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mGluR5 Knockout Mice Display Increased Dendritic Spine Densities

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Abstract

Alterations in dendritic spine densities and morphologies have been correlated with the abnormal functioning of the synapse. Specifically the metabotropic glutamate receptor 5 (mGluR5) has been implicated in dendrogenesis and spineogenesis, since its activation triggers various signaling cascades that have been demonstrated to play roles in synaptic maturation and plasticity. Here we used the Golgi impregnation technique to analyze the dendritic spines of mGluR5$^{-/-}$ knockout mice in comparison to their heterozygote mGluR5$^{+/-}$ littermates. mGluR5$^{-/-}$mice had elevated spine densities irrespective of spine type or location along their dendritic trees in comparison to mGluR5$^{+/-}$ animals. Such anatomical changes may underlie the hyperexcitability observed in mGluR5 total knockout mice.

Keywords

Barrel cortex; mGlur5; dendritic spines

Introduction

The G-protein coupled receptor mGluR5 (metabotropic glutamate receptor-5) belongs to the group-I family that is coupled to Gq, and has been implicated in the modulation of NMDA-receptor activities (Lea et al., 2002). In the normal course of development of the brain, mGluR5 has been implicated in switching from NR2B- to NR2A-containing NMDA receptors, in both the hippocampus and visual cortex (Matta et al., 2011). In the somatosensory cortex, the absence of mGluR5 is correlated with the reduction of NR2B subunits (She et al., 2009; Wijetunge et al., 2008). Furthermore, it has been demonstrated that the proper functioning of mGluR5 receptors are essential in the organization of thalamocortical topographical arrangement during development, as well as neuronal

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cytoarchitectural maturational processes such as dendritogenesis and axonal arborization (Ballester-Rosado et al., 2010; She et al., 2009; Wijetunge et al., 2008).

Recently, it has been proposed that the dysregulation of mGluR5 on the synaptic level is associated with the abnormal state of brain functioning, which may be the neurological underpinning of several developmental disorders such as Fragile X and autism (for a review, see Krueger and Bear, 2011). It has been suggested that diminishing mGluR5 activation may provide alleviation for the abnormal state of dendritic spine functioning in the cerebral cortex (Dölen and Bear, 2008; Bear et al., 2004).) Hence it is essential to understand the role mGluR5 plays in dendritic spine morphology and density, which are anatomical correlates of synaptic functioning. Although several studies have reported the crucial role of mGluR5 in the neurodevelopmental role of the cerebral cortex (Ballester-Rosado et al., 2010; She et al., 2009; Wijetunge et al., 2008), the full spectrum of its impact on the dendritic spine quantities and morphological phenotypes remains elusive. Here, we report the effect of on layer 4 spiny neurons of deliberately knocking out the mGluR5 component in mice . We focus particularly on the basilar dendrites of pyramidal neurons that are known to receive direct sensory inputs from the thalamus (White, 1984).

Material and Methods

Transgenic KO Animals

The selection and treatment of experimental animals were followed in accordance with the Queens College, CUNY Institutional Animal Care and Use Committee and NIH guidelines. MGluR5 genotyping and selection were followed as previously described (She et al. 2009, Ballester-Rosado et al., 2010). Briefly, mGluR5 mice in C57BL/6J background (B6.129-Grm5 ^{tm1Rod}/J, stock number: 003558) were obtained from Jackson Laboratories (Bar Harbor, Maine) and were bred in a mGluR5$^{+/-}$ and mGluR5$^{-/-}$ mating strategy to produce mGluR5$^{+/-}$ (n=3) and mGluR5$^{-/-}$ (n=3) mice. For comparison purposes the heterozygote mGluR5$^{+/-}$ littermates were used and processed in parallel with the mGlur5$^{-/-}$ mice to control for maternal and cage variables and thus there are no comparisons to wild type animals.

Golgi staining and quantification of dendritic protrusions

Brains from P45 mice of mGluR5$^{+/-}$ and mGluR5$^{-/-}$ were processed with the FD Rapid GolgiStain™ Kit (FD Neurotechnologies, Inc.) as previously described (Chen et al., 2009, Chen et al., 2011). In brief, after the establishment of anesthesia, brains were immediately removed, rinsed in double distilled water, and then transferred to a mixture of equal volumes of solution A and B provided in the kit. This mixture of solutions was replaced once after 12 hours of immersion, and stored at room temperature in darkness for two to three weeks. The embedded brains were then transferred into a cryoprotectant solution, and stored at 4°C for at least one week in the dark. Tissues were next rapidly frozen with dry ice and quickly embedded in OCT (ThermoFisher). Brains were cut into 200-250μm thick sections in the coronal plane with a freezing cryostat. Sections were transferred onto triple-dipped gelatin slides. Following air-drying in the dark, sections were rinsed with distilled water reacted in a developing solution (FD Neurotechnologies, Rapid Golgi Stain Kit), and dehydrated with 50%, 75%, 95%, and 100% ethanol, respectively. After defatting with Histoclear™ (ThermoFisher), the slides were mounted and cover slipped with SHUR/Mount (Triangle Biomedial Sciences, Inc.).

