Reduced metabotropic glutamate receptor 5 in the Flinders Sensitive Line of rats, an animal model of depression: An autoradiographic study

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Abstract

Depression is a brain disorder and there is still only a partial understanding of its underlying pathophysiology. Antidepressant medications with a fast onset have not yet been developed. In addition to the monoaminergic systems, the brain glutaminergic system has been implicated in the etiology of depression. Animal studies of depression have gained importance because they permit a more invasive manipulation of the subjects than human studies. In the present study, we measured the densities of the brain regional metabotropic glutaminergic receptor 5 (mGluR5) in the Flinders Sensitive Line (FSL) rat model of depression and two groups of control rats, the Flinders Resistant Line (FRL) and Sprague Dawley (SPD), the parent strain for both the FSL and FRL rats. The FSL rats showed lower densities of mGluR5 in many brain regions compared to either the SPD and/or FRL rats. In addition, the densities in the FRL rats were larger than in the SPD rats, suggesting possible problems in using FRL rats as controls. The presented data suggest that mGluR5 is lower in animal models of depression which could be related to the cognitive and emotional dysfunctions in the FSL rat model of depression and could be relevant to a better understanding of depression in humans.

Keywords

mGluR5 receptors; Depression model; Sprague Dawley rats; Flinders Sensitive Line; Flinders Resistant Line

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1. Introduction

Depression is a debilitating mental disorder with serious societal impact. The chronic, recurring character of depression is illustrated by the fact that 17–20% of the worldwide population suffers from it [33] with 15% of those suffering from major depression committing suicide [7]. The lag period of the therapeutic effect usually takes several weeks (3–6 weeks) and its not achieved in 1/3 of patients [10] which indicates that the therapeutic effect is dependent on the effects beyond the elevation of monoamines [20]. Past results confirm the importance of monoamines in the regulation of depressed mood, but newer data support the idea that other brain neuronal systems may play a more important role in the pathophysiology of depression, specifically excitatory amino acids [40]. The glutamatergic system is a dominant excitatory system in the brain [14] and as such it is not surprising that it might be involved in neurochemical description of the brain depressive state. In the CNS, glutamate is taken back by astrocytes via glutamate transporters and metabolized into glutamine [55].

mGluR5 receptors are broadly distributed throughout the brain, with moderate to high expression in the cerebral cortex, dorsal and ventral striatum, olfactory bulbs and tuberculum, septal area, hippocampus and inferior colliculus [14]. mGluR5 sites are preferentially located perisynaptically on the postsynaptic membrane, resulting in increased calcium release from intracellular stores, cell depolarization, and subsequent increased in cellular excitability and activation of second messenger pathways (e.g., protein kinase A (PKA); protein kinase C (PKC); mitogen activated protein kinase (MPAK))[14]. mGluR5 are coupled to Gq/G11 and activate phospholipase C β and stimulates the generation of IP3 and DAG [17]. Glutamate receptors are divided into two groups: (a) ionotropic; AMPA (α -amino-3-hydroxyl-5-methyl-4-isoxazole-propionate) kainate and NMDA (*N*-methyl D-aspartate); and (b) metabotropic. Three metabotropic (mGluR) receptor groups I, II, III and 8 receptors altogether have been identified so far (mGluR1–mGluR8) [37]. The mGluR1 and mGluR5 receptors belong to group I of the mGluRs [2,14,68]. These receptors can modulate signaling pathways other than Gq and other molecules which are independent of G proteins [17]. The activation of the mGluRs is necessary for the induction of the long-term potentiation (LTD and LTP) in the CA1 and CA3 regions of the hippocampus which is shown to be a cellular mechanism of motor learning and behaviour [17,47]. Recent discoveries that antagonists of mGluR5 act as anxiolytics supports the idea that this receptor is responsible for the modulation of depressive symptoms [65].

A study with 2-methyl-6-(phenylethynyl)pyridine (MPEP; mGluR5 antagonist) performed in the olfactory bulbectomized rats (OBX) restored the learning capacity in this model of depression [55]. Another study showed that prolonged MPEP in OBX rat model of depression produced antidepressant like effects evident by reversion of behavioural parameters [50]. Furthermore, mGluR5 knockout mice exhibited antidepressant-like behaviour [32]. The therapeutic effect of the mGluR5 receptors may account for the modulatory properties of the metabotropic receptors on ionotropic receptors, such as *N*-methyl D-aspartate (NMDA) [26,65], or it can be the result of the activation of other neuronal systems in the brain, such as neuropeptide Y in the amygdala, which has been reported to have anxiolytic properties [65]. The collected data demonstrate that currently

used antidepressants act on the glutamatergic system at several levels (i.e., impact glutamate release [39] by increasing the expression of the glutamate transporter [44] and by changing glutamate receptor activity [52]). Positive allosteric modulators (PAMs) of mGluR5 indirectly increase NMDA function with a non-monoaminergic antipsychotic effect and pro-cognitive effects in animals (e.g., learning and memory) were demonstrated through behavioural changes [14]. The activation of mGluR5 assists with calcium metabolism and the activation of calcium/calmodulin dependent protein kinases [9]. It has been indicated that the mGluR5 receptor is a potential target for drugs that treat other neurological and psychiatric disorders, such as pain, Parkinson's disease, impaired cognition, depression, anxiety and schizophrenia [2,9].

The mGluR5 receptor was previously studied using a labeled antagonist MPEP (2-methyl-6-(phenylethynyl)pyridine) [37], or its analogues labeled with positron emitting radionuclide [69]. Other studies with ABP688 (3-(6-methyl-pyridin-2-ylethynyl)-cyclohex-2-enone-methyl-oxime) have been performed in humans using positron emission tomography (PET) imaging [2,16]. This receptor ligand has a high specificity and characteristics that lend it to research in both animals and humans [27].

The Flinders Sensitive Line (FSL) rats were bred from Sprague Dawley (SPD) rats based on their super sensitivity to diiso-propyl fluorophosphate (DFP), an anticholinesterase agent [49]. The Flinders Resistant Line (FRL) rats represent a control line bred from the same parent strain (SPD). The behavioural characteristics of the FSL rats that resemble many features of depressive disorder in humans are psychomotor retardation, lower weight gain, increased REM sleep, immune abnormalities and anxiety shown in some behavioural tests [48,49], and, as such, the FSL rats are proposed to be a model of retarded depression [48]. An important contribution to the construct validity of the FSL rat model is the alleviation of depressive characteristics following chronic, but not acute, antidepressant treatment [48]. In addition to a variety of animal models of depression, a model based on bacterial meningitis has been proposed [4].

