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MPEP MODULATES METABOTROPIC GLUTAMATE 5 RECEPTORS ENDOGENOUSLY EXPRESSED IN ZEBRAFISH BRAIN

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ABSTRACT

Due to phylogenetic proximity to the human, zebrafish has been recognized as a reliable model to study Alzheimer's disease (AD) and other central nervous system disorders. Furthermore, metabotropic glutamate receptors have been previously reported to be impaired in brain from AD patients. Metabotropic glutamate 5 ($mGlu_5$) receptors are G-protein coupled receptors proposed as potential targets for therapy of different neurodegenerative disorders. Thus, MPEP (2-Methyl-6-(phenylethynyl)pyridine hydrochloride), a selective non-competitive $mGlu_5$ receptors antagonist, has been suggested for pharmacological treatment of AD. The aim of the present work was to quantify $mGlu_5$ receptors in brain from zebrafish and to study the possible modulation of these receptors by MPEP treatment. To this end, radioligand binding assay and open field test were used. Results showed a slightly higher presence of $mGlu_5$ receptors in brain from males than in female zebrafish. However, a significant increase on $mGlu_5$ receptor on male without variation on female was observed after MPEP treatment. This gender specific response was also observed in locomotor behavior being significantly decreased only in male zebrafish. These results confirm the presence of $mGlu_5$ receptors in brain from zebrafish and their gender specific modulation by selective antagonist treatment and suggest a role of these receptors on locomotor activity which is affected in many disorders. In addition, our

data point to zebrafish as a useful model to study mGlu receptors function in both healthy and pathological conditions.

KEYWORDS

mGlu5, MPEP, *Danio rerio*, locomotion, up-regulation

INTRODUCTION

The zebrafish (*Danio rerio*) represents a reliable model for studies dealing with nervous system function in health and disease, due to its phylogenetical proximity to the human[1]. Zebrafish has several advantages, including small size, cheap maintenance and housing, transparency and high fecundity, which make it a suitable model for the study in neuropharmacology and behavior. Furthermore, zebrafish has been also postulated as ideal model for studying Alzheimer Disease (AD)[2]. In this disease, several transduction pathways have been showed to be altered as that mediated by glutamate receptors.

Glutamate, the most abundant excitatory neurotransmitter in the central nervous system, is widely distributed in brain and is involved in learning and memory processes. However, at high concentration it results neurotoxic and can promote degeneration and neuronal death[3]. Glutamate acts through ionotropic and metabotropic receptors. Ionotropic receptors are ion channels activated by glutamate but also by NMDA, AMPA and Kainate and they have been classified following the affinity for these agonists. Metabotropic glutamate (mGlu) receptors are G-proteins coupled receptors divided into three groups. Group I ($mGlu_1$ and $mGlu_5$) are coupled to phospholipase C activity through $G_{q/11}$ proteins and promote the generation of inositol trisphosphate and diacyl glycerol as second messenger. Group II ($mGlu_2$ and $mGlu_3$) and III ($mGlu_4$, $mGlu_6$, $mGlu_7$ and $mGlu_8$) cause a decrease in cAMP level by activating $G_{i/o}$ proteins being directly involved in adenylyl cyclase inhibition[4]. Glutamate modulates neuronal excitability and synaptic transmission through activation of mGlu receptors. Therefore, mGlu receptors can be found in many cell types from both peripheral and central nervous systems. This wide distribution of mGlu receptors could facilitate the development of therapeutic strategies based on the modulation of these receptors, as it has been proposed for neurodegenerative disorders[5-8]. Alfaro and coworkers[9] reported a similar behavior of kainate receptors in zebrafish and rodent models, where the selective non-NMDA antagonist DNQX (6,7-dinitroquinoxaline-2,3-dione) inhibits kainite induced seizures. Recently, cloning and phylogenetic characterization of all

members of the genes of mGlu receptor family (*grm*) has been reported in *Danio rerio*[10]. These authors found a similar group I *grm* expression in larval and adult zebrafish. Moreover, *grms'* expression is also similar to that detected in mammals, which could support the usefulness of zebrafish model to analyze mGlu receptor function through development.

Progress in using zebrafish for modelling human disease has been reviewed elsewhere[11]. Since then, Alzheimer disease[12], cancer[13] and other pathologies, including movement disorders[14], have been explored by means of this vertebrate model. We have previously reported that mGlu receptors are significantly decreased in the frontal cortex from AD brain and the decrease was associated with the progression of pathology[15]. On the other hand, potential of $mGlu_5$ receptor as target for treating AD have been recently proposed[8]. Therefore, the aim of the present work was to study $mGlu_5$ receptors and their possible modulation by MPEP, selective non-competitive antagonist, in whole brain from zebrafish.

RESULTS AND DISCUSSION

Radioligand binding assays were performed to detect and to quantify $mGlu_5$ receptor levels in whole brain membrane preparation from both male and female zebrafish. Each sample (membrane preparation) consisted on a pool of two zebrafish brains to assure a minimum of protein amount suitable for binding assay. Specific binding of [^{3}H]MPEP to $mGlu_5$ receptor was detected in brain membranes and levels in male control animals were slightly higher than that detected in female (73% of male). This specific binding was significantly increased after 24 hours of MPEP treatment in male fish while female levels were not significantly altered, suggesting a gender specific response to MPEP treatment (Fig. 1).

Homo sapiens, *Mus musculus*, *Danio rerio* and *Takifugu rubripes* genomes have been fully sequenced, which allowed other authors to identify over 180 protein predictions belonging to metabotropic glutamate receptors family[16]. Interestingly, these vertebrate genomic databases demonstrate that most of the glutamate receptor subgroups are present in both mammals and bony fishes, indicating a common phylogenetically ancient origin[16-18]. Bjarnadottir and coworkers[17] postulated from gene sequences that eight predicted proteins belonging to mGlu receptor class should be present in zebrafish. Moreover, it has been demonstrated a similar operation of kainate receptors

in zebrafish and rodents, suggesting zebrafish as suitable model for studying glutamate transmission[9].

Expression pattern of mGlu receptors gene (*grm*), including $mGlu_5$ paralogs, was fully and deeply analyzed by Haug and coworkers[10] in adult zebrafish brain at a transcriptional level. A strong expression (presence of transcripts) was detected in hypothalamic structures, dorsal telencephalic regions and the nucleus interpeduncularis. Interestingly, *grm5a* and *grm5b* have no expression and very weak expression, respectively, in cerebellum[10].

The distribution of $mGlu_5$ receptors immunoreactivity (presence of protein) was reported in the synaptic terminal of cones pedicles in the retina of zebrafish[19]. Other members belonging to metabotropic family of glutamate receptors have been identified by immunocytochemistry, such as $mGlu_{6b}$ in retina and other parts of the brain[20] and $mGlu_2$, identified as a synaptic marker of radial glia-derived neurons in adult zebrafish telencephalon[21].

However, at least to our knowledge, detection and quantitation of $mGlu_5$ receptor protein in zebrafish brain by radioligand binding assay have not been published until now. Two compounds, MTEP (3-((2-Methyl-1,3-thiazol-4-yl)ethynyl)pyridine) and MPEP (2-Methyl-6-(phenylethynyl)pyridine) are potent, selective $mGlu_5$ antagonists that easily penetrate blood brain barrier[22] and behave as non-competitive $mGlu_5$ antagonists or negative allosteric modulators (NAMs)[23]. Several radioligands, including [^{3}H]MTEP, [^{3}H]M-MPEP and [^{3}H]MPEP, have been used for the characterization of $mGlu_5$ NAMs binding in *in vivo* and *in vitro* systems[24-26]. One of them, [^{3}H]MPEP, previously utilized as a radioligand by a number of researchers[26-28], has been used in the present work.

