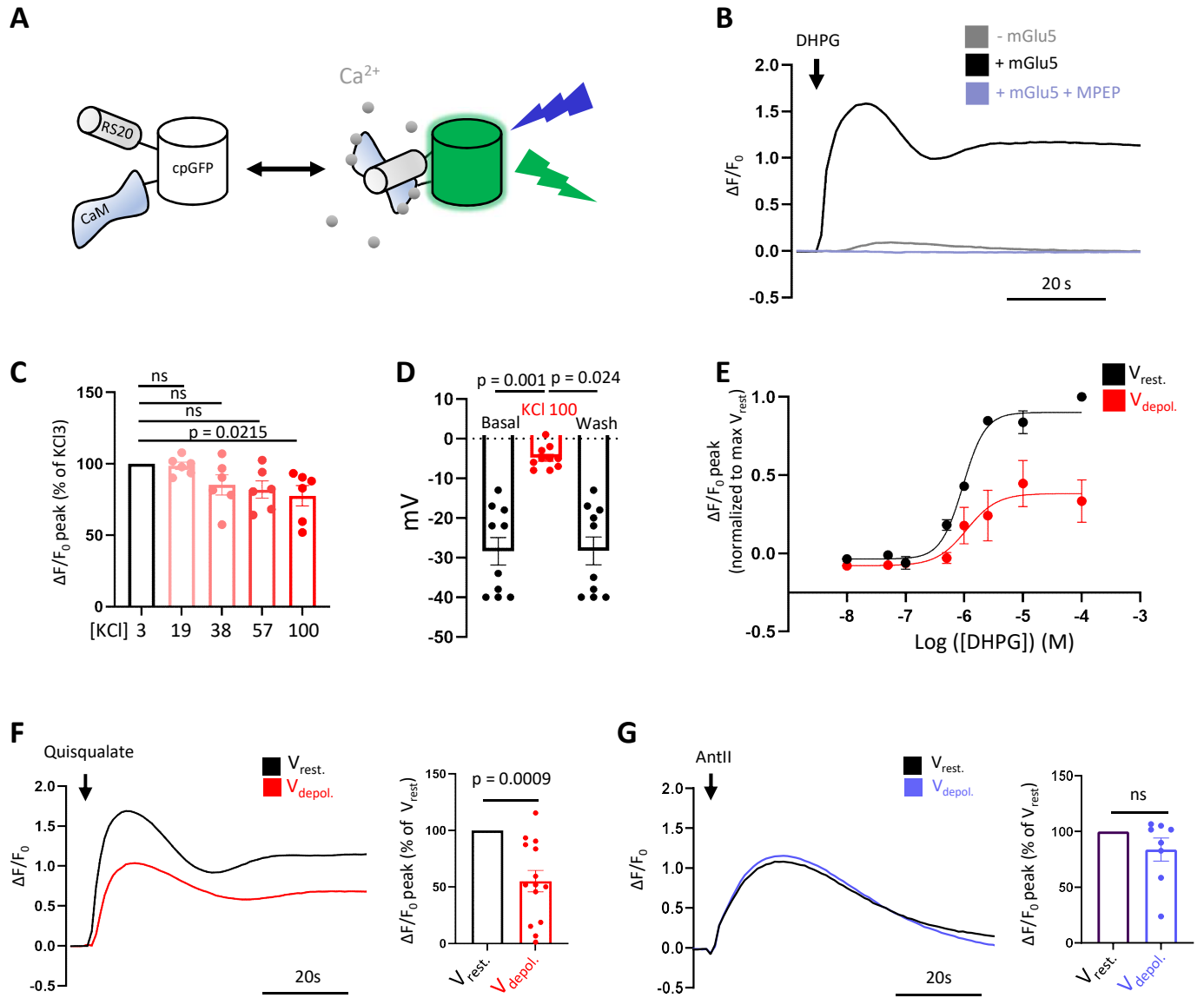


Figure 2

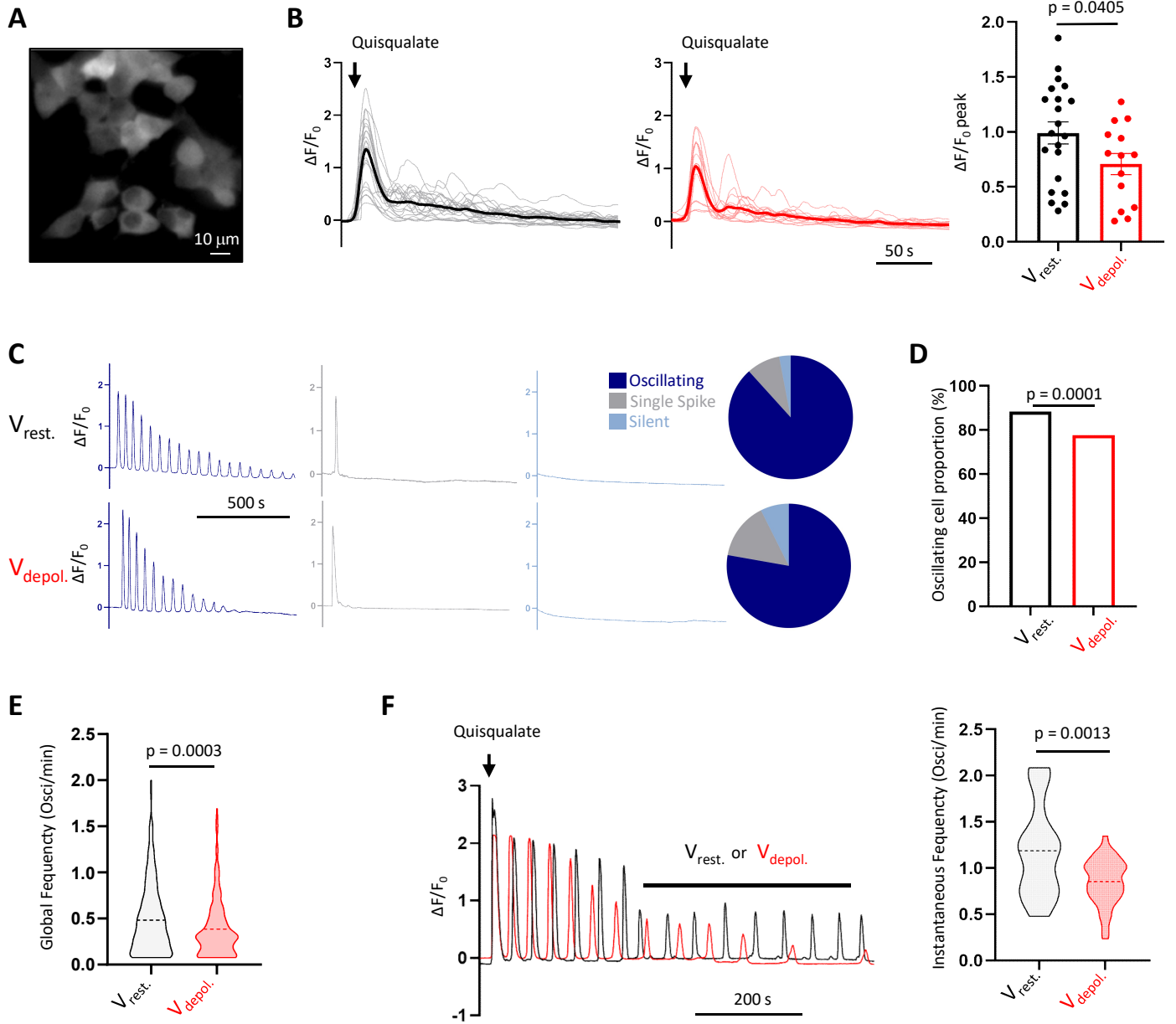