The FSL exhibit many biochemical abnormalities; alteration in 5-HT levels and synthesis [24,70], different 5-HT receptor densities [45,64], differences in response of the 5-HT system to antidepressant treatments [30,31,36,46], increased regional blood glucose utilization [29], and changes in dopaminergic [18,22] and glutaminergic [42] neurotransmission. mGluR3 have been implicated in the control of the Brain-Derived Neurotrophic Factor (BDNF) production, which is lower in the FSL rats [67] and well established in the pathophysiology of depression [6]. FSL rats showed significantly increased glutamate-burst events when measured by an enzyme based microelectrode array in the pre-frontal cortex of 12–15-month old rats [23]. All of this data indicated the glutamatergic system as a possible neurochemical marker of pathophysiological alterations in this animal model of depression.

Based on previously reported data that supports the hypothesis that glutamate is one of the neurochemical systems involved in the development of depressive disorder, the fact that the FSL rat model is a good model of depression, it was hypothesized that the FSL rats will have reduced mGluR5 densities in specific brain regions (mainly the limbic structures) when

compared to the controls (FRL, SPD). In the present studies, both the FRL and SPD rats were used as controls, however, in many previous studies of FSL rats, the FRL rats have been used as controls [48,49].

2. Methods

The studies were performed on Sprague–Dawley (SPD) ($N = 6$), FSL ($N = 6$) and FRL ($N = 6$) male rats weighing approximately 250 g. The SPD (Charles River, St. Constant, Quebec, Canada) rats were housed in the animal facility (room temperature 22 °C and 12 h day–night cycle) for at least 3 days prior to being introduced into the experiment. The FSL and FRL rats were obtained from breeding colonies maintained at the Montreal Neurological Institute of McGill University from virus free breeds kindly supplied by Dr. D. Overstreet (Center for Alcohol Studies, University of North Carolina, Chapel Hill, NC, USA). All of the animal procedures were performed in strict accordance with the Canadian Council on Animal Care guidelines, and were approved by the Animal Care Committee of McGill University. Before being decapitated, the rats were anesthetized with isoflurane. Following decapitation, the brains were frozen in cold isopentane (–20 °C) and put into a freezer at –80 °C until cut for the binding study. The brains were cut to 20 µm thick slices in groups of 4 consecutive coronal sections in a Leica CM3000 (Leica Microsystems, Bensheim, Germany) at –20 °C. The sections were mounted on Gold Plus microscopic slides (Fisher Scientific Company, Ottawa, Canada) and stored at –20 °C in a well-sealed box with a desiccant until used for binding.

To quantify densities of mGluR5 receptors an in vitro autoradiographic measurements with [³H]ABP688 (specific activity 74 Ci/mmol, GE Healthcare, UK) was performed according to the method described by Hintermann et al. [27], with some modifications. Briefly, the slides were warmed up to room temperature and pre-incubated in a buffer containing 30 mM Na HEPES, 110 mM NaCl, 5 mM KCl, 2.5 mM CaCl₂ and 1.2 mM MgCl₂ (pH 7.4) for 20 min to remove endogenous competitors from the tissue. The slides were incubated for 60 min at room temperature in the buffer with the same content as the one used for pre-incubation, except for the addition of 2.5 nM [³H]ABP688. Non-specific binding was determined with the addition of 10 µM 2-methyl-6-(phenylethynyl) pyridine (MPEP) to the medium in the adjacent sections. Following the incubation, the slides were washed (3 × 5 min) in a cold buffer, dipped in cold distilled water and dried under a stream of cool air. After drying, the tissue was mildly fixed and desiccated by exposure to paraformaldehyde powder *in vacuum* overnight [38] and exposed along with [³H] microscalers (GE Healthcare, UK) to tritium-sensitive imaging plates (BAS-TR, Fuji-Film, Japan) for five days. The paraformaldehyde treatment was done to protect the plates from contamination [38]. After the contact, the imaging plates were scanned using BAS 5000 (Fuji-Film, Japan). The quantitative readings of the tracer concentration in nCi/mg-tissue were obtained based on the calibrated system using [³H]labeled standards. There was a linear relationship between the concentrations and the plate image densities. The individual brain regions were identified by the aid of a rat atlas [53]. The mean value of each region was calculated based on the optical densities from four consecutive brain slices bilaterally and converted into a binding quantity expressed in the fmol/mg-tissue utilizing the concentration of the tracer in each bath and the relationship between the B_{max} and B (measured).

The statistical comparisons were performed by using STATISTICA (StatSoft Inc., Tulsa, OK, USA) software. Two factors ANOVA (factor-1; three rat group; factor-2; twenty five brain regions) was used for testing the primary and interaction effects, followed by the Newman–Keuls post hoc test. $p < 0.05$ was taken as significant.

3. Results

There was no significant difference ($F(2,15) = 0.167$; $p > 0.8$) between the weights of rats in the different groups (SPD; 252 ± 28 g; FRL; 246 ± 10 g; FSL 250 ± 11 g).

