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# A role for glia maturation factor dependent activation of mast cells and microglia in MPTP induced dopamine loss and behavioural deficits in mice

**Govindhasamy Pushpavathi Selvakumar**[a,b], **Mohammad Ejaz Ahmed**[a,b], **Ramasamy Thangavel**[a,b], **Duraisamy Kempuraj**[a,b], **Iuliia Dubova**[b], **Sudhanshu P. Raikwar**[a,b], **Smita Zaheer**[b], **Shankar S. Iyer**[a,b], **Asgar Zaheer**[a,b,*]

[a]Harry S. Truman Memorial Veterans Hospital, Columbia, MO 65211, United States

[b]Department of Neurology, and Center for Translational Neuroscience, School of Medicine, University of Missouri, Columbia, MO 65211, United States

## Abstract

The molecular mechanism mediating degeneration of nigrostriatal dopaminergic neurons in Parkinson's disease (PD) is not yet fully understood. Previously, we have shown the contribution of glia maturation factor (GMF), a proinflammatory protein in dopaminergic neurodegeneration mediated by activation of mast cells (MCs). In this study, methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced nigrostriatal neurodegeneration and astro-glial activations were determined by western blot and immunofluorescence techniques in wild type (WT) mice, MC-deficient (MC-KO) mice and GMF-deficient (GMF-KO) mice, with or without MC reconstitution before MPTP administration. We show that GMF-KO in the MCs reduces the synergistic effects of MC and Calpain1 (calcium-activated cysteine protease enzyme)-dependent dopaminergic neuronal loss that reduces motor behavioral impairments in MPTP-treated mouse. Administration of MPTP increase in calpain-mediated proteolysis in nigral dopaminergic neurons further resulting in motor decline in mice. We found that MPTP administered WT mice exhibits oxidative stress due to significant increases in the levels of malondialdehyde, superoxide dismutase and reduction in the levels of reduced glutathione and glutathione peroxidase activity as compared with both MC-KO and GMF-KO mice. The number of TH-positive neurons in the ventral tegmental area, substantia nigra and the fibers in the striatum were significantly reduced while granulocyte macrophage colony-stimulating factor (GM-CSF), MC-Tryptase, GFAP, IBA1, Calpain1 and intracellular adhesion molecule 1 expression were significantly increased in WT mice. Similarly, tyrosine hydroxylase, dopamine transporters and vesicular monoamine transporters 2 proteins expression were significantly reduced in the SN of MPTP treated WT mice. The motor behavior as analyzed by rotarod and hang test was significantly reduced in WT mice as

*Corresponding author at: Department of Neurology, and Center for Translational Neuroscience, University of Missouri – School of Medicine, M741A Medical Science Building, 1 Hospital Drive, Columbia, MO 65211, United States. zaheera@health.missouri.edu (A. Zaheer).

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

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compared with both the MC-KO and GMF-KO mice. We conclude that GMF-dependent MC activation enhances the detrimental effect of astro-glial activation-mediated oxidative stress and neuroinflammation in the midbrain, and its inhibition may slowdown the progression of PD.

**Keywords**

Astro-glial activation; Dopaminergic neurodegeneration; Glia maturation factor; Mast cells; Parkinson's disease

## 1. Introduction

Parkinson's disease (PD) is the most common age associated movement disorder. Clinically, it is characterized by the progressive loss of dopaminergic neurons in the substantia nigra (SN) and ventral tegmental area (VTA) leading to depletion of dopaminergic neurotransmission to the striatum (STR) with resulting motor dysfunction in mid to later stages of PD (Dufty et al., 2007; Esteves et al., 2010; Samantaray et al., 2015; Spillantini et al., 1997). However, the exact pathological mechanism of PD is not fully understood. Accumulating evidences suggest that intracellular mitochondrial dysfunction dependent reactive oxygen species (ROS) production, neuroinflammation, impairment in ubiquitin-proteasomal and autophagy-lysosomal systems along with accumulation of α-synuclein contribute to PD progression (Gandhi and Wood, 2005; Selvakumar et al., 2014, 2015, 2018, 2019). Neuroinflammation is a result of a distinct cascade of events in the brain (Xanthos and Sandkühler, 2014). However, chronic neuroinflammation can result in detrimental effects involving changes in the brain parenchyma, breach of blood brain barrier (BBB), and neuronal hyperexcitability that ultimately leads to neuronal death (Bañuelos-Cabrera et al., 2014; Hendriksen et al., 2017; Lyman et al., 2014; Maas et al., 2008). Neuroinflammation plays a crucial role in PD pathogenesis, due to age, genetics and other sporadic factors that influence immune alterations that can lead to astro-glial activation associated with dopaminergic neurodegeneration (Chung et al., 2010; Kanaan et al., 2010). The neuronal loss is exacerbated when the central inflammatory response is induced with the active peripheral inflammation in PD (Liu and Bing, 2011).

Emerging evidence suggest that the brain's resident immunocompetent macrophages such as astro-glial cells are the major source of inflammatory mediators. Microglia also respond to proinflammatory signals that are released by other type of non-neuronal cells such as mast cells (MCs) (Kempuraj et al., 2018c). Different types of extra and intracellular insults promote neurodegeneration through products, such as proteases, calpains, cytokines, chemokines, ROS and others factors released by astro-glial cells (Crocker et al., 2003; Smith et al., 2012). Furthermore, calpains are unique family of calcium-activated cysteine proteases that include isoforms calpain 1 and alpain 2. Both calpains consist of large and unique subunits with common small regulatory subunits (Crocker et al., 2003; Sorimachi et al., 1997). Activated MCs show increased calpain 1 activity and its deficiency reduces IκB-NF-κB pathway activation without affecting MAPKs or NFAT pathways in MCs (Wu et al., 2014).

MCs are principally bone marrow derived hematopoietic progenitors that enter the brain through BBB, to actively participate in the neuroinflammatory process through their actively stored and newly synthesized inflammatory mediators such as cytokines, chemokines, proteases and ROS (Dong et al., 2014; Hendriksen et al., 2017; Kempuraj et al., 2017; Nelissen et al., 2013; Skaper et al., 2012). During the inflammatory condition, MC release their own mediators acting as enzymatic catalysts and recruiters to initiate, amplify, and propagate to other immune and neuronal cells (Dong et al., 2014; Hendriksen et al., 2017). MC releases granulocyte macrophage colony-stimulating factor (GM-CSF), a monomeric glycoprotein during activation (Kempuraj et al., 2017; Kempuraj et al., 2019; Wodnar-Filipowicz et al., 1989). In addition, MC-Tryptase is an enzyme that secreted by MC along with histamine during the MC activation (Kempuraj et al., 2018b; Kempuraj et al., 2017; Payne and Kam, 2004). However, the molecular interaction between the neuronal cells and MCs particularly in PD progression is still not fully understood (Kempuraj et al., 2018c; Skaper et al., 2014).

Intracellular small molecules are crucial players in several intracellular modifications, which are involved in a large variety of pathological conditions. In recent years, studies have focused on the potential therapeutic use of small agents with strong biological properties. Glia maturation factor (GMF), a 17-kDa neuroinflammatory protein that was first discovered, purified and sequenced in our laboratory (Kaplan et al., 1991; Lim et al., 1989; Zaheer et al., 1993). GMF consists of a 141-amino acid polypeptide chain is abundant in the brain and predominantly expressed by the brain parenchyma cells such as astrocytes, microglia and neurons (Lim et al., 1988; Lim et al., 1989; Lim and Zaheer, 2006). GMF is upregulated under cellular stress condition and is phosphorylated by protein kinases A and C, casein kinase, and ribosomal S6 kinase at multiple phosphorylation sites (Thangavel et al., 2011; Zaheer et al., 2006; Zaheer and Lim, 1997). In the present study, we investigated the role of GMF in the activation of MCs to release MCs proteases that activate the microglial Calpain 1 expression in a MPTP model of PD. Our findings suggest that the presence of GMF in the MCs makes them more reactive to MPTP toxicity by inducing Calpain 1 release from the microglial cells that leads to dopaminergic depletion in nigrostriatal region in the mice midbrain.

## 2. Materials and methods

### 2.1. Mouse primary mast cell culture

Bone marrow-derived MCs (BMMCs) were grown by culturing mouse femur bone marrow cells acquired from WT mice as well as from GMF-knockout (GMF-KO) mice as described previously (Kempuraj et al., 2019; Kim et al., 2011; Sayed et al., 2011; Tagen et al., 2009). Briefly, bone marrow from 5 to 10 mice were pooled together in order to get more number of MCs in the culture. Bone marrow cells were grown in high glucose DMEM (GIBCO, Life Technologies, Grand Island, NY) supplemented with IL-3 (10 ng/ml), 10% heat-inactivated fetal bovine serum (FBS; GIBCO), 1% penicillin-streptomycin (GIBCO), 20 μM 2-mercaptoethanol and1% L-glutamine (GIBCO) for ~4–6 weeks at 37 °C in a 5% $CO_2$ incubator. Cells were counted by a standard hemocytometer as reported previously (Nakano et al., 1985). After ~ 4–6 weeks of culture period, > 98% of the cells in the culture were

MCs as determined by 0.1% toluidine blue staining. BMMCs cultured over 5 weeks were used for these sequential experiments.

### 2.2. Animals

Male C57BL/6 GMF-KO mice was generated in our laboratory and colonies of these mice were always maintained for our studies. The C57BL/6 wild type mice (WT) and WBB6F1/J-KitW/KitW-v/J MCs knockout mice (MC-KO) were obtained from the Jackson Laboratory (Bar Harbor, ME, USA). MC-KO mice shows the stem cell factor receptor CD117 (Kit; c-kit) mutations in $Kit^{W/W\text{-}v}$ mice that impair and markedly reduced levels of MCs (Dahlin et al., 2016; Nautiyal et al., 2008). In contrast, $Kit^{W\text{-}sh/W\text{-}sh}$ mice, bearing the W-sash (Wsh) inversion mutation, have MC deficiency (Dahlin et al., 2016; Grimbaldeston et al., 2005). In addition, these *Kit* ligand mutant mice have pleiotropic defects in MCs formation (Kitamura, 1989; Kitamura et al., 1978; Rodewald and Feyerabend, 2012). Mice carrying certain mutations in the white spotting (W) locus (i.e, c-kit) exhibit reduced c-kit tyrosine kinase-dependent signaling that results in MC deficiency and other phenotypic abnormalities (Nautiyal et al., 2008; Nocka et al., 1990).

### 2.3. Treatment schedule

All experiments using MPTP were performed as previously published safety guidelines (Przedborski et al., 2001). Mice were divided into three experimental groups in each category (WT, MC-KO and GMF-KO; n = 4). Mice were allowed four days for acclimatization and after that for five consecutive day's behavioral training such as rotarod and hang test were performed (Schematic diagram of treatment schedule). At the end of training period, three days before MPTP administration, animals were reconstituted with MCs derived from WT mice and GMF-KO mice via intravenous injection (i.v) as shown in Table 1. MCs were injected with PBS i.v as reported previously (Gaudenzio et al., 2015; Nakano et al., 1985). We injected $5 \times 10^6$ cells in 50 μl of PBS (37° C). Our present findings in this study may be limited because of the non-inclusion of the MC-reconstitution groups without MPTP treatment. We assumed, that since MCs are not found in peripheral circulation under normal conditions (Dahlin et al., 2016); reconstitution with MCs would not alter peripheral or tissue distribution of MCs. A more detailed study of the fate of MCs after reconstitution, specifically brain localization is planned for the future. Another limitation of distinguishing between exogenously added and innate MCs will be explored in future studies. This study evaluated the biological contribution of MCs in dopaminergic neurodegeneration. Currently, we did not evaluate MC numbers in the brain after MC reconstitution. After three days of MC reconstitution, mice were injected with MPTP (18 mg/kg) by intraperitoneal (i.p) as we reported previously (Khan et al., 2013; Khan et al., 2015). At the end of the experimental period (seventh day after the last MPTP injection), behavioral tests were performed and finally the mice were sacrificed by cervical dislocation. Following this mice brains were quickly procured for biochemical estimation and protein expression analysis. These studies were carried out in accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health (NIH). The Committee on the Ethics of Animal Experiments of the University of Missouri-Columbia, MO approved the protocol.

## 2.4. Behavioral assessments

All the behavioral assessments were carried out at the room temperature between 09:00AM and 5:00PM in a calm room without any outside interference (Khan et al., 2015). Trained specific laboratory personnel performed all the behavioral assessments as well as data analysis after the experimental period in a blinded manner.

**2.4.1. Rotarod test—**In the rotarod test, animals were allowed to walk on a Rotamex Rotarod (Columbus, OH, USA), that was widely used to evaluate the motor behavior abnormalities of laboratory rodents. Before MPTP administration, mice were allowed on five consecutive days for training and adaptation in different rpm (5, 10, 15 and 20 rpm) with six trials. In brief, the mice were individually placed on a 30 mm rotating rod suspended a ~30 cm above the floor. The mice underwent three trials per day during the post MPTP behavioral assessments and 90 s is the maximal observation time for each animal trial. Performance of individual mice was measured by the duration that an animal stays upon the rotating rod as a function of rotating rod speed. The length of time of each mice maintain their balance on a rotating rod at speeds of 5, 10, 15 and 20 rpm was automatically recorded. The average retention time on the rotating rod was calculated as previously reported (Khan et al., 2015; Lauretti et al., 2016; Selvakumar et al., 2014).

**2.4.2. Hang test—**Hang test was used to determine the neuromuscular strength and motor functions of the mice. In brief, the apparatus consists of a wire with a 1.6 mm in diameter, 40 cm high and 50 cm long between 2 poles. Mice tend to support themselves with their hind limbs to avoid falling and to aid in progression along the wire. The latency of fall to the floor and the ability to grip in the wire was scored as described previously (Kaizaki et al., 2014; Khan et al., 2015; Selvakumar et al., 2015).

## 2.5. Biochemical analysis

**2.5.1. Determination of lipid peroxidation—**The activity of the lipid peroxidation product malondialdehyde (MDA) was determined by using a thiobarbituric acid reactive assay kit (Cat No. 10009055; Cayman chemical, Ann Arbor, MI), according to the manufacturer instruction. The assay was performed using the homogenates derived from SN of midbrain tissues in RIPA cell lysis buffer (containing protease and phosphatase inhibitor cocktails). The supernatants were prepared by centrifugation at 9, 184×*g* for 10 min at 4°C. The MDA content was calculated and expressed in micromoles per milligram of protein.