Saturation binding assays by using [^{3}H]MPEP (0.08-35 nM) as radioligand were reported in brain membrane homogenates from male Wistar rat[29]. Interestingly, specific [^{3}H]MPEP binding to $mGlu_5$ receptor in hippocampus (Bmax: ca. 230 fmol/mg prot) and cerebral cortex (Bmax: ca. 280 fmol/mg prot) reported by these authors is similar to level of $mGlu_5$ receptor binding in male zebrafish brain detected in the present work (ca. 286 fmol/mg prot) by using 20 nM [^{3}H]MPEP. Also in male Wistar rats, $mGlu_5$ receptor binding sites were determined with 1 nM [^{3}H]MPEP and 10 μM cold MPEP for unspecific binding (similar to our binding assay), and ca. 45 fmol/mg prot where detected in hippocampal synaptic membranes from control rats[28].

Targeting $mGlu_5$ receptor seems to be promising for the development of therapeutic agents[30] for Fragile X syndrome (FXS)[31], Alzheimer's disease[8], Parkinson's disease[32], addiction[33] and other pathologies as anxiety and depression[34, 35]. The mGlu receptor theory of FXS[36] indicate that FMRP (fragile X mental retardation protein) deficit leads to potentiated $mGlu_5$ receptor signaling, which, in turn, results in higher protein synthesis and defective synaptic plasticity including boosted long-term depression. Therefore, $mGlu_5$ receptor blockade could ameliorate the pathology[31]. Likewise, the downregulation or pharmacological blockage of $mGlu_5$ receptor have been reported as neuroprotective in Alzheimer's disease [8]. However, in our study we detected an increased $mGlu_5$ receptor level after short term MPEP treatment which could be considered as a compensatory response to $mGlu_5$ blockade. MPEP extracellular fluid concentration in rat brain reached a peak value of 0.15 μM after 40-60 minutes of a single injection of MPEP (5 mg/kg, i.p.), while plasma concentrations of MPEP lead to 2.6 μM levels 15 min after administration[37]. Such brain concentration generated by the dose of 5 mg/kg would be expected to occupy the $mGlu_5$ receptor completely as an in vivo ED_{50} was in a range of 0.7–0.8 mg/kg[37]. MPEP produces 50% to 80% mGlu5 occupancy at 2.3 to 3.2 mg/kg i.p. and 100% occupancy at doses of 10 mg/kg or higher in rat (reviewed in [33]). As MPEP has similar selectivity and potency for rat $mGlu_5$ receptors in brain tissues as for human recombinant $mGlu_5$ receptors[22, 37], we can guess the same for zebrafish $mGlu_5$ and, therefore, speculate with a similar receptor occupancy. Dose dependency of the effects of MPEP (suppression of addiction-like behaviors) in experimental paradigms employing cocaine, ethanol, and nicotine has been reviewed elsewhere[33] and related to receptor occupancy.

In addition, it has been reported in male Wistar rats that chronic MPEP treatment (3 mg/kg/day, i.p.) for 2 weeks did not change [^{3}H]MPEP specific binding in the striatum[26], while after the same period of time another negative allosteric modulator of $mGlu_5$, MTEP (1 mg/kg/day, i.p.), significantly increased Bmax of [^{3}H]MPEP binding in cerebral cortex (25%) and hippocampus (45%)[29]. Acute and chronic treatments can elicit different effects[38]. However, it have been reported no differences between acute and chronic treatment in the ability of MPEP to induce anxiolytic-like effects in rats after a single dose[35] or repeated (once daily for 7 days) injections of MPEP[39], indicating the lack of tolerance to that effect. Apart from this brain structure- and time- dependent effect of MPEP, this higher level of $mGlu_5$ after acute or chronic treatment would promote the need of also higher levels of antagonist when thinking in a potential therapeutic intervention. Therefore, any knowledge about receptors regulation by

antagonist (or agonist) ligands should be considered on the way to the development of therapeutic strategies targeting these receptors, particularly $mGlu_5$.

Locomotor activity of zebrafish has often been found to be a sensitive measure with which the effects of specific stimuli or of other manipulations may be quantified[40]. Moreover, the zebrafish also represents an alternative model to study some locomotor disorders[14]. Beside this, open field tests are well suited for zebrafish locomotor activity research since they are relatively simple, painless and unconditioned tests that can readily assess spontaneous/natural tendency of an animal to explore a novel environment[41]. The analysis of locomotor activity we studied in the open field is presented in Fig 2 (A–D). Vehicle-treated fish (control group) swam a similar distance as other control zebrafish described before by other authors[40]. Analysis of overall locomotor activity in the open field tank showed a decrease in total distance travelled by the MPEP-treated zebrafish vs vehicle group (figure 2A; 4,153±310 cm vs 5,127±400 cm). Moreover, when analyses were performed separately for males (n = 20) and females (n = 20), male zebrafish treated with MPEP swam significantly lesser distance than vehicle-treated male zebrafish (figure 2A; 3,761±375 cm vs 5,739±694 cm; $p<0.05$) whereas for the female MPEP group distance travelled did not change respect vehicle group over the 10 minutes observation period (figure 2A; 4,545±479 cm vs 4,514±335 cm). Likewise, MPEP-treated male zebrafish showed a significant lower velocity relative to vehicle male zebrafish (figure 2B; $p<0.05$). Moreover, significant differences in swimming speed were detected when fish from both sexes were analyzed together (figure 2B; $p<0.05$). Furthermore, novel environments, such as those experienced in the open field test, can induce an anxious behavior in animals. Anxiety is a state of constant fear of restlessness caused by anticipation of a real or imagined future event[42]. For example, AB wild-type zebrafish manifest anxiety as a hyperactive swimming response[43]. According to this, two animal behaviors have been reported to be a reliable measure of anxiety: Freezing[44] and Thigmotaxis[45]. Freezing was defined as the absence of movement, except of the gills and eyes[46] and it was measured as time spent in immobility (fish velocity < 2 cm/s). On the other hand, thigmotaxis (also called “wall-hugging” or “wall-following” behavior) is the propensity to avoid the center of an arena and stay or move in close proximity to the boundaries of a novel environment, for instance the walls (pheriphery of the tank)[45]. This behavior has been commonly observed in nature but also under laboratory conditions for a wide range of species including fish and humans. Thigmotaxis is believed to be adaptive in nature and meant to facilitate the search for a shelter, protection and/or escape routes[47].

Anxiolytic-or antidepressant-like effects of MPEP in several models of anxiety or depression in rats and mice were first reported after acute oral[48] and intraperitoneal[35] MPEP administration. To test whether the MPEP treatment induced a freezing effect in fish, the percentage of time spent in immobility was analyzed (figure 2C). MPEP treatment did not alter the time spent in immobility in any of the groups assessed. Finally, as a measure of anxiety, the percent of time spent in the periphery of the tank (thigmotaxis) was determined for the four groups. As can be seen in figure 2D, the MPEP treatment did not induce a global effect over anxiety, however, when fish from both sexes were analyzed separately, in the MPEP group were detected significant differences ($p<0.01$), as female fish showed higher % of time in the periphery than males.

The activation of mGlu receptors that modulate the properties and connectivity of spinal neurons can control locomotor activity in mice[49, 50]. In the work of Iwagaki and Miles[51], it was demonstrated that the intensity of locomotor-related motoneuron output can be reduced by group I mGlu receptors activation. Moreover, group I mGlu receptor agonists and antagonist are convulsant or anticonvulsant, respectively, against 3,5-dihydroxyphenylglycine-induced seizures and in other mouse models of generalized motor seizures, suggesting mGlu receptors as possible targets in the treatment of epilepsy. Thus, systemic administration of a noncompetitive antagonist as MPEP could block generalized seizures. However, it would be necessary to identify possible acute and chronic side effects to assess the clinical usefulness of these ligands[52].