A set of autoradiographic images exemplifying the heterogeneous distribution of the mGlu5 receptors in the rat brain is provided in Fig. 1. The highest concentrations of receptors were found in the nucleus accumbens (AN), caudate putamen median part (CPm) and lateral part (CPl), septal nucleus (SepN), hippocampus dorsal (HiD) and ventral part (HiV), visual cortex (VCx), and enthorhinal cortex (EnCx). The quantitative results are expressed as a mean $\pm$ SEM and presented in Table 1. The qualitative distribution of the receptor is very similar in all three groups of rats investigated. Two factor ANOVA on data from all three groups of rats showed a significant difference between the regions [$F(24,360) = 1511.5$, $p < 0.001$] and a group–region interaction (group $\times$ region); [$F(48,360) = 13.7$, $p < 0.001$]. Post hoc analysis with Newman–Keuls correction revealed significant differences ($p < 0.05$) in nine brain regions between the FRL and SPD rats, in ten brain regions between the FRL and FSL rats, and in four brain regions between the FSL and SPD rats. The most significant differences were found, in the limbic and brainstem regions [cingulate cortex (CinCx; difference of 14%), hippocampus dorsal (HiD; difference of 7.8% between FRL and FSL rats and 6.6% between SPD and FRL rats), enthorhinal cortex (EnCx; difference of 14.2% between FRL and FSL rats and 21.1% between SPD and FRL rats), ventral tegmental area (VTA; difference of 20.8% between FRL and FSL rats and 14.8% between SPD and FRL rats), dorsal raphe (DR; difference of 22.5% between FRL and FSL rats and 29.6% between SPD and FRL rats), and median raphe (MR; difference of 24.2% between FRL and FSL rats and 17.2% between SPD and FRL rats); details in Table 1]. In addition the differences in the regional densities are graphically presented in Fig. 2.

The highest density of the mGlu5 receptors was found in the caudate putamen (e.g., in the CPm of SPD rats 1109.0 ± 28.3 fmol/mg; of FRL rats 1104.3 ± 36.9 fmol/mg; and of FSL rats 1010.6 ± 16.9 fmol/mg), cortical structures (between 500 fmol/mg and 600 fmol/mg) and hippocampus (between 670 fmol/mg and 750 fmol/mg) (Table 1). These structures are part of the limbic system or sub-limbic structures which are involved in the regulation of emotions [3]. When the FRL group was compared to the FSL group it was found that the FRL rats have a higher density of mGluR5 in a large majority of the investigated brain regions, but the differences were significant following post hoc correction in only ten brain regions out of twenty-five. When the FSL group was compared to the SPD group, significant differences were found in only four regions. This suggests that with respect to mGluR5, the FSL and SPD rats are very similar, while the FRL rats are different from both of these groups (FSL and SPD).

When comparing the FRL and FSL rats, some regions were found to be elevated in the FSL rats (e.g., CinCx, VTA, ventral thalamus (ThV), frontal cortex (FCx)), while some other brain regions were found to have lower densities of receptors (e.g., AN, SepN, EnCx, DR, MR); detailed in Table 1, and exemplified graphically in Fig. 2. When the FSL rats were compared to the SPD rats, lower densities were found in the caudate putamen lateral part (CPI; difference of 11.5%) and CPm (difference of 8.9%), hypothalamus (Hyp; difference of 11.9%), and SepN (difference of 19.2%) in the FSL rats. Comparatively, when the FRL group was compared to the SPD group, the FRL rats exhibited higher receptor densities in the HiD (difference of 6.6%), EnCx (difference of 21.1%), DR (difference of 29.6%), MR (difference of 17.2%), parietal cortex (PCx; difference of 9.1%), and VCx (difference of 21.1%), and lower densities in the CPI (difference of 9.5%) and VTA (difference of 14.8%). The FRL rats have generally higher densities of the mGluR5 receptors among the brain regions in comparison to the FSL and SPD rats. The FSL and SPD rats have significantly different receptor densities in only four brain regions (SepN, Hyp, CPm, and CPI), making the FSL and SPD rats somewhat similar when mGluR5 is taken as a measure.

4. Discussion

This is the first study in which the mGluR5 regional densities were compared between the FSL, FRL and SPD rats. The main finding of this study is the similarity in the densities between the FSL and SPD rats, with pronounced differences between the FRL and other two strains of rats. This is unexpected because the FSL rats have been found to be a very good rat model of depression [48,49], while the SPD rats are normal. However, the significant differences in the limbic structures might be sufficient for identification of depressive phenotype; the FSL rats. The regional distribution of the receptor densities and differences between different groups can be best appreciated by comparing the quantitative values in Table 1, as well as graphed differences in Fig. 2.

In our study, we reported heterogeneous (increased and decreased) mGluR5 values in different regions of the rat brains with a similar distribution among groups. That finding accords well with others reported previously on glutamate levels or receptor levels in different brain regions and different rat groups. Previous reports also revealed increased plasma levels of glutamate and aspartate in depressed patients [1,25,41,43] as well as in the post-mortem brain tissue (frontal cortex) [25], and in the occipital cortex within a MDD patient studied by proton magnetic resonance spectroscopy [71]. There are also some contradictory reports in which a reduction has been reported; such as reduced serum levels of glutamate and other amino acids following antidepressant treatment [41].

A 3T MRS study showed reduced Glx/creatinine ratios (Glx = glutamate, glutamine and GABA) and glutamine/Cr in the hippocampus of patients with MDD [7]. Also AMPA (GluR1, GluR3), kainate (GluR5), and NMDA (NR2B) were decreased in the perirhinal cortex of the patients with major depression [5], and decreased glutamate and glutamine levels in the frontal cortex of patients with depression were reported [3]. Furthermore, a proton MRS study in depressed patients showed reduced glutamate in the anterior cingulate cortex [54], which was reversed in the responders by electroconvulsive therapy (ECT). Reduced values in glutamatergic system parameters (e.g., receptors, glutamate) are in

accordance with our finding of reduced mGluR5 in the FSL model of depression relative to either control group.

In human PET studies, lower levels of mGluR5 receptors were found in the frontal, temporal, parietal cortices, insula, thalamus, hippocampus of subjects suffering from depression when compared to the normal group. The same study reported lower levels of post-mortem mGluR5 protein expression in the prefrontal cortex [16]. A similar reduction using PET was reported in several studies in patients with MDD [25]. Lower mGluR5 levels may represent a biological trait with increased risk for depression as a possible result of the genetic factors (which is the most likely reason for the mGluR5 reduction in the genetic model of depression; the FSL rats). On the other hand, the receptor may be reduced due to down-regulation, possibly because of increased stress, increased glutamate activity or hormonal impairments (e.g., glucocorticoids) [15,58]. The stress increases the levels of anxiety and depression in offspring rodents and the effect is correlated with age [58,63]. Different receptor densities (increase and decrease) found in the present study, in one group (FSL rats) compared to the other groups (SPD and FRL rats), as opposed to uniform results (only a significant decrease in all brain regions in the FSL relative to SPD rats) supports the idea that the glutamatergic system is differently regulated in distinct brain regions in depressed subjects. Region specific glutamate concentration in the cortex, Hyp and AN of the FSL rats was noted following clomipramine or mirtazapine administration in both sexes [35].

