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MDMA Increases Excitability in the Dentate Gyrus: Role of 5HT2A Receptor Induced PGE2 Signaling

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Abstract

MDMA is a widely abused psychostimulant which causes release of serotonin in various forebrain regions. Recently, we reported that MDMA increases extracellular glutamate concentrations in the dentate gyrus, via activation of 5HT2A receptors. We examined the role of prostaglandin signaling in mediating the effects of 5HT2A receptor activation on the increases in extracellular glutamate and the subsequent long-term loss of parvalbumin interneurons in the dentate gyrus caused by MDMA. Administration of MDMA into the dentate gyrus of rats increased PGE2 concentrations which was prevented by coadministration of MDL100907, a 5HT2A receptor antagonist. MDMA-induced increases in extracellular glutamate were inhibited by local administration of SC-51089, an inhibitor of the EP1 prostaglandin receptor. Systemic administration of SC-51089 during injections of MDMA prevented the decreases in parvalbumin interneurons observed 10 days later. The loss of parvalbumin immunoreactivity after MDMA exposure coincided with a decrease in paired-pulse inhibition and afterdischarge threshold in the dentate gyrus. These changes were prevented by inhibition of EP1 and 5HT2A receptors during MDMA. Additional experiments revealed an increased susceptibility to kainic acid-induced seizures in MDMA treated rats which could be prevented with SC51089 treatments during MDMA exposure. Overall, these findings suggest that 5HT2A receptors mediate MDMA-induced PGE2 signaling and subsequent increases in glutamate. This signaling mediates parvalbumin cell losses as well as physiologic changes in the dentate gyrus, suggesting that the lack of the inhibition provided by these neurons increases the excitability within the dentate gyrus of MDMA treated rats.

Keywords

MDMA; glutamate; parvalbumin; PGE2; hippocampus; serotonin

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Introduction

3,4-Methylenedioxymethamphetamine (MDMA, ecstasy) is a widely abused psychostimulant in the substituted amphetamine class of drugs. Acutely, MDMA causes the release of serotonin (5HT) via reversal of the serotonin transporter (Nichols et al., 1982). In addition to the acute effects of MDMA on 5HT, it has been well documented that MDMA can cause neurotoxicity to 5HT terminals (Schmidt et al., 1986, Schmidt, 1987, Battaglia et al., 1988). Given that the abuse of MDMA has been associated with long-lasting psychiatric effects such as depression and anxiety as well as detrimental effects on learning and memory, it has been suggested that these long-term effects are attributed to the neurotoxic effects of MDMA on the 5HTergic nervous system (Curran and Travill, 1997, Parrott et al., 1998, Parrott et al., 2001).

A growing number of studies support the notion that the GABAergic nervous system within the hippocampus may be impacted by MDMA exposure. Chronic MDMA administration was shown to reduce the binding of [3 H]-flunitrazapam in the hippocampus, suggesting that GABAergic terminals are reduced following MDMA exposure (Armstrong and Noguchi, 2004). In agreement with this finding, Perrine et al. 2010 showed that GABA concentrations within the hippocampus are reduced following chronic MDMA exposure. More recent findings demonstrated that MDMA exposure causes a long-term decrease in parvalbumin (PV) expressing interneurons within the dentate gyrus (Anneken et al., 2013, Abad et al., 2014, Collins et al., 2015).

PV interneurons are a subset of interneurons known to mediate fast GABAergic neurotransmission within the dentate gyrus. These cells receive dense glutamatergic innervations from dentate granule cells and are thought to be critical for feed-back inhibition (Ribak, 1992, Kneisler and Dingledine, 1995a, Blasco-Ibanez et al., 2000). By primarily innervating the somatic and axoaxonic regions of granule cells, PV interneurons exhibit strong control over action potential firing of these cells (Ribak et al., 1993, Freund and Buzsaki, 1996). This control of action potential firing is thought to be an important component in the ability of PV interneurons to synchronize principal cell activity (Bartos et al., 2002). Along these lines, decreases in hippocampal PV interneurons have been reported in both epileptic and schizophrenic patients, which is thought to underlie both cognition dysfunction present in both diseases as well as the imbalance between inhibitory and excitatory function (DeFelipe et al., 1993, Beasley and Reynolds, 1997, Zhang et al., 2002, Arellano et al., 2004).

PV interneurons have been theorized to be susceptible to excitotoxicity as demonstrated by decreases in PV immunoreactivity (PV-IR) following neurotoxic insults in which glutamate neurotransmission is a presumed mechanism (Kwon et al., 1999, Gorter et al., 2001, Moga et al., 2002, Sanon et al., 2005). More recently, we demonstrated that MDMA-induced decreases in PV interneurons were dependent upon activation of NMDA receptors, implicating a role for glutamate in mediating a decrease in these neurons (Collins et al., 2015). Furthermore, MDMA causes an increase in extracellular glutamate concentrations within the hippocampus via increases in serotonin and subsequent activation of 5HT2 receptors (Anneken and Gudelsky, 2012). More recently it was demonstrated that these

increases in glutamate were dependent upon cyclooxygenase-2 (COX2) activity, suggesting a role for prostaglandins in mediating these effects (Anneken et al., 2013). Prostaglandins such as PGE2 have been shown to mediate glutamate release in the hippocampus (Bezzi et al., 1998, Sanzgiri et al., 1999). Furthermore, several studies have suggested that EP1 receptors, which are activated by PGE2, can play a role in excitotoxicity (Ahmad et al., 2006, Ahmad et al., 2008, Mohan et al., 2013).

The goal of this study was to determine whether MDMA mediates increases in PGE2 in the dentate gyrus and whether this was dependent upon activation of 5HT2a receptors. We also investigated the potential role of PGE2 signaling in mediating both the increases in extracellular glutamate, the decreases in PV-IR as well as the long-term effects of these acute changes produced by MDMA on paired-pulse depression and afterdischarge threshold in the dentate gyrus.

Methods

Animals

Adult male Sprague-Dawley rats (200–275 g. Harlan Sprague Dawley, In, USA) were used. Prior to experimentation, rats were allowed at least 5 days to acclimate. The rats were kept on a 12/12-hr light dark cycle in a temperature and humidity controlled room with food and water available ad libitum. Experimental procedures were approved by the Institutional Animal Care and Use Committee at the University of Toledo and University of Cincinnati and were based on NIH Guide for the Care and Use of Laboratory Animals.

Drug Treatments

(±) MDMA-HCl was obtained from the National Institutes of Drug Abuse (NIDA, Research Triangle). For PV interneuron cell counts, electrophysiology and seizure studies, physiological saline or MDMA (7.5 mg/kg) was injected once every 2 hours for a total of 4 i.p. injections. Systemic injections (20 μg/kg, i.p.) of the EP1 receptor antagonist, SC-51089, (Tocris, 0.05% DMSO in water, 1 mL/kg) were given 30 minutes before each administration of MDMA. This dose was based on previous studies depicting the neuroprotective effects of this drug (Abe et al., 2009). Core body temperatures were recorded 1 hour after each injection of saline or MDMA using a rectal probe digital thermometer (Thermalert TH-8, Physitemp Instruments).

Microdialysis

Rats were anesthetized with a xylazine/ketamine hydrochloride mixture (6/70 mg/kg i.p.) and placed in a stereotaxic apparatus (David Kopf Instruments, Tujunga, CA). The exposed dialysis membrane of the microdialysis probe which was made from hollow fiber microdialysis membrane (1 mm of active membrane, 13 KDa cutoff, 216 μm diameter, Spectrum Labs) was placed in the dentate gyrus of the dorsal hippocampus (coordinates: 3.9 mm rostral from bregma, 2.2 mm lateral from the midline and 4.2 mm ventral). Three additional screws were placed in the skull and dental cement was used to anchor the microdialysis probe to the skull.