Neurons were selected from the layer IV barrel as previously described (Chen et al. 2009, 2011). Briefly, the barrel cortex location was identified by observing the characteristic clusters of cells that are typically found in the granular layers, and by matching with a

previously published atlas of Golgi stained mice brain (Valverde, 1998). In the rostral-caudal axis, the barrel cortex was defined by the initial appearance of the anterior commissure. Neurons were viewed and traced using an Olympus BX51 microscope with a 100x (oil immersion, NA 1.4) objective. The scope was equipped with a digital camera (Optronics, Microfire), a mechanical stage (Ludl, Thornwood, NY), and an x-y-z axis encoder connected to a Windows Pentium 4 PC. Neurolucida 8.0 software was used to reconstruct the dendrites and spines. Basilar dendrites of 19 pyramidal neurons from mGluR5$^{+/-}$ mice and 20 pyramidal neurons from mGluR5$^{-/-}$ mice were traced. Layer IV neurons displaying a triangular soma with an apical dendrite, the classical morphology of layer IV star pyramidal neurons, were selected for tracing (Cowan and Stricker 2004). Atypically oriented pyramidal neurons were excluded; only basilar dendrites with a length greater than 80μm were included in the quantification. All reconstructions and dendritic protrusion measurements were performed with the researcher blinded to genotype. Dendritic protrusions included spines and filopodia. The density and location of dendritic protrusions relative to the soma were measured. The statistics were conducted with a Student's t-test and a factorial Analysis of Variance.

Results

To explore the putative role of mGluR5 in dendritic spine and filopodia functioning, we quantitatively compared their density in the basilar dendrites of layer IV pyramidal neurons labeled by the Golgi-staining technique from both mGluR5$^{+/-}$ and mGluR5$^{-/-}$ mice. Distinctive filopodia and spine shapes were observed (Figure1A**)** and classified into four morphological categories: filopodia, stubby, mushrooms with thin neck, and mushrooms with thick neck (Figure 1B). We observed that the overall density of the dendritic protrusions (i.e., filopodia + spines) are significantly elevated in the mGluR5$^{-/-}$ (5.73 ± 1.517, data are expressed mean number of protrusions per 10μm dendritic length a ± SD; n = 20 neurons; mGluR5$^{-/-}$, n = 19 neurons mGluR5$^{+/-}$) over mGluR5$^{+/-}$ mice (3.51 ± 1.297) (Figure 1C). The analysis was also conducted per animal (n=3 mGlur5$^{+/-}$, n=3 mGluR5$^{-/-}$) which resulted in the same finding; mGluR5$^{-/-}$animals had significantly (Independent groups t-test, p<.03) higher density of dendritic protrusions (mean = 5.97±0.61 protrusions/ 10 μm) when compared to the heterozygotes (mean =3.60±0.36 protrusions/10 μm). The increase in densities found in mGluR5$^{-/-}$ neurons was found in all four classes of dendritic protrusions (Figure 2A). Results of a mixed-model ANOVA (dendritic protrusion subtypes vs. genotypes) revealed a significant overall main effect of dendritic protrusion subtypes on dendritic protrusion density ($F(3,81) = 23.08$, $p < 0.001$). Consistent F-ratio data of genotypes on protrusion density were confirmed ($F(1,27) = 17.23$, $p < 0.001$), and there is no significant interaction between dendritic protrusion subtypes and genotypes ($p > 0.40$). Specifically, there was a significant increase in filopodia (all statistical significance levels are reported as $p<0.05$ unless otherwise indicated), stubby, thin, and mushroom types of spines in the mGluR5$^{-/-}$ compared with their mGluR5$^{+/-}$ counterparts (Figure 2A).

To examine if the changes in spine and filopodia density varied with distance from the soma, dendrites were divided into proximal segments (within 40μm from the center of soma) and distal segments (41μm to 80μm away from soma). Regardless of the mouse genotypes, the distal dendritic segments have higher density of dendritic protrusions (Figure 2B). The dendritic protrusions of mGluR$^{-/-}$ neurons were significantly higher than mGluR$^{+/-}$ neurons for both proximal and distal segments of basilar dendrites. In summary, our data provide strong support for the role of mGluR5 in both dendritic morphogenesis and spine formation of layer IV neurons.