Our data showed a reduction in the hippocampal regions (HiD and HiV) which are an important limbic region indicated in the formation of memory and involved in the pathophysiology of depression. Recently, it was shown that FSL rats, when compared to FRL control rats, show reduced volume and number of neurons and synapses in the hippocampus [6,11]. Aging FSL rats have a loss of newly formed cells in the dentate gyrus of the hippocampus, as well as neuropeptide Y cells and shortened 5-HT fibers, which corresponds to altered 5-HT regulation in the hippocampus [28]. A significant portion of depressed patients exhibit a deficit in several memory tasks [20]. Moreover, cognitive dysfunctions, such as reduced ability to concentrate and indecisiveness [13], are included in the diagnostic criteria for major depressive disorder.

The predominant position of the mGluR5 receptor, as also shown in this research, are the limbic and cortical structures, suggesting its involvement in cognition memory and motor function [61]. The FSL rat shows an impairment of emotional memory function in a passive avoidance task with reduced transcription of Arc (activity-regulated cytoskeleton-associated protein), an immediate early gene important for the formation of memory [66], in the prefrontal cortex and hippocampus, the same regions which exhibited lower mGluR5 receptors in the FSL rats, when compared to the SPD rats. Similarly, mice mGluR5 knockout showed learning and memory impairments [17].

There is strong evidence of a glutamatergic system role in pleasure regulation and the pathophysiology of addiction [34] with the implication of a malfunctioned mGluR5s in the formation of depression and addiction disorder [19]. In the present study a reduced mGluR5 receptor in the AN region of FSL rats was found. This region is a part of addiction

regulation, implicated in the formation of anhedonia, one of the main symptoms of depression [62]. Moreover, there is a strong correlation between depression and substance abuse [21,59]. FSL rats showed reduced cocaine intake and less locomotor activation following prolonged (10 days) cocaine administration (30 mg/kg) while microdialysis dopamine sampling from the AN showed no difference between the FSL and control rats [21]. Similarly, it was shown that the mGluR5 knockout mice do not self-administer cocaine, nor do they increase their locomotor activity after repeated cocaine administration despite an increase of dopamine (DA) levels in the nucleus accumbens (AN) [12]. On the other hand, the administration of 2'-methoxyphenyl-(N-2'-pyridinyl)-p-fluoro-benzamidoethylpiperazine (MPPF) (mGluR5 antagonist) results in a dose dependant reduction in cocaine self-administration [12,51]. This indicates that the mGluR5 receptor in AN may play a role in modulating a postsynaptic response to glutamate and dopamine [51,56] and thus has a net effect on disorders involved in pleasure seeking behaviour and pleasure sensation alterations. However, a binge pattern of drug consumption and binge eating may have similar regulatory mechanisms [56].

An alteration in food regulation and intake is well documented in FSL rats. FSL rats weigh less than their control litter mates, and eat less when challenged with a starvation and re-feeding paradigm [17]. The glutamatergic system has recently been a target for the development of binge-eating medication [57]. It was shown that memantine (a NMDA antagonist) and MTEP (a mGluR5 antagonist) reduces highly palatable food consumption in rats and baboons [56,60]. Memantine similarly reduced the effect of highly rewarding stimuli (e.g., sexual interaction and morphine intake) in mice [8,56]. The density of mGluR5 alterations in the Hyp, ThV, cortical, raphe areas and brainstem found in the present study have been shown to be the brain regions implicated in the control of food intake and metabolism [8]. The role of AN in the FSL rat indicates its importance in food regulation as well as the regulation of the pleasure sensation.

5. Conclusion

This study shows an alteration (mostly a reduction) in the mGluR5 receptor density in this rat model of depression (FSL rats), relative to control groups (FRL and to some extent SPD rats). Generally, lower densities in the brain limbic structures in the FSL rats, the rat model of depression, as compared to the normal rats (SPD rats), suggest the involvement of these sites in depression. As such, this data supports the FSL as a solid model for studying depression and it opens up the opportunities to study glutamatergic involvement in depression in this model. Further studies are necessary to better understand the mechanisms that antidepressant drugs have on this particular receptor in the FSL model of depression. In future similar studies in other animal models of depression should be done to establish more definite relationship between densities of mGluR5 receptors and depressive state.

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Abbreviations

5-HT	serotonin
NE	norepinephrine
AMPA	α -amino-3-hydroxyl-5-methyl-4-isoxazole-propionate
NMDA	N-methyl D-aspartate
IP3	inositol 3 phosphate
DAG	diacylglycerol
PKA	protein kinase A
PKC	protein kinase C
MPAK	mitogen-activated protein kinase
ERK	extracellular signal related kinase
MPEP	2-methyl-6-(phenylethynyl)pyridine
PAM	positive allosteric modulators
LTP	long term potentiation
ABP688	3-(6-methyl-pyridin-2-ylethynyl)-cyclohex-2-enone-methyl-oxime
DFP	diisopropyl fluorophosphate
FRL	Flinders Resistant Line
FSL	Flinders Sensitive Line
SPD	Sprague–Dawley rats
BDNF	Brain-Derived Neurotrophic Factor
DA	dopamine
Glx	glutamate, glutamine and GABA
MDD	major depressive disorder
MRS	magnetic resonance spectroscopy
ECT	electroconvulsive therapy
PET	positron emission tomography
MSC	mesenchimal stem cells
AN	nucleus accumbens
CinCx	cingulated cortex

SepN	septal nucleus
Amy	amygdale
HiV	hippocampus ventralis
HiD	hippocampus dorsalis
Hyp	hypothalamus
EnCx	enthorhinal cortex
VTa	ventral tegmental area
ThV	ventral thalamus
DR	dorsal raphe
MR	median raphe
MFB	medial forebrain bundle
MG	medial geniculate
SC	superior colliculus
ThD	dorsal thalamus
CPm	medial caudate putamen
CPl	lateral caudate putamen
SN	substantia nigra
FCx	frontal cortex
SCx	sensory cortex
SMCx	senso-motor cortex
PCx	parietal cortex
ACx	auditory cortex
VCx	visual cortex

