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mGluR5 Positive and Negative Allosteric Modulators Differentially Affect Dendritic Spine Density and Morphology in the Prefrontal Cortex

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Abstract

Positive and negative allosteric modulators (PAMs and NAMs, respectively) of type 5 metabotropic glutamate receptors (mGluR5) are currently being investigated as novel treatments for neuropsychiatric diseases including drug addiction, schizophrenia, and Fragile X syndrome. However, only a handful of studies have examined the effects of mGluR5 PAMs or NAMs on the structural plasticity of dendritic spines in otherwise naïve animals, particularly in brain regions mediating executive function. In the present study, we assessed dendritic spine density and morphology in pyramidal cells of the medial prefrontal cortex (mPFC) after repeated administration of either the prototypical mGluR5 PAM 3-cyano-N-(1,3-diphenyl-1H-pyrazol-5-yl)benzamide (CDPPB, 20 mg/kg), the clinically utilized mGluR5 NAM 1-(3-chlorophenyl)-3-(3-methyl-5-oxo-4H-imidazol-2-yl)urea (fenobam, 20 mg/kg), or vehicle in male Sprague-Dawley rats. Following once daily treatment for 10 consecutive days, coronal brain sections containing the mPFC underwent diolistic labeling and 3D image analysis of dendritic spines. Compared to vehicle treated animals, rats administered fenobam exhibited significant increases in dendritic spine density and the overall frequency of spines with small (<0.2 µm) head diameters, decreases in frequency of spines with medium (0.2–0.4 µm) head diameters, and had no changes in frequency of spines with large head diameters (>0.4 µm). Administration of CDPPB had no discernable effects on dendritic spine density or morphology, and neither CDPPB nor fenobam had any effect on spine length or volume. We conclude that mGluR5 PAMs and NAMs differentially affect mPFC dendritic spine structural plasticity in otherwise naïve animals, and additional studies assessing their effects in combination with cognitive or behavioral tasks are needed.

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Trial Registration

Not applicable.

Conflict of Interest

All authors have no conflicts of interest to declare.

Keywords

allosteric modulator; 3-cyano-N-(1, 3-diphenyl-1H-pyrazol-5-yl)benzamide; dendritic spine; fenobam; medial prefrontal cortex; mGluR5; pyramidal cell; structural plasticity

Introduction

Glutamate is the most prevalent excitatory neurotransmitter in the central nervous system, and mediates the majority of excitatory neurotransmission. Glutamate receptors are classified as either ligand-gated ion channels or metabotropic glutamate receptors (mGluRs). mGluRs are G-protein coupled receptors (GPCRs) that mediate slower, modulatory glutamatergic neurotransmission, and based on their intracellular signaling and pharmacological properties, are categorized into three families: Group I (mGluR1 and mGluR5), Group II (mGluR2 and mGluR3), and Group III (mGluR4, mGluR6, mGluR7 and mGluR8) [1].

Aberrant glutamatergic transmission and receptor signaling has been implicated in numerous neuropsychiatric diseases including drug addiction, schizophrenia, Fragile X syndrome, and neurodegenerative disorders such as Parkinson's and Alzheimer's disease [2–6]. In recent years there has been significant progress towards the development of pharmacological modulators of glutamate transmission, particularly mGluRs, as novel treatments for these afflictions [5, 7–11]. Positive and negative allosteric modulators (PAMs and NAMs, respectively) of GPCR function are ligands that either facilitate or inhibit receptor functioning in the presence of neurotransmitter binding to the orthosteric binding site. mGluR PAMs and NAMs offer several advantages over orthosteric mGluR agonists including increased receptor subtype selectivity, reduced likelihood of receptor desensitization, and more subtle modulatory effects on receptor function [12].

While at least eight mGluR subtypes are known to exist, studies in animals and humans have provided encouraging evidence indicating that allosteric modulators of mGluR5 function may be novel therapeutics for the aforementioned neuropsychiatric diseases. For example, mGluR5 PAMs have been demonstrated to facilitate the extinction of drug-related memories and drug-seeking behavior [13–15], reverse pharmacologically induced cognitive deficits in animal models of schizophrenia and drug addiction [16–20], and improve spatial and recognition memory abilities in otherwise unimpaired animals [21–24]. On the other hand, mGluR5 NAMs have been shown to attenuate the reinforcing effects of various drugs of abuse and produce anti-relapse effects (reviewed in [8, 11, 25]), produce cognitive improvements in rodent models of Fragile X syndrome [26, 27] as well as in Fragile X patients [28], reduce levodopa-induced dyskinesias in animal models of Parkinson's disease [29–31] and in affected human patients [32, 33], and reverse learning and memory deficits in genetic animal models of Alzheimer's disease [34]. Thus, mGluR5 PAMs and NAMs hold significant therapeutic promise for various neuropsychiatric disorders, but their effects on neuronal structural plasticity are not well characterized.

Dendritic spines are the primary receptive regions for excitatory transmission, and their density and structure are highly dynamic and coordinated with synaptic function and

plasticity in both the normal and diseased brain [35–37]. Subtle changes in dendritic spine density or structure can have robust effects on synaptic function and connectivity in neuronal circuits. For example, dendritic spine head diameters are increased following the induction of long-term potentiation (LTP), and are reduced following the induction of long-term depression (LTD) [38–41]. In general, spine morphology is predictive of spine stability and synaptic strength, as spines with larger head diameters tend to form stronger and more lasting synapses, while thinner spines with smaller head diameters tend to be weaker and more transient in nature [42]. In addition, de novo formation of dendritic spines and spine elongation can be rapidly evoked by local application of glutamate [43, 44]. In animal models of neuropsychiatric diseases as well as in postmortem brain tissue from afflicted humans, altered dendritic spine density and altered morphology has been reported, many of which are dependent on the disease, subject age, cell type, and brain region examined [45–50].

Given that mGluR5 PAMs and NAMs are potential novel therapeutics for treatment of various neuropsychiatric diseases, surprisingly few studies have examined the effects of this class of ligands on dendritic spine density and morphology in otherwise unaffected animals. Recent studies indicate that mGluR5 NAMs rescues dendritic spine pathology in the hippocampus [27, 51] and cerebral cortex [26, 52] in mice lacking the fragile X mental retardation protein (FMRP). Embryonic deletion of mGluR5 in mice has been shown to result in increased spine density in pyramidal cells, but only layer IV cells of the barrel cortex were examined [53]. On the other hand, it was recently reported that repeated administration of the mGluR5 PAM 3-cyano-N-(1,3-diphenyl-1H-pyrazol-5-yl)benzamide (CDPPB) following alcohol self-administration in rats facilitated extinction learning, which was accompanied by increases in spine density in the mPFC [14]. However, such effects were observed when treatment was combined with extinction training, and effects of CDPPB in alcohol-naïve animals were not assessed. Given the overwhelming evidence for the importance of the medial prefrontal cortex (mPFC) in the dysfunction of executive control in many of these neuropsychiatric diseases [54], the purpose of the present study was to examine the effects of repeated administration of an mGluR5 PAM or NAM on dendritic spine plasticity in pyramidal cells of the mPFC. To achieve this, we administered either the prototypical mGluR5 PAM CDPPB [55], the clinically utilized mGluR5 NAM 1-(3-chlorophenyl)-3-(3-methyl-5-oxo-4H-imidazol-2-yl)urea (fenobam) [56] or vehicle once daily for 10 consecutive days. Drugs were administered without concomitant behavioral testing in order to more precisely examine the effects of mGluR5 PAMs and NAMs on structural plasticity, without potential interactions with plasticity induced by learning, sensory experience, and cognitive testing [57–59]. We then performed diolistic labeling combined with 3D image analysis to assess potential pharmacological effects on spine density, length, head diameter, and volume in mPFC pyramidal neurons.