Figure 3

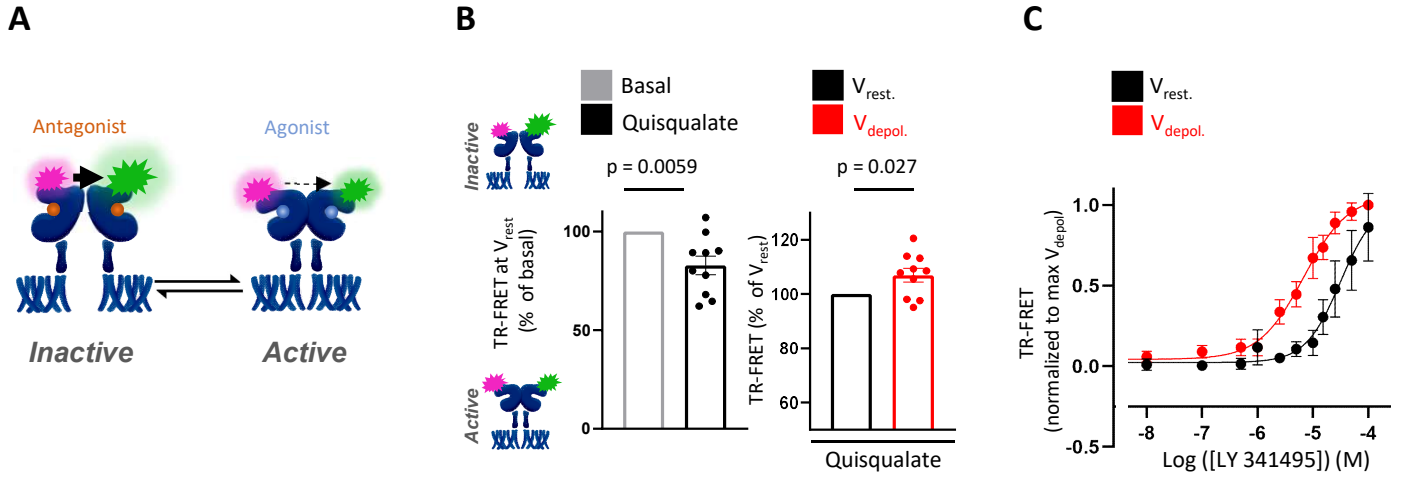


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