**2.5.2. Glutathione (GSH) assay—**The GSH levels were detected in the SN of midbrain tissue homogenate by using GSH assay kit (Cat No. 703002; Cayman chemical), according to the manufacturer's instruction. The level of GSH was calculated in terms of μmol DTNB conjugate formed/mg protein using a molar extinction coefficient of 13.6 $10^3$ $M^{-}1$ $cm^{-}1$ and the results were expressed nanogram (ng) of protein per milligram of protein.

## 2.6. Antioxidant activities

**2.6.1. Glutathione peroxidase (GPx) assay—**GPx catalyzes via the reduction of hydrogen peroxide ($H_2O_2$) and lipid peroxides (ROOH), after which glutathione (GSH) reacts with them, resulting in oxidized glutathione (GSSH) and water ($H_2O$). The activity of

GPx was measured in SN of midbrain tissue homogenate by using GPx assay kit (Cat No. 703102; Cayman chemical), as per manufacturer's instructions. Total activity of GPx was defined as the amount of GPx required to oxidize one mmol of NADPH/min/mg of protein.

**2.6.2. Superoxide dismutase (SOD) assay—**The aforementioned midbrain tissue homogenates-supernatant were also used to study the activity of SOD. Total SOD activity was determined spectrophotometrically using a SOD assay kit (Cat No. 706002; Cayman chemical) and calculated as per the manufacturer's instructions. A SOD unit was defined as the amount of enzyme that reduced the absorbance at 550 nm by 50% and the value is presented as SOD units per milligram of protein.

## 2.7. Western blot

Protein expression levels were determined by Western blot as we reported previously (Selvakumar et al., 2018b). Briefly, after the experimental period animals were sacrificed by cervical dislocation and SN of midbrains tissue were immediately dissected from the whole brain. Midbrain nigral tissues were individually homogenized in a Eppendorf tube contains RIPA cell lysis buffer (containing 20 mM Tris, 1 mM ethylene diamine tetra acetic acid, and 100 mM NaCl with proteinase inhibitor cocktail; Sigma-Aldrich, USA). Homogenates of total SN of midbrains were centrifuged at 21952 × g for 15 min at 4 °C. Protein concentration of the supernatant was measured using the bicinchoninic acid assay (BCA) protein assay kit (Thermo Scientific, Waltham, MA) as per the manufacturer's instructions. After boiling the supernatants at 95 °C for 5 mins they were subjected to 4–12% NuPAGE Bis-Tris gradient gel electrophoresis (Invitrogen, Life Technologies, Carlsbad, CA) at room temprature. Equal amounts of protein samples (ranging from ~ 20 to 35 μg) were loaded in each well along with a lane containing pre-stained protein ladder (Invitrogen). The gel separated proteins were transferred to polyvinylidene fluoride (PVDF) membrane by wet transfer system (Invitrogen). The membranes were blocked in 5% bovine serum albumin (BSA) or non-fat milk powder in Tris-buffered saline-Tween20 detergent (TBST; TBS + 0.05% Tween-20) for 1 h at 4° C, then incubated with the respective primary antibodies diluted in blocking solution: GM-CSF (Santa Cruz biotech, Cat No.13101; 1:150); MC-Tryptase (Millipore Sigma, Cat No. MAB1222; 1:300), Tyrosine hydroxylase (TH) (ThermoScientific, Cat No. P21962; 1:800), dopamine transporters (DAT) (ThermoScientific, Cat No. PA1–4656; 1:800), vesicular monoamine transporters (ThermoScientific, Cat No. PA5–22864; 1:800) Calpain 1 (Cell signaling tech, Cat No. 2556S; 1:500), ICAM 1 (BiosAntibodies, Cat No. BS-0608R; 1:700) and β-actin (Sigma-Aldrich, Cat No. A2228, 1:1000) at 4 °C for overnight with gentle shaking. After the primary antibody incubation followed by washings, membranes were incubated with their corresponding secondary antibodies (anti-rabbit or anti-mouse IgG conjugated with HRP) for 2 h at room temperature. The bands were visualized by adding ECL prime western blotting detection reagent SuperSignal West Pico PLUS Substrate (Cat No. 34580; ThermoScientific). The membranes were stripped and re-probed to detect β-actin expression as a loading control. All densitometry analysis was performed by using ChemiDoc-It2 Imaging System (UVP LLC, Upland, CA) analysis software.

### 2.8. Immunofluorescence staining and stereology counting

Immunofluorescence staining and stereology counting in SN and STR was performed as reported previously (Khan et al., 2013; Khan et al., 2015; Selvakumar et al., 2014). Midbrain tissues were collected from mice injected with MPTP or saline for immunofluorescence detection as previously reported (Chung et al., 2010; Crocker et al., 2003). Briefly, after the experimental period (seventh day after the last MPTP injection), behavioral tests were performed and finally the mice were sacrificed by cervical dislocation. Following this, mice brains were quickly procured and fixed with 4% paraformaldehyde (PFA) solution. After fixation, the brains were changed into 10, 20 and 30% sucrose solution until sink at the bottom of the container. The midbrain region with SN, VTA and STR was identified and dissected out from the whole brain. Optimal cutting temperature (OCT) embedding medium was used to freeze midbrain tissue. Approximately, 3–4 stained tissue sections were utilized per group to prove statistical significance between the groups studied. The latter sections were incubated with 0.3% hydrogen peroxide ($H_2O_2$) for 10 min at room temperature to remove the endogenous peroxidase activity. Coronal sections (25–30 μm-thick) were prepared using a cryostat (Leica Biosystems, Germany) and the sections were washed thrice deeply with 1% PBST (PBS + Triton-X 100). Sections were blocked with blocking buffer (1% BSA in TBST) for 30 mins at 4 °C followed by incubation with the combination of primary antibodies such as anti-rabbit GM-CSF (1:500); anti-rabbit MC-Tryptase (1:500), goat anti-rabbit TH antibody (1:500), ionized calcium-binding adapter molecule 1 (IBA1; 1: 1000), rabbit anti-mouse Calpain 1 and intercellular adhesion molecule 1 (ICAM 1; 1: 300–400) for overnight at 4° C. These sections were then incubated with appropriate secondary antibodies conjugated with green fluorescent dye (Alexa Fluor 488 secondary antibody, Cat No. A-11001, ThermoScientific) and red fluorescent dye (Alexa Fluor 568 secondary antibody, Cat No. A-11004, ThermoScientific) for 1 h at room temperature. Finally, the sections were mounted in gelatin-coated slides with cover slip in VECTASHIELD antifade mounting medium with DAPI (Vector Laboratories). Finally, the coverslips were mounted with Vinol (Sigma) onto microscope slides and cells were examined under a Nikon fluorescent microscope. The total number of TH-positive dopaminergic neurons in SN of the midbrain were counted using the optical fractionator, an unbiased stereological technique of cell counting (Gundersen, 1992; Sugama et al., 2003; West, 1999). To determine the optical density of TH-positive dopaminergic neurons in the VTA and fibers in the STR from immunostained sections, we used NIH ImageJ software. We randomly selected at least five different fields for the analysis, and the results are represented as arbitrary unit (AU) of control.

Confocal microscopic imaging was performed on a Leica TCP SP8 laser scanning confocal microscope (Leica, Biosystems) with a 405-nm diode laser and tunable super continuum white light laser using × 63 oil immersion objective (Molecular Cytology Core Facility, University of Missouri-Columbia). The following excitation and emission bandpass wavelengths were used in confocal microscope: 405/420–480 nm (DAPI), 495/505–550 nm (Alexa Fluor 488) and 570/580–630 nm (Alexa Fluor 568).

### 2.9. Statistical analysis

All the results obtained from the experiments were analyzed by GraphPad InStat 3 software. Results were given as mean ± SEM. Results were analyzed using one-way analysis of variance (ANOVA) followed by post hoc test Tukey-Kramer multiple comparison analysis to determine statistically significant differences. In addition, unpaired *t* test was used when comparing only two groups. The $p < 0.05$ was considered as statistically significant in all the experiments in this study.

## 3. Results

### 3.1. GMF-dependent activation of mast cells amplifies motor behavioral impairments in MPTP treated SN of midbrain

To determine the effect of GMF mediated MC activation on motor behavioral deficits in acute MPTP-treated mice, we performed motor behavioral studies. The latency of falling on the rotarod apparatus and neuromuscular strength and motor functions in hang test at 7 days after the last MPTP administration is shown in Fig. 1. In MPTP-treated mice, robust motor deficits, primary evidence for PD like symptoms is manifested by significantly decreased latency of fall (retention time) in rotarod test at different RPMs such as 5 (A), 10 (B), 15 (C) and 20 (D); along with the hanging time in the hang test (E). MPTP-treated mice when reconstituted with MCs from WT mice show a further decrease in the latency in both behavioral performances as compared with GMF-KO MC reconstituted mice. There are no significant differences between saline treated control from WT, MC-KO and GMF-KO groups.

### 3.2. GMF-dependent activation of mast cells increases oxidative stress in MPTP treated SN of midbrain

Activation of MCs leads to microglial activation, which is a primary source of ROS dependent oxidative stress in the MPTP-treated SN. We sought to determine whether GMF influenced the oxidative stress in the MPTP-treated SN (Fig. 2A–D) through activation of MCs. Oxidative stress is the prime indicator and an early determinant of toxic insults and degeneration of dopaminergic neurons. Accordingly, in this study, we found that acute regimen of MPTP (18 mg/kg) significantly increased lipid peroxidation markers such as the level of MDA (A) and the activity of SOD (B) in SN as compared with saline treated control group. Further, we found that administration of MPTP significantly reduced the level of GSH (C) and the activity of GPx (D) in the SN when compared with saline treated control group. In the SN of MPTP-treated mice that was reconstituted with WT MCs there was further significant increase in the *in vivo* production of MDA, the activity of SOD and reduced levels of GSH and the activity of GPx when compared to reconstitution with GMF-KO MCs to MPTP-treated mice. Controls from WT, MC-KO and GMF-KO using saline alone had no effect on the production of MDA, oxidants and antioxidants.

### 3.3. GMF-dependent activation of mast cells increased GM-CSF and MC-Tryptase and reduced TH-positive expression in the midbrain

To understand the GMF dependent MC activation and it has mediated GM-CSF and MC-Tryptase expression leads to dopaminergic neuronal cell death in SN of midbrain (Fig. 3). We analyzed GM-CSF and MC-Tryptase by western blot (A) and triple immunofluorescence (D and supplementary Fig. 3D a, b and c). Results show that acute administration of MPTP after WT MC reconstitution significantly increased GM-CSF (B) and MC-Tryptase (C) expression in the SN of the midbrain as compared with saline treated control mice. We found that GMF-KO MC reconstituted mice showed a significant reduction in GM-CSF and MC-Tryptase expression when compared with WT MC reconstituted mice. No significant differences were observed between the controls.

In addition, triple immunofluorescence show that GM-CSF (cyan fluorescence) and MC-Tryptase (red fluorescence) qualitatively increased meanwhile TH protein expression (green fluorescence) reduced in the acute administration of MPTP after WT MC reconstituted mice SN of midbrain as compared with saline treated controls. Furthermore, GMF-KO MC reconstituted mice shows a qualitative reduction in GM-CSF and MC-Tryptase expression when compared with WT MC reconstituted mice. These results indicate that GMF augments MPTP induced MCs activation and its mediated dopaminergic neuronal loss in the SN of midbrain.

### 3.4. GMF-dependent activation of mast cells reduced TH, DAT and VMAT2 expressions in the nigrostriatum of midbrain from MPTP treated mice

Next, we examined whether GMF activated MCs upon acute MPTP treatment and further reduced dopaminergic markers expression. We analyzed TH (a rate limiting enzyme of dopamine biosynthesis), dopaminergic transporters DAT (propels the neurotransmitter dopamine into the out of nerve synaptic cleft back into cytosol) and VMAT2 (transports the dopamine from cytosol to nerve synaptic end) as shown in Fig. 4. Results show that acute administration of MPTP after WT MC reconstitution significantly reduced TH (A and B), DAT (A and C) and VMAT2 (A and D) expression in the SN of the midbrain as compared with saline treated control mice. We found that GMF-KO MC reconstituted mice showed a significant enhancement when compared with WT MC reconstituted mice. No significant differences were observed between the controls. These results indicate that GMF augments MPTP induced dopaminergic neuronal markers expression such as TH, DAT and VMAT2 expression.

### 3.5. GMF-dependent activation of mast cells reduced TH-positive immunoreactivity in the midbrain

We performed immunofluorescence to show MPTP mediated toxicity on number of TH-positive neurons in the SN (Fig. 5), VTA (Fig. 6) and in STR (Fig. 7) of the midbrain. Results show that the administration of MPTP significantly reduced number of TH-positive neurons in the SN of WT, MC-KO and GMF-KO (Fig. 5A–L and M) and reduced integrated density of TH immunofluorescence expression in VTA (Fig. 6 A–L and M), and STR (Fig. 7 A–L and M) when compared with saline treated control mice. Furthermore, we found that MCs reconstituted from GMF-KO significantly increased number of TH-positive neurons

and integrated density of TH protein expression when compared with WT mice. No significant differences were observed between the saline treated control mice.

### 3.6. GMF-dependent activation of mast cells synergize with calpain 1 and ICAM 1 expression in the MPTP treated SN of midbrain

To understand the GMF-dependent MC activation-mediated dopaminergic denervation in the SN of MPTP-lesioned WT, MC-KO and GMF-KO mice, we analyzed calpain 1 and ICAM 1 expressions (Fig. 8) in the midbrain regions. Results show that MPTP treatment significantly increased calpain 1 (A and B) as well as ICAM 1 (A and C) expression in the SN of the midbrain when compared with saline treated control mice. We found that mice in all groups reconstituted with WT MCs show further increased calpain 1 and ICAM 1expression when compared with mice reconstituted with GMF-KO MCs. These results indicate that GMF expression in the MCs make them more susceptible to the neurotoxic effects of MPTP. No significant differences were observed between the control mice from WT, MC-KO as well as GMF-KO mice.

