Lipid rafts serve as signaling platforms for mGlu1 receptor-mediated calcium signaling in association with caveolin

Seung-Eon Roh¹,², Yun Hwa Hong¹,³,⁵, Dong Cheol Jang¹,⁴, Jun Kim¹ and Sang Jeong Kim¹,²,³,⁴* 

Abstract

Background: Group I metabotropic glutamate receptors (mGlu1/5 receptors) have important roles in synaptic activity in the central nervous system. They modulate neuronal excitability by mobilizing intracellular Ca²⁺ following receptor activation. Also, accumulating evidence has indicated the association of Ca²⁺ signaling with lipid rafts. Caveolin, an adaptor protein found in a specialized subset of lipid rafts, has been reported to promote the localization of membrane proteins to lipid rafts.

Results: In the present study, we investigated the role of lipid rafts on the mGlu1α receptor-mediated Ca²⁺ transients and lipid rafts localization of the receptor. Furthermore, transfection of mGlu1α receptor with mutated caveolin-binding domain reduced localization of the receptor to lipid rafts. Also, application of a peptide blocker of mGlu1α receptor and caveolin binding reduced the Ca²⁺ signaling and the lipid rafts localization.

Conclusions: Taken together, these results suggest that the binding of mGlu1α receptor to caveolin is crucial for its lipid rafts localization and mGlu1α receptor-mediated Ca²⁺ transients.

Keywords: mGlu1α receptor, Lipid rafts, Caveolin, Calcium

Background

Metabotropic glutamate receptors (mGluRs) are members of the G protein-coupled receptor (GPCR) superfamily. They are activated by glutamate, which is a major excitatory neurotransmitter in the central nervous system (CNS) and regulate brain functions such as memory, motor control, and neuronal development [1]. mGluRs have been classified into three groups according to their sequence similarity, pharmacology and G protein coupling specificity [1,2]. Group 1 mGluRs, which encompass the mGlu1 and mGlu5 receptor, are expressed in several brain regions including the cortex, hippocampus and cerebellum [3]. They are selectively activated by the specific agonist, (S)-3,5,-dihydroxyphenylglycine (DHPG) [4,5]. Exogenous activation by the agonist evokes an elevation of intracellular Ca²⁺ concentration, which contributes to the induction of long-term plasticity [6,7].

Regulation of neurotransmitter signaling has been found to be associated with lipid rafts, which are sphingolipid- and cholesterol-rich domains of the plasma membrane [8]. Several studies have shown that lipid rafts concentrate many of the regulators and ion channels involved in Ca²⁺ signaling, suggesting significant roles of lipid rafts in modulating Ca²⁺ signaling [9,10]. Lipid rafts exist abundantly in dendrites of neurons, in which they associate with glutamate receptors [11-15]. It has also been reported that the signaling of glutamate receptors is dependent on the integrity of lipid rafts. For instance, AMPA receptors localize to lipid rafts, and their residency in rafts is regulated by the NO-mediated signaling pathway [13]. Also, the NMDA receptor is associated with lipid rafts, and their interaction is related to the signaling of NMDA-induced neuronal death [11].

© 2014 Roh et al; licensee BioMed Central Ltd. This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/2.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited. The Creative Commons Public Domain Dedication waiver (http://creativecommons.org/publicdomain/zero/1.0/) applies to the data made available in this article, unless otherwise stated.
Also, it was found that mGluRs co-localize in lipid rafts together with caveolin [16,17]. We have previously reported that mGlu1α receptor interacts with caveolin, a scaffolding protein found in a specialized subset of lipid rafts, which mediates the agonist-induced internalization of receptor [16]. Furthermore, it has been reported that caveolin knockout mice exhibit impaired mGluR-dependent long-term depression (LTD) at CA3-CA1 synapses of the hippocampus [18]. Although lipid rafts have been extensively reported to regulate glutamate receptors, little is known about their contribution to the regulation of glutamate receptor Ca\textsuperscript{2+} signaling.

In the present study, we investigated whether the integrity of lipid rafts is involved in mGlu1 receptor-mediated Ca\textsuperscript{2+} signaling and also whether it affects localization of the receptor to lipid rafts. To this end, we performed Ca\textsuperscript{2+} imaging with the mGlu1 receptor agonist and examined the co-localization of mGlu1 receptor with lipid rafts using a cholesterol extraction drug. We also examined whether the interaction between mGlu1 receptor and caveolin affects mGlu1 receptor-mediated Ca\textsuperscript{2+} signaling and the lipid rafts localization by disrupting binding sites using mutant transfection or peptide blockade.

**Results**

**Disruption of lipid rafts impairs mGlu1α receptor-induced Ca\textsuperscript{2+} signaling and lipid rafts localization of mGlu1 receptor in hippocampal neurons**

To investigate the role of lipid rafts in mGlu1 receptor functionality, we first asked whether the disruption of lipid rafts affects mGlu1 receptor-induced Ca\textsuperscript{2+} transients. For this purpose, we performed Ca\textsuperscript{2+} imaging with hippocampal primary neurons using a ratiometric Ca\textsuperscript{2+} dye, Fura-2/AM in normal Tyrode’s solution (NT solution) using a lipid rafts disturbing drug. Since hippocampal cultured neurons express both mGlu1 and 5 receptors [19,20], we always measured cytosolic Ca\textsuperscript{2+} level ([Ca\textsuperscript{2+}]c) in the presence of 2-Methyl-6-(phenylethynyl)-pyridine (MPEP, 10 μM), the selective antagonist of mGlu5, to specifically observe mGlu1 receptor-mediated responses. As shown in Figure 1A, application of DHPG (50 μM), the selective antagonist of mGlu1 receptor, cells were stained after fixation. Given the validation of antibody specificity of anti-mGlu1 antibody, we observed almost identical expressions of both throughout soma and dendrites. This result contrasts with transferrin receptor, a non-lipid rafts marker, which was not co-localized with CTX-labeled regions (Additional file 1: Figure S1B). Double-labeling of CTX and mGlu1 receptor revealed that mGlu1α receptor co-localizes with lipid rafts throughout the cells including spines (Figure 1C, control: 48.7 ± 2.2%). Upon incubation with 2 mg/ml mβCD treatment, the co-localization was significantly decreased (Figure 1C, mβCD: 33.7 ± 9.3%, P < 0.01 compared to control). However, mβCD/cholesterol complex treatment did not affect the co-localization of mGlu1 receptor with lipid rafts (mβCD/cholesterol: 49.5 ± 9.7%, P = 0.208 compared with control and P < 0.05 compared with treated mβCD). Present results indicate that the integrity of lipid rafts affects the co-localization with mGlu1 receptor. Collectively, we observed disturbance of both lipid rafts localization and Ca\textsuperscript{2+} signaling by mβCD, these data imply the association of lipid rafts localization of mGlu1 receptor with agonist-induced Ca\textsuperscript{2+} transients of the receptor.