Discussion

Deleting the mGluR5 receptor profoundly affected the density and morphology of the dendritic spines and filopodia in Layer IV of the cerebral cortex in the C57/bl6 mice strain. The full-KO animals exhibited significantly elevated densities overall, as well as in every morphological class of dendritic protrusions explored (stubby, filopodia, thin, and thick) compared with their heterozygote littermates. In a previous study it was suggested that knocking out mGluR5 in the C57BL/6JX129 strain resulted in a decreased number of spines within layer IV spiny stellate cells in barrel cortex (Wijetunge et al., 2008). By contrast, our data provides additional information to previous studies (She et al., 2009; Ballester-Rosado et al., 2010) which demonstrated that mGluR5 plays a different role in the functioning of cortical layer IV pyramidal neurons in the C57/BL6 strain. A possible reason for the divergent results is that the animals in our study were P45 whereas in the Wijetunge et al (2008) they utilized younger animals (P21-P23). Specifically we show that the spine densities of pyramidal cells increased following the deletion of the mGlur5 receptor. Interestingly, the frequency of mini excitatory postsynaptic potentials is increased, while their amplitudes are unaffected in these same animals (She et al., 2009). Perhaps the increased synaptic input onto these cells is driving the increased density of dendritic protrusions (Kwon and Sabatini 2011).

It has been suggested that the background strain of the knockout mice might have an impact on the cytoarchitectural makeup of the cerebral cortex (Ramos et al., 2008; Lipoff et al., 2010). The effect of knocking out mGluR5 on the dendritic development seems to be largely depended on the strain of the species investigated (Wijetunge et al., 2008; Ballester-Rosado et al., 2010), in which mGluR5 has very little impact on overall dendritic length in the mixed C57BL/6JX129 strain (Wijetunge et al., 2008), but significantly increased impact in the C57BL/6J strain (Ballester-Rosado et al., 2010). Additionally, the Golgi method has been shown in comparison to intracellular staining of neurons to slightly underestimate overall spine densities (Ruan et al., 2009). It is possible due to the resolution of the light microscope that we are incorrectly estimating the distribution of different dendritic protrusions since protrusions projecting vertically up or down relative to the objective could be obscured by the parent dendrite or mischaracterized (if only their distal end is observed). The resolution of our objective is 0.2 μm in the horizontal plane and ~0.9 μm in the vertical plane and thus it is possible that spines were incorrectly assigned to a particular group (eg. two spines close together may appear as a stubby spine), but such errors, if any, should be consistent across genotypes and independent of protrusion of morphology there was observed an increase in density in the homozygote's compared to the heterozygote animals. Interestingly, despite this limitation the overall distribution of spine morphologies that we observed is comparable to what has been observed using electron microscopy (reviewed in Fiala and Harris, 1999) and also seen with other more modern microscopy techniques (Dumitriu et al., 2010). The increased spine density shown in the current study also correlates well with previously published data which found that completely knocking out mGluR5 components resulted in increased mEPSC frequency (Ballester-Rosado, 2010).

Thus it is likely that the altered spine densities and morphologies observed in these mice underlie the physiological differences and provide a structural basis for their altered receptive fields and behavior (She et al., 2009). Changes in spine densities and morphologies underlie many developmental disorders (see Fortin et al. 2011) and thus understanding the role that specific neurotransmitter systems such as mGlur5 have in influencing spine parameters and, in turn, synaptic functioning provides insights for potential future therapeutic targets. Specifically, it has been proposed in primary auditory cortex that stubby spines are the predominant targets of thalamocortical inputs whereas intracortical inputs do not show preferences (Richardson et al., 2009). Similarly, in

somatosensory cortex (S1) there are some reports that thalamic afferents target larger spines (Kharazia and Wienberg 1994; Benshalom 1989) which is correlated with larger pre-synaptic boutons in primary visual cortex(Ahmed et al. 1994) and larger post-synaptic potentials in S1 (Gil et al. 1999). Alternatively, it has been also observed that spine head size is impacted by AMPA receptor density (McKinney 2010; Holtmaat and Svoboda 2009) and given that the AMPA/NMDA ratio is increased in MGlur5 knockout animals (She et al. 2009) the increase in this ratio may be reflective of increased AMPA receptors in more or larger spines. Given the spine increases in every morphological category, it suggests that no one specific pathway is being impacted differentially. The present results highlight the role that the mGlur5 receptor plays in determining morphological properties as well as densities of dendritic spines, and hence its role in potentially influencing the synaptic functioning in the brain. This highlights the need to take the background strain of the knockout mice into account.