Methods and Materials

Subjects

Male Sprague-Dawley rats (Harlan Laboratories, Livermore, CA) weighing 250–300 g upon arrival were used as subjects. Rats were pair-housed and had *ad libitum* access to food and

water under a 12 hr reversed light/dark cycle (lights off 7 am) throughout the duration of the study. The vivarium was held at a temperature of $22\pm1^{\circ}\text{C}$, and animals were maintained in accordance with the guidelines described in the 8th edition of the National Research Council's Guide for the Care and Use of Laboratory Animals. All procedures and facilities were approved by the Institutional Animal Care and Use Committee at Arizona State University.

Drugs

3-cyano-N-1,3-diphenyl-1H-pyrazol-5-yl)benzamide (CDPPB) and 1-(3-chlorophenyl)-3-(3-methyl-5-oxo-4H-imidazol-2-yl)urea (fenobam) were synthesized by Chemir Evans Analytical Group (Maryland Heights, MO) according to previously published methods [55, 56]. Each drug was suspended in a vehicle consisting of 10% v/v Tween 80 (Sigma-Aldrich, St. Louis, MO) and administered once daily at a dose of 20 mg/kg via the subcutaneous (s.c.) route in a volume of 0.5 ml for ten consecutive days. The doses selected for CDPPB and fenobam were based on previous studies by our laboratory and others that have demonstrated behavioral or cognitive effects of these compounds [15, 18, 20, 21, 23, 56, 60, 61] and a lack of neurotoxicity in the mPFC [15], which has recently been described as an adverse effect of higher doses of mGluR5 PAMs [62]. Animals were randomly assigned to treatment groups receiving either CDPPB, fenobam, or vehicle (n=7–8 per group). Immediately following treatment, animals were returned to the home cage, and general health of the animals was observed daily.

Tissue Preparation

Diolistic labeling of neurons was performed according to previously published procedures [14, 63, 64]. Animals were deeply anesthetized with sodium pentobarbital (150 mg/kg) via the intraperitoneal (i.p.) route and perfused transcardially with 100 mL of phosphate-buffered saline (PBS, pH = 7.4) containing 0.1% w/v heparin followed by 200 mL of 1.5% w/v paraformaldehyde (pH = 7.4) in 0.1 M PBS. After perfusion, brains were extracted and post-fixed in 1.5% paraformaldehyde (PFA) in PBS for one hour at room temperature. Brains were then sectioned on a vibratome (The Vibratome Company, Series 1000, St. Louis, MO) in 150 μm serial coronal sections through the PFC. Sections were stored in 25 mM PBS at 4°C until diolistic labeling procedures.

Preparation of Dye-coated Microparticles

For preparation of dye-coated microparticles, the lipophilic carbocyanine fluorescent dye, 1,1-dioctadecyl-3,3,3-tetramethylindocarbocyanine perchlorate, DiI- C_{18} -(3) (DiI, Molecular Probes, Carlsbad, CA) was dissolved in dichloromethane and applied to 100 mg of 1.3- μm diameter tungsten particles (Bio-Rad, Hercules, CA) on a microscope slide. DiI-coated tungsten particles were scraped from the slide and added to 10 ml of 10 mg/ml polyvinylpyrrolidone (PVP; Sigma-Aldrich). The DiI/PVP solution was sonicated and drawn into Tefzel tubing (Bio-Rad) which was pre-coated with 10 mg/ml PVP. Particles were allowed to settle for 30 minutes, after which the PVP solution was carefully withdrawn from the tubing. The tubing was then dried under 0.4 L/min nitrogen gas for 20 min and then cut into segments and protected from light until use.

Delivery of DiI-coated tungsten particles

A Helios gene gun (Bio-Rad) fitted with a polycarbonate filter (3.0 μm pore size) was used to deliver DiI-coated tungsten particles into the tissue sections via pressurized helium (85 psi). Each tissue section was removed from PBS and diolistically loaded with a single pulse of helium. After labeling, sections were re-suspended in PBS for 24 hours to allow DiI to diffuse into the cell membranes. Next, sections were submerged in a 4% PFA solution for one hour, washed with PBS and mounted on gelatin-coated microscope slides. Sections were covered with anti-fade mounting media to protect against photobleaching, coverslipped, and the edges were sealed to prevent dehydration.

Confocal Imaging

A Leica SP5 (Leica Microsystems, Buffalo Grove, IL) confocal microscope with Leica LAS AF software was used to acquire images of DiI labeled neurons. Sections were visualized at 568 nm excitation using a krypton-argon laser. Images of each labeled neuron were acquired using a 63x/1.4 NA oil immersion objective and an XY pixel resolution of 2048×2048 at a frequency of 200 Hz. Each neuron was first scanned at 1.0 μm increments along the z axis to generate an overall image of the neuron and the dendrite to be analyzed, followed by a z-scan at 0.1 μm intervals focusing on the apical dendrite. Apical dendrites were selected for analysis dendrites due to extensive crossover of labeled basilar dendrite segments. Imaged dendritic segments of identified pyramidal neurons were 70 – 120 μm from the soma. Other criterion for selected dendrites for analysis included being devoid of crossovers from other dendrites, positioned beyond the first branch point since initial dendritic segments are often aspiny [65], and the dendrite segment analyzed was between 50–80 μm in length. All dendritic segments imaged were located in the prelimbic (PL) or infralimbic (ILA) regions of the PFC (N=7–8 per animal, see Figure 1). Localization of cortical layer was noted for each neuron and confirmed by comparison of the position of the neuron analyzed under low (10x) magnification and corresponding sections from a rat brain atlas [66].

Quantification and analysis of dendritic spine density and spine morphology

Z-stacks of scanned dendrite segments were reconstructed using Imaris software (Version 7.5, Bitplane Inc., St. Paul, MN). Dendritic shafts and spines were manually traced using the Auto Depth function of the Filament module. Accurate quantification was achieved using the diameter function. The minimum and maximum values for the dendrite diameter were set at 0.3 μm and 3.7 μm , respectively, and the fluorescence contrast threshold was set at 1.3 μm . The minimum and maximum values of the segment diameter for dendritic spines were set at 0.143 μm and 10.5 μm , respectively, and the fluorescence contrast threshold was set at 3 μm . These settings were adequate to distinguish all fluorescent edges which allowed for accurate representation of the shape and size of dendrites and spines analyzed [14, 63, 64, 67]. All dendrite and spine tracing were performed by an investigator blind to experimental treatment group. Dendritic spines were not classified into morphological categories (e.g., mushroom, thin, stubby, etc.), as the continuum of dendritic spine morphology is highly variable and such classification can overlook spines with inter-category characteristics [65, 68, 69].