**Binding of mGlu1α receptor and caveolin affects lipid rafts-targeting of the receptor**

We have previously described that agonist-induced internalization of mGlu1α receptor is mediated by the association with caveolin, an adaptor protein found in a
subset of specialized lipid rafts. Also, the binding was abolished by mβCD, which, in turn, also blocked the agonist-induced internalization of mGlu1 receptor [16]. Hence, we asked whether interference of the interaction affects lipid localization of mGlu1 receptor. To this end, we used mGlu1α receptor constructs of wild-type (mGlu1αwt) and mutant with disrupted caveolin binding sites (mGlu1αF609,614A; mGlu1αmu), which is reported to impair agonist-induced Ca2+ transients [16]. These constructs were fused with super-ecliptic pHluorins (SEP),
the pH-sensitive variant of GFP, to exclusively visualize mGlu1α receptors on the cell surface. For ultra-fine illustration of the localization, we utilized super-resolution Structured Illumination Microscopy (SIM, Nikon). Hippocampal primary neurons were transfected with SEP-mGlu1αwt or SEP-mGlu1αmu and simultaneously stained with CTX-Alexa 594. SIM imaging and Pearson’s correlation (PC) analysis clearly revealed the significant decrease of co-localization of SEP-mGlu1αmu with lipid rafts compared to SEP-mGlu1αwt at the soma (mGlu1αwt: 0.557 ± 0.079 Pearson’s co-localization coefficient, mGlu1αmu: 0.125 ± 0.123 Pearson’s r, P < 0.05) and at dendrites (mGlu1αwt: 0.415 ± 0.097 Pearson’s co-localization coefficient, mGlu1αmu: 0.099 ± 0.153 Pearson’s co-localization coefficient, P < 0.05) (Figure 2A). The data clearly indicate that the caveolin binding site mutation abolishes lipid rafts targeting of mGlu1α receptor.

To complementarily corroborate the present results, we executed co-localization analysis with Total Internal Reflection Fluorescence (TIRF) imaging to specifically visualize the cell surface in HEK293 cells. HEK293 cells were imaged after transfection with RFP-mGlu1αwt or mGlu1αmu constructs and lipid rafts labeling by CTX-Alexa 488. The result indicated a significant reduction of co-localization between mGlu1αmu and lipid rafts compared to mGlu1αwt (Figure 2B, mGlu1αwt: 42.2 ± 4.8% and mGlu1αmu: 17.1 ± 2.6%, P < 0.01). In conclusion, these results suggest that the interaction of mGlu1α receptor with caveolin is crucial for the lipid rafts localization of the receptor.
Blocking the interaction of mGlu1α receptor and caveolin decreases mGlu1α receptor-induced Ca^{2+} signaling and receptor localization to lipid rafts in hippocampal neurons

Now that we have confirmed the importance of lipid rafts integrity and of the mGlu1α receptor-caveolin interaction in the functionality of mGlu1α receptors, we next investigated whether mGlu1 receptor-induced Ca^{2+} signaling requires the interaction with caveolin in cultured hippocampal neurons. Since hippocampal neurons endogenously express mGlu1α receptors and caveolin, we treated cells with synthetic peptides consisting of the caveolin binding motif of the mGlu1α receptor to abolish the interaction. The peptide sequence was used from our previous study which describes the amino acid sequence of the caveolin binding domain of the mGlu1α receptor [16]. The peptide was made cell-permeable by attaching a cell-penetrating peptide (CPP), human immunodeficiency virus-type 1 Tat sequence (YGRKKRRQRRR). We generated Tat-blocking peptide (Tat-FVTLIFVLA) for interfering with the interaction and Tat-mutant peptide with dual point mutations (Tat-AVTLIAVL A) as a negative control (Figure 3A). First, we demonstrated successful incorporation of both peptides into the hippocampal primary neurons by staining with anti-Tat antibody, as Tat peptides were found to exist throughout the cells including plasma membranes (Figure 3B).

Next, as revealed by co-immunoprecipitation (Co-IP) assay in Figure 3C, preincubation of Tat-blocking peptide was shown to significantly reduce the interaction between mGlu1α receptor and caveolin when immunoprecipitated with anti-caveolin antibody in hippocampal primary neurons (Tat-blocking peptide: 73.3 ± 1.8% of control, P < 0.001 compared to control), while Tat-mutant peptide incubation did not (Tat-mutant peptide: 97.0 ± 1.0% of control, P = 0.369 compared to control, P < 0.001 compared to Tat-blocking peptide). The efficacy of Tat-blocking peptide was also demonstrated by immunoprecipitating with anti-mGlu1α receptor antibody in HEK293 cells after overexpression of mGlu1α receptor (Additional file 1: Figure S2A).

Since the efficacy of the Tat-blocking peptide was validated, we examined whether the peptide impairs mGlu1 receptor-induced Ca^{2+} transients in hippocampal neurons. Here, we also performed Ca^{2+} imaging in the presence of the selective antagonist of mGlu5 receptor, MPEP.
(10 μM) to specifically observe mGlu1 receptor-induced responses. As shown in representative [Ca$^{2+}$]$_c$ traces and ratio change quantification of Figure 4A, application of Tat-blocking peptide (10 μM) for 45 min was sufficient to markedly reduce mGlu1 receptor-induced Ca$^{2+}$ transients (control: 0.28 ± 0.08 ratio, Tat-blocking peptide: 0.02 ± 0.01 ratio, P < 0.01). However, the amplitude was not significantly decreased when incubated with Tat-mutant peptide (Tat-mutant peptide: 0.20 ± 0.05 ratio, P < 0.01 compared to control, P = 0.379 compared to control). A similar result was obtained in HEK cells transfected with mGlu1α receptor (Additional file 1: Figure S2B). Taken together, these results indicate that blockage of interaction of mGlu1α receptor and caveolin impairs agonist-induced Ca$^{2+}$ signaling of mGlu1 receptor.