Endogenous activation of group I mGlu receptors contributing to spinal cord network locomotion regulation has been reported in lampreys[53], *Xenopus* tadpoles[54], and rats[55].There is an endogenous release of glutamate during locomotion in the spinal cord of the lamprey which activates $mGlu_5$ receptor, while a receptor blockade by MPEP causes an increase in the burst frequency. Thus, endogenous $mGlu_5$ receptor activation regulates the activity of locomotor networks through intracellular [Ca^{2+}] oscillations[56], and antagonism with MPEP would clearly reduce the levels of calcium released from internal stores and, in accordance, reduce locomotor activity. We have detected that MPEP (i.p. c.a. 0.8 mg/kg) decreases spontaneous locomotor activity in male fish during open-field test. Similarly, MPEP (10 mg/kg and 30 mg/kg) administered intraperitoneally into mice produced a significant reduction of total locomotor activity[57]. Locomotion and exploration time during exploration of spatial environments and object recognition tests were reduced in rats by i.p. (1-10 mg/kg) but not by prelimbic (1-10 µg) administration of MPEP[58]. In agreement, spontaneous and

cocaine- or amphetamine-induced locomotor activity were decreased in i.p. MPEP treated mice[59].

To analyze whether $mGlu_5$ receptor level and locomotor parameters were related, a correlation study was performed (Table 1). As binding assay results were obtained in brains pooled by pairs, locomotion data were also pooled and averaged in the corresponding individuals before correlation analysis was performed. Results suggest a very weak negative correlation (Pearson r: -0.1918) between $mGlu_5$ level and swim distance in control fishes which is significantly strong (Pearson r: -0.1918, p=0.038) after MPEP treatment. A similar significant (p=0.037) increase in the strength of the correlation between $mGlu_5$ level and mean velocity in control (Pearson r: -0.2655) and MPEP treated fishes (Pearson r: -0.7072) was also observed. Interestingly, the correlation between $mGlu_5$ level and swim distance in control zebrafish changed from very weak (Pearson r: -0.2655) to moderate (Pearson r: -0.5243) when considering only male individuals. Yet MPEP treatment also strengthened this negative correlation (Pearson r: -0.6069). On the other hand, negative correlation between $mGlu_5$ level and mean velocity in male individuals was also strengthened from moderate (Pearson r: -0.5670) to strong (Pearson r: -0.701). Thus, the decrease in swim distance and mean velocity detected after MPEP treatment seems to be related to the increased level of $mGlu_5$.

Our results show that MPEP treatment effect on $mGlu_5$ receptor levels in whole brain membranes is gender dependent. Thus, $mGlu_5$ is upregulated in male while no changes are observed in female individuals. This differential effect could be related to the also different locomotor activity observed in male zebrafish. The higher swimming activity detected in the present work has been previously reported in control zebrafish[60]. Interestingly, there is a negative correlation between $mGlu_5$ level and locomotor activity in male zebrafish.

More than 500 genes, including those related to neurogenesis, cell differentiation, brain and nervous system development, are differentially expressed in males and females, even this gene expression varies during aging[61]. Interestingly, from 15,617 probes obtained through BioMart (http://www.biomart.org/biomart/martview) with the Zebrafish Genome Built (Danio *rerio* Zv9) and compared between male and female data, two probe set corresponding to ionotropic glutamate receptor N-methyl D-aspartate (NMDA) 1a (Dr.12849.1.A1_at) and to Inositol 1,4,5-triphosphate receptor type 3 (Dr.23369.1.S1_at) had significantly lower expression in female zebrafish (76%,

p=0.0097, and 84%, p=0.0481, respectively) (see Additional file 1 in 48). Accordingly, higher expression of NMDA receptors in control males could underlie their also higher swimming activity detected in the present work and reported by other authors in control zebrafish[60]. These authors reported a slight higher decreased swimming activity in males (36%) than in females (29%) during acute (1 hour) blockade of NMDA receptors by MK-801 presence (2 μM) in the tank water, even at 200 μM it was observed a slight increased swimming activity in females[60].

There is supporting evidence from studies on $mGlu_5$ receptor antagonists, which demonstrate that motor and cognitive symptoms induced by NMDA receptor antagonists can get worse by MPEP and MTEP[62]. In fact, the ability of MPEP to change the behavior of zebrafish in an addiction model has been reported[63]. Agonist-mediated $mGlu_5$ receptor activation enhances NMDA receptor sensitivity and activity, likely through PKC phosphorylation of the ion channel associated with NMDA receptors, leading to an enhanced influx of calcium ions[64]. On the other hand, it has been reported in rats the functional interaction between $mGlu_5$ and NMDA receptor antagonists and their effect in locomotion, learning and working memory[65]. Thus, at high dose, MPEP is able to mimic the increase in dopamine release and the cognition impairment elicited by the NMDA antagonist MK-801, while at low dose MPEP enhanced the hyperlocomotion induced by MK-801[65].

In summary, data presented herein show that zebrafish express $mGlu_5$ receptors that can be detected by radioligand binding assay and that are modulated by MPEP in a gender specific manner. This modulation affects also to locomotor activity. All these results in addition with the high resemblance between zebrafish (*grm*) and mammalian (GRM, Grm) transcript expression patterns reinforces the usefulness of zebrafish model to study metabotropic glutamate receptor function in both healthy and pathological conditions.

METHODS

Materials

MPEP (2-Methyl-6-(phenylethynyl)pyridine hydrochloride) was purchased from Tocris (Bristol, UK). The radioligand 2-Methyl-6-([3,5 ^{3}H] phenylethynyl)pyridine ([^{3}H]MPEP, 60 Ci/mmol) was purchased from American Radiolabeled Chemicals (St. Louis, MO,

USA). Liquid scintillation solutions were purchased from Perkin Elmer (Boston, MA, USA). All other products were of analytical grade.

Animals

Male and female adult (4 month-old) zebrafish (AB strain) were used. They were maintained on a constant (14h light / 10h dark) cycle at 26 ± 1 ºC in a recirculating aquarium rack system (Aquaneering, San Diego, CA, USA); water conditioning and environmental quality were maintained following manufacturer's instructions. The experimental protocol was approved by the Neuron Bio Ethics Committee for Animal Research. Animal care was carried out by qualified technicians supervised by veterinarians. Animals were treated in accordance with Spanish and European laws (Real Decreto 53/2013 and Directive 2010/63/EU) and the International guidelines for ethical conduct in the care and use of experimental animals were applied throughout the study.

Treatment

The selective non-competitive $mGlu_5$ receptor antagonist MPEP was diluted in phosphate-buffered saline (PBS) for treatment purposes. Control and treated adult fish were anaesthetized by immersion in 160 µg/mL tricaine and then inoculated intraperitoneally (i.p.) with 300 µM MPEP or PBS. The injection volume was always 10 µL, injected i.p. into the left side of the fish. There is not a validated dose conversion factor from zebrafish to other species including human[66]. However, keeping in mind the differences in pharmacokinetics and pharmacodynamics among species, allometric scaling could be used for such dose extrapolation[67, 68]. Therefore, this treatment would be equivalent to 2,5 mg MPEP/ kg body weight in rat, an animal model were MPEP i.p. injections usually ranges from 1 to 10 mg/kg[68, 69], and occasionally with maximum doses of 30 mg/kg[69, 70]. Assays were conducted with a minimum of ten fish per group.