In addition, we show that MPTP administration to the WT, MC-KO and GMF-KO qualitatively increased calpain 1 and reduced TH immuno-expressions in SN of the midbrain as compared with saline treated control mice (Fig. 9 and supplementary Fig. 9A-La, b and c). As expected, reconstitution with MCs from WT mice shows qualitative reduction in TH and increased calpain 1 expression as compared with mice reconstituted with MCs form GMF-KO mice.

### 3.7. GMF-dependent activation of mast cells potentiates microglial activation in MPTP administered mice

To explore the cross-talk between MCs and microglial cells, we followed GMF dependent MC activation on microglial function through calpain 1 activation. We performed immunofluorescence double-labeling microscopy using IBA1 as the activated microglia marker along with calpain 1staining. Results show that acute MPTP treatment qualitatively enhanced IBA1 (red fluorescence) and calpain 1 (green fluorescence) expression and co-localization in the SN when compared with saline treated control mice (Fig. 10). In addition, we found that in SN of mice reconstituted with MCs from WT mice, MPTP treatment showed cytoplasmic colocalization of IBA1 and calpain 1which exhibited a bright appearance that became more intense when compared with SN of mice reconstituted with GMF-KO. There were no qualitative differences between the control mice.

Double immunoflourecence staining was performed to demonstrate co-expression of IBA1 and ICAM 1 in SN of the midbrain. Representative images show that MPTP administration following MC reconstitution form WT mice shows qualitatively increased expression of IBA1 (red fluorescence) and ICAM 1 (green fluorescence) when compared with saline treated control mice (Fig. 11). We found that reconstitution with MCs from GMF-KO mice shows lesser expression than WT MC reconstituted mice. This indicates that GMF expression dependent MC activation upregulates the microglial activation and ICAM 1 expression. No qualitative differences were found between the control mice in each experimental group.

### 3.8. GMF-dependent activation of mast cells mediates GFAP and cellular adhesion molecule expression in SN of MPTP treated mice

We further investigated the detrimental effects of GMF and its mediated activation of MCs in astrocytic activation and disruption of blood brain barrier integrity in acute MPTP-administered mice SN. Astrocytes are involved in the formation of the BBB (Willis, 2010) and since the BBB is breached by the activation of MCs, we analyzed GFAP (activation marker of astrocytes) and ICAM 1(intracellular adhesion molecule 1) in the SN of the midbrain. The expression of GFAP (green fluorescence intensity) and ICAM-1 (red fluorescence intensity) were significantly increased in mice that received acute MPTP-treatment as compared with saline administered control mice (Fig. 12). In addition, the intensity and the colocalization of these proteins were also qualitatively increased when compared with SN of control mice. The peripheral immune system exerts its immune response through the activation of MCs, breaching the BBB during disease conditions. As expected, MPTP treated mice reconstituted with MCs from WT mice show significantly increased expression of GFAP and ICAM 1 as compared with mice reconstituted with MCs from GMF-KO mice and subjected to MPTP treatment. Taken together, reconstitution with MCs from WT mice accelerates the disruption of BBB, and this is turn could contribute to astrocyte activation that further leads to dopaminergic neurodegeneration in SN of midbrain of the mice.

## 4. Discussion

In the present study, we demonstrate the sequential action of GMF-dependent murine MC activation and calpain 1 expression involved in the dopaminergic neurodegeneration in the SN and STR of midbrain. Murine MC Proteases and calpain 1 mediate the activation of astro-glial cells and this further leads to dopaminergic neurodegeneration and motor behavioral deficits in WT, MC-KO and GMF-KO mice. The salient findings in the current study include a significant oxidative stress, increased calpain 1, ICAM 1, IBA1 and GFAP expressions that lead to depletion of dopaminergic markers such as TH, DAT and VMAT2 expression resulting in motor behavioral deficits, which are causally linked to GMF-dependent MC activation in MPTP lesioned PD mice brain. The MC number used for reconstitution experiments was chosen from previous studies (Nakano et al., 1985) that showed the fate of reconstituted cultured MCs after intravenous and intraperitoneal injections. The study showed that $1 \times 10^6$ cells injected showed a distribution of cells into peritoneal cavity and other organs. Another study showed that $5 \times 10^6$ MCs was sufficient to induce damage of neuronal body and neuronal fibers. From these studies, we proposed our reconstitution experimental design at $5 \times 10^6$ MCs for our animal protocols that was approved by the University of Missouri animal Ethical Committee and the US Department of Veterans Affairs ORD-BLRD.

PD is recognized as the second most common age dependent neurodegenerative disease after Alzheimer’s disease. PD arises due to loss of melanin pigmented dopaminergic neurons in the SN that causes motor and non-motor behavioral deficits (Kanaan et al., 2006; Kanaan et al., 2010). Particularly in PD, dopaminergic neurons in the ventral tier of the SN (vtSN) are more prone to degeneration, although those in the dorsal tier (dtSN) and ventral tegmental

area (VTA) are more resistant to dopaminergic neurodegeneration (Kanaan et al., 2007). As has been shown previously by Phani et al. (2010) certain types of proteins in VTA dopaminergic neurons including protein regulation (Phf6) and ion/metal regulation (PANK2 and Car4) increase the resistance against low dose of MPTP (4 mg/kg). Absence of these proteins in SN dopaminergic neurons increases cell death. However, in the brain, the activity of MC is widely increased when immune or non-immune stimulus signals cause external or internal stimuli including hormones, like corticotrophin releasing hormone (CRH), and various neuropeptides like Substance P and neurotensin (Paus et al., 2006). MC play a crucial role in neurodegenerative disease by their inflammatory response when it is activated by the CRH and such type of neuropeptides during stress conditions (Dong et al., 2014; Hendriksen et al., 2017; Kempuraj et al., 2017; Paus et al., 2006; Skaper et al., 2014). In the present study, we show that high dose of MPTP (28 mg/kg) activates the MCs. Activated MCs release their own pre-stored neuroinflammatory mediators such as GM-CSF, and MC tryptase. Calcium-dependent proteases such as calpain-1and calcium-binding protein calbindin-D abundantly present in SN, ST and VTA regions mediate MPTP toxicity. Our results demonstrate that activation of calpain-1 brought about by MC protein activation mediate dopaminergic degeneration in VTA region.

GMF plays a crucial role in intracellular signal-transduction in neuronal cells, increasing its expression under stress conditions leading to increased proinflammatory cytokine and chemokine expression (Kempuraj et al., 2013; Zaheer et al., 1993; Zaheer et al., 2013). Our recent studies showed that mouse MCs release their own MC proteases (MMCP) such as MMCP-6 and MMCP-7 upon $MPP^+$ and GMF toxicity, which leads to astro-glial activation resulting in aggravation of dopaminergic neurodegeneration. In addition, MC proteases such as MC-Tryptase activate mitogen-activated protein kinases (MAPKs) p38, extracellular signal-regulated kinases 1/2 (ERK1/2), transcription factor nuclear factor-kappa B (NF-κB) in neuronal cells (Kempuraj et al., 2018c). In addition, our previous reports show that GMF is an essential factor for the induction of GM-CSF and GMF is involved in the astro-glial dependent secretion of proinflammatory mediators in the brain (Zaheer et al., 2007). We have previously reported that acute administration of MPTP to WT mice increased various biochemical indices such as TBARS, SOD, GSH and GPx and neurodegeneration in SN region. We recently demonstrated that dopaminergic N27 cells exposed to GMF and $MPP^+$ showed a significant increase in the oxidative stress mediated apoptosis (Selvakumar et al., 2018b). In addition, we also reported that global knockdown of GMF improves the anti-oxidant indices after MPTP administration (Khan et al., 2013; Khan et al., 2015). Earlier findings from our laboratory showed that absence of GMF restricted the motor behavioral impairments (Khan et al., 2015; Lim et al., 2004; Zaheer et al., 2006). In addition, our present study shows that retention time for latency of fall in rotarod and hang test was significantly reduced in mice reconstituted with MCs from WT mice after treatment with MPTP. Reconstitution with MCs from GMF-KO significantly prolonged latency of fall in these behavioral performances.

TH and DAT are the key proteins that regulate catecholamine biosynthesis and transport across the brain neuronal cells including dopamine (Yan et al., 2007). Previous reports showed that increased expression of calpain 1 hastens dopaminergic neurodegeneration in SN as well as in spinal cord and enhances the synaptic impairment (Diepenbroek et al.,

2014; Samantaray et al., 2013). It has been reported that activation of MCs enhances calpain 1 expression, and calpain 1 deficiency significantly reduces MC activation (Wu et al., 2014). In the present study, we found that administration of MPTP significantly increased calpain expression in the neuronal cells brought about by MC activation leading to dopaminergic neuronal cell injury in the mice brain. The IL-33 released by dead cells and recognized by MCs probably elicits an inflammatory response through upregulation of calpain. In confirmation of our earlier findings with MPTP administration (Khan et al., 2013; Khan et al., 2015; Zaheer et al., 2014; Zaheer et al., 2001), a significant reduction of TH-positive dopaminergic neurons were found in the SN, VTA and STR along with calpain 1 expression. Overall, our findings from this study shows a significant reduction of MC activation and improvement in dopaminergic markers such as TH, DAT and VMAT2 expression in the GMF-KO mice administered with MPTP compared with WT MPTP administered mice. Administration of MPTP enhances calpain 1 expression in WT and GMF-KO mice and probably due to neovascularization process, may lead to re-modeling the vessels in damaged brain region (Potz et al., 2016; Prakash and Carmichael, 2015). More importantly, reconstitution with MC from WT mice followed with MPTP administration significantly reduced TH-positive neurons. However, reconstitution with MCs from GMF-KO mice and MPTP administration preserved the dopaminergic denervation, which may be attributed to deficiency in GMF expression.

ICAM 1, IBA1 and GFAP are essential mediators in the initiation of inflammatory sequence in the brain. Previous studies showed that immune cells migrate from the periphery during BBB breach into the brain and exert a protective or detrimental role in SN of the midbrain upon inflammatory stress condition (Choi et al., 2018; Lee et al., 2015; Miklossy et al., 2006). ICAM-1 is a strong immunoglobulin-like cell adhesion molecule, which binds with the leukocyte beta-2 integrin of lymphocyte function-associated antigen 1 (LFA1 or CD11b) through domain 1 (Diamond et al., 1991) and to Mac-1β−2 integrin through domain 3 (Nejentsev et al., 2003b). These interactions of endothelial ICAM-1 with LFA-1 and Mac-1 mediates leukocyte adherence, *trans*-endothelial migration and movement of activated lymphocytes into sites of inflammation. Previously, it was reported that ICAM 1 is an important protein that plays a major role in the immune-mediated cell-to-cell adhesion and an indicator for BBB breach in the brain due to inflammation (Corti et al., 2004; Isogai et al., 2004; Zameer and Hoffman, 2003). ICAM-1 expression in cerebral micro vessels under normal conditions shows limited expression (Wertheimer et al., 1992); conversely, ICAM-1 expression is markedly increased on the luminal surface of endothelial cells in the presence of proinflammatory mediators such as TNF-α, IL-1β, IL-4, and IFN-γ (Meagher et al., 1994). Our previous studies showed that IL-1β, TNF-α and the chemokine (C–C motif) ligand (CCL) 2 expressions were significantly increased in WT mice when compared with GMF-KO and MC-KO mice due to MC activation in brain (Kempuraj et al., 2018a). In addition, the involvement of MC in the BBB breach or degradation may involve the vasoactive and matrix degrading components of MCs, such as heparin, histamine, serotonin, NO, vasoactive intestinal peptide, calcitonin gene-related peptide, VEGF, and cytokines, including TNF-α, which in turn induces the expression of ICAM-1 and permits leukocytes to enter the damaged tissues (Brown and Hatfield, 2012; Dong et al., 2014; Nelissen et al., 2013). Even though other brain resident immune cells such as microglia/macrophages and

endothelial cells can produce TNF-α, the presence and release of TNF-α from MCs precedes BBB breach and aggravates neuroinflammation in brain (Jin et al., 2009). During the inflammatory cascade, activated astrocytes expresses GFAP as well as ICAM 1 thus expanding immune responses, leukocyte accumulation, and microglial migration and activation at the site of inflammation (Akiyama et al., 1993; Choi et al., 2018; Lee et al., 2000). Our earlier findings suggested that MPTP enhanced its toxicity through increases in GMF expression and the production of inflammatory TNF-α, IL-1β, granulocyte macrophage-colony stimulating factor, and the chemokine (C–C motif) ligand (CCL) 2 and MCP-1 expression in SN of the midbrain (Khan et al., 2015). Our current findings demonstrate that activation of MCs and subsequent effects such as oxidative stress enhances dopaminergic neurodegeneration via astro-glial cell activation. Administration of MPTP activates and increases the expression of ICAM 1, IBA1 and GFAP through reconstitution with WT MCs that could lead to reduced TH, DAT and VMAT2 expression resulting in motor deficits. This indicates that under stress conditions WT MC are much more sensitive to MPTP neurotoxicity and enhanced dopaminergic neurodegeneration via BBB breach. These effects were reduced in GMF and MC deficient mice upon MPTP administration.