The morning after surgeries, Dulbecco's phosphate-buffered saline (138 mM NaCl, 2.1 mM KCl, 0.5 mM $MgCl_2$, 1.5 mM KH_2PO4, 8.1 mm $NaHPO_4$, 1.2 mm $CaCl_2$, and 0.5 mM D-glucose, pH 7.4) (Sigma-Aldrich) was perfused at a flow rate of 1.5 μl/min using a model 22 syringe perfusion pump (Harvard Apparatus). An equilibration period of 1 hour was followed by four 30 minute baseline samples. Reverse dialysis of 100 μM MDMA directly into the dentate gyrus began immediately following baseline collections. This dose of MDMA was used in previous studies to determine its effects on local neurochemistry and is meant to mimic brain concentrations seen following neurotoxic doses of MDMA in rodents as well as the resulting neurochemical effects that these doses produce (Nash and Yamamoto 1992, Chu et al. 1996). Reverse dialysis of the selective 5HT2A antagonist MDL100907 (100 nM) or the SC51089 (10 μM) was initiated 30 minutes prior to reverse dialysis of MDMA. These doses were used based on previous findings highlighting selectivity or efficacy of these antagonists for their respective receptors (Kehne et al., 1996, Jones et al., 2009)(Kehne et al., 1996). Brains were sectioned and microdialysis probe placements were verified.

High-performance Liquid Chromatography Analysis of Extracellular Glutamate

Dialysate samples (20μl) were injected onto a C18 column (150 × 2mm, 3μm particle filter, Phenomenex) and glutamate eluted using a mobile phase which consisted of 0.1 M Na_2HPO_4, 0.1mM EDTA and 10% MeOH (pH 6.4). As previously described, glutamate was derivatized using o-pthaldialdehyde and measured using electrochemical detection (LC-4C amperometric detector ((BAS Inc.)(Donzanti and Yamamoto, 1988). In brief, a stock of the derivatization agent was created by dissolving 27 mg o-pthaldialdehyde in 9 mL of 0.1 M sodium tetraborate (pH 9.4). To this stock 1 ml of 100% methanol and 15 μL β-mercaptoethanol was added. This stock solution was then diluted 1:3 with 0.1 M sodium tetraborate and10 μL was added to 20 ul of dialysate or standard, mixed, and allowed to react for 1.5 min before injecting onto the column.

ELISA Analysis of PGE2 Concentrations

Concentrations of extracellular PGE2 in dentate gyrus dialysate samples were measured utilizing a chemiluminescence ELISA kit (Arbor Assays). Dialysate samples were pooled over 2 hours (180 μl total) and 50 μl of the total sample was diluted using 50 μl Dulbecco's phosphate-buffered saline. Chemiluminescence was measured using a CLARIOstar microplate reader (BMG Labtech). Concentrations in diluted dialysate samples were calculated using the standard curve generated from standards prepared in Dulbecco's phosphate-buffered saline.

Immunohistochemistry

Phosphate-buffered saline (0.1 M PBS) was perfused intracardially followed by 4% paraformaldehyde on the 10^{th} day after drug or saline exposure. Brains were cryoprotected, flash frozen and the dorsal hippocampus was sectioned into 50 μm thick slices. Background peroxidase activity was reduced by incubating in 1% H_2O_2, at room temperature for 30 minutes. The sections were blocked for 2 hr at room temperature with 10% normal goat serum (Life Technologies) in 0.1M PBS containing 0.5% Triton-X 100 and Avidin block (4 drops/mL; Vector Laboratories). For PV immunostaining, 50μm thick sections were

incubated for 36 hr at 4°C with a mouse monoclonal PV antibody (1:2000; Swant, cat# PV235) in 0.1 M PBS containing 0.5% Triton-X 100, 1% NGS and Biotin block (4 drops/ml; Vector Laboratories). Sections were then incubated in goat anti-mouse biotinylated secondary antibodies (1:1000, Millipore, cat# AP124b) for 2hr at room temperature followed by incubation in avidin-biotin- horseradish peroxidase (Vectastain Elite ABC Kit; Vector Laboratories) for 2hr at room temperature. Sections were then developed in diaminobenzidine (DAB/Metal Concentrate; Pierce) and mounted on glass slides and coverslipped with Eukitt mounting medium (Sigma- Aldrich). The experimenter was blind to all treatment groups such that a non-experimenter coded all slides and the code was not broken until the end of quantitative analysis.

Stereology

Quatification of PV-IR GABA interneurons in the dorsal hippocampus was made using a modified optical fractionator counting technique (West et al., 1991, Gundersen et al., 1999). Neuronal counts were made using a BX51 Olympus microscope equipped with a DVC camera interfaced with StereoInvestigator 8.21 software (MBF Bioscience). Every fourth section for a total of six sections through the dorsal hippocampus was sampled. The hilus was outlined under a 10x objective and counts were performed using a 20x objective. Grids of 100μm × 100μm were used during counting. The number of grids per section varied from 16 to 27. A Gunderson coefficient of around 0.1 or below were obtained using these stereology experiments, which allows for an accurate estimate of total PV interneurons within the region.

Electrophysiology

On the 10th day after MDMA/saline exposure, rats were anesthetized with an initial injection of ketamine-xylazine anesthesia (20 mg/kg, 6 mg/kg, i.p., respectively). A nose cone containing gauze moistened with isoflurane was used to insure an anesthetic state during tracheotomy. For the remainder of electrophysiology experiments, rats were anesthetized with only isoflurane (0.6% ± 0.2%, 0.5 L/min) delivered with oxygen. Heart rate was kept constant (220–240 BPM) to ensure stable levels of anesthesia. Extracellular field potentials within the upper blade of the dentate gyrus (−4.0 mm A.P., 2.0 mm M.L, 3.2 mm D.V. bregma) were monitored using teflon coated tungsten electrodes (.7–1 MΩ). Stimulation of the perforant path (−7.0 mm A.P., 3.5 mm M.L., 3.5 mm D.V. bregma) was done using a concentric bipolar metal electrode. Stimuli were generated using an A-M systems isolated pulse stimulator. Waveforms were amplified using a BAK electronic amplifier and digitized with Digidata 1322A (Axon. Instruments, Inc.) and stored for analysis using pCLAMP9 software (Molecular Devices). Population spike amplitude (PS) was measured by drawing a tangent to the positive going peaks and from this tangent measuring the amplitude of the negative going peak. Input-output curves were determined using 0.1 ms pulses stepped by 1V intensities (.125 Hz), beginning at 3V. Paired-pulse experiments were performed at stimulus intensities resulting in 60% max PS responses as determined by input-output curves. After-discharge threshold was determined using 1ms stimuli given at 10 Hz for 10 seconds. Stimuli were given at 2 minute intervals with each stimulus exceeding the previous stimulus intensity by .1V. After-discharges were defined as spontaneous discharges lasting at least 5 seconds.

Seizure Susceptibility

Seizure susceptibility was assessed 7 days following MDMA exposure by determining seizure severity in response to kainic acid (KA) (8mg/kg, s.c., dissolved in 0.1M PBS, pH ~7) injections. After KA administration, rats were videotaped for up to 3 h. Seizures were assessed from recorded behaviors using a modified Racine scale: no response (0), frozen posture, staring, and/or facial clonus, (1); myoclonic twitching and tremor (2), forelimb clonus with lordotic posture (3), forelimb clonus with rearing (4), forelimb clonus with rearing, jumping, and falling (5). Only rats which exhibited behaviors consistent with a stage three seizure or above were marked as having seized.