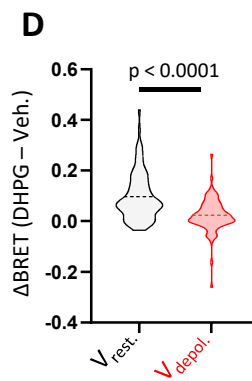
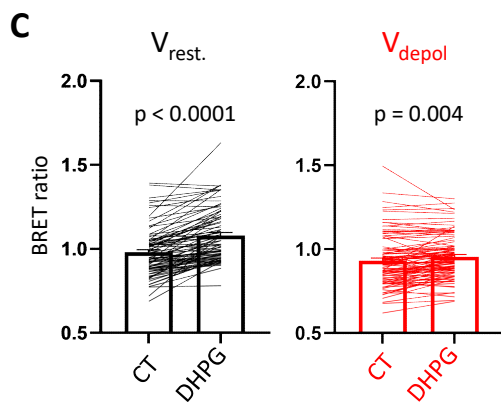
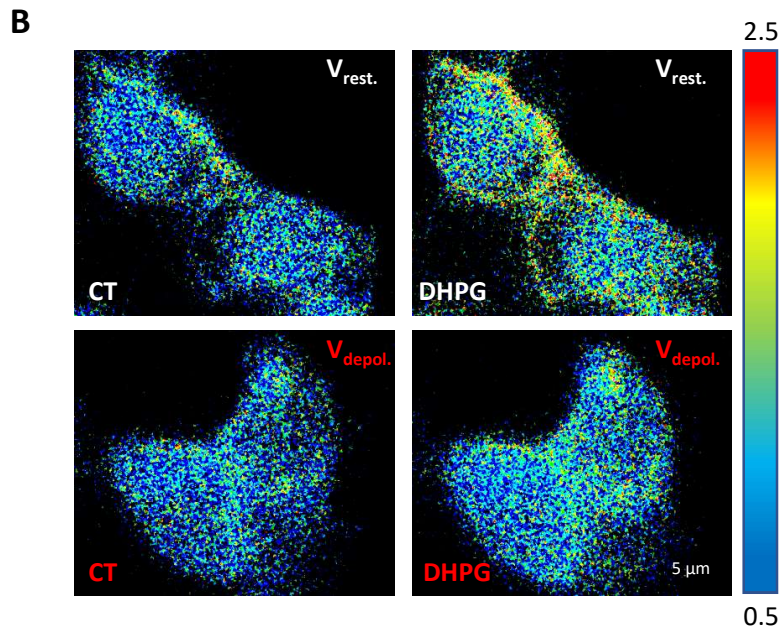
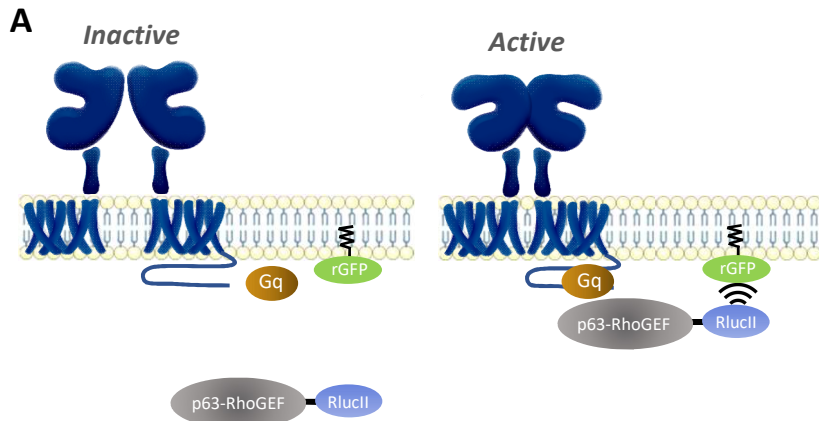