## 5. Conclusions

In summary, the present study demonstrates that reconstitution of MC from WT mice into WT mice, MC-KO mice and GMF-KO mice followed by MPTP administration enhances nigrostriatal dopaminergic degeneration in the midbrain. Administration of MPTP following MC reconstitution enhances oxidative stress by altering levels of MDA, GSH and activities of SOD and GPx, reduces TH, DAT and VMAT2 protein expression. In addition, MPTP reduces number of TH positive dopaminergic neurons in the SN, VTA and STR due to differential expression of GM-CSF, MC-Tryptase, calpain 1 and ICAM 1 in the midbrain. Furthermore, administration of MPTP following MC reconstitution enhances calpain 1 and ICAM 1 expression leading to astro-glial activation-dependent dopaminergic cell death and motor behavior decline. Conversely, these pathological changes were reduced and motor behavioral performances were improved in MC-KO and GMF-KO mice. This represents an important consequence in neurodegenerative disease especially in PD. Our findings and knowledge help us infer that deficiency of GMF is capable of rescuing the dopaminergic neuronal population in the SN via down regulation of MC and its dependent astro-glial responses in an MPTP experimental model of PD. This highlights the therapeutic efficacy of inhibition of GMF in inflammatory disorders.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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## References


Akiyama H, Kawamata T, Yamada T, Tooyama I, Ishii T, McGeer PL, 1993 Expression of intercellular adhesion molecule (ICAM)-1 by a subset of astrocytes in Alzheimer disease and some other degenerative neurological disorders. Acta Neuropathol 85, 628–634. [PubMed: 8337942]

Bañuelos-Cabrera I, Valle-Dorado MG, Aldana BI, Orozco-Suárez SA, Rocha L, 2014 Role of histaminergic system in blood-brain barrier dysfunction associated with neurological disorders. Arch. Med. Res 45, 677–686. [PubMed: 25446620]

Brown MA, Hatfield JK, 2012 Mast cells are important modifiers of autoimmune disease: with so much evidence, why is there still controversy? Front. Immunol 3, 147. [PubMed: 22701454]

Choi JH, Jang M, Nah S-Y, Oh S, Cho I-H, 2018 Multitarget effects of Korean Red Ginseng in animal model of Parkinson's disease: antiapoptosis, antioxidant, antiinflammation, and maintenance of blood–brain barrier integrity. J. Ginseng Res 42, 379–388. [PubMed: 29983619]

Chung YC, Kim SR, Jin BK, 2010 Paroxetine prevents loss of nigrostriatal dopaminergic neurons by inhibiting brain inflammation and oxidative stress in an experimental model of Parkinson's disease. J. Immunol 185, 1230–1237. [PubMed: 20566832]

Corti R, Hutter R, Badimon JJ, Fuster V, 2004 Evolving concepts in the triad of atherosclerosis, inflammation and thrombosis. J. Thromb. Thrombolysis 17, 35–44. [PubMed: 15277786]

Crocker SJ, Smith PD, Jackson-Lewis V, Lamba WR, Hayley SP, Grimm E, Callaghan SM, Slack RS, Melloni E, Przedborski S, Robertson GS, Anisman H, Merali Z, Park DS, 2003 Inhibition of Calpains prevents neuronal and behavioral deficits in an MPTP mouse model of Parkinson's disease. J. Neurosci 23, 4081–4091. [PubMed: 12764095]

Dahlin JS, Ungerstedt JS, Grootens J, Sander B, Gülen T, Hägglund H, Nilsson G, 2016 Detection of circulating mast cells in advanced systemic mastocytosis. Leukemia 30, 1953–1956. [PubMed: 27118407]

Diamond MS, Staunton DE, Marlin SD, Springer TA, 1991 Binding of the integrin Mac-1 (CD11b/CD18) to the third immunoglobulin-like domain of ICAM-1 (CD54) and its regulation by glycosylation. Cell 65, 961–971. [PubMed: 1675157]

Diepenbroek M, Casadei N, Esmer H, Saido TC, Takano J, Kahle PJ, Nixon RA, Rao MV, Melki R, Pieri L, Helling S, Marcus K, Krueger R, Masliah E, Riess O, Nuber S, 2014 Overexpression of the calpain-specific inhibitor calpastatin reduces human alpha-Synuclein processing, aggregation and synaptic impairment in [A30P]αSyn transgenic mice. Hum. Mol. Genet 23, 3975–3989. [PubMed: 24619358]

Dong H, Zhang X, Qian Y, 2014 Mast cells and neuroinflammation. Med. Sci. Monit. Basic Res 20, 200–206. [PubMed: 25529562]

Dufty BM, Warner LR, Hou ST, Jiang SX, Gomez-Isla T, Leenhouts KM, Oxford JT, Feany MB, Masliah E, Rohn TT, 2007 Calpain-cleavage of alpha-synuclein: connecting proteolytic processing to disease-linked aggregation. Am. J. Pathol 170, 1725–1738. [PubMed: 17456777]

Esteves AR, Arduino DM, Swerdlow RH, Oliveira CR, Cardoso SM, 2010 Dysfunctional mitochondria uphold calpain activation: contribution to Parkinson's disease pathology. Neurobiol. Dis 37, 723–730. [PubMed: 20034566]

Gandhi S, Wood NW, 2005 Molecular pathogenesis of Parkinson's disease. Hum. Mol. Genet 14, 2749–2755.

Gaudenzio N, Sibilano R, Starkl P, Tsai M, Galli SJ, Reber LL, 2015 Analyzing the functions of mast cells in vivo using 'Mast Cell Knock-in' mice. J Vis Exp e52753. [PubMed: 26068439]

Grimbaldeston MA, Chen C-C, Piliponsky AM, Tsai M, Tam S-Y, Galli SJ, 2005 Mast cell-deficient W-sash c-kit mutant Kit W-sh/W-sh mice as a model for investigating mast cell biology in vivo. Am. J. Pathol 167, 835–848. [PubMed: 16127161]

Gundersen HJ, 1992 Stereology: the fast lane between neuroanatomy and brain function–or still only a tightrope? Acta Neurol. Scand. Suppl 137, 8–13. [PubMed: 1357908]

Hendriksen E, van Bergeijk D, Oosting RS, Redegeld FA, 2017 Mast cells in neuroinflammation and brain disorders. Neurosci. Biobehav. Rev 79, 119–133. [PubMed: 28499503]

Isogai N, Tanaka H, Asamura S, 2004 Thrombosis and altered expression of intercellular adhesion molecule-1 (ICAM-1) after avulsion injury in rat vessels. J. Hand Surgery: Br. Eur 29, 230–234.

Jin Y, Silverman AJ, Vannucci SJ, 2009 Mast cells are early responders after hypoxia-ischemia in immature rat brain. Stroke 40, 3107–3112. [PubMed: 19520991]

Kaizaki A, Tanaka S, Yoshida T, Numazawa S, 2014 Maternal MDMA administration in mice leads to neonatal growth delay. J. Toxicol. Sci 39, 33–39. [PubMed: 24418707]

Kanaan NM, Collier TJ, Marchionini DM, McGuire SO, Fleming MF, Sortwell CE, 2006 Exogenous erythropoietin provides neuroprotection of grafted dopamine neurons in a rodent model of Parkinson's disease. Brain Res 1068, 221–229. [PubMed: 16368081]

Kanaan NM, Kordower JH, Collier TJ, 2007 Age-related accumulation of Marinesco bodies and lipofuscin in rhesus monkey midbrain dopamine neurons: Relevance to selective neuronal vulnerability. J. Compar. Neurol 502, 683–700.

Kanaan NM, Kordower JH, Collier TJ, 2010 Age-related changes in glial cells of dopamine midbrain subregions in rhesus monkeys. Neurobiol. Aging 31, 937–952. [PubMed: 18715678]

Kaplan R, Zaheer A, Jaye M, Lim R, 1991 Molecular cloning and expression of biologically active human glia maturation factor-beta. J. Neurochem 57, 483–490. [PubMed: 1712830]

Kempuraj D, Khan MM, Thangavel R, Xiong Z, Yang E, Zaheer A, 2013 Glia maturation factor induces interleukin-33 release from astrocytes: implications for neurodegenerative diseases. J. Neuroimmune Pharmacol 8, 643–650. [PubMed: 23397250]

Kempuraj D, Selvakumar GP, Thangavel R, Ahmed ME, Zaheer S, Kumar KK, Yelam A, Kaur H, Dubova I, Raikwar SP, Iyer SS, Zaheer A, 2018 Glia Maturation factor and mast cell-dependent expression of inflammatory mediators and proteinase activated receptor-2 in neuroinflammation. J. Alzheimer's Dis 66, 1117–1129. [PubMed: 30372685]

Kempuraj D, Selvakumar GP, Thangavel R, Ahmed ME, Zaheer S, Raikwar SP, Iyer SS, Bhagavan SM, Beladakere-Ramaswamy S, Zaheer A, 2017 Mast Cell activation in brain injury, stress, and post-traumatic stress disorder and Alzheimer's disease pathogenesis. Front. Neurosci 11.

Kempuraj D, Selvakumar GP, Zaheer S, Thangavel R, Ahmed ME, Raikwar S, Govindarajan R, Iyer S, Zaheer A, 2018c Cross-talk between glia, neurons and mast cells in neuroinflammation associated with Parkinson's disease. J. Neuroimmune Pharmacol 13, 100–112. [PubMed: 28952015]

Kempuraj D, Thangavel R, Selvakumar GP, Ahmed ME, Zaheer S, Raikwar SP, Zahoor H, Saeed D, Dubova I, Giler G, Herr S, Iyer SS, Zaheer A, 2019 Mast cell proteases activate astrocytes and glia-neurons and release interleukin-33 by activating p38 and ERK1/2 MAPKs and NF-kappaB. Mol. Neurobiol 56, 1681–1693. [PubMed: 29916143]

Khan MM, Kempuraj D, Thangavel R, Zaheer A, 2013 Protection of MPTP-induced neuroinflammation and neurodegeneration by Pycnogenol. Neurochem. Int 62, 379–388. [PubMed: 23391521]

Khan MM, Zaheer S, Thangavel R, Patel M, Kempuraj D, Zaheer A, 2015 Absence of glia maturation factor protects dopaminergic neurons and improves motor behavior in mouse model of parkinsonism. Neurochem. Res 40, 980–990. [PubMed: 25754447]

Kim DY, Hong GU, Ro JY, 2011 Signal pathways in astrocytes activated by cross-talk between of astrocytes and mast cells through CD40-CD40L. J Neuroinflammation 8, 25. [PubMed: 21410936]

Kitamura Y, 1989 Heterogeneity of Mast Cells and Phenotypic Change Between Subpopulations. Ann. Rev. Immunol 7, 59–76. [PubMed: 2653378]

Kitamura Y, Go S, Hatanaka K, 1978 Decrease of mast cells in W/Wv mice and their increase by bone marrow transplantation. Blood 52, 447–452. [PubMed: 352443]

Lauretti E, Di Meco A, Merali S, Praticò D, 2016 Chronic behavioral stress exaggerates motor deficit and neuroinflammation in the MPTP mouse model of Parkinson's disease. Transl. Psychiatry 6, e733. [PubMed: 26859816]

Lee SJ, Drabik K, Van Wagoner NJ, Lee S, Choi C, Dong Y, Benveniste EN, 2000 ICAM-1-induced expression of proinflammatory cytokines in astrocytes: involvement of extracellular signal-regulated kinase and p38 mitogen-activated protein kinase pathways. J. Immunol 165, 4658–4666. [PubMed: 11035109]

Lee YY, Park J-S, Lee E-J, Lee S-Y, Kim D-H, Kang JL, Kim H-S, 2015 Anti-inflammatory mechanism of Ginseng Saponin Metabolite Rh3 in Lipopolysaccharide-Stimulated Microglia: Critical Role of 5′-Adenosine Monophosphate-Activated Protein Kinase Signaling Pathway. J. Agric. Food. Chem 63, 3472–3480. [PubMed: 25798758]

Lim R, Hicklin DJ, Ryken TC, Miller JF, Bosch EP, 1988 Endogenous immunoreactive glia maturation factor-like molecule in cultured rat Schwann cells. Brain Res 468, 277–284. [PubMed: 3382960]

Lim R, Miller JF, Zaheer A, 1989 Purification and characterization of glia maturation factor beta: a growth regulator for neurons and glia. PNAS 86, 3901–3905. [PubMed: 2726756]

Lim R, Zaheer A, 2006 Glia maturation factor in brain function In: Lajtha A, Lim R (Eds.), Handbook of Neurochemistry and Molecular Neurobiology: Neuroactive Proteins and Peptides Springer, US, Boston, MA, pp. 203–222.

Lim R, Zaheer A, Khosravi H, Freeman JH Jr., Halverson HE, Wemmie JA, Yang B, 2004 Impaired motor performance and learning in glia maturation factor-knockout mice. Brain Res 1024, 225–232. [PubMed: 15451385]

Liu M, Bing G, 2011 Lipopolysaccharide animal models for Parkinson's disease. Parkinson's disease 2011 327089–327089.

Lyman M, Lloyd DG, Ji X, Vizcaychipi MP, Ma D, 2014 Neuroinflammation: The role and consequences. Neurosci. Res 79, 1–12. [PubMed: 24144733]

Maas AIR, Stocchetti N, Bullock R, 2008 Moderate and severe traumatic brain injury in adults. Lancet Neurol 7, 728–741. [PubMed: 18635021]

Meagher L, Mahiouz D, Sugars K, Burrows N, Norris P, Yarwood H, Becker-Andre M, Haskard DO, 1994 Measurement of mRNA for E-selectin, VCAM-1 and ICAM-1 by reverse transcription and the polymerase chain reaction. J. Immunol. Methods 175, 237–246. [PubMed: 7523527]

Miklossy J, Doudet DD, Schwab C, Yu S, McGeer EG, McGeer PL, 2006 Role of ICAM-1 in persisting inflammation in Parkinson disease and MPTP monkeys. Exp. Neurol 197, 275–283. [PubMed: 16336966]

Nakano T, Sonoda T, Hayashi C, Yamatodani A, Kanayama Y, Yamamura T, Asai H, Yonezawa T, Kitamura Y, Galli SJ, 1985 Fate of bone marrow-derived cultured mast cells after intracutaneous, intraperitoneal, and intravenous transfer into genetically mast cell-deficient W/Wv mice. Evidence that cultured mast cells can give rise to both connective tissue type and mucosal mast cells. J. Exp. Med 162, 1025–1043. [PubMed: 3897446]

Nautiyal KM, Ribeiro AC, Pfaff DW, Silver R, 2008 Brain mast cells link the immune system to anxiety-like behavior. Proc. Natl. Acad. Sci 105, 18053. [PubMed: 19004805]

Nelissen S, Lemmens E, Geurts N, Kramer P, Maurer M, Hendriks J, Hendrix S, 2013 The role of mast cells in neuroinflammation. Acta Neuropathol 125, 637–650. [PubMed: 23404369]

Nocka K, Tan JC, Chiu E, Chu TY, Ray P, Traktman P, Besmer P, 1990 Molecular bases of dominant negative and loss of function mutations at the murine c-kit/white spotting locus: W37, Wv, W41 and W. EMBO J 9, 1805–1813. [PubMed: 1693331]

Paus R, Theoharides TC, Arck PC, 2006 Neuroimmunoendocrine circuitry of the 'brain-skin connection'. Trends Immunol 27, 32–39. [PubMed: 16269267]