Statistical Analyses

Overall differences in each variable (spine density, volume, length, and head diameter) across treatment groups were analyzed using SAS (SAS Institute Inc., Research Triangle Park, NC) using a mixed model (SAS Proc Mixed) procedure as we have used previously [14, 70]. The mixed model procedure uses a first-order autoregressive covariance matrix across the sequential slices within each rat. Morphological characteristics of dendritic spines (length, volume, and head diameter) were also binned for frequency analyses [67] where the number of observations in each bin was expressed as a percent of the total number of observations for that particular variable. The following bin sizes were used: spine volume ($0.05 \mu\text{m}^3$), length ($0.5 \mu\text{m}$), and head diameter ($0.2 \mu\text{m}$). Binned data were analyzed by a two-way ANOVA with bin and treatment group as main factors, followed by Bonferroni post hoc tests using Prism version 5.0 (GraphPad, La Jolla, CA). P values < 0.05 were considered statistically significant for all analyses.

Results

General Animal Health

Animals were not subjected to any type of behavioral testing to eliminate potential confounds of these assessments on drug-induced effects on mPFC dendritic spine density or morphology. Therefore, body weight and general health were assessed prior to and following each daily drug administration. All animals showed normal rates of weight gain, and no adverse physical signs of distress were observed.

Localization of Labeled Neurons

Figure 1 shows the regions of the mPFC in which labeled pyramidal neurons were localized, which includes both the prelimbic and infralimbic subregions. Figure 2 shows a representative labeled pyramidal neuron with a dendritic segment selected for analysis. Due to variability in the location of labeled neurons, effects of pharmacological treatment on dendritic spine density and morphology in specific cortical layers could not be assessed. However, all pyramidal cells analyzed were verified to be localized in layers II-V of the mPFC. There were no group differences in the length of the dendrite segment analyzed ($p>0.05$).

Spine Density

A significant effect of treatment on dendritic spine density ($F(2,21)=6.07$, $p<0.01$) was observed, such that rats treated with fenobam exhibited significantly increased spine density as compared to rats treated with vehicle (Figure 3A). However, there was no evidence of an effect of CDPPB on spine density as compared to vehicle treated animals ($p>0.05$).

Spine Head Diameter

A significant effect of treatment on dendritic spine head diameter was also observed ($F(2,21)=6.93$, $p<0.005$), such that rats treated with fenobam exhibited significantly decreased spine head diameter as compared to rats treated with vehicle (Figure 3B). Binned frequency analysis revealed that the overall reduction of spine head diameter by fenobam

was characterized by an increase in frequency of occurrence of spine heads with diameters $<0.2\ \mu\text{m}$ ($p<0.001$) and a decrease in frequency of occurrence of spine heads with diameters between 0.2 and $0.4\ \mu\text{m}$ in medium sized spine heads ($p<0.01$). No group differences were observed in the frequency of occurrence of spine heads with diameters $>0.4\ \mu\text{m}$. In addition, rats treated with CDPPB showed no evidence of effects on spine head diameter as compared to vehicle treated animals ($p>0.05$).

Spine Length and Volume

No effect of treatment on either spine length or volume were observed, either as overall treatment group differences or when data were analyzed by binned frequency of occurrence (Figure 3C and 3D, $p>0.05$).

Discussion

The overall findings of this study are that repeated administration of the mGluR5 NAM fenobam increases dendritic spine density in pyramidal neurons of the rat mPFC, while repeated treatment with the mGluR5 PAM CDPPB has no effect on spine density. Furthermore, repeated fenobam treatment produced an overall reduction in spine head diameter, and binned frequency analysis revealed that fenobam treatment increased the frequency of occurrence of spine head diameters $<0.2\ \mu\text{m}$, decreased the frequency of occurrence of spine head diameters between 0.2 and $0.4\ \mu\text{m}$, while leaving spine head diameters $>0.4\ \mu\text{m}$ unaffected. Rats treated repeatedly with CDPPB showed no evidence of changes in spine head diameter, and neither CDPPB nor fenobam treatment altered spine volume or length. Taken together, these findings suggest that in otherwise normal animals, negative but not positive allosteric modulation of mGluR5 receptors alters the structural plasticity of dendritic spines in pyramidal neurons of the mPFC.

The lack of observed effects of CDPPB were somewhat surprising. In vitro studies have shown that mGluR5 ligands can induce de novo spine growth and elongation in cortical and hippocampal neurons [43, 71]. In addition, studies by our laboratory and others have shown that CDPPB and other mGluR5 PAMs alter performance in mPFC-dependent tasks including the facilitation of extinction of drug-related memories and drug-seeking behavior [13–15], rescue of pharmacologically induced deficits in attentional set-shifting and reversal learning [16–20, 72, 73], and improvements in spatial and recognition memory in otherwise unimpaired animals [21–24]. However, the aforementioned effects were observed when mGluR5 PAMs were administered in conjunction with behavioral testing. In the present study, we examined the effects of CDPPB or fenobam alone on structural plasticity to avoid potential interactions with plasticity induced by learning and cognitive performance [57–59]. It is therefore possible that mGluR5 PAM-induced structural plasticity may require engagement of mPFC pyramidal activity during cognitive or behavioral performance. For example, we recently demonstrated that following operant alcohol self-administration, repeatedly daily administration of CDPPB (30 mg/kg) facilitated extinction learning and reduced subsequent cue-induced reinstatement of alcohol-seeking [14]. When pyramidal neurons in the mPFC of these animals were labeled and analyzed via methods similar to the present study, rats treated with CDPPB showed increases in dendritic spine density in both

the prelimbic and infralimbic subregions only when CDPPB was combined with extinction training; rats treated with CDPPB undergoing forced abstinence in the home cage did not show such alterations in spine density [14]. In addition, animals treated with CDPPB in combination with extinction training demonstrated increases in the number of long (filopodia-like) spines in the prelimbic cortex, and increased mushroom-shaped spines in the infralimbic cortex, whereas animals treated with CDPPB during forced abstinence showed no morphological changes in dendritic spines. Thus, although we observed no effects of treatment with CDPPB on spine density or morphology in the present study, it is possible that mGluR5 PAMs may alter dendritic spine density and morphology in pyramidal neurons in the mPFC either in the presence of other pharmacological manipulations or performance in behavioral tasks. Recent electrophysiological data support this notion, as local application of an mGluR5 PAM onto mPFC slices had no effect on pyramidal neuronal firing rates alone, but altered activity when combined with the cholinergic agonist carbachol [74]. However, other studies have demonstrated that acute mGluR5 PAM or orthosteric agonist administration increases mPFC spontaneous neuronal activity [75–78]. It is also unclear as to what regions other than the mPFC might demonstrate structural plasticity in response to repeated mGluR5 PAM or NAM administration, since the expression of mGluR5 receptors is high in other regions such as the striatum and hippocampus, and mGluR5 PAMs affect other measures of neuronal plasticity such as LTP and LTD in regions such as the hippocampus [21, 74]. Clearly, future studies incorporating other pharmacological manipulations or behavioral tasks are needed to determine under what conditions and brain regions mGluR5 PAMs are able to modulate dendritic spine density and/or morphology.