To examine whether the Tat-blocking peptide is also responsible for disrupting lipid rafts localization of the receptor in hippocampal neurons, we performed double-labeling immunocytochemistry of endogenous mGlu1α receptor and lipid rafts. Following 45 min treatment of Tat-blocking or -mutant peptide (10 μM), cells were stained with CTX-Alexa 594 and anti-mGlu1α receptor antibody. As shown in Figure 4B, Tat-blocking peptide significantly reduced the localization of the mGlu1α receptor to lipid rafts (control: 54.4 ± 8.5%, Tat-blocking peptide: 28.5 ± 4.7%, P < 0.01), indicating the importance of the interaction of both proteins in lipid rafts localization of mGlu1α receptor. Tat-mutant peptide had no noticeable effect on the localization of the receptor to lipid rafts (Tat-mutant peptide: 51.3 ± 7.0%, P = 0.38 compared to control, P < 0.01 compared to Tat-blocking peptide). In conclusion, these data strongly suggest that the interaction of mGlu1α receptor and caveolin is critical for lipid rafts localization and agonist-induced Ca$^{2+}$ signaling of the endogenous mGlu1α receptor.

Discussion

In this study, we provide a series of evidences for the importance of lipid rafts as a signaling platform for mGlu1α receptor. Using multiple measures, we demonstrated that the integrity of lipid rafts, localization of mGlu1 receptor to lipid rafts and the interaction with caveolin are crucial for agonist-induced mGlu1 receptor

![Figure 4](http://www.molecularbrain.com/content/7/1/9)
intracellular Ca\(^{2+}\) homeostasis is tightly regulated and disturbances in Ca\(^{2+}\) homeostasis have been implicated in several neurodegenerative diseases [30,31]. It is not at all surprising that disturbances in Ca\(^{2+}\) signaling pathways underlie neuronal loss, since many factors involved in neuronal function are dependent on Ca\(^{2+}\) signaling [32]. Although lipid rafts regulate Ca\(^{2+}\) signaling in cardiac myocytes and other tissues, this is yet to be well established in the nervous system. A recent study in astrocytes shows several proteins that form the inositol 1,4,5-triphosphate (IP\(_3\)) dependent Ca\(^{2+}\) cascade – metabotropic receptor P2Y1, Gq, IP\(_3\) receptor (IP3R), phospholipase C \(\beta\) (PLC\(\beta\)) and protein kinase Ca (PKC\(\alpha\)) – are all enriched in lipid rafts. Stimulation of cells with a purinergic agonist recruited PLC\(\beta\) and PKC\(\alpha\) to rafts fractions, whereas lipid raft disruption showed inhibition of agonist-evoked Ca\(^{2+}\) waves [33]. It has also been reported that various membrane receptors localize in lipid rafts and their signaling is dependent on lipid raft integrity in nervous systems [34-38]. Although lipid rafts regulate glutamate receptor signaling, little is known about their contribution to the Ca\(^{2+}\) signaling of glutamate receptors. This study shows that lipid rafts regulate Ca\(^{2+}\) signaling of glutamate receptor in nervous system.

One hypothesis to explain the decrease in mGlu1\(\alpha\) receptor-induced Ca\(^{2+}\) transients depending on lipid raft availability is the reduced interaction of Ca\(^{2+}\) signaling proteins [34-36,38]. Well-established pathways of group I mGluR Ca\(^{2+}\) signaling are the G-protein-dependent (Gaq) intracellular Ca\(^{2+}\) release via PLC and IP\(_3\) activation pathway and the Ca\(^{2+}\) influx via transient receptor potential canonical (TRPC) channel pathway. Interestingly, not only group I mGluRs but also Gq/11, PLC, IP3R, and TRPC, are now known to be present in lipid raft domains [34-36,38]. Thus, it is conceivable that the co-localization of mGlu1\(\alpha\) receptors and their Ca\(^{2+}\) signaling partners, facilitated by their residence in lipid rafts, might regulate coupling of the receptor signaling. It would be interesting further study to examine the function of these molecules after impairing the lipid rafts integrity. A different hypothesis is that mGluRs exist in different affinity states for glutamate, depending on the membrane composition. The receptor is in a high-affinity state when associated with sterol-rich lipid rafts, and in a low-affinity state outside of rafts [39]. Enrichment of the membranes with cholesterol shifts the receptor into the high-affinity state, and induces its association with rafts. It is also possible that differences of agonist binding affinity according to differential lipid raft localization might regulate the coupling of the receptor signaling. Another recent study suggests that mGlu1\(\alpha\) is recruited by agonist to lipid rafts and this is supported in part by intact cholesterol recognition/interaction amino acid consensus (CRAC) motif [40]. The data are in line with our result which showed impairment of Ca\(^{2+}\) signaling by cholesterol extracting drug, m\(\beta\)CD, implying the importance of cholesterol in the receptor function. Further, several studies have suggested MAPK (ERK1/2) and PI3K-Akt-mTOR pathways as down-stream effectors of mGlu1 receptor activation which are involved in long term depression (LTD) [17,41]. It might be interesting to investigate whether m\(\beta\)CD or caveolin binding blockade could affect the activation of these pathways, ultimately influencing synaptic plasticity.

But the function of mGlu1 receptor outside the lipid rafts is still unknown. At least, however, we propose that mGlu1 receptors within lipid rafts display higher activity than those outside lipid rafts as revealed by our data (Figure 1). It is also supported by the previous report which shows transition from resting state to active state of ligand binding domain when the receptor moves to lipid rafts [39]. Also, it may be explained that the mGlu1 receptors may have redundancy in order that only a small fraction of receptors remain active, leaving resting molecules ready to instantly participate in responses to external stimuli. Hence, the activity control by lipid rafts localization might be thought as an important mechanism for mGlu1 receptor function.