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Highlights

- mGluR5$^{-/-}$ mice show overall elevated spine densities in their somatosensory cortex.
- mGluR5$^{-/-}$ mice display increases in spine density for every morphological phenotype.
- This elevated spine density is observed in both distal and proximal sections of the dendrite.

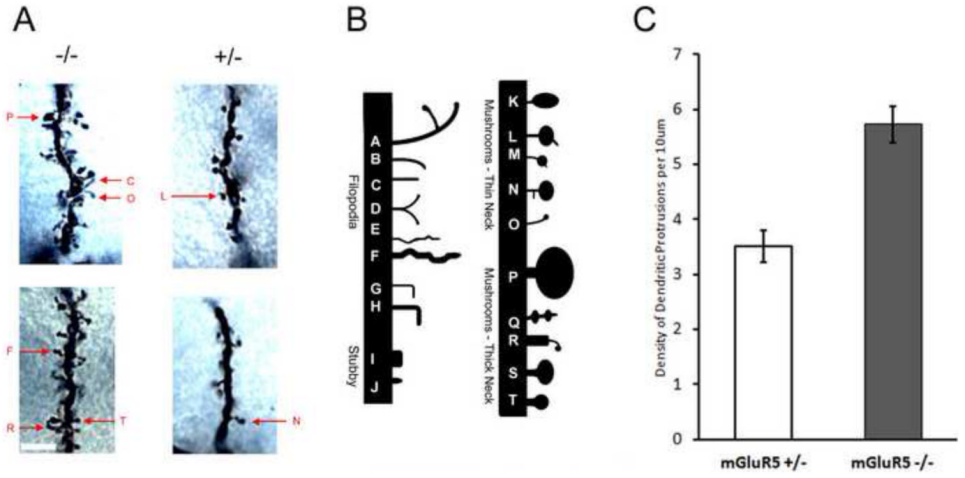

Figure 1.

(A): Microphotographs of Golgi impregnated dendritic protrusions in the mouse barrel layer IV pyramidal neurons. Note the morphological heterogeneity of dendritic spines Stereotyped spines were indicated by arrows and labeled with the letters corresponding to spine subtypes shown in **B**. (**B)** The distinctive dendritic spines observed were categorized and grouped into four categories: filopodia, stubby, thin-neck mushrooms, and thick-neck mushrooms. **(C):** The complete loss of mGluR5 receptor functioning resulted in the significant elevation of dendritic protrusions overall. Asterisks indicate significant difference. Scale bars = 5μm.

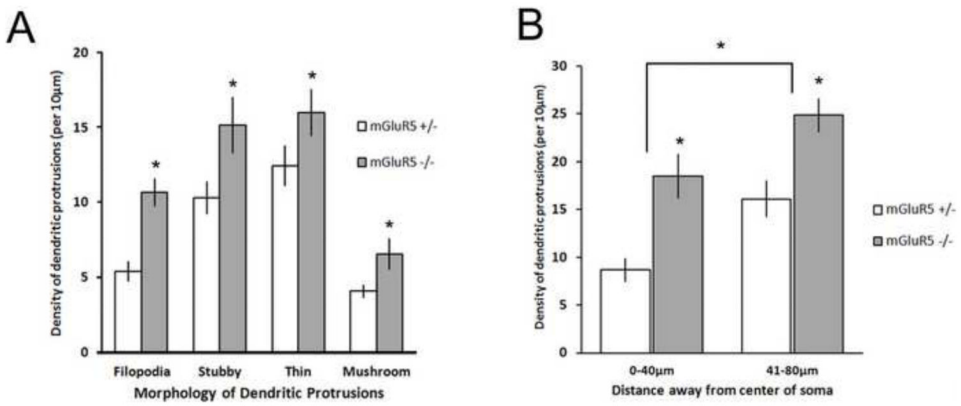

Figure 2.

(A): Summary of spine densities on different types of dendritic spines for mGluR5$^{+/-}$and mGluR5$^{-/-}$ neurons. The loss of mGluR5 resulted in elevation of every morphological category of dendritic spines examined. (**B):** The elevation of dendritic spine density in the mGluR5$^{-/-}$ mice is observed in both proximal (defined as 0-40μm away from soma) and distal (defined as 41-80μm away from soma) segments.