Open-Field Test

The open field apparatus consisted of a cylindrical plastic tank (20 cm diameter, 20 cm height) filled with 2.5 L of water, to a height of 8 cm). The bottom of the tank was virtually divided in two zones: center and periphery (the area within 3.3 cm from the walls). Moreover, 3 light sources were used to indirectly light the maze.

After 24 hours of treatment, four zebrafish (one of each group) were individually placed in the center of the open field (one tank per zebrafish) and their behavior was recorded for 10 minutes after 1 minute of habituation period. The temperature of the water was

maintained at 26 ± 1 ºC throughout the experiment. The order in which animals were tested was randomized. Locomotor activity in zebrafish has been shown to exhibit a diurnal cycle that is regulated by circadian rhythms[71]. To minimize the effect of circadian rhythms on the experimental outcome, all experiments were carried out between 11:00 and 15:00 hours. The experimenter was located outside the testing room during the recording to avoid disturbance of behavioral responses.

Each behavioral session was filmed by a single HD video camera placed above the center of the 4 open field tanks and analyzed later with the software SMART v2.5. The endpoints measured included: (1) total distance moved (cm), (2) average speed (cm/s), (3) percentage of time spent in immobility (absence of movement was considered when speed <2 cm/s) and (4) global pattern of locomotor activity and zone preference (% time in zone).

Whole brain plasma membrane isolation

Zebrafish brains were extracted and frozen at -80 °C until membrane isolation[72]. Briefly, for each sample (membrane preparation), a pool of two zebrafish brains was homogenized on ice-cold isolation buffer (50 mM Tris-HCl pH 7.4, 10 mM $MgCl_2$ containing protease inhibitors) and centrifuged at 4 °C for 5 min at 1000xg in a Beckman JA 21 centrifuge. The supernatant was centrifuged at 4 °C for 20 min at 27000xg and the pellet was resuspended in isolation buffer. Protein concentration was measured by Lowry method, using bovine serum albumin as standard.

Radioligand binding assay

Metabotropic glutamate 5 receptors in plasma membrane were determined by using the selective mGlu5 antagonist [^{3}H]MPEP as radioligand, as described previously with modifications[73]. Briefly, membranes (60 µg of protein) were incubated for 60 min at 25 °C with 20 nM [^{3}H]MPEP in assay buffer (15 mM Tris-HCl, 25 mM $MgCl_2$, 120 mM NaCl, 100 mM KCl, 2 mM $CaCl_2$, pH 7.4). Nonspecific binding was obtained in the presence of unlabeled MPEP at 20 µM. Binding assay was stopped by rapid filtration through Whatman GF/B filters, which were immediately washed and counted in a Microbeta Trilux liquid scintillation counter (Wallac).

Statistical and data analysis

Data are presented as mean ± standard error of the mean (SEM). One-way ANOVA followed by Newman-Keuls post-hoc study and Student's t-test statistical analyses

were performed using Prism GraphPad software (version 3.03). Differences between mean values were considered statistically significant at $p<0.05$.

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Author Contributions

† J.L.A. and S.S.: equal contribution, should be considered as first coauthors. M.M. and J.S.B. planned the studies. J.L.A., D.L. and M.M. performed radioligand binding assays. S.S., F.G-S. and J.S.B. performed locomotor activity analysis. J.L.A., M.M. and S.S. wrote the manuscript.

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REFERENCES

[1] Guo, S. (2004) Linking genes to brain, behavior and neurological diseases: what can we learn from zebrafish?, *Genes Brain Behav 3*, 63-74.

[2] Santana, S., Rico, E. P., and Burgos, J. S. (2012) Can zebrafish be used as animal model to study Alzheimer's disease?, *Am J Neurodegener Dis 1*, 32-48.

[3] Lewerenz, J., and Maher, P. (2015) Chronic Glutamate Toxicity in Neurodegenerative Diseases-What is the Evidence?, *Front Neurosci 9*, 469.

[4] Kritis, A. A., Stamoula, E. G., Paniskaki, K. A., and Vavilis, T. D. (2015) Researching glutamate - induced cytotoxicity in different cell lines: a comparative/collective analysis/study, *Front Cell Neurosci 9*, 91.

[5] Byrnes, K. R., Loane, D. J., and Faden, A. I. (2009) Metabotropic glutamate receptors as targets for multipotential treatment of neurological disorders, *Neurotherapeutics 6*, 94-107.

[6] Luscher, C., and Huber, K. M. (2010) Group 1 mGluR-dependent synaptic long-term depression: mechanisms and implications for circuitry and disease, *Neuron 65*, 445-459.

[7] Niswender, C. M., and Conn, P. J. (2010) Metabotropic glutamate receptors: physiology, pharmacology, and disease, *Annu Rev Pharmacol Toxicol 50*, 295-322.

[8] Kumar, A., Dhull, D. K., and Mishra, P. S. (2015) Therapeutic potential of mGluR5 targeting in Alzheimer's disease, *Front Neurosci 9*, 215.

[9] Alfaro, J. M., Ripoll-Gomez, J., and Burgos, J. S. (2011) Kainate administered to adult zebrafish causes seizures similar to those in rodent models, *Eur J Neurosci 33*, 1252-1255.

[10] Haug, M. F., Gesemann, M., Mueller, T., and Neuhauss, S. C. (2013) Phylogeny and expression divergence of metabotropic glutamate receptor genes in the brain of zebrafish (Danio rerio), *J Comp Neurol 521*, 1533-1560.

[11] Lieschke, G. J., and Currie, P. D. (2007) Animal models of human disease: zebrafish swim into view, *Nat Rev Genet 8*, 353-367.

[12] Newman, M., Ebrahimie, E., and Lardelli, M. (2014) Using the zebrafish model for Alzheimer's disease research, *Front Genet 5*, 189.

[13] Zhao, S., Huang, J., and Ye, J. (2015) A fresh look at zebrafish from the perspective of cancer research, *J Exp Clin Cancer Res 34*, 80.

[14] Flinn, L., Bretaud, S., Lo, C., Ingham, P. W., and Bandmann, O. (2008) Zebrafish as a new animal model for movement disorders, *J Neurochem 106*, 1991-1997.

[15] Albasanz, J. L., Dalfo, E., Ferrer, I., and Martin, M. (2005) Impaired metabotropic glutamate receptor/phospholipase C signaling pathway in the cerebral cortex in Alzheimer's disease and dementia with Lewy bodies correlates with stage of Alzheimer's-disease-related changes, *Neurobiol Dis 20*, 685-693.

[16] Bjarnadottir, T. K., Fredriksson, R., and Schioth, H. B. (2005) The gene repertoire and the common evolutionary history of glutamate, pheromone (V2R), taste(1) and other related G protein-coupled receptors, *Gene 362*, 70-84.

[17] Bjarnadottir, T. K., Schioth, H. B., and Fredriksson, R. (2005) The phylogenetic relationship of the glutamate and pheromone G-protein-coupled receptors in different vertebrate species, *Ann N Y Acad Sci 1040*, 230-233.

[18] Fredriksson, R., and Schioth, H. B. (2005) The repertoire of G-protein-coupled receptors in fully sequenced genomes, *Mol Pharmacol 67*, 1414-1425.

[19] Yazulla, S., and Studholme, K. M. (2001) Neurochemical anatomy of the zebrafish retina as determined by immunocytochemistry, *J Neurocytol 30*, 551-592.

[20] Huang, Y. Y., Haug, M. F., Gesemann, M., and Neuhauss, S. C. (2012) Novel expression patterns of metabotropic glutamate receptor 6 in the zebrafish nervous system, *PLoS One 7*, e35256.