The ability of the mGluR5 NAM fenobam to increase dendritic spine density in the mPFC is consistent with a previous report in which it was demonstrated that mice with a targeted embryonic deletion of mGluR5 receptors show increased dendritic spine density in layer IV pyramidal neurons of the mouse barrel cortex [53]. This study also demonstrated that mice lacking mGluR5 receptors exhibit increase densities of filopodia, stubby, thin, and mushroom shaped spines [53]. However, comparisons of findings from animals with embryonic gene deletion with those following relatively brief (e.g., 10 day) pharmacological treatments in adulthood must be made with caution, as numerous developmental and genetic factors can contribute to any convergent or divergent results [79]. In other pharmacological studies, it has been demonstrated that repeated administration of the mGluR5 NAM AFQ056/mavoglurant increases dendritic spine density in the hippocampus of wild-type mice [51], and administration of fenobam increases cortical glutamatergic synapse levels of synaptopodin, a regulator of mGluR-mediated spine plasticity [52]. Both of these sets of findings are also consistent with the present results. However, other studies have reported that repeated administration of fenobam or other mGluR5 NAMs, or genetic reduction of mGluR5 expression, leaves cortical dendritic spine density unaltered in wild-type animals [26, 52, 80]. Yet, these studies examined dendritic spine plasticity in the visual or somatosensory cortices as opposed to the mPFC in the present study.

Dendritic spine density and morphology are highly dynamic, correlated with synaptic strength, and regulated by glutamatergic transmission [35, 36, 38–42, 81]. It is well established that mGluR5 receptors are structurally and biochemically coupled to NMDA receptor function, such that potentiation of mGluR5 increases the probability of NMDA

receptor opening, while mGluR5 blockade indirectly decreases NMDA receptor opening [12, 82, 83]. Thus, as a result of direct blockade of mGluR5 receptors and subsequent indirect attenuation of NMDA receptor activity, fenobam may induce the formation of dendritic spines in order to maintain normal synaptic function. Indeed, other studies have demonstrated that pharmacological blockade of NMDA receptors increases the density of cortical dendritic spines, though these effects can be dependent on duration of drug administration, brain region, and ligand utilized [84, 85]. In the present study, rats treated with fenobam showed an overall decrease in dendritic spine head diameter in the mPFC. However, binned frequency analysis revealed that fenobam treatment actually caused an increase in the frequency of spines with small ($<0.2\ \mu\text{m}$) head diameters, decreased the frequency of spines with medium ($0.2\text{--}0.4\ \mu\text{m}$) head diameters, and had no effect on the frequency of spines with large head diameters ($>0.4\ \mu\text{m}$). De novo spine formation is often characterized by an initial appearance of thin and filopodia-like spines which have small head diameters, and subsequent synaptic stabilization and maturation is accompanied by the formation of spines with larger heads (i.e., mushroom-shaped) [35, 36, 38–42]. Although we did not classify dendritic spines into distinct categories in the present study, our results suggest that fenobam treatment was associated with the formation of thin, immature spines with narrow head diameters and a reduction in the frequency of more mature spines with larger spine heads. We did not observe any significant effects of either CDPPB or fenobam on dendritic spine length or volume in mPFC pyramidal neurons. Although changes in spine head diameter were observed, these findings suggest that the effects of allosteric modulation of mGluR5 on spine morphology are subtle, and when administered in the absence of behavioral testing or assessment of cognitive performance, may not be sufficient to induce changes in other aspects of spine morphology.

The precise molecular mechanisms underlying the ability of repeated fenobam administration to increase mPFC pyramidal cell spine density and frequency of thin spines were not investigated in the present study, as we only employed peripheral mGluR5 ligand administration. However, given the role of glutamate and various downstream glutamate receptor-mediated intracellular signaling processes that regulate spine morphogenesis and plasticity (for reviews see [39, 86–89]), some speculation can be made. Early studies demonstrated that stimulation of Group I mGluRs increases local mRNA translation and protein synthesis in dendritic spines [90]. However, it was subsequently revealed that Group I mGluR-mediated protein synthesis is highly regulated by fragile X mental retardation protein (FMRP), the expression of which is epigenetically suppressed in Fragile X syndrome and leads to excess mRNA translation and internalization of (e.g., α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid, AMPA) glutamate receptors, resulting in loss of synaptic strength [91, 92]. We hypothesize that fenobam reduces basal levels of glutamate signaling at mGluR5 receptors in layer 5 mPFC pyramidal cells, which not only reduces postsynaptic NMDA receptor function, but also downstream signaling events including the ability of FMRP to regulate protein synthesis (Fig. 4). The hypothesized end result is an increase in F-actin polymerization leading to increased spine density and the frequency of thin spines in order to achieve normal glutamate synaptic function. This hypothesis is preliminary, however, and requires empirical evaluation.

It should be noted that while the present study assessed the effects of selective mGluR5 ligands, other mGluR subtypes such as mGluR2/3 and mGluR7 are highly expressed in this region [93–95], and their pharmacological activation or inhibition may also alter mPFC dendritic spine plasticity. In addition, mGluR1 receptors, which like mGluR5 are also Group I mGluRs that have a predominantly postsynaptic distribution [96], are expressed to a lesser degree in the mPFC than mGluR5 [97–99]. However, there is evidence that mPFC mGluR1 receptors are primarily expressed on non-pyramidal cells such as interneurons [100–104] where they contribute to feedforward inhibition of pyramidal cells to regulate decision making and nociception [105–107]. There is also evidence for species differences in the cell type distribution of Group I mGluRs in the mPFC, with rodent mGluR1 primarily localized to interneurons [100–104] and mGluR5 primarily localized to pyramidal neurons [97, 108], whereas in primates mGluR1 and mGluR5 appear to be expressed by both of these excitatory and inhibitory neuronal populations [109]. It is therefore important to take potential species- and receptor-specific effects into consideration when assessing the clinical implications of mGluR ligand-induced structural plasticity.

Conclusion

In summary, the present study showed that repeated administration of the clinically utilized mGluR5 NAM fenobam in rats resulted in increased spine density and frequency of spines with smaller head diameter in mPFC pyramidal neurons, while showing no evidence of effect on spine length or volume. In contrast, repeated administration of the prototypical mGluR5 PAM CDPBPB produced no detectable effects on dendritic spine density or morphology in mPFC pyramidal neurons. These findings suggest that mGluR5 NAMs may cause selective compensatory increases in dendritic spine density as well as the frequency of thin, immature spines in order to stabilize synaptic function and excitatory transmission. However, future studies utilizing other doses, durations of treatment, and various animal models of neuropsychiatric diseases are clearly warranted to further characterize effects of mGluR5 PAMs or NAMs on dendritic spine plasticity. Studies are also needed to determine whether such mGluR5 PAM or NAM induced changes mediate the cognitive effects of these classes of potential therapeutic compounds.