References

1. Altamura CA, Mauri MC, Ferrara A, Moro AR, D'Andrea G, Zamberlan F. Plasma and platelet excitatory amino acids in psychiatric disorders. *Am J Psychiatry*. 1993; 150:1731–1733. [PubMed: 8214185]
2. Ametamey SM, Treyer V, Streffer J, Wyss MT, Schmidt M, Blagoev M, Hintermann S, Auberson Y, Gasparini F, Fischer UC, Buck A. Human PET studies of metabotropic glutamate receptor subtype 5 with 11C-ABP688. *J Nucl Med*. 2007; 48:247–252. [PubMed: 17268022]

3. Auer DP, Putz B, Kraft E, Lipinski B, Schill J, Holsboer F. Reduced glutamate in the anterior cingulate cortex in depression: an in vivo proton magnetic resonance spectroscopy study. *Biol Psychiatry*. 2000; 47:305–313. [PubMed: 10686265]
4. Barichello T, Dos Santos I, Savi GD, Simoes LR, Generoso JS, Comim CM, Sachs D, Teixeira AL, Quevedo J. Depressive-like-behavior and proinflammatory interleukine levels in the brain of rats submitted to pneumococcal meningitis. *Brain Res Bull*. 2010; 82:243–246. [PubMed: 20450961]
5. Beneyto M, Kristiansen LV, Oni-Orisan A, McCullumsmith RE, Meador-Woodruff JH. Abnormal glutamate receptor expression in the medial temporal lobe in schizophrenia and mood disorders. *Neuropsychopharmacology*. 2007; 32:1888–1902. [PubMed: 17299517]
6. Bjornebekk A, Mathe AA, Brene S. The antidepressant effect of running is associated with increased hippocampal cell proliferation. *Int J Neuropsychopharmacol*. 2005; 8:357–368. [PubMed: 15769301]
7. Block W, Traber F, von Widdern O, Metten M, Schild H, Maier W, Zobel A, Jessen F. Proton MR spectroscopy of the hippocampus at 3 T in patients with unipolar major depressive disorder: correlates and predictors of treatment response. *Int J Neuropsychopharmacol*. 2009; 12:415–422. [PubMed: 18845018]
8. Broberger C. Brain regulation of food intake and appetite: molecules and networks. *J Intern Med*. 2005; 258:301–327. [PubMed: 16164570]
9. Carroll FI. Antagonists at metabotropic glutamate receptor subtype 5: structure activity relationships and therapeutic potential for addiction. *Ann N Y Acad Sci*. 2008; 1141:221–232. [PubMed: 18991960]
10. Charney DS. Monoamine dysfunction and the pathophysiology and treatment of depression. *J Clin Psychiatry*. 1998; 59(Suppl 14):11–14.
11. Chen F, Madsen TM, Wegener G, Nyengaard JR. Imipramine treatment increases the number of hippocampal synapses and neurons in a genetic animal model of depression. *Hippocampus*. 2010; 20:1376–1384. [PubMed: 19921703]
12. Chiamulera C, Epping-Jordan MP, Zocchi A, Marcon C, Cottiny C, Tacconi S, Corsi M, Orzi F, Conquet F. Reinforcing and locomotor stimulant effects of cocaine are absent in mGluR5 null mutant mice. *Nat Neurosci*. 2001; 4:873–874. [PubMed: 11528416]
13. Clark L, Chamberlain SR, Sahakian BJ. Neurocognitive mechanisms in depression: implications for treatment. *Annu Rev Neurosci*. 2009; 32:57–74. [PubMed: 19400725]
14. Cleva RM, Olive MF. Positive allosteric modulators of type 5 metabotropic glutamate receptors (mGluR5) and their therapeutic potential for the treatment of CNS disorders. *Molecules*. 2011; 16:2097–2106. [PubMed: 21368721]
15. Deschwanden A, Karolewicz B, Feyissa AM, Treyer V, Ametamey SM, Johayem A, Burger C, Auberson YP, Sovago J, Stockmeier CA, Buck A, Hasler G. Reduced metabotropic glutamate receptor 5 density in major depression determined by [¹¹C]ABP688 PET and Postmortem Study. *Am J Psychiatry*. 2011; 168:727–734. [PubMed: 21498461]
16. Deschwanden A, Karolewicz B, Feyissa AM, Treyer V, Ametamey SM, Johayem A, Burger C, Auberson YP, Sovago J, Stockmeier CA, Buck A, Hasler G. Reduced metabotropic glutamate receptor 5 density in major depression determined by [(11)C]ABP688 PET and postmortem study. *Am J Psychiatry*. 2011; 168:727–734. [PubMed: 21498461]
17. Dhimi GK, Ferguson SS. Regulation of metabotropic glutamate receptor signaling, desensitization and endocytosis. *Pharmacol Ther*. 2006; 111:260–271. [PubMed: 16574233]
18. Dremencov E, Newman ME, Kinor N, Blatman-Jan G, Schindler CJ, Overstreet DH, Yadid G. Hyperfunctionality of serotonin-2C receptor-mediated inhibition of accumbal dopamine release in an animal model of depression is reversed by antidepressant treatment. *Neuropharmacology*. 2005; 48:34–42. [PubMed: 15617725]
19. Enz R. Metabotropic glutamate receptors and interacting proteins: evolving drug target. *Curr Drug Targets*. 2011
20. Eriksson TM, Delagrange P, Spedding M, Popoli M, Mathe AA, Ogren SO, Svenningsson P. Emotional memory impairments in a genetic rat model of depression: involvement of 5-HT/MEK/Arc signaling in restoration. *Mol Psychiatry*. 2011