[21] Kroehne, V., Freudenreich, D., Hans, S., Kaslin, J., and Brand, M. (2011) Regeneration of the adult zebrafish brain from neurogenic radial glia-type progenitors, *Development 138*, 4831-4841.

[22] Gasparini, F., Lingenhohl, K., Stoehr, N., Flor, P. J., Heinrich, M., Vranesic, I., Biollaz, M., Allgeier, H., Heckendorn, R., Urwyler, S., Varney, M. A., Johnson, E. C., Hess, S. D., Rao, S. P., Sacaan, A. I., Santori, E. M., Velicelebi, G., and Kuhn, R. (1999) 2-Methyl-6-(phenylethynyl)-pyridine (MPEP), a potent, selective and systemically active mGlu5 receptor antagonist, *Neuropharmacology 38*, 1493-1503.

[23] Schoepp, D. D., Jane, D. E., and Monn, J. A. (1999) Pharmacological agents acting at subtypes of metabotropic glutamate receptors, *Neuropharmacology 38*, 1431-1476.

[24] Anderson, J. J., Rao, S. P., Rowe, B., Giracello, D. R., Holtz, G., Chapman, D. F., Tehrani, L., Bradbury, M. J., Cosford, N. D., and Varney, M. A. (2002) [3H]Methoxymethyl-3-[(2-methyl-1,3-thiazol-4-yl)ethynyl]pyridine binding to metabotropic glutamate receptor

subtype 5 in rodent brain: in vitro and in vivo characterization, *J Pharmacol Exp Ther 303*, 1044-1051.

[25] Gasparini, F., Andres, H., Flor, P. J., Heinrich, M., Inderbitzin, W., Lingenhohl, K., Muller, H., Munk, V. C., Omilusik, K., Stierlin, C., Stoehr, N., Vranesic, I., and Kuhn, R. (2002) [(3)H]-M-MPEP, a potent, subtype-selective radioligand for the metabotropic glutamate receptor subtype 5, *Bioorg Med Chem Lett 12*, 407-409.

[26] Domenici, M. R., Potenza, R. L., Martire, A., Coccurello, R., Pezzola, A., Reggio, R., Tebano, M. T., and Popoli, P. (2005) Chronic treatment with the mGlu5R antagonist MPEP reduces the functional effects of the mGlu5R agonist CHPG in the striatum of 6-hydroxydopamine-lesioned rats: possible relevance to the effects of mGlu5R blockade in Parkinson's disease, *J Neurosci Res 80*, 646-654.

[27] Matosin, N., Frank, E., Deng, C., Huang, X. F., and Newell, K. A. (2013) Metabotropic glutamate receptor 5 binding and protein expression in schizophrenia and following antipsychotic drug treatment, *Schizophr Res 146*, 170-176.

[28] Nagaraja, R. Y., Grecksch, G., Reymann, K. G., Schroeder, H., and Becker, A. (2004) Group I metabotropic glutamate receptors interfere in different ways with pentylenetetrazole seizures, kindling, and kindling-related learning deficits, *Naunyn Schmiedebergs Arch Pharmacol 370*, 26-34.

[29] Nowak, G., Pomierny-Chamiolo, L., Siwek, A., Niedzielska, E., Pomierny, B., Palucha-Poniewiera, A., and Pilc, A. (2014) Prolonged administration of antidepressant drugs leads to increased binding of [(3)H]MPEP to mGlu5 receptors, *Neuropharmacology 84*, 46-51.

[30] Hovelso, N., Sotty, F., Montezinho, L. P., Pinheiro, P. S., Herrik, K. F., and Mork, A. (2012) Therapeutic potential of metabotropic glutamate receptor modulators, *Curr Neuropharmacol 10*, 12-48.

[31] Pop, A. S., Gomez-Mancilla, B., Neri, G., Willemsen, R., and Gasparini, F. (2014) Fragile X syndrome: a preclinical review on metabotropic glutamate receptor 5 (mGluR5) antagonists and drug development, *Psychopharmacology (Berl) 231*, 1217-1226.

[32] Finlay, C., and Duty, S. (2014) Therapeutic potential of targeting glutamate receptors in Parkinson's disease, *J Neural Transm (Vienna) 121*, 861-880.

[33] Mihov, Y., and Hasler, G. (2016) Negative Allosteric Modulators of Metabotropic Glutamate Receptors Subtype 5 in Addiction: a Therapeutic Window, *Int J Neuropsychopharmacol 19*.

[34] Li, G., Jorgensen, M., and Campbell, B. M. (2013) Metabotropic glutamate receptor 5-negative allosteric modulators for the treatment of psychiatric and neurological disorders (2009-July 2013), *Pharm Pat Anal 2*, 767-802.

[35] Tatarczynska, E., Klodzinska, A., Chojnacka-Wojcik, E., Palucha, A., Gasparini, F., Kuhn, R., and Pilc, A. (2001) Potential anxiolytic- and antidepressant-like effects of MPEP, a potent, selective and systemically active mGlu5 receptor antagonist, *Br J Pharmacol 132*, 1423-1430.

[36] Bear, M. F., Huber, K. M., and Warren, S. T. (2004) The mGluR theory of fragile X mental retardation, *Trends Neurosci 27*, 370-377.

[37] Nagel, J., Greco, S., Parsons, C. G., Flik, G., Tober, C., Klein, K. U., and Danysz, W. (2015) Brain concentrations of mGluR5 negative allosteric modulator MTEP in relation to receptor occupancy--Comparison to MPEP, *Pharmacol Rep 67*, 624-630.

[38] Tran, S., Nowicki, M., Chatterjee, D., and Gerlai, R. (2015) Acute and chronic ethanol exposure differentially alters alcohol dehydrogenase and aldehyde dehydrogenase activity in the zebrafish liver, *Prog Neuropsychopharmacol Biol Psychiatry 56*, 221-226.

[39] Pilc, A., Klodzinska, A., Branski, P., Nowak, G., Palucha, A., Szewczyk, B., Tatarczynska, E., Chojnacka-Wojcik, E., and Wieronska, J. M. (2002) Multiple MPEP administrations evoke anxiolytic- and antidepressant-like effects in rats, *Neuropharmacology 43*, 181-187.