Statistical Analyses

Stereological counts, PGE2 measurements and electrophysiology experiments were analyzed using a two-way ANOVA to compare the effects of saline or MDMA and determine an interaction with SC-51089 and MDL100907. Extracellular glutamate concentrations were compared between groups using a two-way ANOVA with repeated measures. Post-hoc analysis was performed using Tukey's test. Statistical significance was set at $p<0.05$. Chi-square and Fischer exact tests were used to compare the effects of MDMA and SC-51089 on seizure susceptibility.

Results

MDMA-induced increases in PGE2 concentrations within the dentate gyrus

PGE2 concentrations in the dentate gyrus were measured in dialysate samples in which 100 μM MDMA or aCSF was reverse dialyzed directly into the dentate gyrus. As shown in Fig. 1, reverse dialysis of MDMA+Vehicle resulted in a significant increase in PGE2 concentrations compared to aCSF+vehicle in the 0–2 and 4–6 hour collection samples. Two-way ANOVA analysis revealed a significant main-effect of MDMA on PGE2 concentrations at the 0–2 and 4–6 hour time points ($F_{(1,29)}=5.385$ (0–2 hr), $F_{(1,29)}=6.542$ (4–6 hr) $p<0.05$). PGE2 concentrations from the MDMA+MDL100907 group were significantly reduced compared to the MDMA+Vehicle group ($p<0.05$). Furthermore, the PGE2 concentrations from the MDMA+MDL100907 treated group were not significantly different than the aCSF +Vehicle treated group.

EP1 receptor inhibition and local perfusion of MDMA into the dentate gyrus

The potential role of local EP1 receptor activation in mediating increases in extracellular glutamate concentrations caused by reverse dialysis of 100 μM MDMA into the dentate gyrus was determined. SC-51089 was reverse dialyzed 30 minutes prior to and during MDMA administration. A two-way repeated measures ANOVA comparing MDMA+vehicle and aCSF+vehicle groups revealed a significant effect of MDMA treatment ($F_{(1,84)}=5.379$; $p<0.05$) and time ($F_{(6,84)}=4.489$; $p<0.05$) as well as a significant interaction ($F_{(6,84)}=3.369$; $p<0.05$). Reverse dialysis of MDMA+vehicle resulted in a significant increase in extracellular glutamate concentrations compared to aCSF+vehicle ($p<0.05$, Fig.2). A significant effect of SC-51089 treatment ($F_{(1,113)}=5.424$; $p<0.05$) was also revealed when comparing MDMA+vehicle and MDMA+SC-51089 treated groups. Extracellular glutamate

concentrations were not significantly different between MDMA+SC-51089 and aCSF +vehicle groups.

Effect of SC-51089 on MDMA-induced PV cell losses

The ability of SC-51089 coadministration to prevent MDMA-induced decreases in PV cell counts was determined. Two-way ANOVA analysis revealed a main effect of MDMA ($F_{(1,22)}$=9.384; p<0.05) as well as an interaction between MDMA and SC-51089 ($F_{(1,22)}$=5.892; p<0.05) on the number of PV-positive cells. As shown in Fig. 3, MDMA +vehicle treated animals had significantly fewer PV interneurons in the dentate gyrus compared with saline+vehicle treated rats (p<0.05). MDMA+vehicle treated rats had significantly fewer parvalbumin cells than MDMA+SC-51089 rats, which were not significantly different from saline vehicle controls. The body temperatures of MDMA +SC-51089 treated rats did not differ significantly from those of MDMA+vehicle (mean temperatures were 38.8 °C and 38.6 °C. for MDMA+vehicle and MDMA+SC-51089, respectively).

Effect of MDMA on input-output function in the dentate gyrus

To determine whether MDMA has any effect on the excitability of dentate gyrus granule cells, we monitored the PS amplitude of granule cells in response to perforant path stimulation. These experiments were performed 10 days following MDMA exposure, which corresponds to the time when MDMA-induced PV interneuron decreases are evident. Fig. 4a shows a representative trace of an evoked response in the dentate gyrus, noting the PS, which is an indication action potential firing in granule cells. Input-ouput curves, as shown in Fig. 4b, were calculated in order to determine baseline synaptic transmission in the dentate gyrus. There was no significant change in input-output function mediated by MDMA treatment.

The effects of MDL100907 and SC-51089 on decreases in paired-pulse depression in the dentate gyrus caused by MDMA

To determine potential changes in GABAergic inhibition caused by MDMA, field potential recordings were performed in the dentate gyrus 10 days after MDMA exposure. Perforant path induced paired-pulse responses were monitored at interstimulus intervals known to mediate paired-pulse depression (20–80 ms). Two-way ANOVA analysis revealed a significant main-effect of MDMA at the 40, 50 and 65 ms intervals ($F_{(1,24)}$=4.616 (40ms), $F_{(1,24)}$=8.906 (50ms), $F_{(1,24)}$=5.740 (65ms); p<0.05). As shown in Fig. 5b & 5c, MDMA +Vehicle treated rats exhibited a significant reduction in paired-pulse depression at 40, 50 and 65 ms interstimulus intervals compared to Saline+Vehicle treated rats (p<0.05). Two-way ANOVA analysis also revealed a significant interaction of MDL100907 and SC-51089 and MDMA treatment at the 40 and 50 ms interstimulus intervals ($F_{(1,24)}$=6.686 (SC-51089, 40ms), $F_{(1,24)}$=4.902 (SC-51089, 50ms), $F_{(1,24)}$=6.272 (MDL100907, 40ms), $F_{(1,24)}$=4.945 (MDL100907, 50ms); p<0.05). Furthermore, there was no difference between paired-pulse depression at any interstimulus intervals between either MDL100907+MDMA or SC-51089+MDMA treated rats and Saline+Vehicle treated rats.

Effects of MDL100907 and SC-51089 on MDMA-induced reductions in perforant path induced afterdischarge threshold in the dentate gyrus

Potential changes in the excitatory/inhibitory balance in the dentate gyrus caused by MDMA were determined by measuring perforant path stimulus intensities needed to drive spontaneous afterdischarges in the dentate gyrus, 10 days after MDMA exposure. Two-way ANOVA analysis revealed a significant main effect of MDMA treatment on reducing afterdischarge threshold ($F_{(1,24)}$=15.187; p<0.05). These experiments revealed a significant reduction in the stimulus intensity required to drive spontaneous afterdischarges in the dentate gyrus of MDMA+Vehicle treated rats compared to Saline+Vehicle treated rats (p<0.05). Further analysis revealed a significant interaction between either MDL100907 or SC-51089 treatment and MDMA treatment ($F_{(1,24)}$=9.188 (SC-51089), $F_{(1,23)}$=4.663 (MDL100907); p<0.05). There was no significant difference in the afterdischarge threshold between either MDL100907+MDMA or SC-51089 treated rats and Saline+Vehicle treated rats.

SC-51089 prevents MDMA-induced increases in kainic acid-induced seizure susceptibility

Kainic acid-induced seizure behavior was monitored 7 days after MDMA exposure in order to further gauge changes in excitability in the brains of MDMA treated rats. MDMA +vehicle treated rats were significantly more likely to exhibit stage 3 seizures or greater in response to a single injection of 8 mg/kg kainic acid than saline+vehicle treated rats (p<0.05). Additionally, MDMA+SC-51089 treated rats were less likely to exhibit stage 3 or higher seizures than MDMA+vehicle treated rats (p<0.05), but had similar susceptibility to saline+vehicle controls.