21. Fagergren P, Overstreet DH, Goiny M, Hurd YL. Blunted response to cocaine in the Flinders hypercholinergic animal model of depression. *Neuroscience*. 2005; 132:1159–1171. [PubMed: 15857718]
22. Friedman A, Friedman Y, Dremencov E, Yadid G. VTA dopamine neuron bursting is altered in an animal model of depression and corrected by desipramine. *J Mol Neurosci*. 2008; 34:201–209. [PubMed: 18197479]
23. Hascup KN, Hascup ER, Stephens ML, Glaser PE, Yoshitake T, Mathe AA, Gerhardt GA, Kehr J. Resting glutamate levels and rapid glutamate transients in the prefrontal cortex of the Flinders Sensitive Line rat: a genetic rodent model of depression. *Neuropsychopharmacology*. 2011; 36:1769–1777. [PubMed: 21525860]
24. Hasegawa S, Nishi K, Watanabe A, Overstreet DH, Diksic M. Brain 5-HT synthesis in the Flinders Sensitive Line rat model of depression: an autoradiographic study. *Neurochem Int*. 2006; 48:358–366. [PubMed: 16427159]
25. Hashimoto K. Emerging role of glutamate in the pathophysiology of major depressive disorder. *Brain Res Rev*. 2009; 61:105–123. [PubMed: 19481572]
26. Heidinger V, Manzerra P, Wang XQ, Strasser U, Yu SP, Choi DW, Behrens MM. Metabotropic glutamate receptor 1-induced upregulation of NMDA receptor current: mediation through the Pyk2/Src-family kinase pathway in cortical neurons. *J Neurosci*. 2002; 22:5452–5461. [PubMed: 12097497]
27. Hintermann S, Vranesic I, Allgeier H, Brulisauer A, Hoyer D, Lemaire M, Moenius T, Urwyler S, Whitebread S, Gasparini F, Auberson YP. ABP688, a novel selective and high affinity ligand for the labeling of mGlu5 receptors: identification, in vitro pharmacology, pharmacokinetic and biodistribution studies. *Bioorg Med Chem*. 2007; 15:903–914. [PubMed: 17110115]
28. Husum H, Aznar S, Hoyer-Hansen S, Larsen MH, Mikkelsen JD, Moller A, Mathe AA, Wortwein G. Exacerbated loss of cell survival, neuropeptide Y-immunoreactive (IR) cells, and serotonin-IR fiber lengths in the dorsal hippocampus of the aged flinders sensitive line depressed rat: implications for the pathophysiology of depression? *J Neurosci Res*. 2006; 84:1292–1302. [PubMed: 17099915]
29. Kanemaru K, Diksic M. The Flinders Sensitive Line of rats, a rat model of depression, has elevated brain glucose utilization when compared to normal rats and the Flinders Resistant Line of rats. *Neurochem Int*. 2009; 55:655–661. [PubMed: 19560506]
30. Kanemaru K, Hasegawa S, Nishi K, Diksic M. Acute citalopram has different effects on regional 5-HT synthesis in FSL, FRL, and SDP rats: an autoradiographic evaluation. *Brain Res Bull*. 2008; 77:214–220. [PubMed: 18674602]
31. Kanemaru K, Nishi K, Hasegawa S, Diksic M. Chronic citalopram treatment elevates serotonin synthesis in flinders sensitive and flinders resistant lines of rats, with no significant effect on Sprague–Dawley rats. *Neurochem Int*. 2009; 54:363–371. [PubMed: 19418630]
32. Karolewicz B, Johnson L, Szebeni K, Stockmeier CA, Ordway GA. Glutamate signaling proteins and tyrosine hydroxylase in the locus coeruleus of alcoholics. *J Psychiatr Res*. 2008; 42:348–355. [PubMed: 17481661]
33. Keller MB, Lavori PW, Rice J, Coryell W, Hirschfeld RM. The persistent risk of chronicity in recurrent episodes of nonbipolar major depressive disorder: a prospective follow-up. *Am J Psychiatry*. 1986; 143:24–28. [PubMed: 3942283]
34. Kenny PJ, Paterson NE, Boutrel B, Semenova S, Harrison AA, Gasparini F, Koob GF, Skoubis PD, Markou A. Metabotropic glutamate 5 receptor antagonist MPEP decreased nicotine and cocaine self-administration but not nicotine and cocaine-induced facilitation of brain reward function in rats. *Ann N Y Acad Sci*. 2003; 1003:415–418. [PubMed: 14684476]
35. Kokras N, Antoniou K, Polissidis A, Papadopoulou-Daifoti Z. Antidepressants induce regionally discrete, sex-dependent changes in brain's glutamate content. *Neurosci Lett*. 2009; 464:98–102. [PubMed: 19666087]
36. Kovacevic T, Skelin I, Diksic M. Chronic fluoxetine treatment has a larger effect on the density of a serotonin transporter in the Flinders Sensitive Line (FSL) rat model of depression than in normal rats. *Synapse*. 2010; 64:231–240. [PubMed: 19924693]