[40] Tran, S., and Gerlai, R. (2013) Individual differences in activity levels in zebrafish (Danio rerio), *Behav Brain Res 257*, 224-229.
[41] Sousa, N., Almeida, O. F., and Wotjak, C. T. (2006) A hitchhiker's guide to behavioral analysis in laboratory rodents, *Genes Brain Behav 5 Suppl 2*, 5-24.
[42] Blaser, R. E., Chadwick, L., and McGinnis, G. C. (2010) Behavioral measures of anxiety in zebrafish (Danio rerio), *Behav Brain Res 208*, 56-62.
[43] Lopez-Patino, M. A., Yu, L., Cabral, H., and Zhdanova, I. V. (2008) Anxiogenic effects of cocaine withdrawal in zebrafish, *Physiol Behav 93*, 160-171.
[44] Norton, W., and Bally-Cuif, L. (2010) Adult zebrafish as a model organism for behavioural genetics, *BMC Neurosci 11*, 90.
[45] Champagne, D. L., Hoefnagels, C. C., de Kloet, R. E., and Richardson, M. K. (2010) Translating rodent behavioral repertoire to zebrafish (Danio rerio): relevance for stress research, *Behav Brain Res 214*, 332-342.
[46] Grossman, L., Utterback, E., Stewart, A., Gaikwad, S., Chung, K. M., Suciu, C., Wong, K., Elegante, M., Elkhayat, S., Tan, J., Gilder, T., Wu, N., Dileo, J., Cachat, J., and Kalueff, A. V. (2010) Characterization of behavioral and endocrine effects of LSD on zebrafish, *Behav Brain Res 214*, 277-284.
[47] Sharma, S., Coombs, S., Patton, P., and Burt de Perera, T. (2009) The function of wall-following behaviors in the Mexican blind cavefish and a sighted relative, the Mexican tetra (Astyanax), *J Comp Physiol A Neuroethol Sens Neural Behav Physiol 195*, 225-240.
[48] Spooren, W. P., Vassout, A., Neijt, H. C., Kuhn, R., Gasparini, F., Roux, S., Porsolt, R. D., and Gentsch, C. (2000) Anxiolytic-like effects of the prototypical metabotropic glutamate receptor 5 antagonist 2-methyl-6-(phenylethynyl)pyridine in rodents, *J Pharmacol Exp Ther 295*, 1267-1275.
[49] El Manira, A., Kettunen, P., Hess, D., and Krieger, P. (2002) Metabotropic glutamate receptors provide intrinsic modulation of the lamprey locomotor network, *Brain Res Brain Res Rev 40*, 9-18.
[50] Nistri, A., Ostroumov, K., Sharifullina, E., and Taccola, G. (2006) Tuning and playing a motor rhythm: how metabotropic glutamate receptors orchestrate generation of motor patterns in the mammalian central nervous system, *J Physiol 572*, 323-334.
[51] Iwagaki, N., and Miles, G. B. (2011) Activation of group I metabotropic glutamate receptors modulates locomotor-related motoneuron output in mice, *J Neurophysiol 105*, 2108-2120.
[52] Moldrich, R. X., Chapman, A. G., De Sarro, G., and Meldrum, B. S. (2003) Glutamate metabotropic receptors as targets for drug therapy in epilepsy, *Eur J Pharmacol 476*, 3-16.
[53] Krieger, P., Grillner, S., and El Manira, A. (1998) Endogenous activation of metabotropic glutamate receptors contributes to burst frequency regulation in the lamprey locomotor network, *Eur J Neurosci 10*, 3333-3342.
[54] Chapman, R. J., and Sillar, K. T. (2007) Modulation of a spinal locomotor network by metabotropic glutamate receptors, *Eur J Neurosci 26*, 2257-2268.
[55] Taccola, G., Marchetti, C., and Nistri, A. (2004) Modulation of rhythmic patterns and cumulative depolarization by group I metabotropic glutamate receptors in the neonatal rat spinal cord in vitro, *Eur J Neurosci 19*, 533-541.
[56] Kettunen, P., Krieger, P., Hess, D., and El Manira, A. (2002) Signaling mechanisms of metabotropic glutamate receptor 5 subtype and its endogenous role in a locomotor network, *J Neurosci 22*, 1868-1873.
[57] Yan, Q. J., Rammal, M., Tranfaglia, M., and Bauchwitz, R. P. (2005) Suppression of two major Fragile X Syndrome mouse model phenotypes by the mGluR5 antagonist MPEP, *Neuropharmacology 49*, 1053-1066.
[58] Christoffersen, G. R., Simonyi, A., Schachtman, T. R., Clausen, B., Clement, D., Bjerre, V. K., Mark, L. T., Reinholdt, M., Schmith-Rasmussen, K., and Zink, L. V. (2008) MGlu5

antagonism impairs exploration and memory of spatial and non-spatial stimuli in rats, *Behav Brain Res 191*, 235-245.

[59] McGeehan, A. J., Janak, P. H., and Olive, M. F. (2004) Effect of the mGluR5 antagonist 6-methyl-2-(phenylethynyl)pyridine (MPEP) on the acute locomotor stimulant properties of cocaine, D-amphetamine, and the dopamine reuptake inhibitor GBR12909 in mice, *Psychopharmacology (Berl) 174*, 266-273.

[60] Swain, H. A., Sigstad, C., and Scalzo, F. M. (2004) Effects of dizocilpine (MK-801) on circling behavior, swimming activity, and place preference in zebrafish (Danio rerio), *Neurotoxicol Teratol 26*, 725-729.

[61] Arslan-Ergul, A., and Adams, M. M. (2014) Gene expression changes in aging zebrafish (Danio rerio) brains are sexually dimorphic, *BMC Neurosci 15*, 29.

[62] Krystal, J. H., Mathew, S. J., D'Souza, D. C., Garakani, A., Gunduz-Bruce, H., and Charney, D. S. (2010) Potential psychiatric applications of metabotropic glutamate receptor agonists and antagonists, *CNS Drugs 24*, 669-693.

[63] Tucker, B., Richards, R. I., and Lardelli, M. (2006) Contribution of mGluR and Fmr1 functional pathways to neurite morphogenesis, craniofacial development and fragile X syndrome, *Hum Mol Genet 15*, 3446-3458.

[64] Kanuma, K., Aoki, T., and Shimazaki, Y. (2010) Recent patents on positive allosteric modulators of the metabotropic glutamate 5 receptor as a potential treatment for schizophrenia, *Recent Pat CNS Drug Discov 5*, 23-34.

[65] Homayoun, H., Stefani, M. R., Adams, B. W., Tamagan, G. D., and Moghaddam, B. (2004) Functional Interaction Between NMDA and mGlu5 Receptors: Effects on Working Memory, Instrumental Learning, Motor Behaviors, and Dopamine Release, *Neuropsychopharmacology 29*, 1259-1269.

[66] Liang, J., Jin, W., Li, H., Liu, H., Huang, Y., Shan, X., Li, C., Shan, L., and Efferth, T. (2016) In Vivo Cardiotoxicity Induced by Sodium Aescinate in Zebrafish Larvae, *Molecules 21*, 190.

[67] Nair, A. B., and Jacob, S. (2016) A simple practice guide for dose conversion between animals and human, *J Basic Clin Pharm 7*, 27-31.

[68] Sharma, V., and McNeill, J. H. (2009) To scale or not to scale: the principles of dose extrapolation, *Br J Pharmacol 157*, 907-921.

[69] Kumar, J., Hapidin, H., Bee, Y. T., and Ismail, Z. (2013) Effects of the mGluR5 antagonist MPEP on ethanol withdrawal induced anxiety-like syndrome in rats, *Behav Brain Funct 9*, 43.

[70] Ballard, T. M., Woolley, M. L., Prinssen, E., Huwyler, J., Porter, R., and Spooren, W. (2005) The effect of the mGlu5 receptor antagonist MPEP in rodent tests of anxiety and cognition: a comparison, *Psychopharmacology (Berl) 179*, 218-229.

[71] Hurd, M. W., Debruyne, J., Straume, M., and Cahill, G. M. (1998) Circadian rhythms of locomotor activity in zebrafish, *Physiol Behav 65*, 465-472.

[72] Gonzalez-Nunez, V., Barrallo, A., Traynor, J. R., and Rodriguez, R. E. (2006) Characterization of opioid-binding sites in zebrafish brain, *J Pharmacol Exp Ther 316*, 900-904.

[73] Malherbe, P., Kratochwil, N., Zenner, M. T., Piussi, J., Diener, C., Kratzeisen, C., Fischer, C., and Porter, R. H. (2003) Mutational analysis and molecular modeling of the binding pocket of the metabotropic glutamate 5 receptor negative modulator 2-methyl-6-(phenylethynyl)-pyridine, *Mol Pharmacol 64*, 823-832.

[74] Evans, J. D. (1996) *Straightforward statistics for the behavioral sciences*, Pacific Grove, CA: Brooks/Cole Publishing.