Previously, mechanisms of caveolin recruiting receptors to lipid rafts have been proposed [42,43]. As shown through \textit{in vitro} studies, caveolin is sufficient to recruit soluble Ras, the class of signal transducing GTPase, onto lipid membranes [43]. In our previous study, we showed that mGlu1\(\alpha\) receptor contains the putative caveolin binding motifs spanning from the first transmembrane domain to the first inner loop, and from the seventh transmembrane domain to the C-terminal domain of the receptor In HEK293 cells, mGlu1\(\alpha\) receptor mutant which has mutations in only the first transmembrane
domain sufficiently reduced lipid rafts localization of receptor. In hippocampal neurons, we also showed that a blocking peptide impairs the interaction of mGlu1α receptor with caveolin, resulting in a disruption to the lipid raft localization of the receptor. Incubation time (45 min) of the blocking peptide was sufficient to interrupt the interaction between receptor and caveolin, considering the rapid constitutive recycling of mGlu1α receptor [17]. A key finding in this study is that interactions between mGlu1α receptor and caveolin are necessary for lipid rafts localization of the receptor and these ultimately form the Ca\(^{2+}\) signaling pathway. As for the seemingly discrepancy of the effect of Tat-blocking peptide on the Ca\(^{2+}\) transient and immunoprecipitation of mGlu1 receptor, it is considered that the Tat-blocking peptide significantly perturbs the receptor function while retaining some part of the interaction between mGlu1 receptor and caveolin. At any rate, our data are consistent with a study which showed that ATP-induced Ca\(^{2+}\) increases originated in specific areas of the caveolin-enriched plasma membrane in endothelial cells, suggesting that caveolin may be involved in the initiation of agonist-stimulated Ca\(^{2+}\) signaling [44]. However, another report suggests that the interaction of mGlu1 receptor and caveolin-1 is not required for its localization to lipid rafts [40]. It showed that mGlu1 receptor mutated with two caveolin-1 binding sites displayed comparable agonist binding affinity and agonist-induced localization to lipid rafts with wild-type. However, since the mutant itself has significantly reduced surface expression as they discussed, it is difficult to directly compare these measures between wild-type and mutant, while we used a mutant for one caveolin binding site which has normal surface expression level. Also, the experimental conditions such as cell type and agonist are different with ours. Another in vivo result from caveolin\(^{-/}\) brain cortex containing glial tissues does not directly implicate it as neuronal tissues. Still, caveolin should be functionally involved with mGlu1 receptor since caveolin knockout mice show impaired mGlu receptor LTD [18]. Overall, since we observed decreased calcium transients when using mutant mGlu1 receptor [16] and when treated with caveolin binding blocking peptides in our present study, we suggest that caveolin affects, rather than agonist binding affinity of mGlu1 receptor, lipid rafts localization and coupling with downstream effector.

mGlu1 receptor has been implicated in several neurological disorders. For example, disturbances in Ca\(^{2+}\) homeostasis in hippocampal cells have been implicated in neurodegenerative diseases such as Alzheimer's disease (AD) [45]. Specifically, the impairment of declarative memory coincides with the extracellular accumulation of amyloid-β protein (Aβ) [46] and Aβ-enhanced LTD is mediated by mGluR activity and requires an influx of extracellular Ca\(^{2+}\) [47]. It has been shown that enhanced mGluR signaling and Ca\(^{2+}\) release regulated by IP3R were identified as underlying causes of the age-dependent cognitive phenotypes observed [48]. Also, chronic pain is a disease caused by plasticity changes in synapses of nociceptive center and such process is mediated by mGluRs including mGlu1 receptor [49]. Indeed, antagonism of the receptor has emerged as a potential treatment target of pain [50]. Further, there are several evidence which implicate mGlu1 receptor in ataxia [51,52], and anxiety [53,54]. As such, our findings hold significance in that manipulation of lipid rafts and caveolin binding sites which substantially affect the mGlu1 receptor function could modulate the states of diseases described above.

Conclusions
In conclusion, the findings described here suggest that lipid rafts regulate Ca\(^{2+}\) signaling of mGlu1 receptors, and caveolin is required for receptor residency in lipid rafts, suggesting lipid rafts and caveolin as modulation targets for related diseases.

Methods
Cell culture, transfection and DNA constructs
Primary hippocampal neurons were prepared from postnatal day 1 C57BL/6 mouse. In brief, hippocampi were isolated, stripped of meninges, and enzymatically dissociated with trypsin (Invitrogen, USA). After washing, cells were plated on 12 mm coverslips (0.5 × 10\(^4\) cells) for intracellular Ca\(^{2+}\) measurement or immunocytochemistry. Cultures were incubated at 5% CO\(_2\) and 37°C with Neurobasal media (Invitrogen, CA, USA) supplemented with B27 (Invitrogen) and 0.5 mM glutamine (Invitrogen). At DIV3, cells were treated with 1-β-D-Arabinofuranosylcytosine (5 μM, Calbiochem, USA) and fed twice a week with new media until DIV10-14 for experiments including Ca\(^{2+}\) imaging, microscopy and western blotting. The use and care of animals employed in this study followed the guidelines of the National Institutes of Health Animal Research Advisory Committee. We have followed ARRIVE (Animal Research: Reporting In Vivo Experiments) guidelines.

Human embryonic kidney 293 (HEK293) cells were grown in Dulbecco’s Modified Eagle Medium (Invitrogen) supplemented with 10% (v/v) fetal bovine serum and 1% antibiotics (Invitrogen). For the transient expression of mGlu1α receptor, cells growing on 12 mm cover slips or in 35 mm dishes were transfected with 0.5 or 3 μg of DNA, respectively, using FuGENE 6 transfection reagent (Roche Molecular Biochemicals, IN, USA) according to manufacturer’s instructions. Wild-type receptor (mGlu1α receptor\(^{wt}\)) DNA constructs containing red fluorescent protein (RFP-mGlu1α receptor in pRK5 vector) in the extracellular N-terminus were used as previously described [16]. Mutants of mGlu1α receptor (mGlu1α...
receptor\textsuperscript{\textmu}m; mGlu1\textalpha receptor\textsuperscript{F609,614A} were generated using the QuikChange site-directed mutagenesis kit (Stratagene, La Jolla, CA, USA) following the manufacturer’s instructions, using forward primer (22 mer): 5'-cctcgtagcc tgcgctctc tcccacgcc gttcttgacc gg-3' and reverse primer (22 mer): 5'cctcgtagca acgagctga gttgtag ccgcc cagcctgac ag-3'. These constructs were further fused with super ecliptic pHluorin (SEP), the pH-sensitive variant of GFP, for SIM imaging.

Antibodies
Rabbit anti-mGlu1\textalpha receptor was obtained from Dr. C. H. Kim (Department of Pharmacology, Yonsei University College of Medicine, Korea). Rabbit anti-caveolin, rabbit anti-transferrin receptor, rabbit anti-mGlu1 receptor were purchased from BD Bioscience (Lexington, KY, USA). Mouse monoclonal anti-Tat was purchased from Immuno College of Medicine, Korea). Rabbit anti-caveolin, rabbit antibodies, and rabbit anti-monosialotetrahexosylganglioside were purchased from Stressgen (Collegeville, PA, USA) was immunoblotted. Cholera toxin B subunit which binds specifically to ganglioside GM1 was used as marker of surface lipid rafts.