Payne V, Kam PCA, 2004 Mast cell tryptase: a review of its physiology and clinical significance. Anaesthesia 59, 695–703. [PubMed: 15200544]

Phani S, Gonye G, Iacovitti L, 2010 VTA neurons show a potentially protective transcriptional response to MPTP. Brain Res 1343, 1–13. [PubMed: 20462502]

Potz BA, Abid MR, Sellke FW, 2016 Role of calpain in pathogenesis of human disease processes. J. Nat. Sci 2, e218. [PubMed: 27747292]

Prakash R, Carmichael ST, 2015 Blood-brain barrier breakdown and neovascularization processes after stroke and traumatic brain injury. Curr. Opin. Neurol 28, 556–564. [PubMed: 26402408]

Przedborski S, Jackson-Lewis V, Naini AB, Jakowec M, Petzinger G, Miller R, Akram M, 2001 The parkinsonian toxin 1-methyl-4-phenyl-1,2,3,6-tetra-hydropyridine (MPTP): a technical review of its utility and safety. J. Neurochem 76, 1265–1274. [PubMed: 11238711]

Rodewald H-R, Feyerabend Thorsten B., 2012 Widespread immunological functions of mast cells: fact or fiction? Immunity 37, 13–24. [PubMed: 22840840]

Samantaray S, Knaryan VH, Shields DC, Banik NL, 2013 Critical role of calpain in spinal cord degeneration in Parkinson's disease. J. Neurochem 127 (6), 880–890. [PubMed: 23875735]

Samantaray S, Knaryan VH, Shields DC, Cox AA, Haque A, Banik NL, 2015 Inhibition of calpain activation protects MPTP-induced nigral and spinal cord neurodegeneration, reduces

inflammation, and improves gait dynamics in mice. Mol. Neurobiol 52, 1054–1066. [PubMed: 26108182]

Sayed BA, Walker ME, Brown MA, 2011 Cutting edge: mast cells regulate disease severity in a relapsing-remitting model of multiple sclerosis. J. Immunol 186, 3294–3298. [PubMed: 21325623]

Selvakumar GP, Iyer SS, Kempuraj D, Ahmed ME, Thangavel R, Dubova I, Raikwar SP, Zaheer S, Zaheer A, 2019 Molecular association of glia maturation factor with the autophagic machinery in rat dopaminergic neurons: a role for endoplasmic reticulum stress and MAPK activation. Mol. Neurobiol 56 (6), 3865–3881. [PubMed: 30218400]

Selvakumar GP, Iyer SS, Kempuraj D, Raju M, Thangavel R, Saeed D, Ahmed ME, Zahoor H, Raikwar SP, Zaheer S, Zaheer A, 2018b Glia maturation factor dependent inhibition of mitochondrial PGC-1α triggers oxidative stress-mediated apoptosis in N27 rat dopaminergic neuronal cells. Mol. Neurobiol 55, 7132–7152. [PubMed: 29383690]

Selvakumar GP, Janakiraman U, Essa MM, Justin Thenmozhi A, Manivasagam T, 2014 Escin attenuates behavioral impairments, oxidative stress and inflammation in a chronic MPTP/probenecid mouse model of Parkinson's disease. Brain Res 1585, 23–36. [PubMed: 24657313]

Selvakumar GP, Manivasagam T, Rekha KR, Jayaraj RL, Elangovan N, 2015 Escin, a novel triterpene, mitigates chronic MPTP/p-induced dopaminergic toxicity by attenuating mitochondrial dysfunction, oxidative stress, and apoptosis. J. Mol. Neurosci 55, 184–197. [PubMed: 24788336]

Skaper SD, Facci L, Giusti P, 2014 Mast cells, glia and neuroinflammation: partners in crime? Immunology 141, 314–327. [PubMed: 24032675]

Skaper SD, Giusti P, Facci L, 2012 Microglia and mast cells: two tracks on the road to neuroinflammation. FASEB J 26, 3103–3117. [PubMed: 22516295]

Smith JA, Das A, Ray SK, Banik NL, 2012 Role of pro-inflammatory cytokines released from microglia in neurodegenerative diseases. Brain Res. Bull 87, 10–20. [PubMed: 22024597]

Sorimachi H, Ishiura S, Suzuki K, 1997 Structure and physiological function of calpains. Biochem. J 328 (Pt 3), 721–732. [PubMed: 9396712]

Spillantini MG, Schmidt ML, Lee VMY, Trojanowski JQ, Jakes R, Goedert M, 1997 α-Synuclein in Lewy bodies. Nature 388, 839–840. [PubMed: 9278044]

Sugama S, Yang L, Cho BP, DeGiorgio LA, Lorenzl S, Albers DS, Beal MF, Volpe BT, Joh TH, 2003 Age-related microglial activation in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced dopaminergic neurodegeneration in C57BL/6 mice. Brain Res 964, 288–294. [PubMed: 12576189]

Tagen M, Elorza A, Kempuraj D, Boucher W, Kepley CL, Shirihai OS, Theoharides TC, 2009 Mitochondrial uncoupling protein 2 inhibits mast cell activation and reduces histamine content. J. Immunol 183, 6313–6319. [PubMed: 19846869]

Thangavel R, Stolmeier D, Yang X, Anantharam P, Zaheer A, 2011 Expression of glia maturation factor in neuropathological lesions of Alzheimer's disease. Neuropathol. Appl. Neurobiol 38, 572–581.

Wertheimer SJ, Myers CL, Wallace RW, Parks TP, 1992 Intercellular adhesion molecule-1 gene expression in human endothelial cells. Differential regulation by tumor necrosis factor-alpha and phorbol myristate acetate. J. Biological Chem 267, 12030–12035.

West MJ, 1999 Stereological methods for estimating the total number of neurons and synapses: issues of precision and bias. Trends Neurosci 22, 51–61. [PubMed: 10092043]

Willis CL, 2010 Glia-induced reversible disruption of blood-brain barrier integrity and neuropathological response of the neurovascular unit. Toxicol. Pathol 39, 172–185. [PubMed: 21189317]

Wodnar-Filipowicz A, Heusser CH, Moroni C, 1989 Production of the haemopoietic growth factors GM-CSF and interleukin-3 by mast cells in response to IgE receptor-mediated activation. Nature 339, 150–152. [PubMed: 2524008]

Wu Z, Chen X, Liu F, Chen W, Wu P, Wieschhaus AJ, Chishti AH, Roche PA, Chen WM, Lin TJ, 2014 Calpain-1 contributes to IgE-mediated mast cell activation. J. Immunol 192, 5130–5139. [PubMed: 24760147]

Xanthos DN, Sandkühler J, 2014 Neurogenic neuroinflammation: Inflammatory CNS reactions in response to neuronal activity. Nat. Rev. Neurosci 15, 43–53. [PubMed: 24281245]

Yan HQ, Ma X, Chen X, Li Y, Shao L, Dixon CE, 2007 Delayed increase of tyrosine hydroxylase expression in rat nigrostriatal system after traumatic brain injury. Brain Res 1134, 171–179. [PubMed: 17196177]

Zaheer A, Fink BD, Lim R, 1993 Expression of glia maturation factor beta mRNA and protein in rat organs and cells. J. Neurochem 60, 914–920. [PubMed: 8436977]

Zaheer A, Haas JT, Reyes C, Mathur SN, Yang B, Lim R, 2006 GMF-knockout mice are unable to induce brain-derived neurotrophic factor after exercise. Neurochem. Res 31, 579–584. [PubMed: 16758368]

Zaheer A, Kempuraj D, Khan M, Javed H, Zaheer S, 2014 Mast cell activation by glia maturation factor, 1-methyl-4-phenylpyridinium and α-synuclein: implications for Parkinson's disease (596.5). FASEB J 28, 596.595.

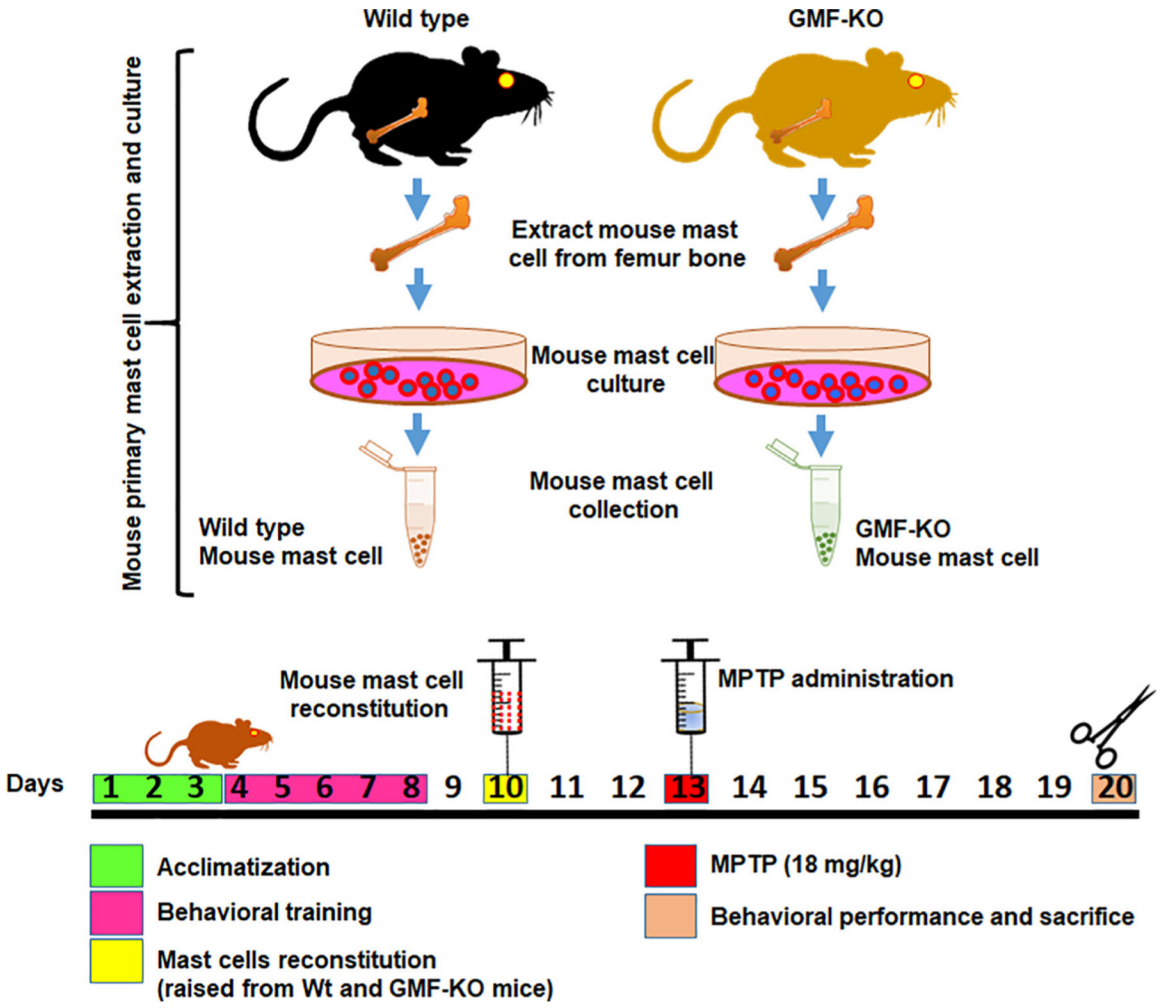
Zaheer A, Lim R, 1997 Protein kinase A (PKA)- and protein kinase C-phosphorylated glia maturation factor promotes the catalytic activity of PKA. J. Biol. Chem 272, 5183–5186. [PubMed: 9030586]

Zaheer A, Yorek MA, Lim R, 2001 Effects of glia maturation factor overexpression in primary astrocytes on MAP kinase activation, transcription factor activation, and neurotrophin secretion. Neurochem. Res 26, 1293–1299. [PubMed: 11885780]

Zaheer A, Zaheer S, Sahu SK, Knight S, Khosravi H, Mathur SN, Lim R, 2007 A novel role of glia maturation factor: induction of granulocyte-macrophage colony-stimulating factor and pro-inflammatory cytokines. J. Neurochem 101, 364–376. [PubMed: 17250654]

Zaheer S, Thangavel R, Wu Y, Khan MM, Kempuraj D, Zaheer A, 2013 Enhanced expression of glia maturation factor correlates with glial activation in the brain of triple transgenic Alzheimer's disease mice. Neurochem. Res 38, 218–225. [PubMed: 23086473]

Zameer A, Hoffman SA, 2003 Increased ICAM-1 and VCAM-1 expression in the brains of autoimmune mice. J. Neuroimmunol 142, 67–74. [PubMed: 14512165]




**Schematic diagram 1.**

Mice were acclimatized in the procedure room for the first 3 days (1–3). Behavioral training was started on the 4th day and continued until the 8th day. After a day, animals were injected with MC (i.v), which were obtained from the bone marrow of WT or GMF-KO mice. After 3 days the animals were injected with MPTP (18 mg/kg, i,p) for a total of four injections at 2 h intervals in a day. Behavioral studies were performed after 7 days following the last MPTP injection on the 20th day of our experimental period. After this, the mice were sacrificed and brain tissues were acquired for the biochemical and protein expression studies.