Figure 1

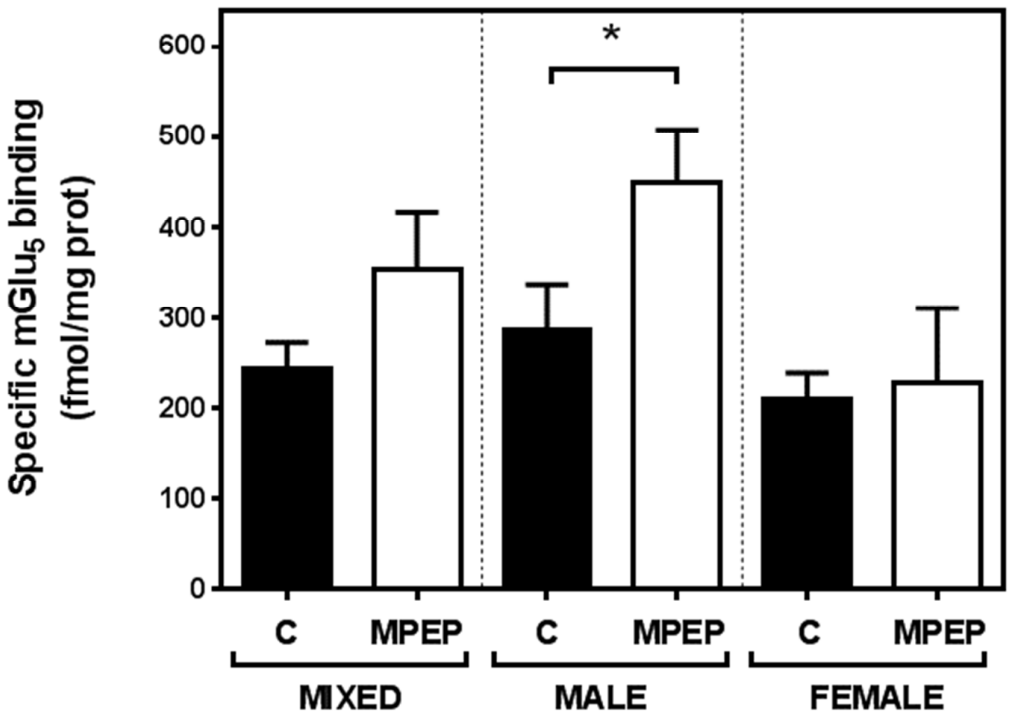
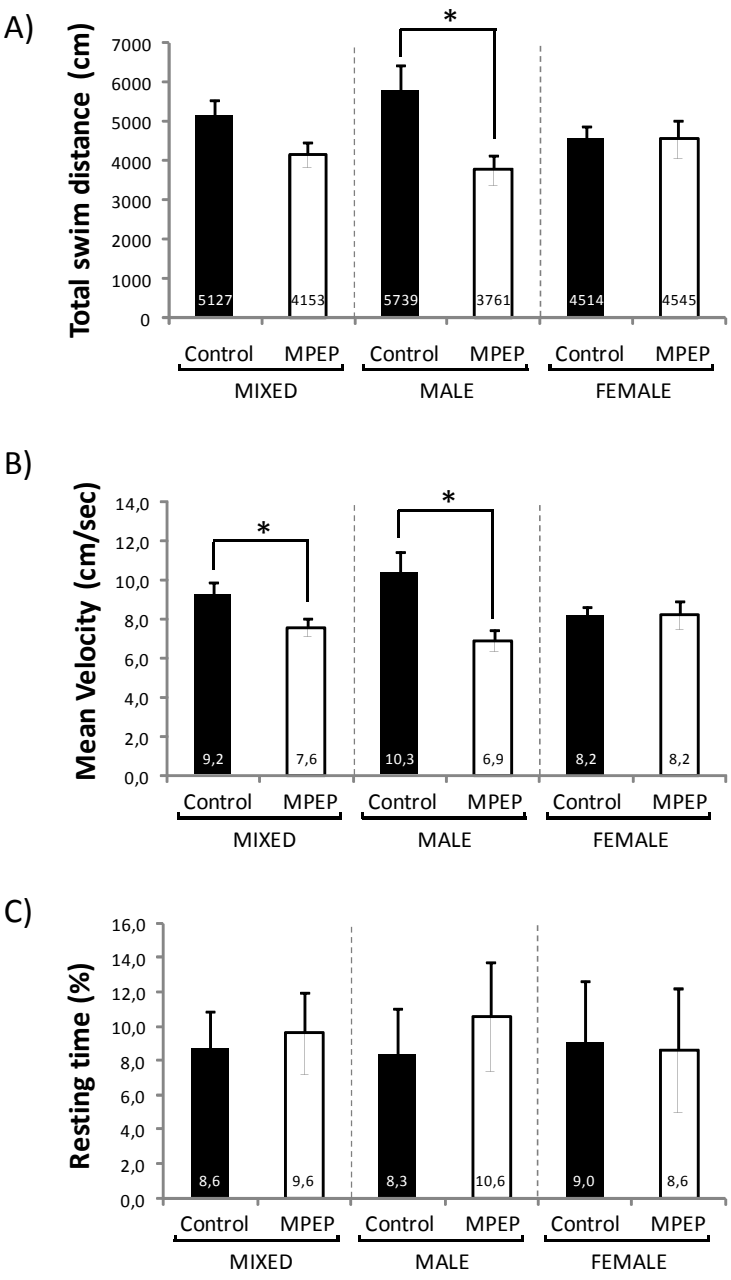


Figure 1. Metabotropic glutamate receptor 5 level is increased in brain membrane from male MPEP treated zebrafish. Binding assay for specific $mGlu_5$ measurement in zebrafish brain membrane by using the radioligand [^{3}H]MPEP. Data from male, female or mixed sexes are mean ± SEM values from 9 control and 7 MPEP treated samples (two animals each), each measured in duplicated. *$p<0.05$ significantly different as compared with control samples using Student's t test.

Figure 2


A)
Total swim distance (cm)
7000
6000
5000
4000
3000
2000
1000
0
*
5127
4153
5739
3761
4514
4545
Control
MPEP
Control
MPEP
Control
MPEP
MIXED
MALE
FEMALE
B)
Mean Velocity (cm/sec)
14,0
12,0
10,0
8,0
6,0
4,0
2,0
0,0
*
*
9,2
7,6
10,3
6,9
8,2
8,2
Control
MPEP
Control
MPEP
Control
MPEP
MIXED
MALE
FEMALE
C)
Resting time (%)
16,0
14,0
12,0
10,0
8,0
6,0
4,0
2,0
0,0
8,6
9,6
8,3
10,6
9,0
8,6
Control
MPEP
Control
MPEP
Control
MPEP
MIXED
MALE
FEMALE