Figure 5

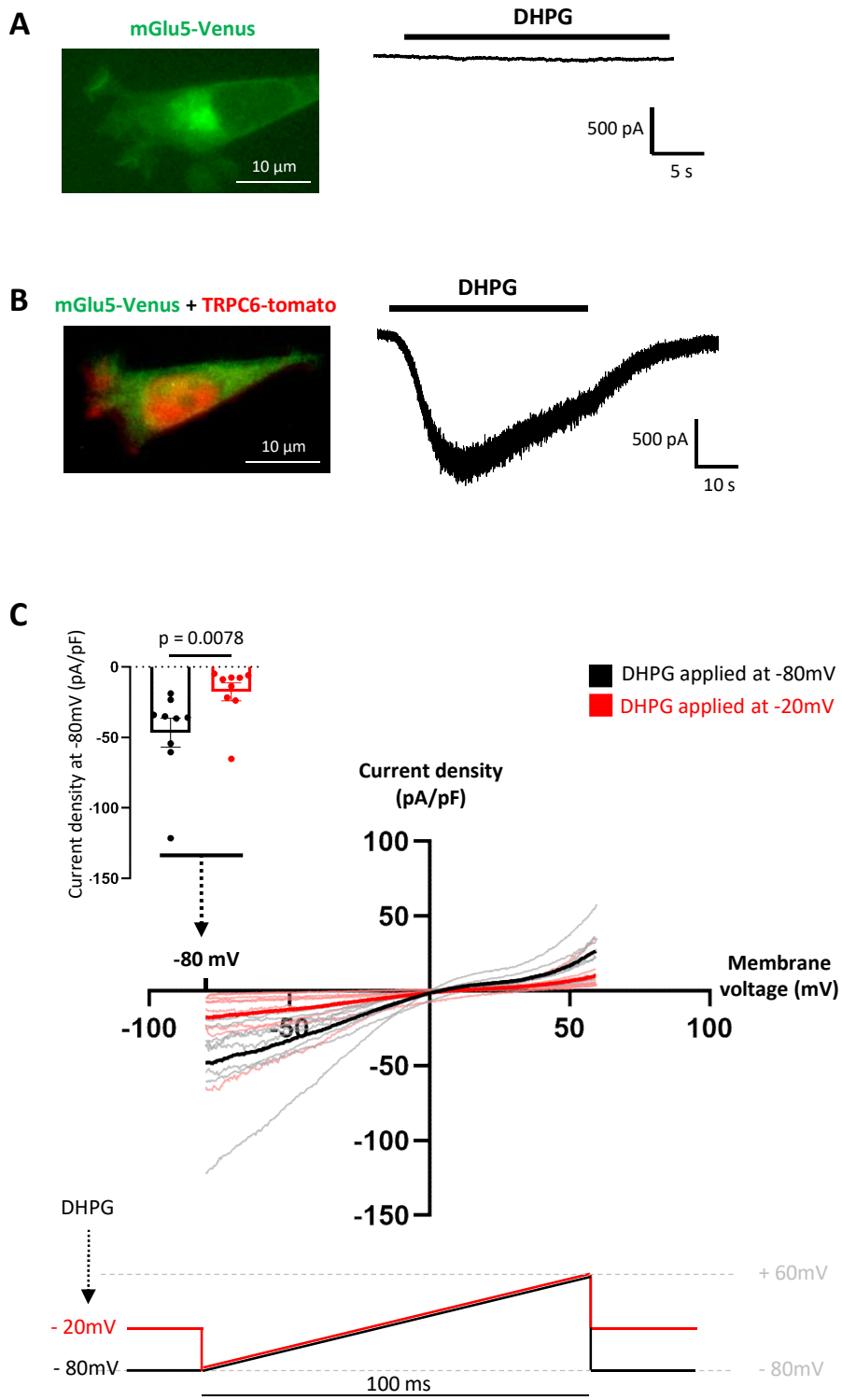
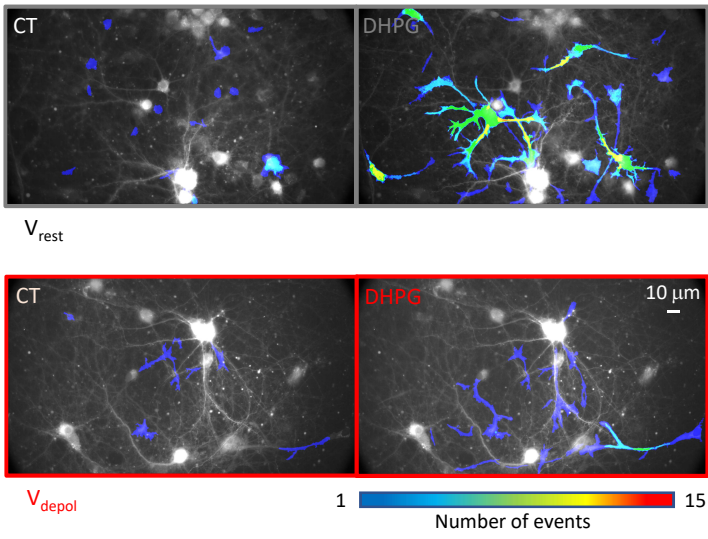
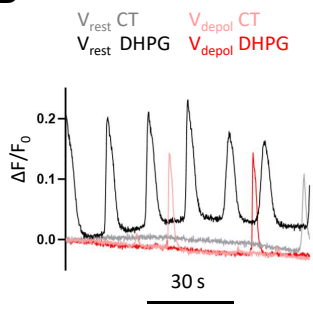