Intracellular Ca\textsuperscript{2+} measurements
Cells on coverslips were loaded for 30 min at 37°C with acetoxy-methyl-ester Fura-2 (Fura-2/AM; Molecular Probes, Carlsbad, CA, USA) in normal Tyrode’s solution (NT; 140 mM NaCl, 5 mM KCl, 2 mM CaCl\textsubscript{2}, 1 mM MgCl\textsubscript{2}, 10 mM glucose, and 10 mM HEPES, pH 7.4) supplemented with 0.01% pluronic acid (Sigma, USA). Ca\textsuperscript{2+} imaging experiments were performed using a confocal microscope (Olympus BX50) with 40 x UV objective (Olympus, Tokyo, Japan). For Fura-2/AM excitation, a monochromator polychrome-II (TILL-Photonics, Munich BioRegio, Germany) was controlled by Axon Imaging Workbench software 6.0 (AIW; Axon Instruments, CA, USA) to provide sequential illumination at two alternating wavelengths, 340 and 380 nm. Fluorescence of Fura-2/AM was detected at an emission wavelength of 510 nm. Video images were acquired using an intensified CCD camera (LUCA; Andor Technology, Belfast, UK). Fluorescence emission ratios following excitation at 340 and 380 nm were calculated. The values were exported from AIW to Origin Pro 8.0 software (OriginLab, MA, USA) for additional analysis and plotting. After randomly selecting an imaging field, all the individual cells in the field were selected as ROI, 10-15 cells each. All experiments were independently performed at least three times.

Immunocytochemistry and cholera toxin (CTX) cell-surface labeling
Cholera toxin B subunit which binds specifically to ganglioside GM1 was used as marker of surface lipid rafts. Following a rinse with PBS (137 mM NaCl, 2.7 mM KCl, 10 mM Na\textsubscript{2}HPO\textsubscript{4} • 2 H\textsubscript{2}O, and 2.0 mM KH\textsubscript{2}PO\textsubscript{4}, pH 7.4), cells were fixed with 4% paraformaldehyde (PFA) and incubated with 2 μg/ml Alexa 488 or 594 conjugated cholera toxin B subunit (CTX-Alexa 488 or 594) in PBS at room temperature for 15 min. Cells were then washed with PBS and further fixed with 4% paraformaldehyde (PFA) for 30 min at room temperature. For double labeling of the lipid rafts makers and mGlu1α receptor, neurons were permeabilized with 0.2% saponin for 10 min at RT and blocked with 5% normal goat serum for 1 h at RT. Rabbit anti-mGlu1α receptor was exposed to neurons overnight at 4°C. Neurons were then treated with anti-rabbit Alexa 594 conjugated secondary antibody for 45 min at RT and mounted on slides using ProLong Gold Anti-fade reagent (Invitrogen). Images for mGlu1α receptor co-localization with lipid rafts markers were acquired by Olympus FV-1000 confocal microscope (Olympus, Japan) equipped with 100x oil-immersion lens (1.35 NA). Cells were excited with 488 nm (from an argon laser) and 559 nm light (from a diode laser).

Co-localization analysis
Co-localization of mGlu1α receptor to CTX-Alexa 488 was analyzed in the soma and dendrites up to 50 μm away from the soma of the neurons. Images were acquired from 3-4 dendrite branches per neuron from several neurons in each condition, and the co-localization scores were obtained as the percentage of overlapping pixels of CTX with mGlu1 receptor or other proteins including flotillin, caveolin, and transferrin receptor after exclusion of background fluorescence. We manually selected soma or dendritic regions of interest for analysis and subtracted the mean intensity of background, outside of soma or dendrites. Co-localization was generally quantified using the MetaMorph 6.0 software (Molecular Devices, Downingtown, PA, USA). For more detailed analysis of co-localization accordingly with SIM imaging in Figure 2, the analysis was performed using Pearson’s correlation test by Nikon NIS-Element-AR software (Nikon, Japan) and data was presented as Pearson’s correlation coefficient, where the coefficient between 0 and -1 implies no co-localization, whereas a value 1 corresponds a perfect co-localization. The specificity of lipid rafts and mGlu1 receptor staining was tested by negative control staining, transferrin and IgG, respectively (Additional file 1: Figure S1).

Structured illumination microscopy
To analyze the co-localization of mGlu1 receptor and lipid rafts in hippocampal primary neurons, we utilized the super-resolution structured illumination microscopy (SIM; Nikon N-SIM). Images were obtained by Eclipse...
Ti-E inverted microscope equipped with Nikon’s legendary CFI Apo TIRF 100× oil objective lens (NA 1.49) and iXon DU-897 EMCCD camera (Andor Technology). Specimens were excited with a diode laser (488 nm and iXon DU-897 EMCCD camera (Andor Technology). Specimens were excited with a diode laser (488 nm and 512×512, 16-bit; Andor Technologies) and analyzed (Olympus Corp., Tokyo, Japan). Images were captured with an IX-71 inverted microscope fitted with a X60, 1.45 N.A. TIRF lens under the control by Cell TM software (Olympus Corp., Tokyo, Japan). Images were captured with an IX-71 inverted microscope fitted with a X60, 1.45 N.A. TIRF lens under the control by Cell TM software. Immunofluorescence images were subject to 16-bit deconvolution using NIS-Element-AR software (Nikon).

**Total internal reflection fluorescence (TIRF) imaging**

TIRF imaging was performed to precisely observe cell surface proteins according to our previous procedures [16]. In brief, RFP-mGlu1α receptor or -mGlu1α receptor expressing HEK cells were washed with PBS and stained with CTX-Alexa 488. Cells were then washed with PBS, fixed with 4% PFA for 30 min at room temperature and mounted on slides using ProLong Gold Anti-fade reagent (Invitrogen). Imaging was performed using Olympus IX-71 inverted microscope fitted with a X60, 1.45 N.A. TIRF lens under the control by Cell TM software (Olympus Corp., Tokyo, Japan). Images were captured with a back-illuminated Andor iXon887 EMCCD camera (512×512, 16-bit; Andor Technologies) and analyzed using MetaMorph 6.0 software.

**mGlu1α receptor-derived synthetic peptides**

Peptides were synthesized to disturb the interaction between mGlu1α receptor and caveolin for Ca2+ imaging and co-localization study. For readily incorporation into the cells, peptides were made cell-permeable utilizing an arginine-enriched cell membrane transduction domain of the HIV-1 Tat protein (YGRKKRRQRRR) [14]. Two peptides were synthesized from AnyGen Co. Ltd. (Kwangju, Korea): Tat-blocking peptides [Tat peptide fused with caveolin binding site of mGlu1α receptor; YGRKKRRQRRR-FVTTLIFVLA] and Tat-mutant peptides that do not interfere with the interaction [Tat peptide fused with caveolin binding site of mGlu1α receptor with dual point mutation; YGRKKRRQRRR-AVTLIAVLA] as a negative control. To validate the incorporation of the peptides into the plasma membrane, cells incubated with vehicle or the peptides for 45 min were stained with anti-Tat antibody (Immuno Diagnostics, MA, USA) for confocal imaging.