Discussion

The findings of the current paper demonstrate that the acute effects of MDMA on glutamatergic neurotransmission mediate long-term changes in hippocampal function. These findings support a link between 5HT2A receptor activation and prostaglandin signaling in mediating the increases in extracellular glutamate concentrations within the dentate gyrus caused by MDMA. This signaling induced by MDMA mediates the long-term changes in PV interneurons within the dentate gyrus and reduced inhibition within the region.

Prior studies have shown that systemic neurotoxic doses of MDMA increase extracellular glutamate concentrations within the hippocampus via 5HT release (Anneken and Gudelsky, 2012) and 5HT2A activation within the dentate gyrus (Collins et al., 2015). However, it was unclear whether this was a direct effect of the local activation of 5HT2A receptors on glutamate terminals by MDMA or an indirect effect of 5HT2A receptor activation. Moreover, previous findings by Anneken et al (2013) demonstrated that MDMA-induced increases in glutamate could be prevented by inhibition of COX2, suggesting a potential role for prostaglandins in mediating the increases in glutamate (Anneken et al., 2013) but the relationship if any, between prostaglandins, 5HT2A receptors, glutamate, and the loss of PVir remained to be established. As an initial step in testing this relationship, the current findings show that the local perfusion of MDMA into the dentate gyrus caused an increase in the extracellular concentrations of prostaglandin, PGE2 (Fig. 1), which was dependent

upon 5HT2A receptor activation. Consistent with the current findings, activation of 5HT2A receptors has been shown to mediate increases in arachidonic acid, primarily via increases in activity of phospholipase A2 (Felder et al., 1990). It remains to be determined if the increases in PGE2 are mediated via 5HT2A receptor-induced increases in phospholipase A2 activity and subsequent arachidonic acid production. It remains unknown which cell type in the dentate gyrus is responsible for this signaling, however, it is known that 5HT2A receptors within the DG are expressed by astrocytes and granule cells (Xu and Pandey, 2000).

More recently, studies have demonstrated a potential excitotoxic role of PGE2-induced activation of EP1 receptors (Ahmad et al., 2008, Mohan et al., 2013). The current findings now demonstrate a relation between PGE2 and glutamate such that the reverse dialysis of SC-51089 into the dentate gyrus prevented the increases in glutamate caused by the local administration of MDMA (Fig. 2). Although the location of EP1 receptors involved in MDMA-induced glutamate release is not known, previous studies have implicated astrocytes as a likely source of PGE2-induced glutamate increases in the hippocampus (Bezzi et al., 1998, Sanzgiri et al., 1999). These studies linked increases in calcium within astrocytes to glutamate release caused by PGE2. Thus, EP1 receptors, which are known to mediate increases in intracellular calcium and are found on astrocytes may be mediating glutamate release from astrocytes via increases in intracellular calcium (Fiebich et al., 2001, Zonta et al., 2003, Rojas et al., 2014). Although PGE2 has been shown to mediate K^+-induced glutamate release from cortical nerve terminals via calcium increases induced by EP2 receptor signaling (Lin et al., 2014), glutamate increases caused by MDMA are not action potential dependent (Anneken and Gudelsky, 2012). Therefore, it is likely that MDMA-induced glutamate increases within the dentate gyrus are by astrocytes.

Previous reports have shown that the decrease in PV interneurons in the hippocampus following MDMA could be prevented by inhibition of COX-2 during MDMA exposure (Anneken et al., 2013). More recently, we showed that the decreases in PV immunoreactivity were dependent upon activation of NMDA receptors (Collins et al., 2015) but the relationship between the long-term decreases in PV interneurons and the observed changes in extracellular glutamate and EP1 receptor activation remained to be determined. As seen in Figure 3, SC-51089 administration 30 minutes prior to each MDMA injection prevented the loss of PV immunoreactivity observed 10 days after MDMA exposure. SC-51089 treatments had no effect on MDMA-induced hyperthermia. These data provides additional evidence supporting the role of prostaglandins in mediating decreases in PV cells. Furthermore, as SC-51089 also prevented the increases in glutamate, the results indicate that the loss of PV interneurons within the dentate gyrus are mediated through increases in glutamate as a consequence of PGE2-induced activation of EP1 receptors. Previous studies have suggested that PV interneurons may be more susceptible to excitotoxicity due to the expression profile of glutamate receptors linked to excitotoxicity (Kwon et al., 1999, Gorter et al., 2001, Moga et al., 2002, Sanon et al., 2005), however, there is a possibility that other neurons within the hippocampus are damaged or impaired as a result of MDMA-induced glutamate increases within the hippocampus.

PV interneurons receive glutamatergic efferents from both granule cells and the perforant path (Kneisler and Dingledine, 1995b, a). Upon stimulation of the perforant path, PV interneurons provide strong inhibition of dentate gyrus granule cells via inhibitory synapses onto the somatic and axoaxonic regions of granule cells (Freund and Buzsaki, 1996, Miles et al., 1996). Thus, deficits in PV interneuron function may significantly alter the feed-forward and feedback inhibition in the dentate gyrus. As shown in Figure 4b, MDMA did not produce a shift in the input-output curves produced by MDMA suggesting that baseline neurotransmission is not altered. To help gauge whether inhibition within the dentate gyrus is affected, the paired-pulse experiments were performed and showed a significant decrease in paired-pulse inhibition at the 40, 50 and 65 ms intervals of MDMA treated rats (Fig. 4b & 4c). Paired-pulse depression in dentate gyrus has been main attributed to GABAergic inhibition (Sloviter, 1991, DiScenna and Teyler, 1994). Thus, the MDMA-induced changes in paired-pulse inhibition could be mediated by decreases in PV interneurons in the dentate gyrus following MDMA exposure, which would reduce GABAergic inhibition region. It is however possible that these changes may be mediated by increases in granule cell excitability. In other words, MDMA may result in an increased ability of dentate gyrus granule cells to fire successive action potentials. This would increase the chance that any given granule cell would fire an action potential at P2. Another possible explanation for the MDMA-induced decrease in paired-pulse depression would be through changes in glutamate release probability at the perforant path inputs. In light of these possibilities, patch clamp experiments investigating MDMA-induced changes in granule cell membrane characteristics or alterations in presynaptic forms of plasticity at perforant path inputs are warranted. Our results from figure 4c also demonstrate that the decrease in paired-pulse inhibition was prevented with SC-51089 or MDL100907 treatments during MDMA exposure, and extends the relationship between EP1, 5HT2A receptors and extracellular glutamate to the long-term physiological functioning of the dentate gyrus.

To further investigate changes in excitatory/inhibitory signaling in the dentate gyrus caused by MDMA, we measured afterdischarge threshold in the dentate gyrus 10 days following MDMA exposure. MDMA treatment caused a significant reduction in the threshold stimulus intensity needed to drive afterdischarges in the dentate gyrus (Fig. 6). Furthermore, the changes in afterdischarge threshold could be prevented by inhibition of 5HT2A and EP1 during MDMA exposure, suggesting that decreases in PV interneurons may play a role in the afterdischarge threshold changes caused by MDMA. Several studies and reviews have highlighted the dentate gyrus as being involved in the generation of seizure activity (Lothman et al., 1992, Scharfman, 1994). Furthermore, GABAergic inhibition mediated by PV interneurons is known to reduce action potential firing of granule cells and has been suggested to play a central role in regulating seizure activity (Freund and Buzsaki, 1996, Miles et al., 1996). Our findings in figure 7 show that exposure to MDMA 7 days earlier, increases the susceptibility to kainic acid-induced seizures. This finding is in agreement with previous studies showing that MDMA treated mice are more susceptible to kainic acid induced seizures (Giorgi et al., 2005, Abad et al., 2014). Importantly, we show that this increased susceptibility to kainic acid-induced seizures could be prevented when rats were treated with SC-51089 during MDMA treatment. This further supports the potential role of

the EP1 receptor in the PV-interneuron decreases and the increased excitability in the brains of rodents exposed to MDMA.