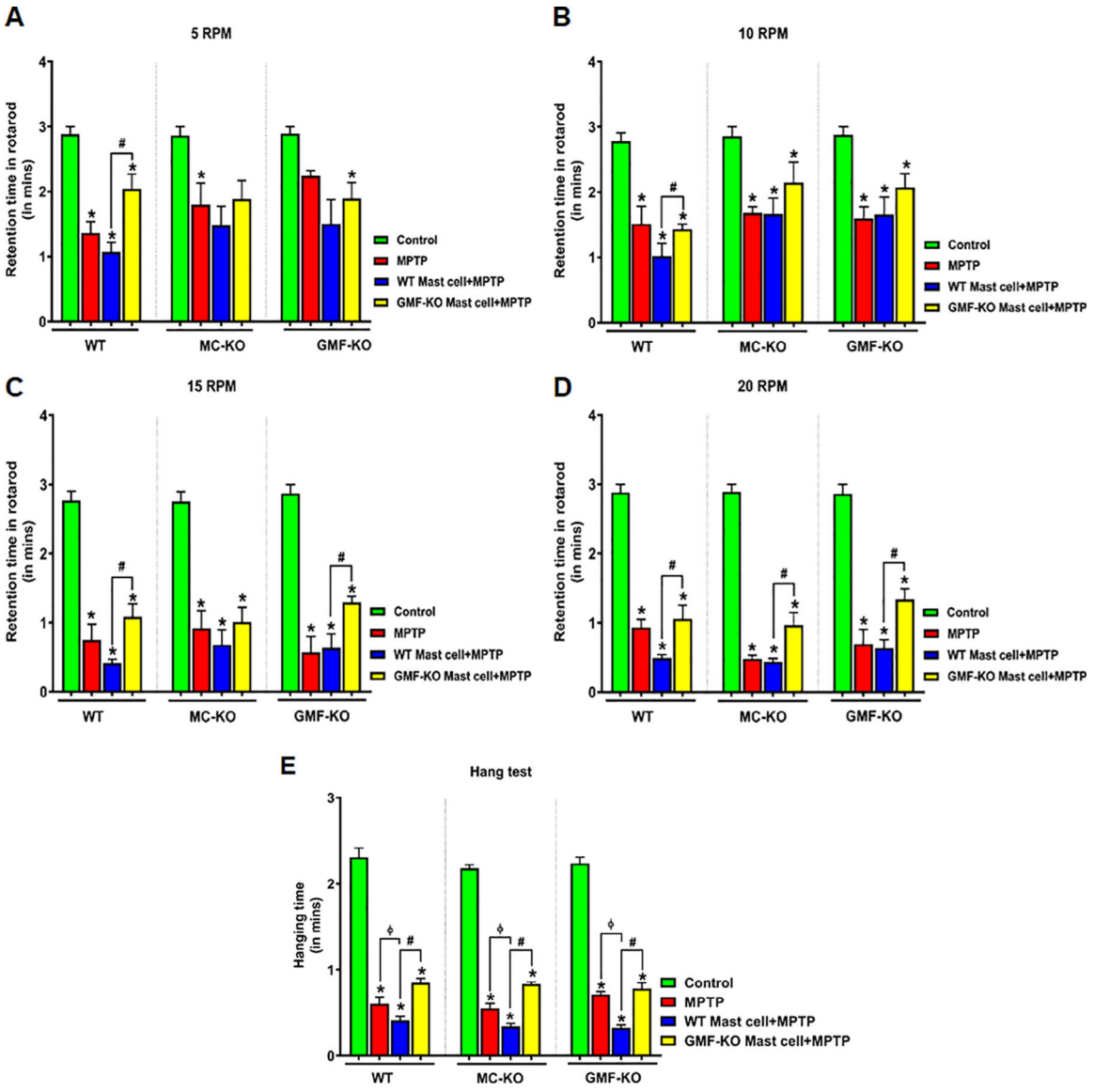



**Fig. 1.**

MPTP-induced motor behavioral impairments were assessed by rotarod and hang test performances. MPTP (18 mg/kg by i.p) was administered with and without reconstitution of MC from WT, MC-KO and GMF-KO mice. Mice reconstituted with MC from WT mice shows significant motor behavioral impairments in rotarod by reducing retention time at different RPM (A:5 RPM, B: 10 RPM, C: 15 RPM and D:20 RPM) and hanging time in hang test (E) compared with saline treated control mice, and these impairments were significantly reduced in MC-KO and GMF-KO mice treated with MPTP. Values are presented as mean ± SEM (n = 4). $^{*}p < 0.05$ saline treated control mice vs MPTP only treated mice. $^{\phi}p < 0.05$ MPTP only treated mice vs WT and GMF-KO MCs reconstituted mice. $^{\#}p < 0.05$ wt MC reconstituted mice vs GMF-KO MC reconstituted mice.

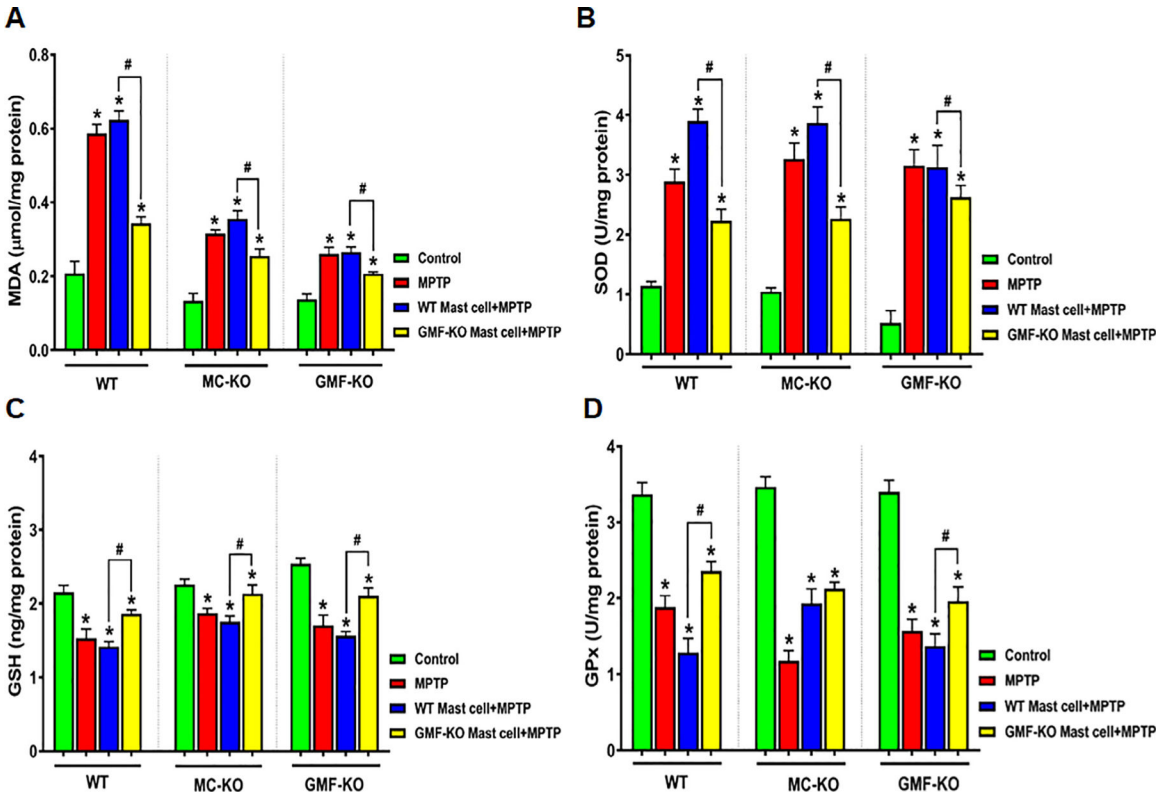



**Fig. 2.**

MPTP induced oxidative stress was determined by analyzing the levels of TBARS (MDA), GSH, activities of SOD and GPx in the SN of the midbrain. Acute neurotoxicity of MPTP (18 mg/kg by i.p) causes significant oxidative stress in WT mice as seen by increasing levels of MDA (A) and the activities of SOD (B), reductions in the levels of GSH (C) and the activities of GPx (D) when compared with control mice. Deficiency of GMF and MC significantly improves these oxidative stress markers expressions when compared with MPTP treated WT mice. Values are presented as mean ± SEM (n = 4). ∗$p < 0.05$ saline treated control mice vs MPTP treated mice. $^{\#}p < 0.05$ wt MC reconstituted mice vs GMF-KO MC reconstituted mice. MDA levels expressed as µmol/mg of protein; GSH level expressed as ng/mg of protein; SOD expressed as units/mg of protein; GPx units-micrograms of glutathione consumed/min.

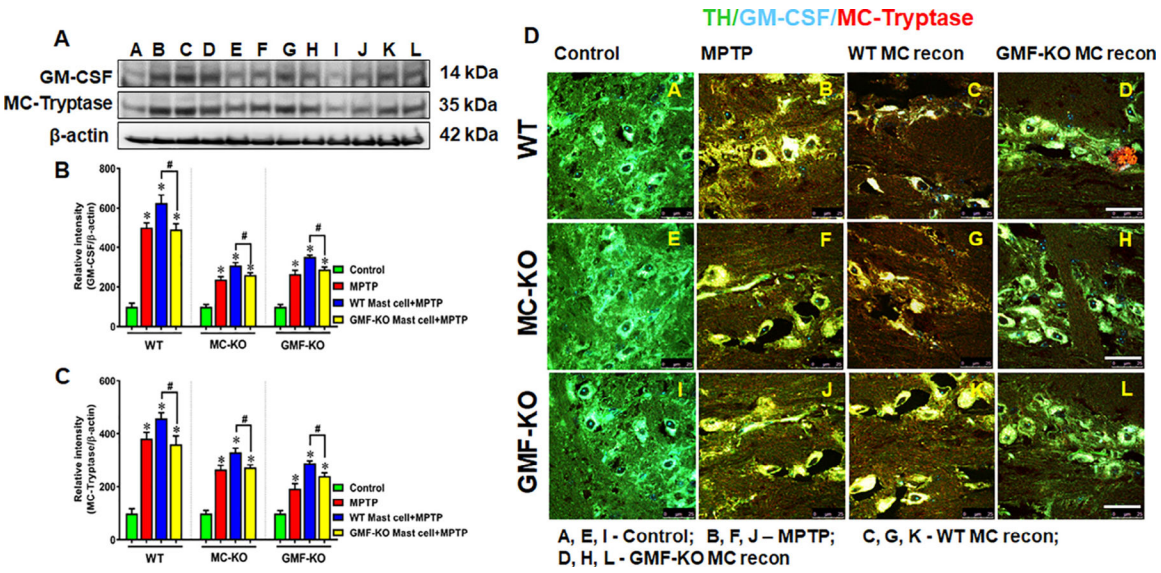



**Fig. 3.**

MPTP induce to attenuate TH, and increases GM-CSF and MC-Tryptase expressions in SN of midbrain. Acute administration of MPTP (18 mg/kg) after reconstitution with WT MC significantly reduced TH-protein expression, increased GM-CSF and MC-Tryptase expression in SN of the midbrain as compared with saline control mice as determined by western blot (A) and immunofluorescence (A-L). Mice reconstituted with MC from GMF-KO mice show significant higher TH-protein and reduced GM-CSF and MC-Tryptase expression in dopaminergic neurons in SN of the midbrain. Bar graphs show the effect of MPTP exposure on the GM-CSF and MC-Tryptase expression as compared to the controls (B and C). Values are presented as mean ± SEM (n = 4). $^{*}p < 0.05$ saline treated control mice vs MPTP treated mice. $^{\#}p < 0.05$ wt MC reconstituted mice vs GMF-KO MC reconstituted mice. Scale bar 100 μm.

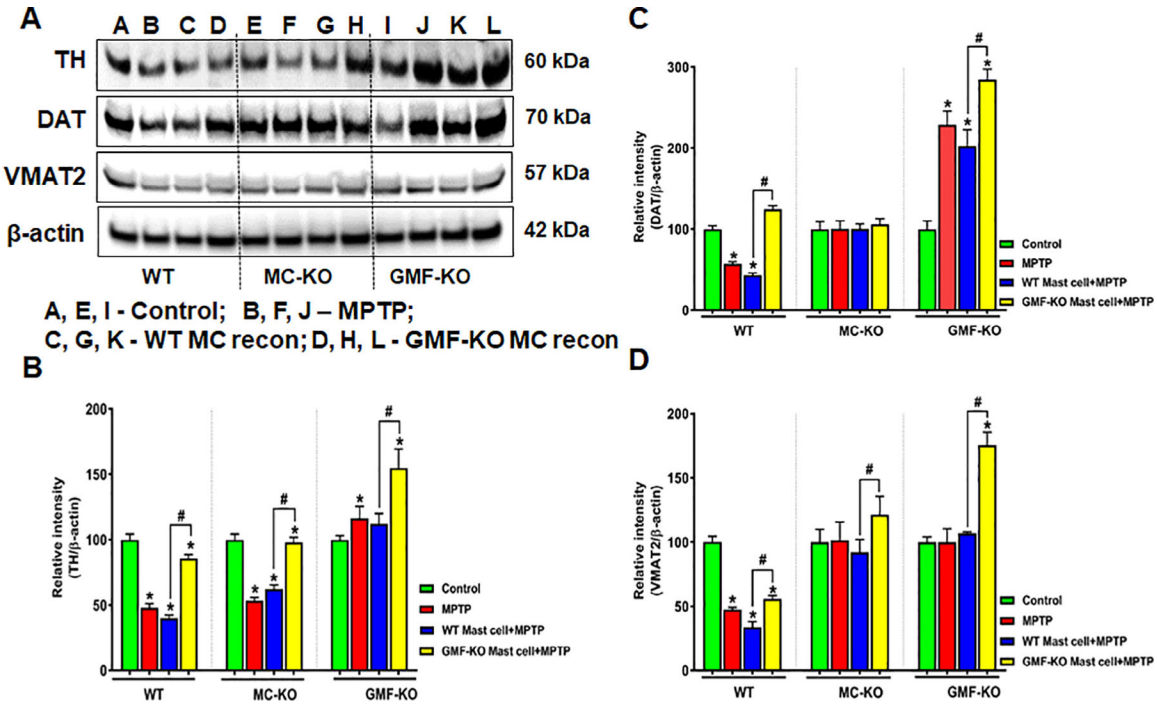



**Fig. 4.**

MPTP induced attenuation of TH, DAT and VMAT2 expressions in SN of midbrain. Acute administration of MPTP (18 mg/kg) and reconstitution with WT MC significantly reduced TH, DAT and VMAT2 protein expression when compared with saline control mice as determined by western blot (A). Mice with MC reconstituted from GMF-KO mice and MPTP treatment shows significantly higher expressions of TH, DAT and VMAT2 proteins when compared with reconstitution with WT MC and MPTP treatment. Bar graphs show the effect of MPTP administration on the relative intensity to the control and shows the densitometry of the bands (B, C and D). Values are presented as mean ± SEM (n = 4). ∗$p < 0.05$ saline treated control mice vs MPTP treated mice. $^{\#}p < 0.05$ wt MC reconstituted mice vs GMF-KO MC reconstituted mice.

