J Chin Med Assoc

ORIGINAL ARTICLE

Dextromethorphan moderates reward deficiency associated with central serotonin transporter availability in 3,4-methylenedioxy-methamphetamine-treated animals

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Abstract

Background: The neurotoxicity of 3,4-methylenedioxy-methamphetamine (MDMA) to the serotonergic system is well-documented. Dextromethorphan (DM), an antitussive drug, decreased morphine- or methamphetamine (MA)-induced reward in rats and may prevent MDMA-induced serotonergic deficiency in primates, as indicated by increased serotonin transporter (SERT) availability. We aimed to investigate the effects of DM on reward, behavioral sensitization, and neurotoxicity associated with loss of SERT induced by chronic MDMA administration in rats.

Methods: Conditioned place preference (CPP) and locomotor activity tests were used to evaluate drug-induced reward and behavioral sensitization; 4-[^{18}F]-ADAM/animal-PET and immunohistochemistry were used to explore the effects of DM on MDMA-induced loss of SERT.

Results: MDMA significantly reduced SERT binding in the rat brain; however, co-administration of DM significantly restored SERT, enhancing the recovery rate at day 14 by an average of ~23% compared to the MDMA group. In confirmation of the PET findings, immunochemistry revealed MDMA reduced SERT immunoactivity in all brain regions, whereas DM markedly increased the serotonergic fiber density after MDMA induction.

Conclusion: Behavioral tests and in vivo longitudinal PET imaging demonstrated the CPP indexes and locomotor activities of the reward system correlate negatively with PET 4-[^{18}F]ADAM SERT activity in the reward system. Our findings suggest MDMA induces functional abnormalities in a network of brain regions important to decision-making processes and the motivation circuit. DM may exert neuroprotective effects to reverse MDMA-induced neurotoxicity.

Keywords: Dextromethorphan; MDMA; PET; Reward; Serotonin transporter

1. INTRODUCTION

As an analog of methamphetamine (MA), the drug 3,4-methylenedioxy-MA (MDMA), also commonly known as "ecstasy," is an addictive psychostimulant that induces euphoria, increases pleasant feelings, sociability, and confidence, and elevates empathy.[1–3] MDMA has been widely abused all over the world, especially by adolescents and young adults.[2] Furthermore, the neurotoxicity of MDMA has been demonstrated in many studies. MDMA given either acutely or chronically is toxic to serotonergic neurons in rats, monkeys, and humans[4–7]; however, the mechanisms underlying MDMA-induced serotonergic neurotoxicity are not well-understood. Many studies have found that oxidative stress and inflammation are involved in this neurotoxicity.[8] Activation of N-methyl-D-aspartate (NMDA) receptors has been suggested to contribute to MDMA-induced oxidative stress[9,10] and pretreatment with the NMDA receptor antagonist MK-801 was found to attenuate MDMA-induced serotonergic neurotoxicity.[11] In MDMA-induced inflammation, MDMA appears to increase the activity of microglia,[12] which may lead to the release of proinflammatory cytokines, prostaglandins, nitric oxide (NO), and peroxides (superoxide) and thereby promote nerve tissue damage.[8,13]

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Author contributions: Dr. Skye Hsin-Hsien Yeh, Dr. Kuo-Hsing Ma, and Dr. Eagle Yi-Kung Huang contributed equally to this work.

Conflicts of interest: The authors declare that they have no conflicts of interest related to the subject matter or materials discussed in this article

Journal of Chinese Medical Association. (2024) 87: 538-549.

Received October 27, 2023; accepted December 15, 2023.

doi: 10.1097/JCMA.0000000000001087

Dextromethorphan (DM) is a D-isomer of levorphanol that does not exert any action on the opioid receptors and is used in the clinic as an antitussive. DM has been shown to exert anticonvulsant and neuroprotective properties by antagonizing NMDA receptors.[14–16] DM is particularly attractive for clinical use as an NMDA antagonist because it has been dispensed as a nonprescription antitussive drug for more than 50 years and has a wide margin of safety.[17] We previously found that DM may prevent MDMA-induced serotonergic deficiency, as indicated by increased uptake and availability of 4-[^{18}F]-ADAM serotonin transporter (SERT), a selective SERT radiotracer, but not volumetric changes in the primate brain.[18]