The dentate gyrus plays a key role in the formation of episodic memories. It receives inputs conveying different modalities of sensory information but the most recognized inputs are those which relay spatial information (Hargreaves et al., 2005). Thus, damage to the dentate gyrus by MDMA may impair spatial memory performance. In fact, several studies have reported significant impairments in spatial memory performance in rodents treated with MDMA (Robinson et al., 1993, Sprague et al., 2003, Williams et al., 2003, Vorhees et al., 2004, Skelton et al., 2006, Arias-Cavieres et al., 2010). Our results demonstrate changes in physiology within the dentate gyrus which likely alter the processing of spatial information by this region and explain the spatial memory deficits caused by MDMA. Further studies are warranted to further characterize the potential impact that MDMA-induced changes in GABAergic inhibition may have on dentate gyrus function and consequent behavioral changes.

In conclusion, our findings highlight a potential novel signaling mechanism mediated by MDMA in which 5HT2A receptor activation elicits PGE2 signaling, ultimately leading to increases in glutamatergic neurotransmission. This signaling appears to mediate both the changes in PV interneurons as well as changes in inhibition within the dentate gyrus. The significance of these findings are evident in light of previous studies highlighting spatial memory deficits as well as seizure susceptibility seen in animals treated with MDMA as well as human MDMA users.

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Abbreviations

5HT	serotonin
AMPA	α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
COX-2	cyclooxygenase 2
GABA	*gamma*-Aminobutyric acid
MDMA	3,4-Methylenedioxymethamphetamine

NMDA	N-Methyl-D-aspartate
PBS	phosphate buffered saline
PS	population spike
PV	parvalbumin
PV-IR	parvalbumin immunoreactive

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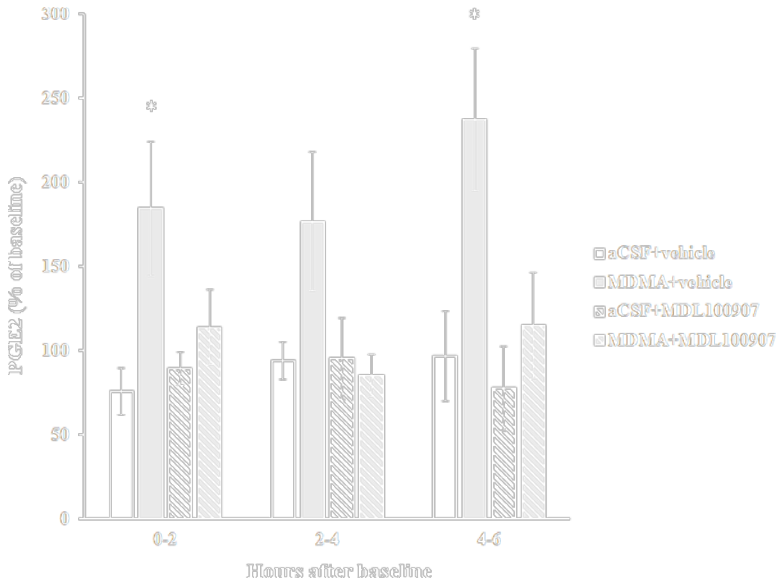

Figure 1.

MDL100907 prevents increases in PGE2 within the dentate gyrus caused by reverse dialysis 100μM MDMA. PGE2 concentrations were measured in dialysate samples from the dentate gyrus in which 100μM of MDMA was perfused for 6 hours. MDL100907 (100nM) or vehicle was reverse dialysed 30 minutes prior to and during MDMA. n=6–11 per group. Two-way repeated measures ANOVA revealed a significant main effect of MDMA ($p<0.05$). MDL100907 prevented MDMA-induced increases in PGE2 ($p<0.05$). * denotes values statistically significant from aCSF+vehicle controls ($p<0.05$).

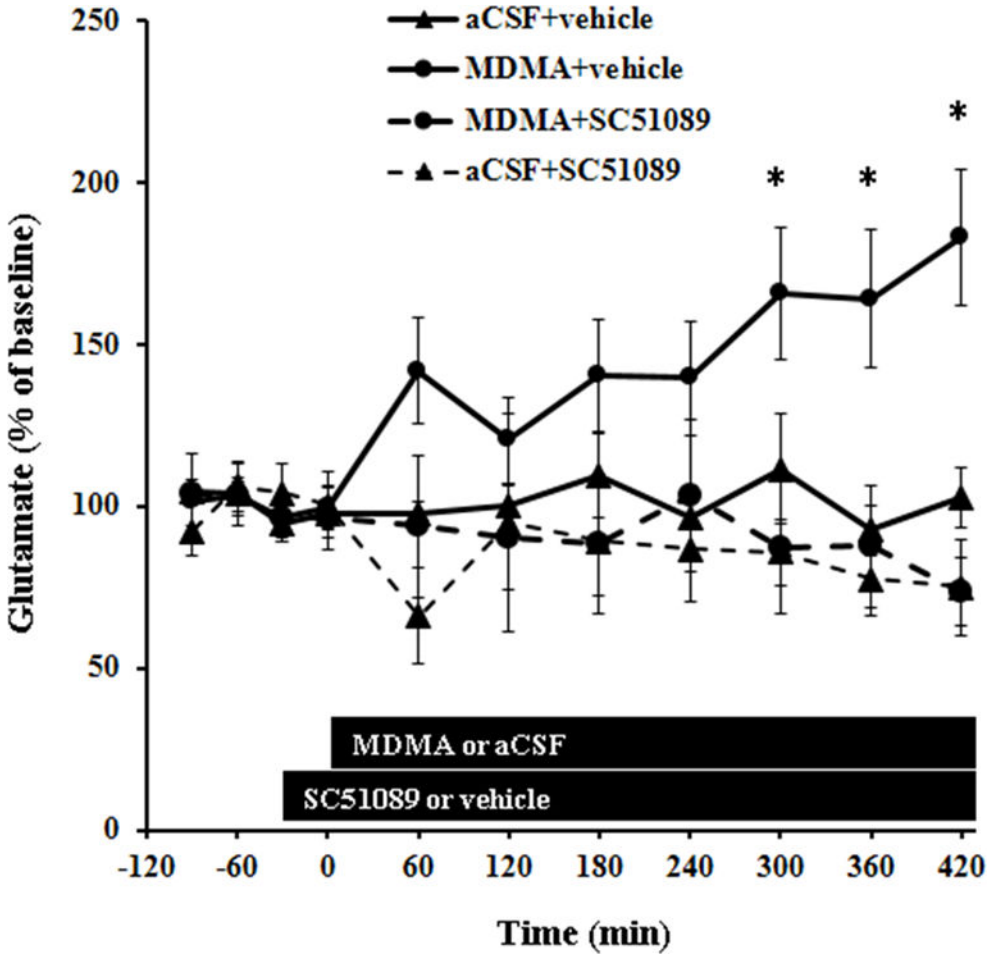

Figure 2.

SC-51089 prevents increases in extracellular glutamate within the dentate gyrus caused by reverse dialysis of 100μM MDMA. Extracellular glutamate concentrations were measured via microdialysis in the dentate gyrus during which time 100μM of MDMA was perfused for 7 hours (noted by bar). SC-51089 (100nM) or vehicle was reverse dialysed 30 minutes prior and during MDMA. n=6–11 per group. Two-way repeated measures ANOVA revealed a significant main effect of MDMA ($p<0.05$). SC-51089 prevented MDMA-induced increases in glutamate ($p<0.05$). * denotes values statistically significant from aCSF+vehicle controls ($p<0.05$).