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List of Abbreviations

AMPA	α -Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
CDPBPB	3-cyano-N-(1,3-diphenyl-1H-pyrazol-5-yl)benzamide
fenobam	1-(3-chlorophenyl)-3-(3-methyl-5-oxo-4H-imidazol-2-yl)urea
FMRP	fragile X mental retardation protein

GPCR	G-protein coupled receptor
mGluR5	type 5 metabotropic glutamate receptor
mPFC	medial prefrontal cortex
NAM	negative allosteric modulator
NMDA	N-methyl-D-aspartate
PAM	positive allosteric modulator

References

1. Conn PJ, Pin J-P. Pharmacology and functions of metabotropic glutamate receptors. *Annu Rev Pharmacol Toxicol.* 1997; 37:205–237. [PubMed: 9131252]
2. Schwartz TL, Sachdeva S, Stahl SM. Glutamate neurocircuitry: theoretical underpinnings in schizophrenia. *Front Pharmacol.* 2012; 3:195. [PubMed: 23189055]
3. Kalivas PW, Lalumiere RT, Knackstedt L, Shen H. Glutamate transmission in addiction. *Neuropharmacology.* 2009; 56 (Suppl):169–173. [PubMed: 18675832]
4. Gass JT, Olive MF. Glutamatergic substrates of drug addiction and alcoholism. *Biochem Pharmacol.* 2008; 75:218–265. [PubMed: 17706608]
5. Pop AS, Gomez-Mancilla B, Neri G, Willemsen R, Gasparini F. Fragile X syndrome: a preclinical review on metabotropic glutamate receptor 5 (mGluR5) antagonists and drug development. *Psychopharmacology.* 2014; 231:1217–1226. [PubMed: 24232444]
6. Caraci F, Battaglia G, Sortino MA, Spampinato S, Molinaro G, Copani A, et al. Metabotropic glutamate receptors in neurodegeneration/neuroprotection: still a hot topic? *Neurochem Int.* 2012; 61:559–565. [PubMed: 22306345]
7. Dickerson JW, Conn PJ. Therapeutic potential of targeting metabotropic glutamate receptors for Parkinson's disease. *Neurodegener Dis Manag.* 2012; 2:221–232. [PubMed: 23526920]
8. Pomierny-Chamiolo L, Rup K, Pomierny B, Niedzielska E, Kalivas PW, Filip M. Metabotropic glutamatergic receptors and their ligands in drug addiction. *Pharmacol Ther.* 2014; 142:281–305. [PubMed: 24362085]
9. Herman EJ, Bubser M, Conn PJ, Jones CK. Metabotropic glutamate receptors for new treatments in schizophrenia. *Handb Exp Pharmacol.* 2012:297–365. [PubMed: 23027420]
10. Laruelle M. Schizophrenia: from dopaminergic to glutamatergic interventions. *Curr Opin Pharmacol.* 2014; 14C:97–102. [PubMed: 24524997]
11. Olive MF. Metabotropic glutamate receptor ligands as potential therapeutics for drug addiction. *Curr Drug Abuse Rev.* 2009; 2:83–98. [PubMed: 19630739]
12. Niswender CM, Conn PJ. Metabotropic glutamate receptors: physiology, pharmacology, and disease. *Annu Rev Pharmacol Toxicol.* 2010; 50:295–322. [PubMed: 20055706]
13. Olive MF. Cognitive effects of Group I metabotropic glutamate receptor ligands in the context of drug addiction. *Eur J Pharmacol.* 2010; 639:47–58. [PubMed: 20371237]
14. Gass JT, Trantham-Davidson H, Kassab AS, Glen WBd, Olive MF, Chandler LJ. Enhancement of extinction learning attenuates ethanol-seeking behavior and alters plasticity in the prefrontal cortex. *J Neurosci.* 2014; 334:7562–7574. [PubMed: 24872560]
15. Gass JT, Olive MF. Positive allosteric modulation of mGluR5 receptors facilitates extinction of a cocaine contextual memory. *Biol Psychiatry.* 2009; 65:717–720. [PubMed: 19100966]
16. Darrah JM, Stefani MR, Moghaddam B. Interaction of N-methyl-D-aspartate and group 5 metabotropic glutamate receptors on behavioral flexibility using a novel operant set-shift paradigm. *Behav Pharmacol.* 2008; 19:225–234. [PubMed: 18469540]
17. Gastambide F, Gilmour G, Robbins TW, Tricklebank MD. The mGlu₅ positive allosteric modulator LSN2463359 differentially modulates motor, instrumental and cognitive effects of

- NMDA receptor antagonists in the rat. *Neuropharmacology*. 2013; 64:240–247. [PubMed: 22884612]
18. Stefani MR, Moghaddam B. Activation of type 5 metabotropic glutamate receptors attenuates deficits in cognitive flexibility induced by NMDA receptor blockade. *Eur J Pharmacol*. 2010; 639:26–32. [PubMed: 20371234]
 19. Reichel CM, Schwendt M, McGinty JF, Olive MF, See RE. Loss of object recognition memory produced by extended access to methamphetamine self-administration is reversed by positive allosteric modulation of metabotropic glutamate receptor 5. *Neuropsychopharmacology*. 2011; 36:782–792. [PubMed: 21150906]
 20. LaCrosse AL, Burrows BT, Angulo RM, Conrad PR, Himes SM, Mathews N, et al. mGluR5 positive allosteric modulation and its effects on MK-801 induced set-shifting impairments in a rat operant delayed matching/non-matching-to-sample task. *Psychopharmacology*. 2015; 232:251–258. [PubMed: 24973895]
 21. Ayala JE, Chen Y, Banko JL, Sheffler DJ, Williams R, Telk AN, et al. mGluR5 positive allosteric modulators facilitate both hippocampal LTP and LTD and enhance spatial learning. *Neuropsychopharmacology*. 2009; 34:2057–2071. [PubMed: 19295507]
 22. Balschun D, Zuschratter W, Wetzel W. Allosteric enhancement of metabotropic glutamate receptor 5 function promotes spatial memory. *Neuroscience*. 2006; 142:691–702. [PubMed: 16890368]
 23. Uslaner JM, Parmentier-Batteur S, Flick RB, Surles NO, Lam JS, McNaughton CH, et al. Dose-dependent effect of CDPPB, the mGluR5 positive allosteric modulator, on recognition memory is associated with GluR1 and CREB phosphorylation in the prefrontal cortex and hippocampus. *Neuropharmacology*. 2009; 57:531–538. [PubMed: 19627999]
 24. Xu J, Zhu Y, Kraniotis S, He Q, Marshall JJ, Nomura T, et al. Potentiating mGluR5 function with a positive allosteric modulator enhances adaptive learning. *Learn Mem*. 2013; 20:438–445. [PubMed: 23869026]
 25. Bird MK, Lawrence AJ. The promiscuous mGlu5 receptor - a range of partners for therapeutic possibilities? *Trends Pharmacol Sci*. 2009; 30:617–623. [PubMed: 19892412]
 26. Michalon A, Sidorov M, Ballard TM, Ozmen L, Spooren W, Wettstein JG, et al. Chronic pharmacological mGlu5 inhibition corrects fragile X in adult mice. *Neuron*. 2012; 74:49–56. [PubMed: 22500629]
 27. Levenga J, Hayashi S, de Vrij FM, Koekkoek SK, van der Linde HC, Nieuwenhuizen I, et al. AFQ056, a new mGluR5 antagonist for treatment of fragile X syndrome. *Neurobiol Dis*. 2011; 42:311–317. [PubMed: 21316452]
 28. Berry-Kravis EM, Hessel D, Coffey S, Hervey C, Schneider A, Yuhas J, et al. A pilot open-label single-dose trial of fenobam in adults with fragile X syndrome. *J Med Genet*. 2009; 46:266–271. [PubMed: 19126569]
 29. Rylander D, Iderberg H, Li Q, Dekundy A, Zhang J, Li H, et al. A mGluR5 antagonist under clinical development improves L-DOPA-induced dyskinesia in parkinsonian rats and monkeys. *Neurobiol Dis*. 2010; 39:352–361. [PubMed: 20452425]
 30. Petrov D, Pedros I, de Lemos ML, Pallas M, Canudas AM, Lazarowski A, et al. Mavoglurant as a treatment for Parkinson's disease. *Expert Opin Investig Drugs*. 2014; 23:1165–1179.
 31. Gregoire L, Morin N, Ouattara B, Gasparini F, Bilbe G, Johns D, et al. The acute antiparkinsonian and antidyskinetic effect of AFQ056, a novel metabotropic glutamate receptor type 5 antagonist, in L-Dopa-treated parkinsonian monkeys. *Parkinson Relat Disord*. 2011; 17:270–276.
 32. Stocchi F, Rascol O, Destee A, Hattori N, Hauser RA, Lang AE, et al. AFQ056 in Parkinson patients with levodopa-induced dyskinesia: 13-week, randomized, dose-finding study. *Mov Disord*. 2013; 28:1838–1846. [PubMed: 23853029]
 33. Berg D, Godau J, Trenkwalder C, Eggert K, Csoti I, Storch A, et al. AFQ056 treatment of levodopa-induced dyskinesias: results of 2 randomized controlled trials. *Mov Disord*. 2011; 26:1243–1250. [PubMed: 21484867]
 34. Um JW, Kaufman AC, Kostylev M, Heiss JK, Stagi M, Takahashi H, et al. Metabotropic glutamate receptor 5 is a coreceptor for Alzheimer Ab oligomer bound to cellular prion protein. *Neuron*. 2013; 79:887–902. [PubMed: 24012003]