**Co-immunoprecipitation (Co-IP)**

Co-IP was performed according to our previous procedure [16]. In brief, lysates were incubated with 2.5 μg/mL rabbit anti-caveolin or mouse anti-mGlu1α receptor (BD Bioscience) antibody for 16 h. They are then incubated with 10 μL of protein G-agarose (Santa Cruz Biotechnology, CA, USA) for 3 h at 4°C. Immunoprecipitates were extensively washed in washing buffer (25 mM Tris, pH 7.4, 10 mM NaCl, 1% Triton X-100), resuspended in 250 mM Tris, pH 6.8, 357.7 mM β-mercaptoethanol, 10% sodium dodecyl sulfate (SDS), 0.5% bromophenol blue, and 50% glycerol (5× SDS sample buffer) and then subjected to immunoblotting.

**Statistics**

Data were analyzed as mean ± standard error of mean (SEM). All statistical analyses were performed as Student’s t-test or one way ANOVA with post-hoc analysis where there are more than two variants using OriginPro 8 software. Statistical tests were indicated in figure legends. The differences between groups were considered to be significant when p < 0.05.

**Additional file**

**Additional file 1: Figure S1.** Specificity of mGlu1 receptor antibody and CTX-Alexa 488 labeling lipid rafts in hippocampal neurons. (A) Negative control images for the specificity of mGlu1α receptor antibody. Hippocampal neurons were stained with mGlu1α receptor antibody or IgG (Red) with DAPI (Blue). Scale bar = 10 μm. The data are representative from at least 3 separate experiments. (B) Cells were labeled with CTX-Alexa 488 (green) together with antibodies recognizing transferrin receptor (negative control; red) or ganglioside GM1 (positive control, red). White boxes in the upper images are enlarged in lower images. Overlapping region (yellow) shows co-localization of green and red signals. Scale bar = 10 μm. Quantification of co-localization was presented on right. The data is shown as mean ± SEM.

**Figure S2.** Tat-blocking peptides disturb mGlu1 receptor–caveolin interaction and affect mGlu1 receptor-mediated Ca2+ transients in HEK293 cells. (A) Co-immunoprecipitation (Co-IP) of mGlu1α receptor with caveolin in cells treated with Tat peptides (10 μM for 45 min) is shown. The Co-IP of mGlu1α receptor with caveolin was significantly reduced by Tat-blocking peptide but not by Tat-mutant peptide n=3. (B) Effects of Tat peptides on the intracellular Ca2+ transients induced by DHPG. HEK293 cells transfected with RFP-mGlu1α receptor construct were incubated with Tat-blocking/mutant peptides and loaded with Fura-2/AM. Cells were perfused with DHPG (50 μM for 60 s). Arrows indicate the DHPG applications. n = 16 (Control), 30 (Tat-blocking peptide), 12 (Tat-mutant peptide). The data is shown as mean ± SEM from at least independent three experiments.

**Abbreviations**

AMP A: 3-amino-3-(5-methyl-3-oxo-1,2- oxazol-4-yl) propanoic acid; DHPG: Dihydroxyphenylglycolylglycol; GM1: Monosialotetrahexosylganglioside; HEK293: Human embryonic kidney 293; IP3R: Inositol 1,4,5-triphosphate receptor; [Ca2+]i: Cytosolic Ca2+ level; NT solution: Normal Tyrode’s solution; MBCD: Methyl-beta-cyclohextrim; mGluR: Metabotropic glutamate receptor; MPEP: 2-methyl-6-(phenylethynyl)-pyridine; NMDA: N-Methyl-D-aspartate; PBS: Phosphate bufferd saline; PFA: PFA; Paraformaldehyde; PKCa: Protein kinase Ca; PLCβ: Phospholipase C β; RFP: Red fluorescent protein; SEP: Super ecliptic pHluorin; SDS: Sodium dodecyl sulfate; TIRF: Total internal reflection fluorescence; SIM: Structured illumination microscopy.

**Competing interests**

All authors declare that they have no conflict of interests.

**Authors’ contributions**

SER, YHH, JK and SJK participated in the design of the study. SER and YHH carried out the molecular studies, calcium imaging, and immunoassays. DCJ carried out the immunoblotting. Authors participated in immunoassays. SER, YHH and SJK drafted the manuscript. All authors read and approved the final manuscript.

**Acknowledgements**

This study was supported by a grant of the Korea Healthcare technology R&D Project, Ministry of Health, Welfare and Family Affairs, Republic of Korea (A102054, A110861 and A120476), National Research Foundation of Korea (NRF) grant funded by the Korea government (MEST) (2009-0080939 and 2011-0030737). Confocal, TIRF microscopy and SIM were carried out in the Biomedical Imaging Center at Seoul National University College of Medicine.
Received: 30 September 2013  Accepted: 30 January 2014
Published: 10 February 2014

Author details
1 Department of Physiology, Seoul National University College of Medicine, 28, Yeongeon-dong, Jongno-gu, Seoul 110-799, Korea. 2 Department of Biomedical Science, Seoul National University College of Medicine, Seoul, Korea. 3 Department of Brain and Cognitive Sciences, College of Natural Science, Seoul National University, Seoul, Korea. 4 Department of Biomedical Engineering, Huree University of Information and Communication Technology, Ulaanbaatar, Mongolia.