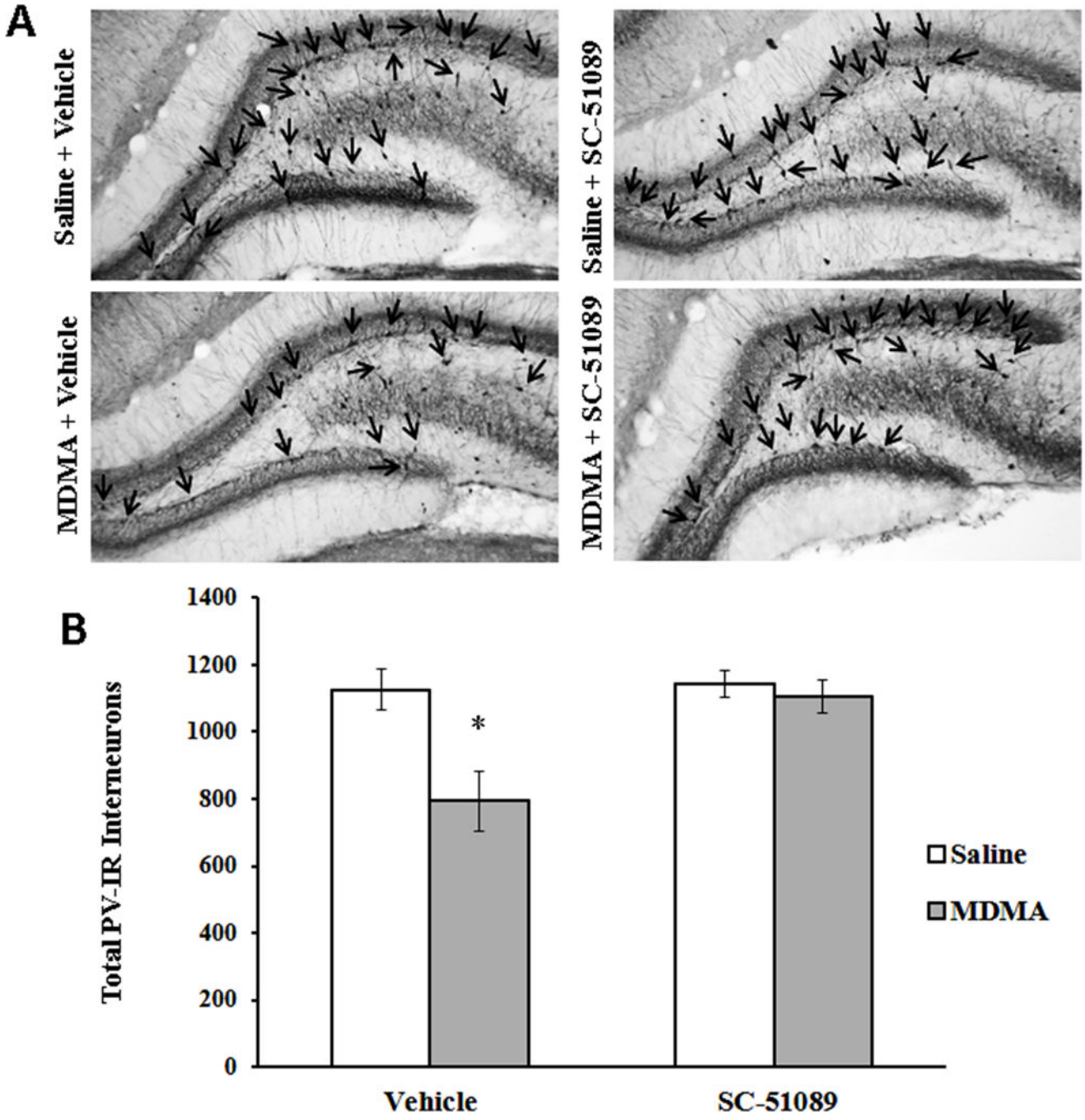

Figure 3.

Effect of SC-51089 on MDMA-induced PV-ir interneuron decreases in the dentate gyrus. Rats were treated with SC- 51089 (20 μg/kg, i.p.) 30 minutes prior to each MDMA (7.5mg/kg every 2h × 4 i.p injections) or saline injection. Stereologic cell counts of PV-IR interneurons was performed 10 days after drug exposure. A) Representative staining of PV-IR for treatment groups with PV positive cells labeled with black arrows. B) Quantitative assessment of PV-IR (n=6–7 per group). MDMA+vehicle treatment resulted in a significant decrease in PV-IR neurons in the dentate gyrus compared to saline+vehicle treatments

($p<0.05$). SC-51089 pretreatment prevented MDMA-induced decreases in PV-IR ($p<0.05$). *=statistically significant from saline+vehicle controls ($p<0.05$).

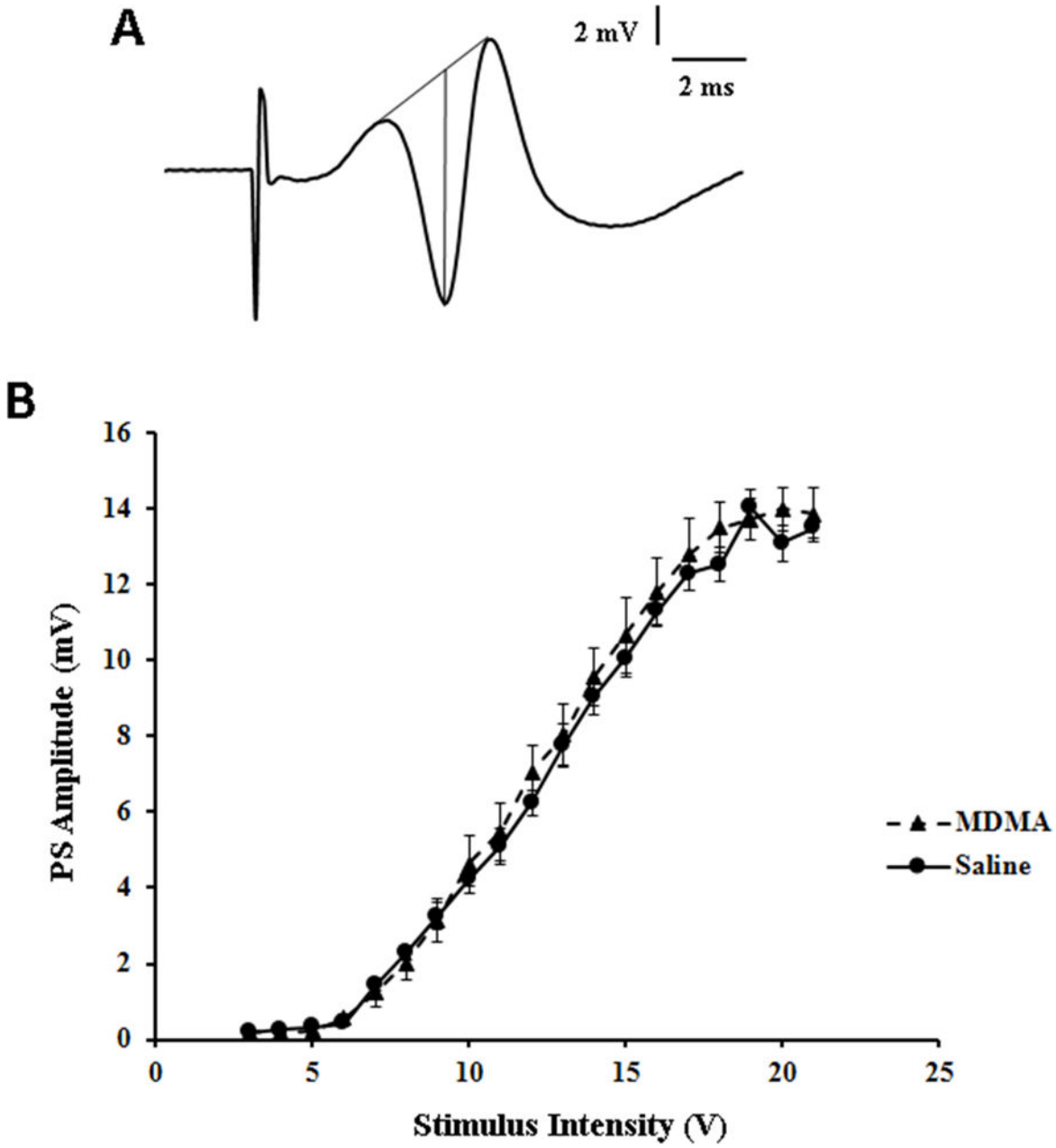

Figure 4.