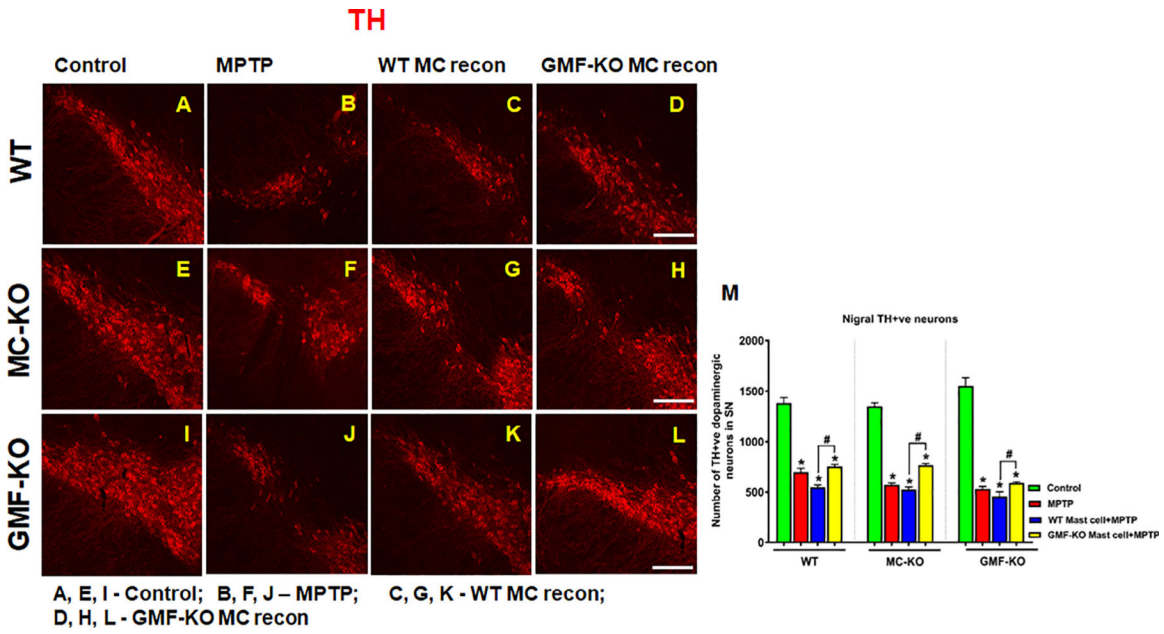



**Fig. 5.**

MPTP induced attenuation of TH protein in SN of midbrain. Acute administration of MPTP (18 mg/kg) after reconstitution with WT MC significantly reduced the number of TH-positive dopaminergic neurons and protein expression in SN of the midbrain as compared with saline control mice as determined by immunofluorescence (A-L). Mice reconstituted with MC from GMF-KO mice show a significant higher number of TH-positive dopaminergic neurons in SN of the midbrain. Bar graphs show the effect of MPTP exposure on the number of TH-positive expressing neurons compared to the controls (M). Values are presented as mean ± SEM (n = 4). ∗$p < 0.05$ saline treated control mice vs MPTP treated mice. $^{\#}p < 0.05$ wt MC reconstituted mice vs GMF-KO MC reconstituted mice. Scale bar 100 μm.

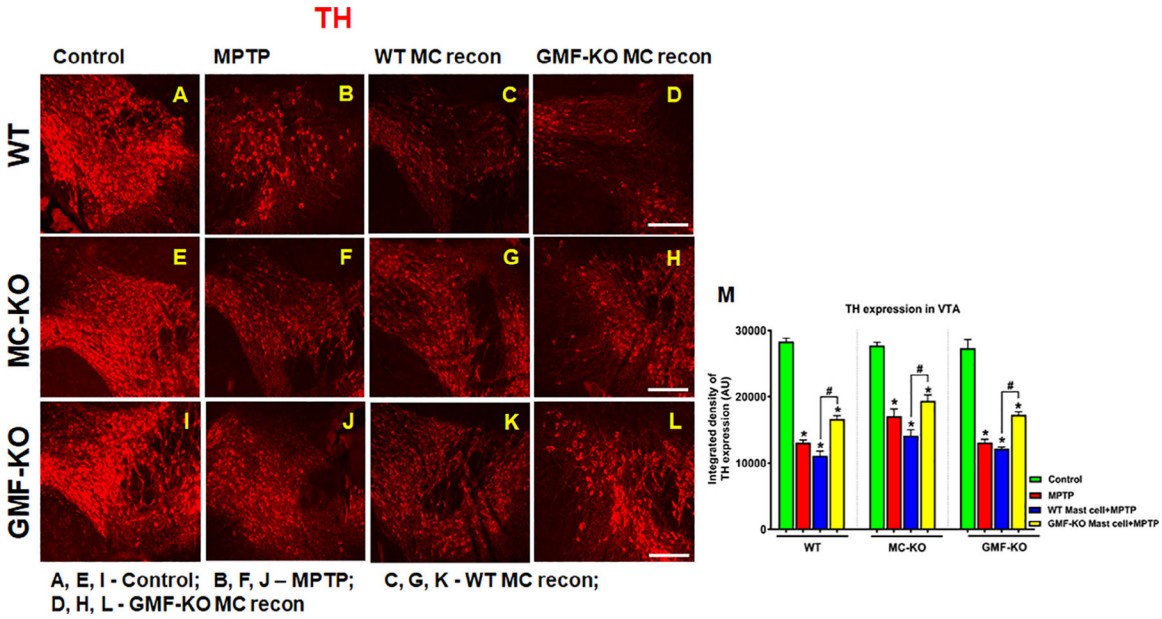



**Fig. 6.**

MPTP induced attenuation of TH protein in VTA of midbrain. Acute treatment with MPTP (18 mg/kg) following reconstitution with WT MC significantly reduced integrated density of TH protein expression in VTA of the midbrain as compared with saline control mice as determined by immunofluorescence (A-L). In sections from mice reconstituted with MCs from GMF-KO mice show a significantly higher integrated density of TH protein expression in VTA of the midbrain when compared with MC reconstitution from WT. Bar graphs show the effect of MPTP exposure on the integrated density of TH expression in VTA to the control (M). Values are presented as mean ± SEM (n = 4). $^{*}p < 0.05$ saline treated control mice vs MPTP treated mice. $^{\#}p < 0.05$ wt MC reconstituted vs GMF-KO MC reconstituted mice. Scale bar 100 μm.

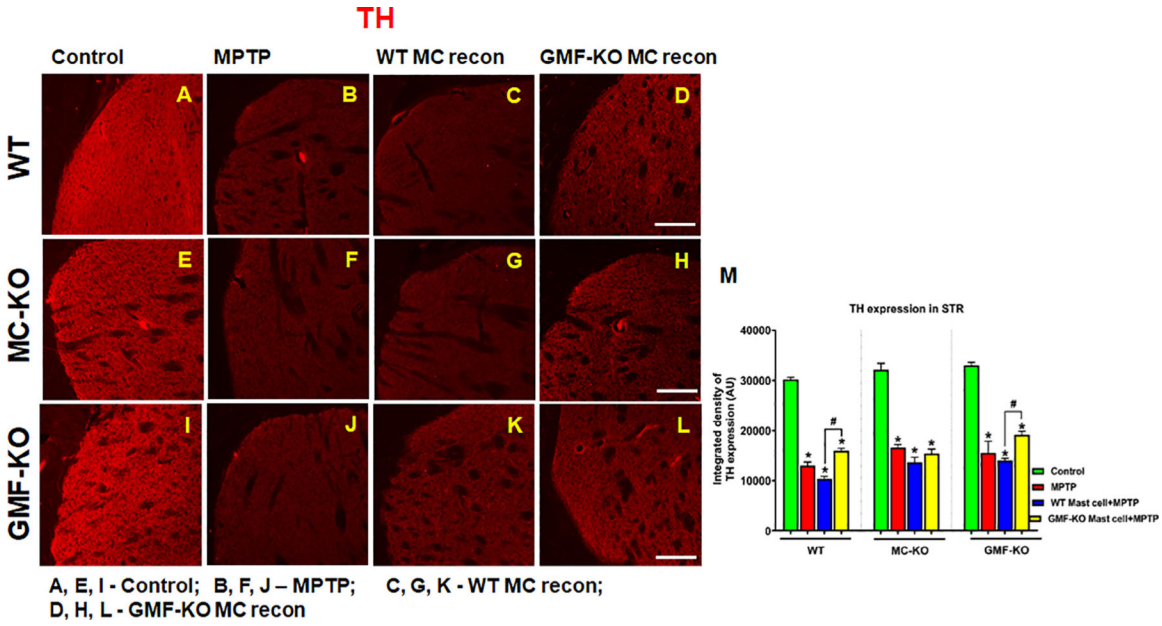



**Fig. 7.**

MPTP induced attenuation of TH protein in STR of midbrain was determined by immunofluorescence. Acute treatment with MPTP (18 mg/kg) and reconstitution with WT MC significantly reduced integrated density of TH protein expression in STR of the midbrain as compared with control mice. MC reconstitution from GMF-KO mice show a significant higher integrated density of TH protein expression in STR of the midbrain. Bar graphs show the effect of MPTP exposure on the integrated density of TH expression in STR to the control. Values are presented as mean ± SEM (n = 4). $^{*}p < 0.05$ saline treated control mice vs MPTP treated mice. $^{\#}p < 0.05$ wt MC reconstituted mice vs GMF-KO MC reconstituted mice. Scale bar 100 μm.

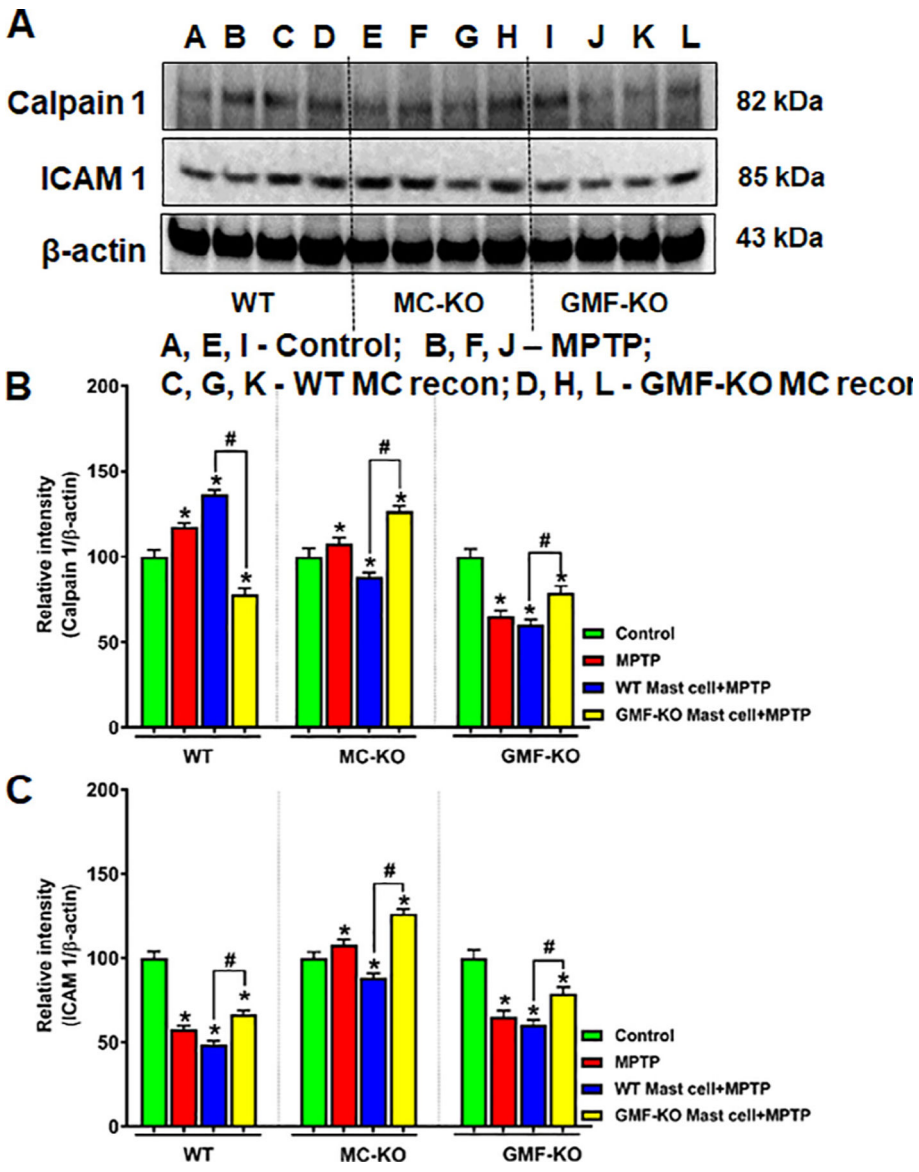



**Fig. 8.**

MPTP induced calpain 1 and ICAM 1 expression in SN of the midbrain. Administration of MPTP (18 mg/kg) and WT MC reconstitution significantly increased calpain 1 and ICAM 1 expression in SN of WT mice when compared with saline-treated control mice as determined by western blotting (A). Mice reconstituted with MC from GMF-KO mice followed by MPTP treatment show a significant reduction in calpain 1 and ICAM 1 protein expressions when compared with those from WT MC reconstitution. Bar graphs show the effect of MPTP administration on the relative intensity to the control and shows the densitometry of the bands (B and C). Values are presented as mean ± SEM (n = 4). $^{*}p < 0.05$ saline treated control mice vs MPTP treated mice. $^{\#}p < 0.05$ wt MC reconstituted mice vs GMF-KO MC reconstituted mice.