References
1. Nakanishi S. Metabotropic glutamate receptors: synaptic transmission, modulation, and plasticity. Neuroreport 1994, 13(5):1031–1037.
2. Conn PJ, Pin JP. Pharmacology and functions of metabotropic glutamate receptors. Annu Rev Pharmacol Toxicol 1997, 37:205–237.
3. Ferraguti F, Shigemoto R. Metabotropic glutamate receptors. Cell Tissue Res 2006, 326(2):483–504.
4. Pin JP, Duvoisin R. The metabotropic glutamate receptors: structure and functions. Neuropharmacology 1995, 34(1):1–26.
5. Hermans E, Challiss RA: Structural, signalling and regulatory properties of the group I metabotropic glutamate receptors: prototypic family C G-protein-coupled receptors. Biochem J 2001, 359(Pt 1):465–484.
6. Borotto ZA, Collingridge GL. Characterisation of LTP induced by the activation of glutamate metabotropic receptors in area CA1 of the hippocampus. Neuropharmacology 1993, 32(1):9–11.
7. Topolnik I, Azzi M, Morin F, Kougoulioutzakis A, Lalacce JC: mGluR1/S subtype-specific calcium signalling and induction of long-term potentiation in rat hippocampal oriens/alveus interneurons. The Journal of physiology 2006, 575(1):115–131.
8. Allen JA, Halwerson-Tamboli RA, Rasenick MM. Lipid raft microdomains and neurotransmitter signalling. Nat Rev Neuroscience 2006, 8(2):128–140.
9. Ishimi M, Anderson RG. Calcium signal transduction from caveole. Cell Calcium 1999, 26(5):201–208.
10. Pani B, Singh BB. Lipid rafts/caveole as microdomains of calcium signalling. Cell Calcium 2009, 45(4):625–633.
11. Beshoff S, Bawa D, Teves L, Wallace MC, Gurd JM. Increased phosphorylation and redistribution of NMDA receptors between synaptic lipid rafts and postsynaptic densities following transient global ischaemia in the rat brain. J Neurochem 2005, 93(1):186–194.
12. Dalient-Ramboson I, Salcedo-Tello P, Bermudez-Rattoni F. Spatial memory formation induces recruitment of NMDA receptor and PSD-95 to synaptic lipid rafts. J Neurochem 2008, 106(4):1658–1668.
13. Hou Q, Huang Y, Amato S, Snyder SH, Huganir RL, Man HY: Regulation of AMPA receptor localization in lipid rafts. Mol Cell Neurosci 2008, 38(2):213–223.
14. Irwin-Dodds PG, Shapiro ME, Yi Z, Chang K, Wenthold RJ: NMDA receptors interact with ftoflavin-1 and -2, lipid raft-associated proteins. FEBS Lett 2009, 583(8):1226–1230.
15. Hering H, Lin CC, Sheng M. Lipid rafts in the maintenance of synapses, dendritic spines, and surface AMPA receptor stability. J Neurosci 2003, 23(8):3262–3271.
16. Hong YH, Kim JY, Lee JH, Chae HG, Jang SS, Jeon JH, Kim CH, Kim J, Kim SJ: Agonist-induced internalization of mGluR1A is mediated by caveolin. J Neurosci 2009, 111(1):61–71.
17. Francesconi A, Kumari R, Zukin RS. Regulation of group I metabotropic glutamate receptor trafficking and signaling by the caveolin/lipid raft pathway. J Neurosci 2009, 29(11):3590–3602.
18. Takayasu Y, Takeuchi K, Kumari R, Bennett ML, Zukin RS, Francesconi A: Caveolin-1 knockout mice exhibit impaired induction of mGluR-dependent long-term depression at CA3-CA1 synapses. Proc Natl Acad Sci USA 2010, 107(50):21778–21783.
19. Shigemoto R, Nakamichi S, Mizuno N: Distribution of the mRNA for a metabotropic glutamate receptor (mGluR1) in the central nervous system: an in situ hybridization study in adult and developing rat. J Comp Neurol 1992, 322(1):121–135.
20. Shigemoto R, Nomura S, Oshii H, Sugihara H, Nakanishi S, Mizuno N: Immunohistochemical localization of a metabotropic glutamate receptor, mGlUR5, in the rat brain. Neurosci Lett 1993, 163(1):53–57.
21. Kilsdonk EP, Yancey PG, Stoudt GW, Bangert FR, Johnson WL, Phillips MC, Rothblat GH: Cellular cholesterol efflux mediated by cyclodextrins. J Biol Chem 1995, 270(29):17250–17256.
22. Argot JM, de la Llana MM, Stoudt GW, Rodriguez-W, Phillips MC, Rothblat GH: Cyclodextrins as catalysts for the removal of cholesterol from macrophage foam cells. J Clin Invest 1997, 99(4):773–780.
23. Christian AE, Haynes MP, Phillips MC, Rothblat GH: Use of cyclodextrins for manipulating cellular cholesterol content. J Lipid Res 1997, 38(11):2264–2272.
24. Ablan S, Rawat SS, Maid M, Wang JM, Puril A, Blumenthal R: The role of cholesterol and sphingolipids in chemokine receptor function and HIV-1 envelope glycoprotein-mediated fusion. Viral J 2006, 3:104.
25. Launikonis BS, Stephenson DG: Effects of membrane cholesterol manipulation on excitation-contraction coupling in skeletal muscle of the toad. J Physiol 2001, 534(Pt 1):71–85.
26. Harder T, Scheiffele P, Verkade P, Simonis K: Lipid domain dynamics of the plasma membrane revealed by patching of membrane components. J Cell Biol 1998, 141(4):929–942.
27. Malenka RC, Kauer JA, Perkel DJ, Nicoll RA: The impact of postsynaptic calcium on synaptic transmission–its role in long-term potentiation. Trends Neurosci 1989, 12(1):444–450.
28. Marty A: The physiological role of calcium-dependent channels. Trends Neurosci 1989, 12(1):40–44.
29. Dubinsky JM: Intracellular calcium levels during the period of delayed excitotoxicity. J Neurosci 1993, 13(2):623–631.
30. Bezprozvanny I: Calcium signaling and neurodegenerative diseases. Trends Mol Med 2005, 11(4):89–100.
31. Marambaud P, Drees-Weninger O, Vinguez V: Calcium signaling in neurodegeneration. Mol Neurodegener 2009, 4:20.
32. Aarts MM, Timmusk TR: MTP7 and ischemic CNS injury. Neuroscientist 2005, 11(2):116–123.
33. Weerth SH, Holzclaw LA, Russell JT: Signaling proteins in raft-like microdomains are essential for Ca2+ wave propagation in glial cells. Cell Calcium 2007, 41(2):155–167.
34. Fujimoto T, Nakade S, Miyawaki A, Mikoshika K, Ogawa K. Localization of Insol 1,4,5-trisphosphate receptor-like protein in plasmaembrenal caveolae. J Cell Biol 1992, 119(5):1507–1513.
35. Fujimoto T, Miyawaki A, Mikoshika K: Insol 1,4,5-trisphosphate receptor-like protein in plasmaembrenal caveolae is linked to actin filaments. J Cell Sci 1995, 108(17):15–17.
36. Lockwich TP, Liu X, Singh BB, Jadowiec J, Weiland S, Ambudkar IS: Assembly of Trp1 in a signaling complex associated with caveolin-scaffolding lipid raft domains. J Biol Chem 2001, 276(16):11944–11942.
37. Dunphy JT, Greenstreet WK, Linder ME: Enrichment of G-protein palmitoyltransferase activity in low density membranes: in vitro reconstitution of Gpalt to these domains requires palmitoyltransferase activity. J Biol Chem 2001, 276(4):34300–34304.
38. Bhatnagar A, Sheffer DJ, Kroese WK, Compton-Tooth B, Roth BL: Caveolin-1 interacts with S-HT2A serotonin receptors and profoundly modulates the signaling of selected Galphag-coupled protein receptors. J Biol Chem 2004, 279(33):34614–34623.
39. Erogul C: Glutamate-binding affinity of Drosophila metabotropic glutamate receptor is modulated by association with lipid rafts. Proc Natl Acad Sci USA 2003, 100(18):10219–10224.
40. Kumari R, Castillo C, Francesconi A: Agonist-dependent signaling by group I metabotropic glutamate receptors is regulated by association with lipid domains. J Biol Chem 2013, 288(4):32004–32019.
41. Antion MD, Hou L, Wong H, Hoeffer CA, Nunn E: mGluR-dependent long-term depression is associated with increased phosphorylation of S6 and synthesis of elongation factor 1A but remains expressed in Sk6-deficient mice. Mol Cell Biol 2008, 28(9):3996–3907.
42. Okamoto T, Schlegel A, Scherer PE, Lisanti MP: Caveolins, a family of scaffolding proteins for organizing “preassembled signaling complexes” at the plasma membrane. J Biol Chem 1998, 273(10):5419–5422.
43. Song KS, Li S, Okamoto T, Quilliam LA, Sargiacomo M, Lisanti MP: Co-purification and direct interaction of Ras with caveolin, an integral membrane protein of caveolae microdomains. Detergent-free purification of caveolae microdomains. J Biol Chem 1996, 271(16):9690–9697.
Endothelial Ca\(^{2+}\) waves preferentially originate at specific loci in caveolin-rich cell edges. 