35. Rochefort NL, Konnerth A. Dendritic spines: from structure to in vivo function. *EMBO Rep.* 2012; 13:699–708. [PubMed: 22791026]
36. Shepherd GM. The dendritic spine: a multifunctional integrative unit. *J Neurophysiol.* 1996; 75:2197–2210. [PubMed: 8793734]
37. Piers TM, Kim DH, Kim BC, Regan P, Whitcomb DJ, Cho K. Translational concepts of mGluR5 in synaptic diseases of the brain. *Front Pharmacol.* 2012; 3:199. [PubMed: 23205012]
38. Kasai H, Fukuda M, Watanabe S, Hayashi-Takagi A, Noguchi J. Structural dynamics of dendritic spines in memory and cognition. *Trends Neurosci.* 2010; 33:121–129. [PubMed: 20138375]
39. Murakoshi H, Yasuda R. Postsynaptic signaling during plasticity of dendritic spines. *Trends Neurosci.* 2012; 35:135–143. [PubMed: 22222350]
40. Yuste R. Dendritic spines and distributed circuits. *Neuron.* 2011; 71:772–781. [PubMed: 21903072]
41. De Roo M, Klausner P, Garcia PM, Poglia L, Muller D. Spine dynamics and synapse remodeling during LTP and memory processes. *Prog Brain Res.* 2008; 169:199–207. [PubMed: 18394475]
42. Yasumatsu N, Matsuzaki M, Miyazaki T, Noguchi J, Kasai H. Principles of long-term dynamics of dendritic spines. *J Neurosci.* 2008; 28:13592–13608. [PubMed: 19074033]
43. Kwon HB, Sabatini BL. Glutamate induces de novo growth of functional spines in developing cortex. *Nature.* 2011; 474:100–104. [PubMed: 21552280]
44. Mattison HA, Popovkina D, Kao JP, Thompson SM. The role of glutamate in the morphological and physiological development of dendritic spines. *Eur J Neurosci.* 2014; 39:1761–1770. [PubMed: 24661419]
45. Glausier JR, Lewis DA. Dendritic spine pathology in schizophrenia. *Neuroscience.* 2013; 251:90–107. [PubMed: 22546337]
46. He CX, Portera-Cailliau C. The trouble with spines in fragile X syndrome: density, maturity and plasticity. *Neuroscience.* 2013; 251:120–128. [PubMed: 22522472]
47. Villalba RM, Smith Y. Differential striatal spine pathology in Parkinson's disease and cocaine addiction: A key role of dopamine? *Neuroscience.* 2013; 251:2–20. [PubMed: 23867772]
48. Robinson TE, Kolb B. Structural plasticity associated with exposure to drugs of abuse. *Neuropharmacology.* 2004; 47 (Suppl 1):33–46. [PubMed: 15464124]
49. Penzes P, Cahill ME, Jones KA, VanLeeuwen JE, Woolfrey KM. Dendritic spine pathology in neuropsychiatric disorders. *Nat Neurosci.* 2011; 14:285–293. [PubMed: 21346746]
50. Konopaske GT, Lange N, Coyle JT, Benes FM. Prefrontal cortical dendritic spine pathology in schizophrenia and bipolar disorder. *JAMA Psychiatry.* 2014; 71:1323–1331. [PubMed: 25271938]
51. Pop AS, Levenga J, de ECE, Buijsen RA, Nieuwenhuizen IM, Li T, et al. Rescue of dendritic spine phenotype in *Fmr1* KO mice with the mGluR5 antagonist AFQ056/Mavoglurant. *Psychopharmacology.* 2014; 231:1227–1235. [PubMed: 23254376]
52. Wang GX, Smith SJ, Mourrain P. *Fmr1* KO and fenobam treatment differentially impact distinct synapse populations of mouse neocortex. *Neuron.* 2014; 84:1273–1286. [PubMed: 25521380]
53. Chen CC, Lu HC, Brumberg JC. mGluR5 knockout mice display increased dendritic spine densities. *Neurosci Lett.* 2012; 524:65–68. [PubMed: 22819970]
54. Diamond A. Executive functions. *Annu Rev Psychol.* 2013; 64:135–168. [PubMed: 23020641]
55. Kinney GG, O'Brien JA, Lemaire W, Burno M, Bickel DJ, Clements MK, et al. A novel selective positive allosteric modulator of metabotropic glutamate receptor subtype 5 has in vivo activity and antipsychotic-like effects in rat behavioral models. *J Pharmacol Exp Ther.* 2005; 313:199–206. [PubMed: 15608073]
56. Porter RH, Jaeschke G, Spooren W, Ballard T, Buettelmann B, Kolczewski S, et al. Fenobam: a clinically validated non-benzodiazepine anxiolytic is a potent, selective and non-competitive mGlu5 receptor antagonist with inverse agonist activity. *J Pharmacol Exp Ther.* 2005; 315:711–721. [PubMed: 16040814]
57. Garin-Aguilar ME, Diaz-Cintra S, Quirarte GL, Aguilar-Vazquez A, Medina AC, Prado-Alcala RA. Extinction procedure induces pruning of dendritic spines in CA1 hippocampal field depending on strength of training in rats. *Front Behav Neurosci.* 2012; 6:12. [PubMed: 22438840]