Effect of a neurotoxic dose of MDMA on input-output curves in the dentate gyrus. 10 days after MDMA (7.5mg/kg every 2h × 4 i.p injections) or saline injections local field potentials in the dentate gyrus were recorded. A) Representative trace from electrophysiology recordings depicting a population response recorded in the granular cell layer in response to perforant path stimulation. Population spikes (PS) were measured as the negative peak amplitude drawn from a line tangent to the two positive peaks. B) Input-output curves depicting PS amplitudes as a function of stimulus intensity. No significant difference in input-output curves was noted between MDMA and Saline treated rats.

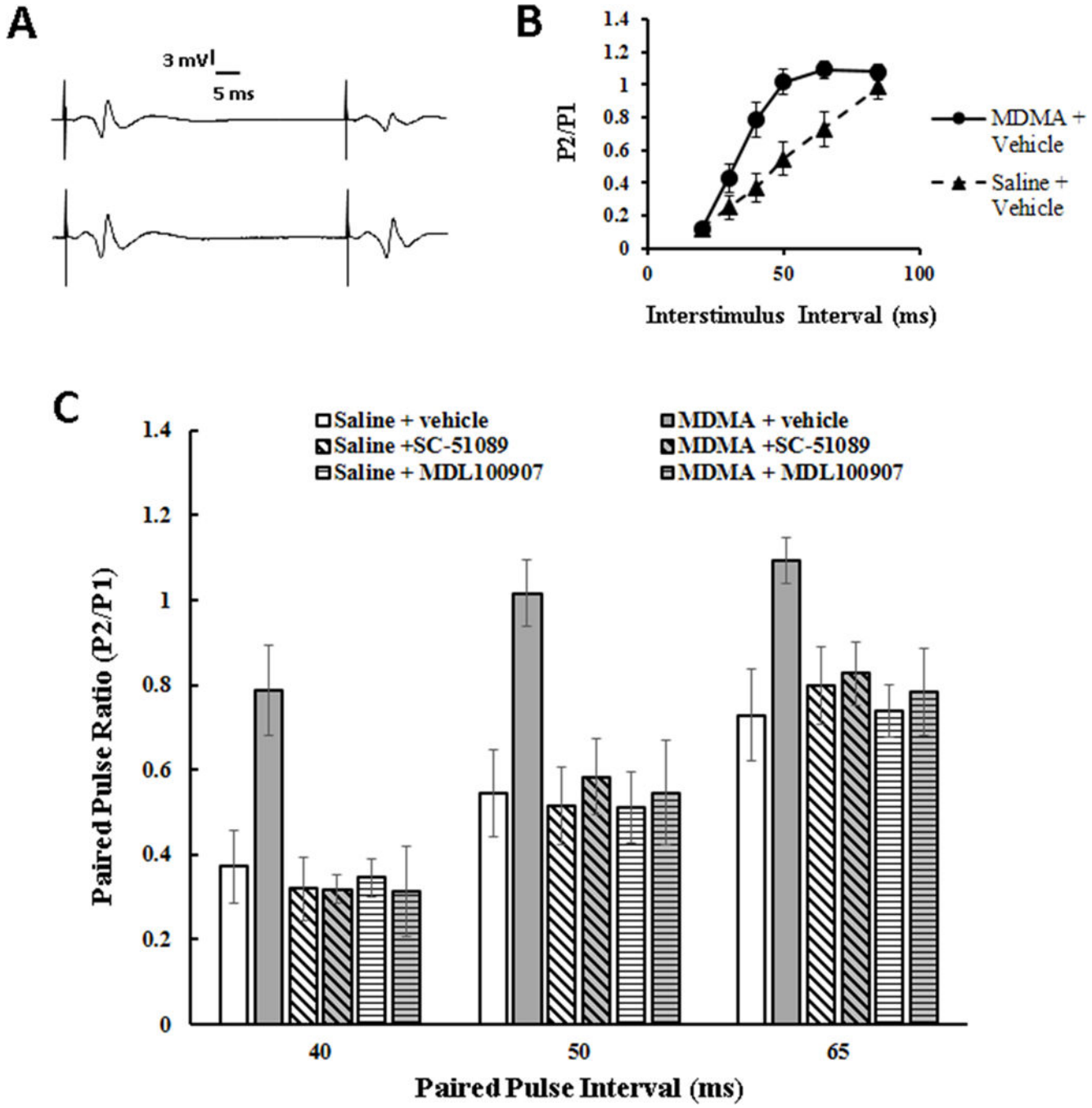

Figure 5.

MDL100907 and SC-51089 prevent MDMA-induced reductions in paired-pulse depression in the dentate gyrus. Rats were treated with MDMA (7.5mg/kg every 2h × 4 i.p injections) 10 days prior to electrophysiology recordings. MDL100907 or SC-51089 were given 30 minutes prior to each injection of MDMA. A) Representative traces from a MDMA + vehicle and Saline + Vehicle treated rat at 50 ms inter-pulse interval. B) Summary of the paired-pulse responses (PS2/PS1) for MDMA + vehicle and Saline + Vehicle treated rats at varying interstimulus intervals of 20–80 ms. C) Summary of 40, 50 and 65 ms interstimulus

interval paired-pulse responses for all treatment groups. Paired-pulse depression was significantly reduced in MDMA + vehicle treated rats compared to Saline + Vehicle treated rats. MDL100907 and SC-51089 pretreatment prevented MDMA-induced decreases in paired-pulse depression. *=statistically significant from saline+vehicle controls ($p<0.05$).