37. Kuhn R, Pagano A, Stoehr N, Vranesic I, Flor PJ, Lingenhoel K, Spooren W, Gentsch C, Vassout A, Pilc A, Gasparini F. In vitro and in vivo characterization of MPEP, an allosteric modulator of the metabotropic glutamate receptor subtype 5: review article. *Amino Acids*. 2002; 23:207–211. [PubMed: 12373539]
38. Liberatore GT, Wong JY, Krenus D, Jeffreys BJ, Porritt MJ, Howells DW. Tissue fixation prevents contamination of tritium-sensitive storage phosphor imaging plates. *Biotechniques*. 1999; 26:432–434. [PubMed: 10090979]
39. Lorenc-Koci E, Smialowska M, Antkiewicz-Michaluk L, Golembiowska K, Bajkowska M, Wolfarth S. Effect of acute and chronic administration of 1,2,3,4-tetrahydroisoquinoline on muscle tone, metabolism of dopamine in the striatum and tyrosine hydroxylase immunocytochemistry in the substantia nigra, in rats. *Neuroscience*. 2000; 95:1049–1059. [PubMed: 10682712]
40. Machado-Vieira R, Salvatore G, Ibrahim LA, Diaz-Granados N, Zarate CA Jr. Targeting glutamatergic signaling for the development of novel therapeutics for mood disorders. *Curr Pharm Des*. 2009; 15:1595–1611. [PubMed: 19442176]
41. Maes M, Verkerk R, Vandoolaeghe E, Lin A, Scharpe S. Serum levels of excitatory amino acids, serine, glycine, histidine, threonine, taurine, alanine and arginine in treatment-resistant depression: modulation by treatment with antidepressants and prediction of clinical responsiveness. *Acta Psychiatr Scand*. 1998; 97:302–308. [PubMed: 9570492]
42. Matriciano F, Caruso A, Orlando R, Marchiafava M, Bruno V, Battaglia G, Gruber SH, Melchiorri D, Tatarelli R, Girardi P, Mathe AA, Nicoletti F. Defective group-II metabotropic glutamate receptors in the hippocampus of spontaneously depressed rats. *Neuropharmacology*. 2008; 55:525–531. [PubMed: 18590921]
43. Mattson MP. Glutamate and neurotrophic factors in neuronal plasticity and disease. *Ann N Y Acad Sci*. 2008; 1144:97–112. [PubMed: 19076369]
44. Moutsimilli L, Farley S, Dumas S, El Mestikawy S, Giros B, Tzavara ET. Selective cortical VGLUT1 increase as a marker for antidepressant activity. *Neuropharmacology*. 2005; 49:890–900. [PubMed: 16111724]
45. Nishi K, Kanemaru K, Diksic M. A genetic rat model of depression, Flinders sensitive line, has a lower density of 5-HT(1A) receptors, but a higher density of 5-HT(1B) receptors, compared to control rats. *Neurochem Int*. 2009; 54:299–307. [PubMed: 19121358]
46. Nishi K, Kanemaru K, Hasegawa S, Watanabe A, Diksic M. Both acute and chronic buspirone treatments have different effects on regional 5-HT synthesis in Flinders Sensitive Line rats (a rat model of depression) than in control rats. *Neurochem Int*. 2009; 54:205–214. [PubMed: 19084042]
47. Orzel-Gryglewska J, Kusmierczak M, Jurkowlanec E. Involvement of GABAergic transmission in the midbrain ventral tegmental area in the regulation of hippocampal theta rhythm. *Brain Res Bull*. 2010; 83:310–320. [PubMed: 20851748]
48. Overstreet DH. Behavioral characteristics of rat lines selected for differential hypothermic responses to cholinergic or serotonergic agonists. *Behav Genet*. 2002; 32:335–348. [PubMed: 12405515]
49. Overstreet DH, Friedman E, Mathe AA, Yadid G. The Flinders Sensitive Line rat: a selectively bred putative animal model of depression. *Neurosci Biobehav Rev*. 2005; 29:739–759. [PubMed: 15925699]
50. Palucha A, Pilc A. Metabotropic glutamate receptor ligands as possible anxiolytic and antidepressant drugs. *Pharmacol Ther*. 2007; 115:116–147. [PubMed: 17582504]
51. Paterson NE, Semenova S, Gasparini F, Markou A. The mGluR5 antagonist MPEP decreased nicotine self-administration in rats and mice. *Psychopharmacology (Berl)*. 2003; 167:257–264. [PubMed: 12682710]
52. Paul IA, Layer RT, Skolnick P, Nowak G. Adaptation of the NMDA receptor in rat cortex following chronic electroconvulsive shock or imipramine. *Eur J Pharmacol*. 1993; 247:305–311. [PubMed: 7905831]
53. Paxinos, G., Watson, C. *The Rat Brain Atlas in Stereotaxic Coordinates*. 3. Academic Press; New York: 1997. Compact
54. Pfleiderer B, Michael N, Erfurth A, Ohrmann P, Hohmann U, Wolgast M, Fiebich M, Arolt V, Heindel W. Effective electroconvulsive therapy reverses glutamate/glutamine deficit in the left

- anterior cingulum of unipolar depressed patients. *Psychiatry Res.* 2003; 122:185–192. [PubMed: 12694892]
55. Pilc A, Klodzinska A, Branski P, Nowak G, Palucha A, Szewczyk B, Tatarczynska E, Chojnacka-Wojcik E, Wieronska JM. Multiple MPEP administrations evoke anxiolytic- and antidepressant-like effects in rats. *Neuropharmacology.* 2002; 43:181–187. [PubMed: 12213272]
 56. Popik P, Kos T, Zhang Y, Bisaga A. Memantine reduces consumption of highly palatable food in a rat model of binge eating. *Amino Acids.* 2011; 40:477–485. [PubMed: 20571841]
 57. Popik P, Wrobel M, Rygula R, Bisaga A, Bessalov AY. Effects of memantine, an NMDA receptor antagonist, on place preference conditioned with drug and nondrug reinforcers in mice. *Behav Pharmacol.* 2003; 14:237–244. [PubMed: 12799526]
 58. Quan M, Zheng C, Zhang N, Han D, Tian Y, Zhang T, Yang Z. Impairments of behavior, information flow between thalamus and cortex, and prefrontal cortical synaptic plasticity in an animal model of depression. *Brain Res Bull.* 2011; 85:109–116. [PubMed: 21396989]
 59. Regier DA, Farmer ME, Rae DS, Locke BZ, Keith SJ, Judd LL, Goodwin FK. Comorbidity of mental disorders with alcohol and other drug abuse. Results from the Epidemiologic Catchment Area (ECA) Study. *JAMA.* 1990; 264:2511–2518. [PubMed: 2232018]
 60. Reus GZ, Stringari RB, Kirsch TR, Fries GR, Kapczinski F, Roesler R, Quevedo J. Neurochemical and behavioural effects of acute and chronic memantine administration in rats: further support for NMDA as a new pharmacological target for the treatment of depression? *Brain Res Bull.* 2010; 81:585–589. [PubMed: 19954760]
 61. 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]
 62. Salamone JD, Cousins MS, Snyder BJ. Behavioral functions of nucleus accumbens dopamine: empirical and conceptual problems with the anhedonia hypothesis. *Neurosci Biobehav Rev.* 1997; 21:341–359. [PubMed: 9168269]
 63. Salari AA, Enayati M, Hosseini MH, Shahi HR, Saki G. Maternal infection during late pregnancy increases anxiety- and depression-like behaviors with increasing age in male offspring. *Brain Res Bull.* 2011
 64. Shayit M, Yadid G, Overstreet DH, Weller A. 5-HT(1A) receptor subsensitivity in infancy and supersensitivity in adulthood in an animal model of depression. *Brain Res.* 2003; 980:100–108. [PubMed: 12865163]
 65. Spooren W, Gasparini F. mGlu5 receptor antagonists: a novel class of anxiolytics? *Drug News Perspect.* 2004; 17:251–257. [PubMed: 15334174]
 66. Vazdarjanova A, McNaughton BL, Barnes CA, Worley PF, Guzowski JF. Experience-dependent coincident expression of the effector immediate-early genes *arc* and *Homer 1a* in hippocampal and neocortical neuronal networks. *J Neurosci.* 2002; 22:10067–10071. [PubMed: 12451105]
 67. Venero JL, Santiago M, Tomas-Camardiel M, Matarredona ER, Cano J, Machado A. DCG-IV but not other group-II metabotropic receptor agonists induces microglial BDNF mRNA expression in the rat striatum. Correlation with neuronal injury. *Neuroscience.* 2002; 113:857–869. [PubMed: 12182892]
 68. Wieronska JM, Smialowska M, Branski P, Gasparini F, Klodzinska A, Szewczyk B, Palucha A, Chojnacka-Wojcik E, Pilc A. In the amygdala anxiolytic action of mGlu5 receptors antagonist MPEP involves neuropeptide Y but not GABAA signaling. *Neuropsychopharmacology.* 2004; 29:514–521. [PubMed: 14666119]
 69. Wyss MT, Ametamey SM, Treyer V, Bettio A, Blagoev M, Kessler LJ, Burger C, Weber B, Schmidt M, Gasparini F, Buck A. Quantitative evaluation of ¹¹C-ABP688 as PET ligand for the measurement of the metabotropic glutamate receptor subtype 5 using autoradiographic studies and a beta-scintillator. *Neuroimage.* 2007; 35:1086–1092. [PubMed: 17320417]
 70. Zangen A, Overstreet DH, Yadid G. High serotonin and 5-hydroxyindoleacetic acid levels in limbic brain regions in a rat model of depression: normalization by chronic antidepressant treatment. *J Neurochem.* 1997; 69:2477–2483. [PubMed: 9375680]