58. Lai CS, Franke TF, Gan WB. Opposite effects of fear conditioning and extinction on dendritic spine remodelling. *Nature*. 2012; 483:87–91. [PubMed: 22343895]
59. Chen X, Leischner U, Rochefort NL, Nelken I, Konnerth A. Functional mapping of single spines in cortical neurons in vivo. *Nature*. 2011; 475:501–505. [PubMed: 21706031]
60. Horio M, Fujita Y, Hashimoto K. Therapeutic effects of metabotropic glutamate receptor 5 positive allosteric modulator CDPPB on phencyclidine-induced cognitive deficits in mice. *Fundam Clin Pharmacol*. 2013; 27:483–488. [PubMed: 22594375]
61. Watterson LR, Kufahl PR, Nemirovsky NE, Sewalia K, Hood LE, Olive MF. Attenuation of reinstatement of methamphetamine-, sucrose-, and food-seeking behavior in rats by fenobam, a metabotropic glutamate receptor 5 negative allosteric modulator. *Psychopharmacology*. 2013; 225:151–159. [PubMed: 22820868]
62. Parmentier-Batteur S, Hutson PH, Menzel K, Uslaner JM, Mattson BA, O'Brien JA, et al. Mechanism based neurotoxicity of mGlu5 positive allosteric modulators - development challenges for a promising novel antipsychotic target. *Neuropharmacology*. 2014; 82:161–173. [PubMed: 23291536]
63. Staffend NA, Meisel RL. DiOlistic labeling of neurons in tissue slices: a qualitative and quantitative analysis of methodological variations. *Front Neuroanat*. 2011; 5:14. [PubMed: 21427781]
64. Staffend NA, Meisel RL. DiOlistic labeling in fixed brain slices: phenotype, morphology, and dendritic spines. *Curr Prot Neurosci*. 2011; Chapter 2(Unit 2):13.
65. Peters A, Kaiserman-Abramof IR. The small pyramidal neuron of the rat cerebral cortex. The perikaryon, dendrites and spines. *Am J Anat*. 1970; 127:321–355. [PubMed: 4985058]
66. Swanson, LW. Brain maps: structure of the rat brain. Elsevier; New York: 1998.
67. Shen H, Sesack SR, Toda S, Kalivas PW. Automated quantification of dendritic spine density and spine head diameter in medium spiny neurons of the nucleus accumbens. *Brain Struct Funct*. 2008; 213:149–157. [PubMed: 18535839]
68. Arellano JI, Benavides-Piccione R, Defelipe J, Yuste R. Ultrastructure of dendritic spines: correlation between synaptic and spine morphologies. *Front Neurosci*. 2007; 1:131–143. [PubMed: 18982124]
69. Spacek J, Hartmann M. Three-dimensional analysis of dendritic spines. I. Quantitative observations related to dendritic spine and synaptic morphology in cerebral and cerebellar cortices. *Anat Embryol (Berl)*. 1983; 167:289–310. [PubMed: 6614508]
70. Kroener S, Mulholland PJ, New NN, Gass JT, Becker HC, Chandler LJ. Chronic alcohol exposure alters behavioral and synaptic plasticity of the rodent prefrontal cortex. *PLoS One*. 2012; 7:e37541. [PubMed: 22666364]
71. Vanderklish PW, Edelman GM. Dendritic spines elongate after stimulation of group I metabotropic glutamate receptors in cultured hippocampal neurons. *Proc Natl Acad Sci U S A*. 2002; 99:1639–1644. [PubMed: 11818568]
72. Gastambide F, Cotel MC, Gilmour G, O'Neill MJ, Robbins TW, Tricklebank MD. Selective remediation of reversal learning deficits in the neurodevelopmental MAM model of schizophrenia by a novel mGlu5 positive allosteric modulator. *Neuropsychopharmacology*. 2012; 37:1057–1066. [PubMed: 22129780]
73. Gilmour G, Broad LM, Wafford KA, Britton T, Colvin EM, Fivush A, et al. In vitro characterisation of the novel positive allosteric modulators of the mGlu5 receptor, LSN2463359 and LSN2814617, and their effects on sleep architecture and operant responding in the rat. *Neuropharmacology*. 2013; 64:224–239. [PubMed: 22884720]
74. Pollard M, Bartolome JM, Conn PJ, Steckler T, Shaban H. Modulation of neuronal microcircuit activities within the medial prefrontal cortex by mGluR5 positive allosteric modulator. *J Psychopharmacol*. 2014; 28:935–946. [PubMed: 25031220]
75. Lecourtier L, Homayoun H, Tamagnan G, Moghaddam B. Positive allosteric modulation of metabotropic glutamate 5 (mGlu5) receptors reverses N-methyl-D-aspartate antagonist-induced alteration of neuronal firing in prefrontal cortex. *Biol Psychiatry*. 2007; 62:739–746. [PubMed: 17511968]

76. Marek GJ, Zhang C. Activation of metabotropic glutamate 5 (mGlu5) receptors induces spontaneous excitatory synaptic currents in layer V pyramidal cells of the rat prefrontal cortex. *Neurosci Lett*. 2008; 442:239–243. [PubMed: 18621097]
77. Kiritoshi T, Sun H, Ren W, Stauffer SR, Lindsley CW, Conn PJ, et al. Modulation of pyramidal cell output in the medial prefrontal cortex by mGluR5 interacting with CB₁. *Neuropharmacology*. 2013; 66:170–178. [PubMed: 22521499]
78. Fontanez-Nuin DE, Santini E, Quirk GJ, Porter JT. Memory for fear extinction requires mGluR5-mediated activation of infralimbic neurons. *Cereb Cortex*. 2011; 21:727–735. [PubMed: 20705895]
79. Wijetunge LS, Till SM, Gillingwater TH, Ingham CA, Kind PC. mGluR5 regulates glutamate-dependent development of the mouse somatosensory cortex. *J Neurosci*. 2008; 28:13028–13037. [PubMed: 19052194]
80. Dolen G, Osterweil E, Rao BS, Smith GB, Auerbach BD, Chattarji S, et al. Correction of fragile X syndrome in mice. *Neuron*. 2007; 56:955–962. [PubMed: 18093519]
81. McKinney RA. Excitatory amino acid involvement in dendritic spine formation, maintenance and remodelling. *J Physiol*. 2010; 588:107–116. [PubMed: 19933758]
82. Pisani A, Gubellini P, Bonsi P, Conquet F, Picconi B, Centonze D, et al. Metabotropic glutamate receptor 5 mediates the potentiation of N-methyl-D-aspartate responses in medium spiny striatal neurons. *Neuroscience*. 2001; 106:579–587. [PubMed: 11591458]
83. Awad H, Hubert GW, Smith Y, Levey AI, Conn PJ. Activation of metabotropic glutamate receptor 5 has direct excitatory effects and potentiates NMDA receptor currents in neurons of the subthalamic nucleus. *J Neurosci*. 2000; 20:7871–7879. [PubMed: 11050106]
84. Li N, Lee B, Liu RJ, Banasr M, Dwyer JM, Iwata M, et al. mTOR-dependent synapse formation underlies the rapid antidepressant effects of NMDA antagonists. *Science*. 2010; 329:959–964. [PubMed: 20724638]
85. Ruddy RM, Chen Y, Milenkovic M, Ramsey AJ. Differential effects of NMDA receptor antagonism on spine density. *Synapse*. 2015; 69:52–56. [PubMed: 25220437]
86. Bosch M, Hayashi Y. Structural plasticity of dendritic spines. *Curr Opin Neurobiol*. 2012; 22:383–388. [PubMed: 21963169]
87. Fortin DA, Srivastava T, Soderling TR. Structural modulation of dendritic spines during synaptic plasticity. *Neuroscientist*. 2012; 18:326–341. [PubMed: 21670426]
88. Lamprecht R. The actin cytoskeleton in memory formation. *Prog Neurobiol*. 2014; 117C:1–19. [PubMed: 24530292]
89. Tada T, Sheng M. Molecular mechanisms of dendritic spine morphogenesis. *Curr Opin Neurobiol*. 2006; 16:95–101. [PubMed: 16361095]
90. Weiler II, Greenough WT. Metabotropic glutamate receptors trigger postsynaptic protein synthesis. *Proc Natl Acad Sci U S A*. 1993; 90:7168–7171. [PubMed: 8102206]
91. Bear MF, Huber KM, Warren ST. The mGluR theory of fragile X mental retardation. *Trends Neurosci*. 2004; 27:370–377. [PubMed: 15219735]
92. Bassell GJ, Warren ST. Fragile X syndrome: loss of local mRNA regulation alters synaptic development and function. *Neuron*. 2008; 60:201–214. [PubMed: 18957214]
93. Kinoshita A, Shigemoto R, Ohishi H, van der Putten H, Mizuno N. Immunohistochemical localization of metabotropic glutamate receptors, mGluR7a and mGluR7b, in the central nervous system of the adult rat and mouse: a light and electron microscopic study. *J Comp Neurol*. 1998; 393:332–352. [PubMed: 9548554]
94. Petralia RS, Wang YX, Niedzielski AS, Wenthold RJ. The metabotropic glutamate receptors, mGluR2 and mGluR3, show unique postsynaptic, presynaptic and glial localizations. *Neuroscience*. 1996; 71:949–976. [PubMed: 8684625]
95. Ohishi H, Neki A, Mizuno N. Distribution of a metabotropic glutamate receptor, mGluR2, in the central nervous system of the rat and mouse: an immunohistochemical study with a monoclonal antibody. *Neurosci Res*. 1998; 30:65–82. [PubMed: 9572581]
96. Lujan R, Roberts JD, Shigemoto R, Ohishi H, Somogyi P. Differential plasma membrane distribution of metabotropic glutamate receptors mGluR1a, mGluR2 and mGluR5, relative to neurotransmitter release sites. *J Chem Neuroanat*. 1997; 13:219–241. [PubMed: 9412905]