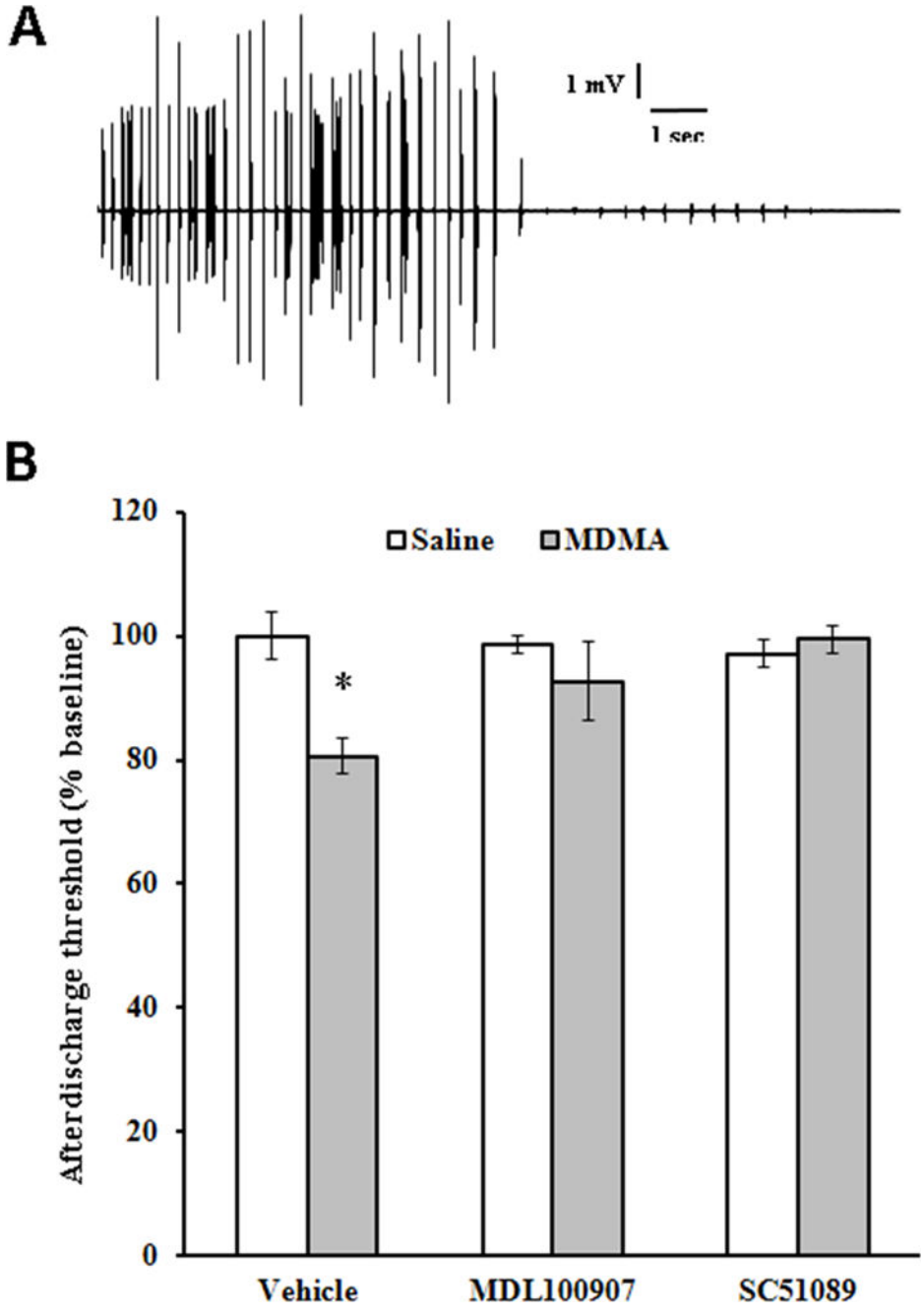

Figure 6.

Changes in afterdischarge threshold of the dentate gyrus mediated in MDMA-pretreated rats. Afterdischarge thresholds were performed in the dentate gyrus of anesthetized animals, 10 days after drug treatments. MDL100907 or SC-51089 was given 30 minutes prior to each injection of MDMA. A) Example extracellular field potential recording in the dentate gyrus displaying afterdischarges followed by a recovery to baseline activity in response to threshold electrical stimulation of the perforant path. B) Mean thresholds for inducing afterdischarges. MDMA + vehicle treated rats had significantly reduced afterdischarge thresholds compared to saline + vehicle controls. MDL100907 and SC-51089 pretreatment

prevented MDMA-induced decreases in afterdischarge threshold. *=statistically significant from saline+vehicle controls ($p<0.05$).

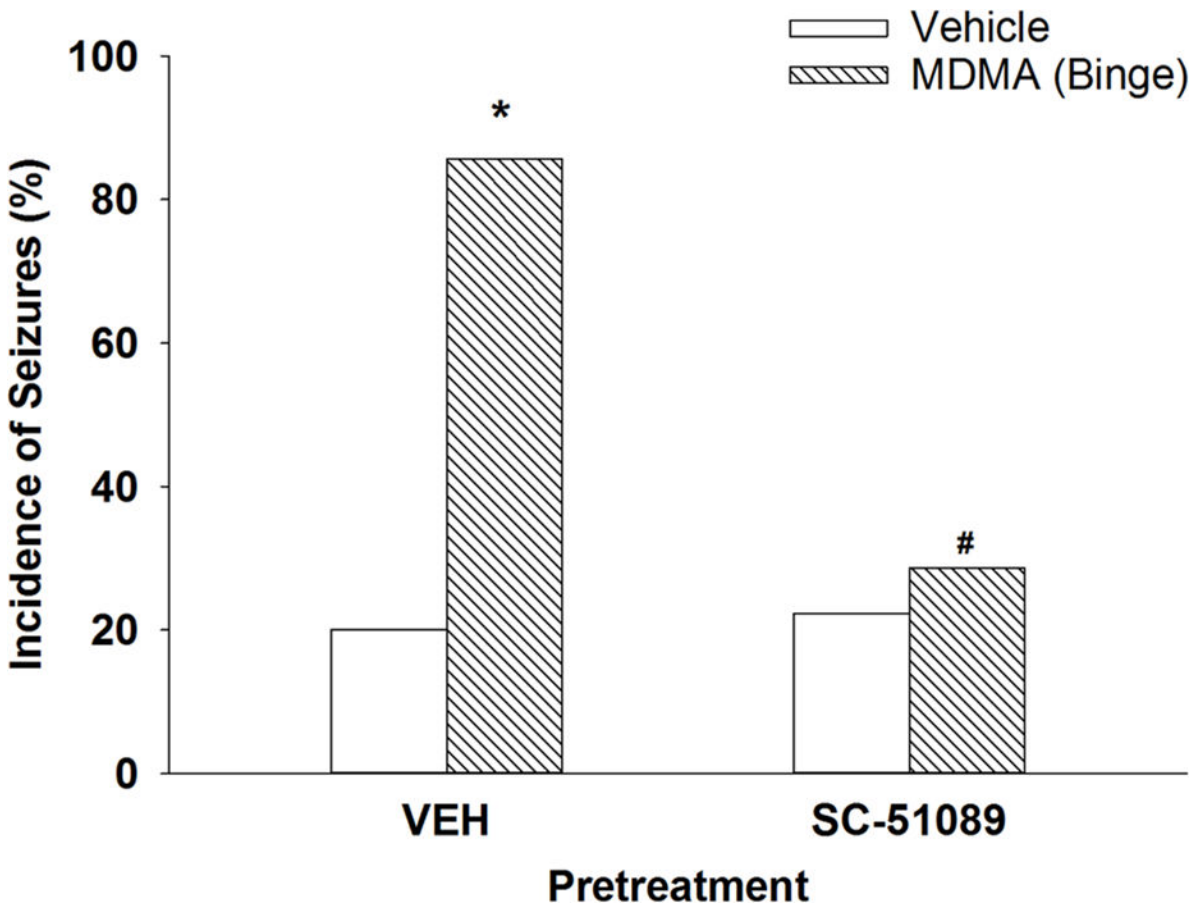

Figure 7.

The effects of MDMA on seizure susceptibility in rats. Rats were treated with either SC-51089 (20 μg/kg, sc) or vehicle 30 minutes prior to each injection of either MDMA (4 × 7.5 mg/kg, ip) or vehicle. Kainic acid (8mg/kg, sc) was administered 7 days after treatment. The percentage of rats exhibiting stage 3–5 seizures was recorded. N=5–9 rats/group. *=statistically significant from saline+vehicle controls ($p<.05$). #= statistically significant from MDMA+vehicle values ($p<0.05$)