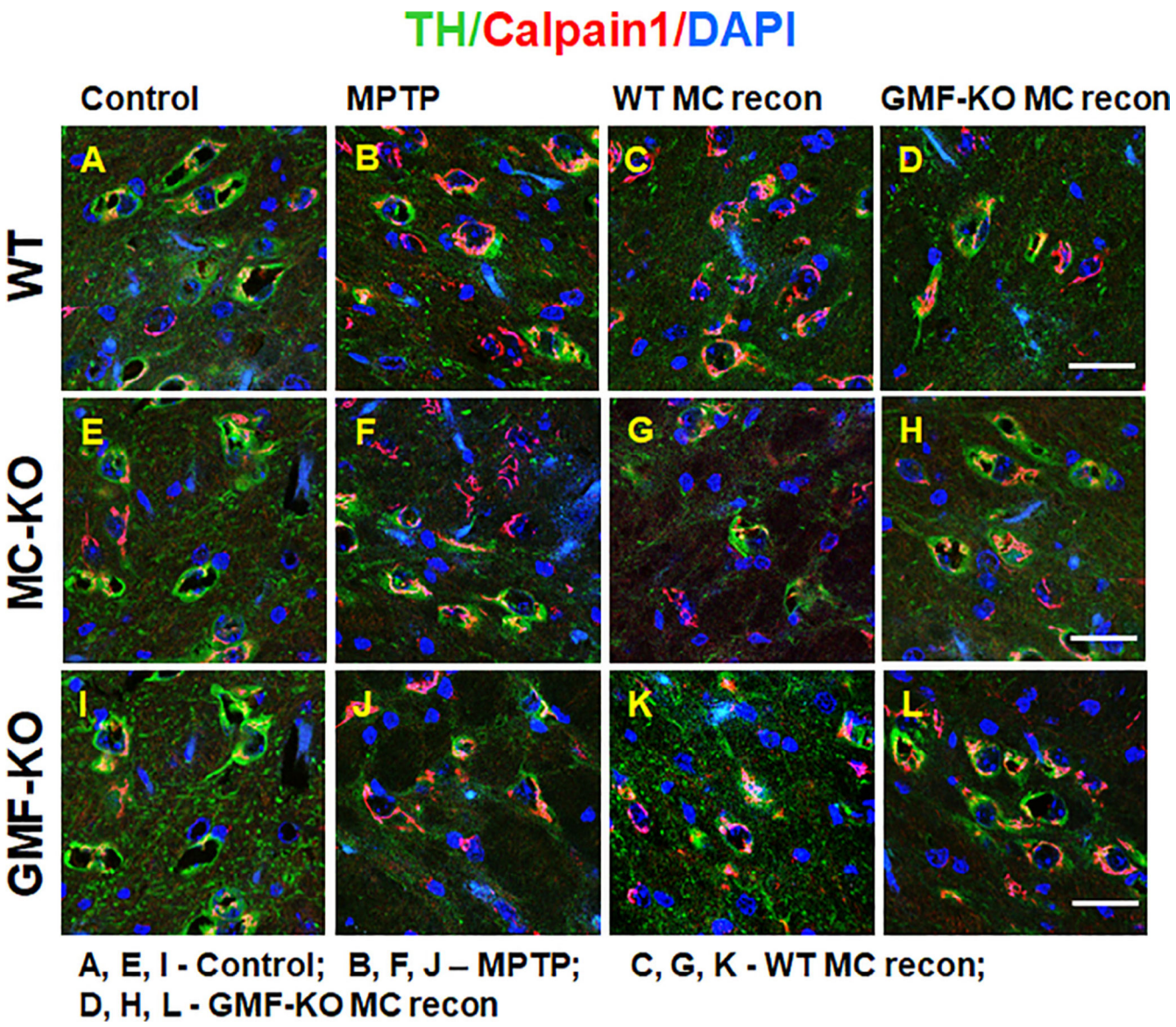



**Fig. 9.**

MPTP decreases TH and enhances calpain 1 expression in dopaminergic neurons of SN of the midbrain. Administration of MPTP (18 mg/kg) following MC reconstituted from WT qualitatively increased TH (green fluorescent) and calpain 1 (red fluorescent) expression when compared with saline control mice, GMF-KO MC reconstituted mice show qualitatively lesser expression of these proteins as compared with WT MC reconstituted mice. Representative images: A, E and I saline treated control mice; B, F and J MPTP (18 mg/kg) only treated mice; C, G and K reconstituted with WT MC and MPTP treated mice; D, H and L reconstituted with GMF-KO MC and MPTP treated mice. Scale bar 25 μm. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

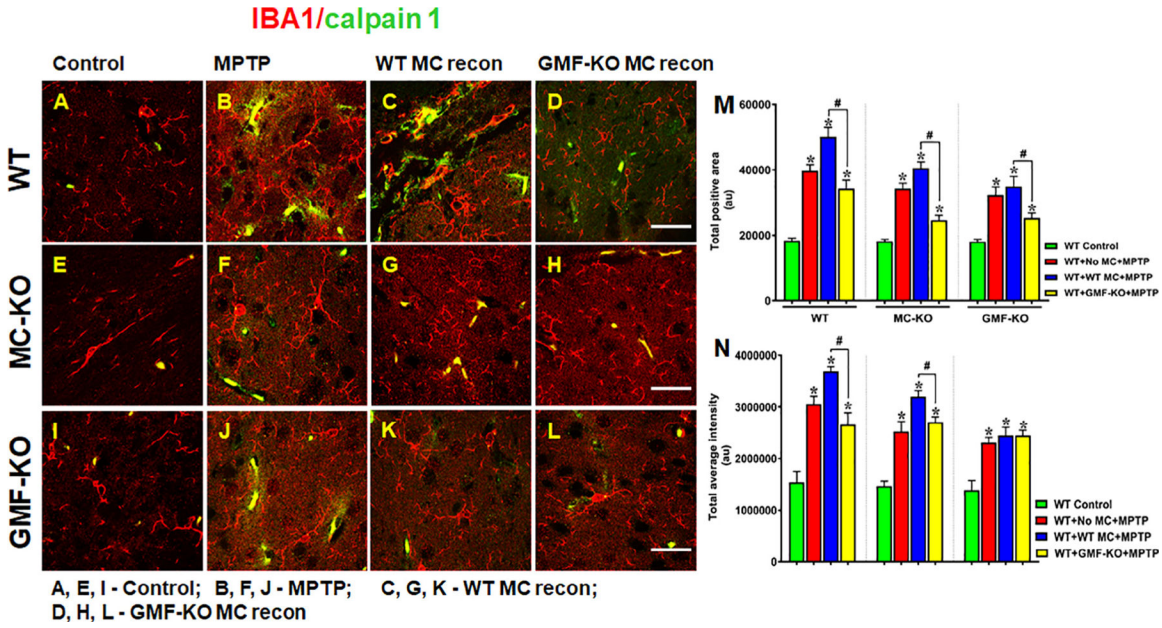



**Fig. 10.**

MPTP enhances IBA1 and calpain 1 expression in the SN of the midbrain. Administration of MPTP (18 mg/kg) after reconstitution with MC from WT mice qualitatively increased IBA1 (red fluorescence) and calpain 1 (green fluorescence) expression when compared with saline control mice, With mice reconstituted with GMF-KO MC qualitatively lesser expression of these proteins was seen when compared with WT MC reconstituted mice. Bar graphs shows the total positive area (M) and total average intensity (N) of GFAP and ICAM 1 expressions were quantitatively increased in mice reconstituted with MC from WT. Representative images: A, E and I saline treated control mice; B, F and J MPTP (18 mg/kg) only treated mice; C, G and K reconstituted with WT MC and MPTP treated mice; D, H and L reconstituted with GMF-KO MC and MPTP treated mice. Scale bar 25 μm; au arbitrary units. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

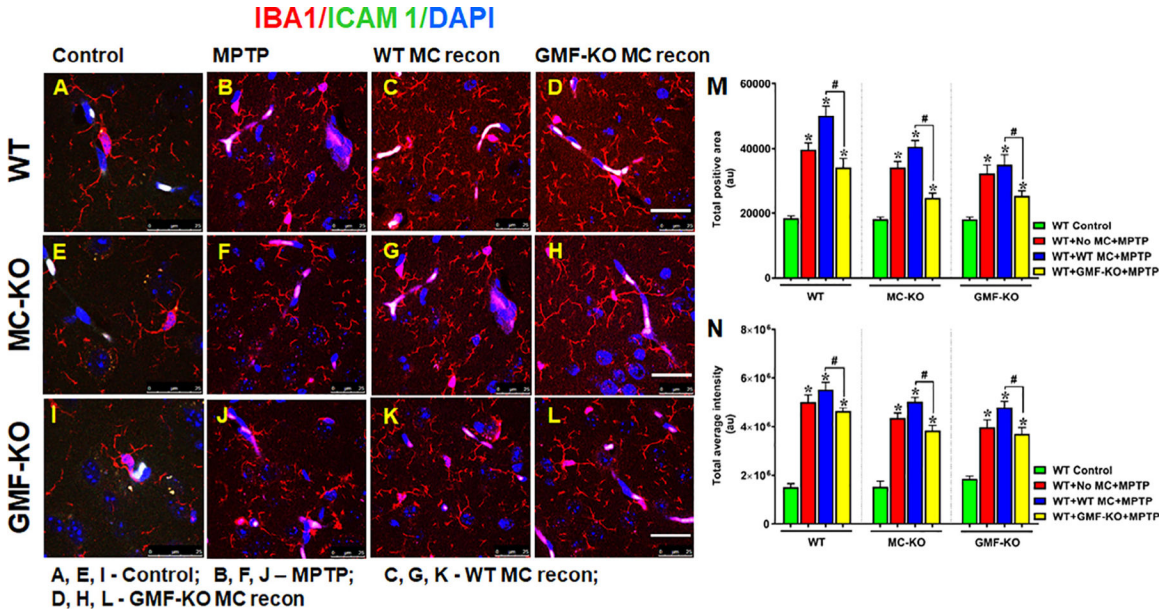



**Fig. 11.**

MPTP enhances IBA1 and ICAM 1 expression in SN of the midbrain. Administration of MPTP (18 mg/kg) after MC reconstituted from WT mice qualitatively increased IBA1 (red fluorescence) and ICAM 1 (green fluorescence) expression when compared with saline control mice, Mice reconstituted with GMF-KO MC show qualitatively lesser expression of these proteins as compared with WT MC reconstituted mice. Bar graphs shows the total positive area (M) and total average intensity (N) of IBA1 and ICAM 1 expressions were quantitatively increased in mice reconstituted with MC from WT. Representative images: A, E and I saline treated control mice; B, F and J MPTP (18 mg/kg) only treated mice; C, G and K reconstituted with WT MC and MPTP treated mice; D, H and L reconstituted with GMF-KO MC and MPTP treated mice. Scale bar 25 μm; au arbitrary units. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

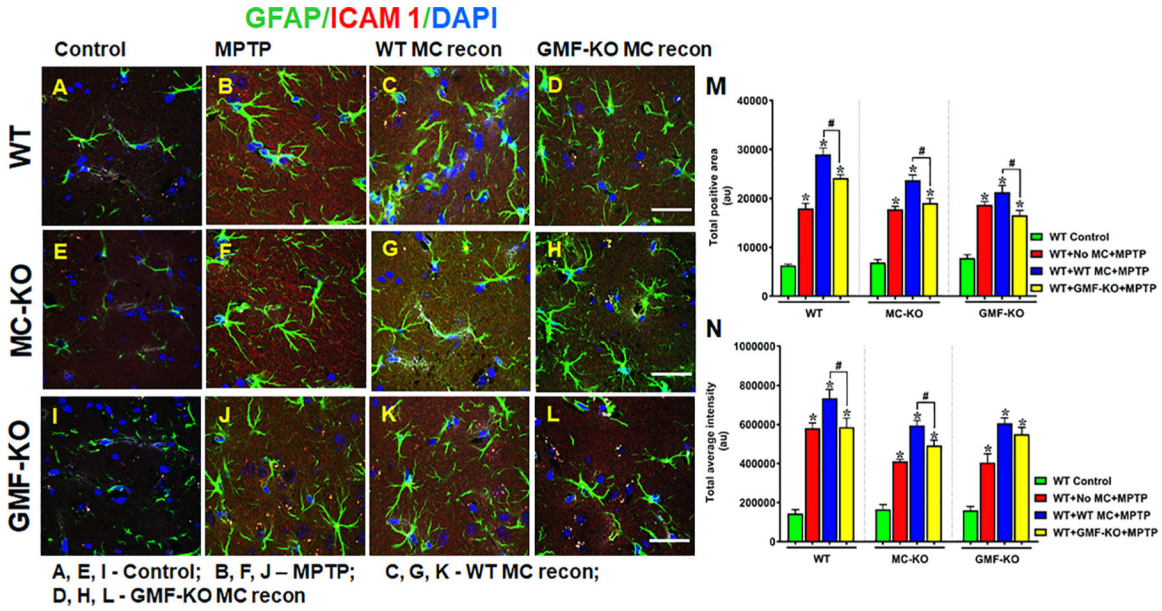



**Fig. 12.**

MPTP enhances GFAP and ICAM 1 expression in SN of the midbrain. Administration of MPTP (18 mg/kg) to mice reconstituted with MC from WT qualitatively increased GFAP (green fluorescence) and ICAM 1 (red fluorescence) expression when compared with saline control mice, mice reconstituted with GMF-KO MC show qualitatively lesser expression of these proteins as compared with WT MC reconstituted mice. Bar graphs shows the total positive area (M) and total average intensity (N) of GFAP and ICAM 1 expressions were quantitatively increased in mice reconstituted with MC from WT. Representative images: A, E and I saline treated control mice; B, F and J MPTP (18 mg/kg) only treated mice; C, G and K reconstituted with WT MC and MPTP treated mice; D, H and L reconstituted with GMF-KO MC and MPTP treated mice. Scale bar 25 μm; au arbitrary units. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

**Table 1**

MPTP administration and mast cells reconstitution schedule.

| Mice type | Mice groups and treatments (n = 4) |
|---|---|
| WT mice | a. Control (Phosphate buffered saline; PBS) |
| | b. MPTP (18 mg/kg; i.p) |
| | c. Mast cell reconstitution from WT mice (i.v) + MPTP (18 mg/kg) |
| | d. Mast cell reconstitution from GMF-KO mice (i.v) + MPTP (18 mg/kg) |
| MC-KO | a. Control (PBS) |
| | b. MPTP (18 mg/kg; i.p) |
| | c. Mast cell reconstitution from WT mice (i.v) + MPTP (18 mg/kg) |
| | d. Mast cell reconstitution from GMF-KO mice (i.v) + MPTP (18 mg/kg) |
| GMF-KO | a. Control (PBS) |
| | b. MPTP (18 mg/kg; i.p) |
| | c. Mast cell reconstitution from WT mice (i.v) + MPTP (18 mg/kg) |
| | d. Mast cell reconstitution from GMF-KO mice (i.v) + MPTP (18 mg/kg) |