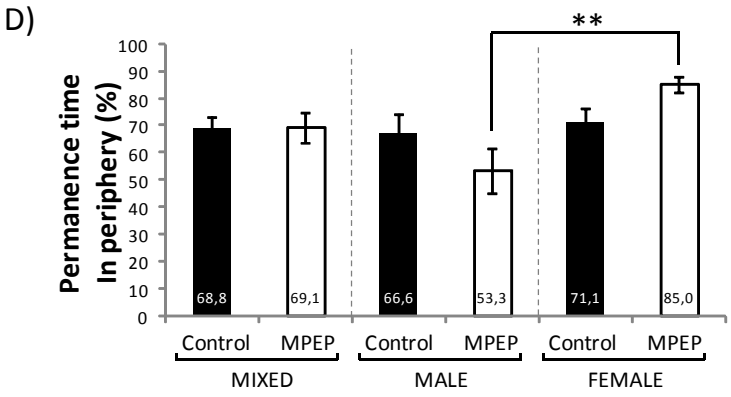


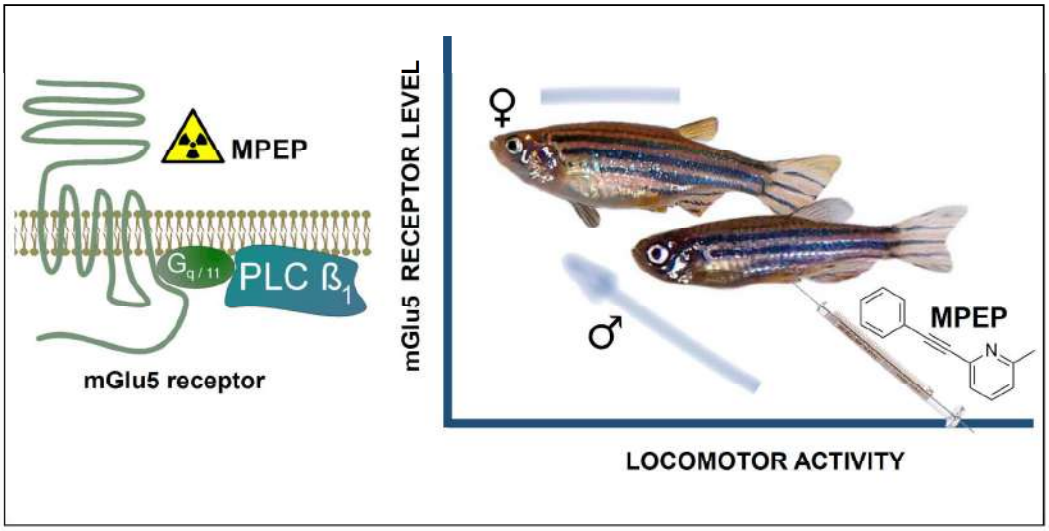
Figure 2. Effect of MPEP treatment in locomotor activity in zebrafish. Forty adult zebrafish of both sexes were distributed as follows: 10 female treated with Vehicle (female control group); 10 male treated with Vehicle (male control group); 10 female treated with MPEP (female MPEP group); 10 male treated with MPEP (male MPEP group. Vehicle and MPEP (300 µM) were administered via i.p. in 10 µL of volume solution. 24 hours after treatment fish were individually placed in the center of the Open Field and their swim activity recorded during 10 minutes after a habituation period of 1 minute. It is shown the mean±SEM of (A) total distance moved (cm), (B) average speed (cm/s) (mean velocity when speed > 2 cm/sec), (C) percentage of time spent in immobility and (D) percentage of time in periphery (thigmotaxis; % time in periphery). Significance was set when*p<0.05; **p<0.01 in student t-test and One-Way ANOVA followed by Newman-Keuls post-hoc study.

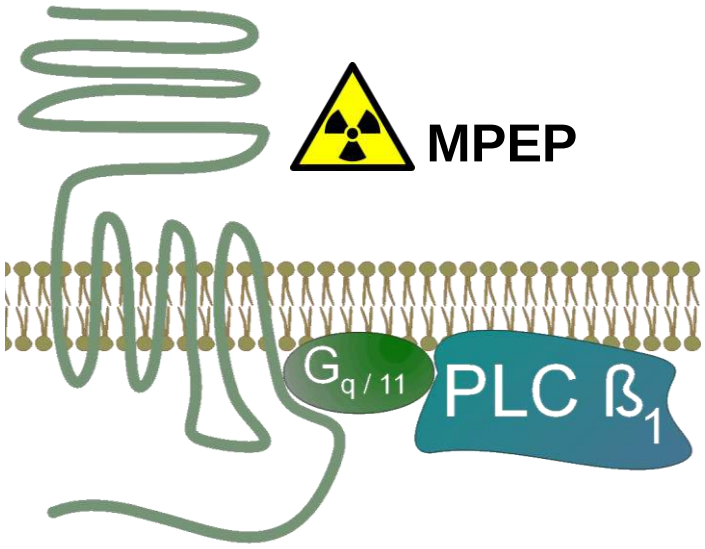
Animal group	Locomotor parameter	n	Pearson r	Strength of the correlation	95% confidence interval	P value (one tailed)	P value summary	Is the correlation significant? (alpha=0.05)	R square
CONTROL mixed	Total swim distance	9	-0,1918	very weak	-0,7592 to 0,5413	0,3106	ns	No	0,0368
	Mean velocity	9	-0,2655	weak	-0,7903 to 0,4840	0,2450	ns	No	0,0705
	Resting time	9	-0,2428	weak	-0,7810 to 0,5023	0,2645	ns	No	0,0590
	Permanence time in periphery	9	-0,6179	strong	-0,9090 to 0,07838	0,0381	*	Yes	0,3818
MPEP mixed	Total swim distance	7	-0,6855	strong	-0,9488 to 0,1396	0,0446	*	Yes	0,4699
	Mean velocity	7	-0,7072	strong	-0,9528 to 0,09802	0,0378	*	Yes	0,5002
	Resting time	7	0,2910	weak	-0,5918 to 0,8564	0,2633	ns	No	0,0847
	Permanence time in periphery	7	-0,6348	strong	-0,9390 to 0,2265	0,0628	ns	No	0,4030
CONTROL male	Total swim distance	4	-0,5243	moderate	-0,9877 to 0,8804	0,2378	ns	No	0,2749
	Mean velocity	4	-0,5670	moderate	-0,9891 to 0,8660	0,2165	ns	No	0,3215
	Resting time	4	-0,0348	very weak	-0,9637 to 0,9583	0,4826	ns	No	0,0012
	Permanence time in periphery	4	-0,4556	moderate	-0,9853 to 0,8992	0,2722	ns	No	0,2076
MPEP male	Total swim distance	4	-0,6069	strong	-0,9903 to 0,8499	0,1965	ns	No	0,3684
	Mean velocity	4	-0,7401	strong	-0,9941 to 0,7655	0,1300	ns	No	0,5477
	Resting time	4	0,0610	very weak	-0,9561 to 0,9655	0,4695	ns	No	0,0037
	Permanence time in periphery	4	-0,4051	moderate	-0,9833 to 0,9105	0,2974	ns	No	0,1641
CONTROL female	Total swim distance	5	-0,3076	weak	-0,9359 to 0,7887	0,3073	ns	No	0,0947
	Mean velocity	5	-0,6864	strong	-0,9770 to 0,4966	0,1003	ns	No	0,4712
	Resting time	5	-0,3617	weak	-0,9430 to 0,7645	0,2749	ns	No	0,1308
	Permanence time in periphery	5	-0,9293	very strong	-0,9954 to -0,2612	0,0112	*	Yes	0,8636
MPEP female	Total swim distance	3	-0,7889	strong	0	0,2107	ns	No	0,6223
	Mean velocity	3	-0,3720	weak	0	0,3787	ns	No	0,1384
	Resting time	3	0,8791	very strong	0	0,1581	ns	No	0,7729
	Permanence time in periphery	3	0,0202	very weak	0	0,4936	ns	No	0,0004

Table 1. Correlation analysis between mGlu5 receptor level and locomotion related values.

Table 1. Correlation analysis between specific $mGlu_5$ binding and locomotion parameters. Binding and locomotion data from each sample preparation were analyzed in the different animal groups and the strength of the correlation calculated (Pearson r) and defined[74] as "*very weak*" (0.00-0.19), "*weak*" (0.20-0.39), "*moderate*" (0.40-0.59), "*strong*·" (0.60-0.79) and "*very strong*" (0.80-1.00).

Graphic for the Table of Contents





mGlu5 receptor