71. Sanacora G, Gueorguieva R, Epperson CN, Wu YT, Appel M, Rothman DL, Krystal JH, Mason GF. Subtype-specific alterations of gamma-aminobutyric acid and glutamate in patients with major depression. *Arch Gen Psychiatry*. 2004; 61:705–713. [PubMed: 15237082]

Fig. 1.

A representative set of autoradiograms obtained at different cross sections exemplifying the distribution of mGluR5 sites in the control SPD rats and in the FRL and FSL rats.

Fig. 2.

A graphical presentation of the receptor differences, expressed as percent of control group in selected brain regions (those having differences greater than few percent) between FRL and FSL rats (bars stripped under angle), SPD and FSL rats (bars stripped horizontally), and FRL and SPD rats (solid black bars with whit dots).

Table 1

The mGluR5 receptor densities (mean [fmol/mg] $\pm$ SEM (standard error of the mean)) in naïve SPD, FRL and FSL rats. There were 6 rats in each of the groups.

Limbic areas	SPD	FRL	FSL
AN	883.1 $\pm$ 16.0	913.6 $\pm$ 42.2 *	822.6 $\pm$ 25.3
CinCx	728.9 $\pm$ 23.1 Ω	623.4 $\pm$ 29.0 *	716.1 $\pm$ 9.1
SepN	978.8 $\pm$ 56.9	867.2 $\pm$ 33.8	790.6 $\pm$ 42.5 #
Amy	498.5 $\pm$ 17.2	468.8 $\pm$ 9.0	472.2 $\pm$ 10.8
HiV	747.9 $\pm$ 37.2	751.8 $\pm$ 33.9	673.9 $\pm$ 25.9
HiD	689.6 $\pm$ 13.0 Ω	735.1 $\pm$ 18.2 *	678.0 $\pm$ 11.2
Hyp	395.6 $\pm$ 13.7	370.8 $\pm$ 13.4	348.5 $\pm$ 15.7 #
EnCx	703.5 $\pm$ 19.6 Ω	851.8 $\pm$ 39.6 *	731.2 $\pm$ 25.0
Brainstem			
VTA	321.3 $\pm$ 17.6 Ω	273.7 $\pm$ 13.8 *	330.5 $\pm$ 12.9
ThV	332.1 $\pm$ 18.8	315.3 $\pm$ 9.3 *	361.3 $\pm$ 15.7
DR	228.7 $\pm$ 14.4 Ω	296.4 $\pm$ 20.3 *	229.6 $\pm$ 10.2
MR	190.9 $\pm$ 6.2 Ω	223.6 $\pm$ 8.3 *	169.5 $\pm$ 8.9
MFB	358.9 $\pm$ 6.2	326.8 $\pm$ 10.9	359.9 $\pm$ 24.6
Sensory nuclei			
MG	379.0 $\pm$ 7.7	397.4 $\pm$ 21.2	372.0 $\pm$ 12.1
SC	416.0 $\pm$ 14.1	400.7 $\pm$ 12.7	424.3 $\pm$ 17.9
Motor structures and basal ganglia			
ThD	388.9 $\pm$ 23.1	366.5 $\pm$ 9.2	406.4 $\pm$ 13.4
CPm	1109.0 $\pm$ 28.3	1104.3 $\pm$ 36.9 *	1010.6 $\pm$ 16.9 #
CPI	1079.4 $\pm$ 33.2 Ω	976.7 $\pm$ 24.4	954.9 $\pm$ 11.2 #
SN	289.8 $\pm$ 7.3	283.6 $\pm$ 8.0	283.8 $\pm$ 12.0
Cortices			
FCx	594.0 $\pm$ 18.5	580.5 $\pm$ 13.0	593.6 $\pm$ 11.4
SCx	553.8 $\pm$ 18.3	530.6 $\pm$ 14.2	528.3 $\pm$ 11.0
SMCx	566.0 $\pm$ 14.2	579.1 $\pm$ 8.7	564.2 $\pm$ 8.4
PCx	507.0 $\pm$ 12.1 Ω	553.1 $\pm$ 13.3 *	496.9 $\pm$ 8.0
ACx	584.9 $\pm$ 6.5	602.7 $\pm$ 23.8	607.5 $\pm$ 22.2
VCx	500.9 $\pm$ 47.7 Ω	606.7 $\pm$ 24.6	545.7 $\pm$ 16.5

* Statistically significant difference between FRL and FSL.

Statistically significant difference between FSL and SPD.

Ω Statistically significant difference between FRL and SPD.