Bading H, Ginty DD, Greenberg ME: Regulation of gene expression in hippocampal neurons by distinct calcium signaling pathways. Science 1993, 260(5105):181–186.

Lue LF, Kuo YM, Rohrer AE, Brachova L, Shen Y, Sue L, Beach T, Kurth JH, Rydel RE, Rogers J: Soluble amyloid beta peptide concentration as a predictor of synaptic change in Alzheimer’s disease. Am J Pathol 1999, 153(3):853–862.

Li S, Hong S, Shepardson NE, Walsh DM, Shankar GM, Selkoe D: Soluble oligomers of amyloid \(\beta\) protein facilitate hippocampal long-term depression by disrupting neuronal glutamate uptake. Neuron 2009, 62(6):788–801.

soluble oligomers of amyloid \(\beta\) protein facilitate hippocampal long-term depression by disrupting neuronal glutamate uptake. Neuron 2009, 62(6):788–801.

Bading H, Ginty DD, Greenberg ME: Regulation of gene expression in hippocampal neurons by distinct calcium signaling pathways. Science 1993, 260(5105):181–186.

Lue LF, Kuo YM, Rohrer AE, Brachova L, Shen Y, Sue L, Beach T, Kurth JH, Rydel RE, Rogers J: Soluble amyloid beta peptide concentration as a predictor of synaptic change in Alzheimer’s disease. Am J Pathol 1999, 153(3):853–862.

Li S, Hong S, Shepardson NE, Walsh DM, Shankar GM, Selkoe D: Soluble oligomers of amyloid \(\beta\) protein facilitate hippocampal long-term depression by disrupting neuronal glutamate uptake. Neuron 2009, 62(6):788–801.

Bading H, Ginty DD, Greenberg ME: Regulation of gene expression in hippocampal neurons by distinct calcium signaling pathways. Science 1993, 260(5105):181–186.

Lue LF, Kuo YM, Rohrer AE, Brachova L, Shen Y, Sue L, Beach T, Kurth JH, Rydel RE, Rogers J: Soluble amyloid beta peptide concentration as a predictor of synaptic change in Alzheimer’s disease. Am J Pathol 1999, 153(3):853–862.

Li S, Hong S, Shepardson NE, Walsh DM, Shankar GM, Selkoe D: Soluble oligomers of amyloid \(\beta\) protein facilitate hippocampal long-term depression by disrupting neuronal glutamate uptake. Neuron 2009, 62(6):788–801.

Bading H, Ginty DD, Greenberg ME: Regulation of gene expression in hippocampal neurons by distinct calcium signaling pathways. Science 1993, 260(5105):181–186.

Lue LF, Kuo YM, Rohrer AE, Brachova L, Shen Y, Sue L, Beach T, Kurth JH, Rydel RE, Rogers J: Soluble amyloid beta peptide concentration as a predictor of synaptic change in Alzheimer’s disease. Am J Pathol 1999, 153(3):853–862.

Li S, Hong S, Shepardson NE, Walsh DM, Shankar GM, Selkoe D: Soluble oligomers of amyloid \(\beta\) protein facilitate hippocampal long-term depression by disrupting neuronal glutamate uptake. Neuron 2009, 62(6):788–801.

Bading H, Ginty DD, Greenberg ME: Regulation of gene expression in hippocampal neurons by distinct calcium signaling pathways. Science 1993, 260(5105):181–186.

Lue LF, Kuo YM, Rohrer AE, Brachova L, Shen Y, Sue L, Beach T, Kurth JH, Rydel RE, Rogers J: Soluble amyloid beta peptide concentration as a predictor of synaptic change in Alzheimer’s disease. Am J Pathol 1999, 153(3):853–862.

Li S, Hong S, Shepardson NE, Walsh DM, Shankar GM, Selkoe D: Soluble oligomers of amyloid \(\beta\) protein facilitate hippocampal long-term depression by disrupting neuronal glutamate uptake. Neuron 2009, 62(6):788–801.

Bading H, Ginty DD, Greenberg ME: Regulation of gene expression in hippocampal neurons by distinct calcium signaling pathways. Science 1993, 260(5105):181–186.

Lue LF, Kuo YM, Rohrer AE, Brachova L, Shen Y, Sue L, Beach T, Kurth JH, Rydel RE, Rogers J: Soluble amyloid beta peptide concentration as a predictor of synaptic change in Alzheimer’s disease. Am J Pathol 1999, 153(3):853–862.

Li S, Hong S, Shepardson NE, Walsh DM, Shankar GM, Selkoe D: Soluble oligomers of amyloid \(\beta\) protein facilitate hippocampal long-term depression by disrupting neuronal glutamate uptake. Neuron 2009, 62(6):788–801.

Bading H, Ginty DD, Greenberg ME: Regulation of gene expression in hippocampal neurons by distinct calcium signaling pathways. Science 1993, 260(5105):181–186.

Lue LF, Kuo YM, Rohrer AE, Brachova L, Shen Y, Sue L, Beach T, Kurth JH, Rydel RE, Rogers J: Soluble amyloid beta peptide concentration as a predictor of synaptic change in Alzheimer’s disease. Am J Pathol 1999, 153(3):853–862.

Li S, Hong S, Shepardson NE, Walsh DM, Shankar GM, Selkoe D: Soluble oligomers of amyloid \(\beta\) protein facilitate hippocampal long-term depression by disrupting neuronal glutamate uptake. Neuron 2009, 62(6):788–801.