Figure 6

A



B



C

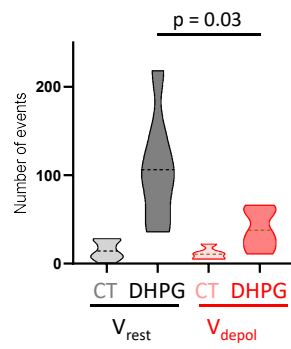


Figure 7

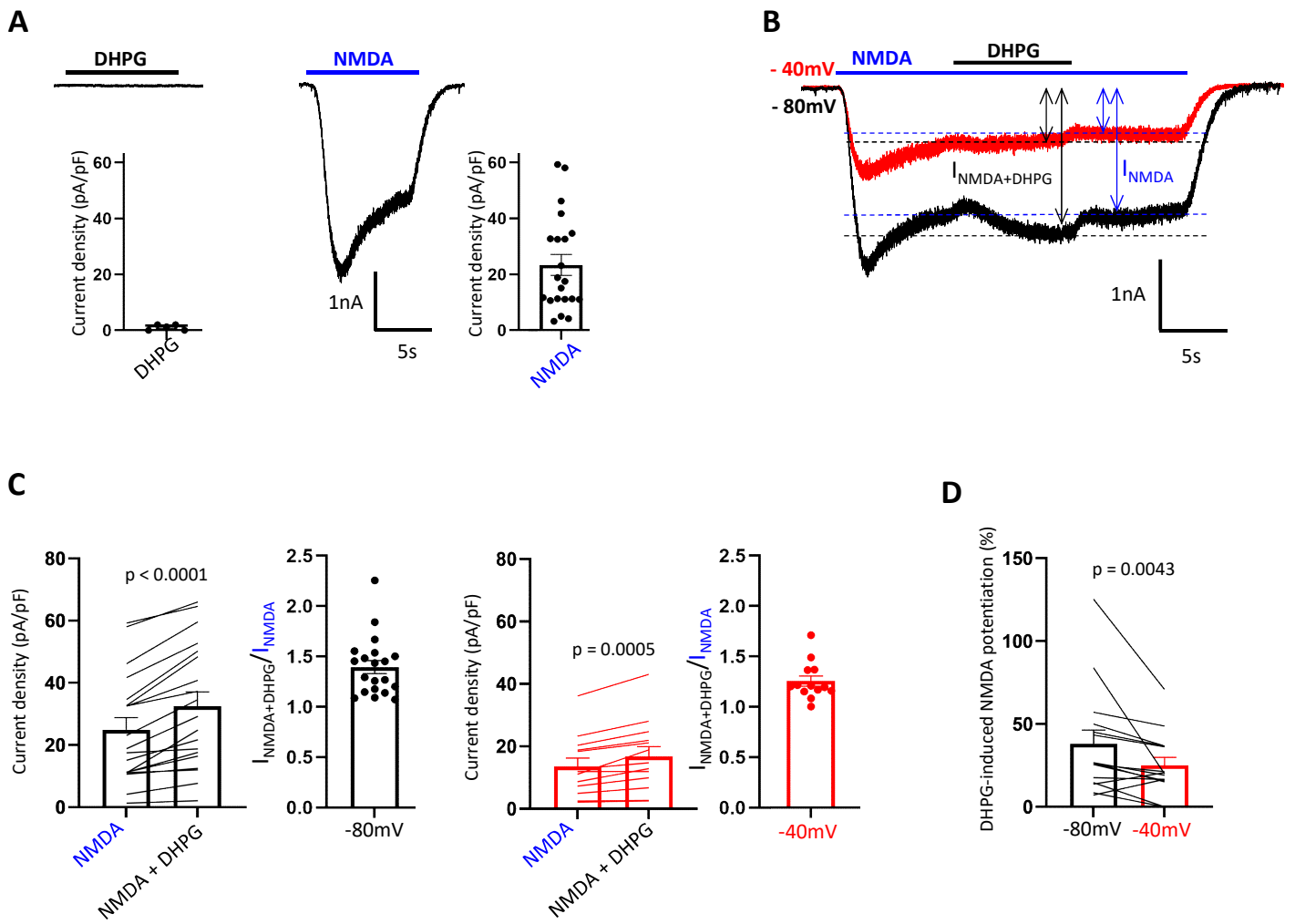


Figure 8