97. Kerner JA, Standaert DG, Penney JBJ, Young AB, Landwehrmeyer GB. Expression of group one metabotropic glutamate receptor subunit mRNAs in neurochemically identified neurons in the rat neostriatum, neocortex, and hippocampus. *Mol Brain Res.* 1997; 48:259–269. [PubMed: 9332723]
98. Fotuhi M, Sharp AH, Glatt CE, Hwang PM, von Krosigk M, Snyder SH, et al. Differential localization of phosphoinositide-linked metabotropic glutamate receptor (mGluR1) and the inositol 1,4,5-trisphosphate receptor in rat brain. *J Neurosci.* 1993; 13:2001–2012. [PubMed: 8386753]
99. Testa CM, Standaert DG, Young AB, Penney JBJ. Metabotropic glutamate receptor mRNA expression in the basal ganglia of the rat. *J Neurosci.* 1994; 14:3005–3018. [PubMed: 8182455]
100. Baude A, Nusser Z, Roberts JD, Mulvihill E, McIlhinney RA, Somogyi P. The metabotropic glutamate receptor (mGluR1a) is concentrated at perisynaptic membrane of neuronal subpopulations as detected by immunogold reaction. *Neuron.* 1993; 11:771–787. [PubMed: 8104433]
101. Petralia RS, Wang YX, Singh S, Wu C, Shi L, Wei J, et al. A monoclonal antibody shows discrete cellular and subcellular localizations of mGluR1 alpha metabotropic glutamate receptors. *J Chem Neuroanat.* 1997; 13:77–93. [PubMed: 9285353]
102. Ferraguti F, Conquet F, Corti C, Grandes P, Kuhn R, Knopfel T. Immunohistochemical localization of the mGluR1b metabotropic glutamate receptor in the adult rodent forebrain: evidence for a differential distribution of mGluR1 splice variants. *J Comp Neurol.* 1998; 400:391–407. [PubMed: 9779943]
103. Lopez-Bendito G, Shigemoto R, Fairen A, Lujan R. Differential distribution of group I metabotropic glutamate receptors during rat cortical development. *Cereb Cortex.* 2002; 12:625–638. [PubMed: 12003862]
104. Cauli B, Porter JT, Tsuzuki K, Lambolez B, Rossier J, Quenet B, et al. Classification of fusiform neocortical interneurons based on unsupervised clustering. *Proc Natl Acad Sci U S A.* 2000; 97:6144–6149. [PubMed: 10823957]
105. Sun H, Neugebauer V. mGluR1, but not mGluR5, activates feed-forward inhibition in the medial prefrontal cortex to impair decision making. *J Neurophysiol.* 2011; 106:960–973. [PubMed: 21613584]
106. Ji G, Neugebauer V. Pain-related deactivation of medial prefrontal cortical neurons involves mGluR1 and GABA_A receptors. *J Neurophysiol.* 2011; 106:2642–2652. [PubMed: 21880942]
107. Luongo L, de Novellis V, Gatta L, Palazzo E, Vita D, Guida F, et al. Role of metabotropic glutamate receptor 1 in the basolateral amygdala-driven prefrontal cortical deactivation in inflammatory pain in the rat. *Neuropharmacology.* 2013; 66:317–329. [PubMed: 22796105]
108. Romano C, Sesma MA, McDonald CT, O'Malley K, van den Pol AN, Olney JW. Distribution of metabotropic glutamate receptor mGluR5 immunoreactivity in rat brain. *J Comp Neurol.* 1995; 355:455–469. [PubMed: 7636025]
109. Muly EC, Maddox M, Smith Y. Distribution of mGluR1a and mGluR5 immunolabeling in primate prefrontal cortex. *J Comp Neurol.* 2003; 467:521–535. [PubMed: 14624486]

Figure 1. Location of labeled mPFC pyramidal neurons analyzed in the present study. Pyramidal neurons were selected for confocal imaging and dendritic spine analysis if they were located in either the prelimbic or infralimbic regions (gradient shaded region). Numbers above each coronal section represent the distance (in mm) of the section from bregma. Figure adapted from [62].

Figure 2.
Representative labeled mPFC pyramidal neuron with dendrite segment selected for analysis.
Scale bar = 15 μm .

Figure 3.

Effects of repeated administration of CDPPB or fenobam on dendritic spine density and morphology in rat mPFC pyramidal neurons. **A.** Rats treated with fenobam exhibited significantly increased spine density ($***p < 0.01$ vs. vehicle treated animals). **B.** Inset: Overall spine head diameter was significantly decreased in rats treated with fenobam ($*p < 0.005$). Binned frequency analysis revealed that rats treated with fenobam exhibited increased frequency of smaller ($< 0.2 \mu\text{m}$) head diameters ($***p < 0.001$ vs. vehicle) and decreased the frequency of medium head diameters ($0.2\text{--}0.4 \mu\text{m}$; $**p < 0.01$ vs. vehicle). **C,** **D.** No differences were observed in spine length (**C**) or volume (**D**) in rats treated with either CDPPB or fenobam vs. vehicle. $n = 7\text{--}8/\text{group}$.

Figure 4.

Hypothesized mechanism by which fenobam increases dendritic spine density and the frequency of spines with smaller head diameters. Fenobam produces reductions in basal levels of signaling at mGluR5 receptor in layer 5 mPFC pyramidal cells, which reduces both NMDA receptor function as well as downstream signaling, including the ability of FMRP to regulate protein synthesis. As a result, F-actin polymerization is increased leading to increased spine density and the frequency of thin spines in order to achieve normal glutamate synaptic function. Abbreviations: AMPA, α -Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor; FMRP, fragile X mental retardation protein; GluR5, type 5 metabotropic glutamate receptor; NAM, negative allosteric modulator; NMDA, N-methyl-D-aspartate receptor.