The same therapeutic effects of DM have also been observed in morphine-treated rats,[19] which implies that DM may reduce the reward effect of addictive drugs via a common mechanism. Recently, the results of a double-blinded, placebo-controlled trial of DM combined with clonidine for the treatment of heroin withdrawal suggested that DM exerted some beneficial effects in attenuating the severity of opioid withdrawal symptoms and could thus be used as an adjunct medication in the treatment of opioid withdrawal.[20] Another clinical study showed that DM attenuated inflammation and opioid use in humans undergoing methadone maintenance treatment.[21] Therefore, the therapeutic potential of DM for the treatment of MDMA addiction and neurotoxicity is worthy of further study.

The aim of this study was to evaluate (1) the effects of DM on MDMA-induced reward and behavioral sensitization in rats by using the conditioned place preference (CPP)-rewarding effect and locomotor activity tests; (2) to assess the effects of DM on SERT availability after MDMA induction using N,N-dimethyl-2-(2-amino-4-[^{18}F]-fluorophenylthio)-benzylamine (4-[^{18}F]-ADAM) PET; and (3) to determine the associations between behavior tests (reward effect and locomotor sensitization) and SERT availability after MDMA induction in rats treated with DM.

2. METHODS

2.1. Animals

Male Sprague-Dawley (SD) rats, weighing 220 to 380 g, were purchased from the National Laboratory Animal Center, Taipei, Taiwan, ROC. All rats were housed in an animal room under a 12-hour light/dark cycle, temperature of 25°C ± 2°C, 55% humidity, with two to three animals per cage and ad libitum access to a standard diet and water at the National Defense Medical Center's Animal Center, which is accredited by AAALAC International. The animals were acclimated for at least 1 week before the experiments. All animal care was conducted in accordance with institutional and international standards (Principles of Laboratory Animal Care, NIH). The experimental protocol was approved by the Institutional Animal Care and Use Committee (IACUC) of the National Defense Medical Center, Taiwan, ROC.

2.2. Experimental design

2.2.1. Animal groups and drug preparation

Rats were randomly allocated to one of two experiments and then randomized into three groups (n = 8 per group) for each experiment. MDMA (purity, 98%) was obtained from the Investigation Bureau of Taiwan and DM was purchased from Sigma-Aldrich (St. Louis, MO). MDMA and DM were dissolved in saline (0.9% NaCl) at a final concentration of 10 and 20 mg/mL, respectively.

2.2.1.1. Experiment 1: co-administration of DM. Group 1 control received saline injections (i.p.) throughout the CPP conditioning and withdrawal periods; group 2 MDMA received MDMA (5 mg/kg, s.c.) during CPP conditioning and saline during withdrawal; group 3 MDMA ± DM received MDMA (5 mg/kg, s.c.) during CPP conditioning and DM (10 mg/kg, i.p.) during withdrawal; and (4) group 4 DM received DM (10 mg/kg, i.p.) during CPP conditioning and saline during withdrawal.

2.2.1.2. Experiment 2: postadministration of DM. Group 5 MDMA ± post-DM10 received MDMA (5 mg/kg, s.c.) during CPP conditioning and posttreatment with DM (10 mg/kg, i.p.) during withdrawal, and group 6 MDMA post-DM-20 received MDMA (5 mg/kg, s.c.) during CPP conditioning and were post-treated with DM (20 mg/kg, i.p.) during withdrawal.

2.2.2. Schedule of drug administration and behavioral and imaging tests

2.2.2.1. Experiment 1: co-administration of DM. The aim of experiment 1 was to determine whether MDMA could induce a rewarding effect and behavioral sensitization and whether co-administration of MDMA with DM could reduce MDMA-induced neurotoxicity. The 16-day experimental schedule is shown in Fig. 1A. On day 1, the animals were placed in an isolated dark room for 60 minutes to habituate them to the CPP. The extent of MDMA-induced behavioral sensitization was assessed using locomotor activity tests performed on the afternoon of day 0 after the saline injection (as baseline to determine the control saline effect) and after injection of MDMA (to determine the acute effects of MDMA) on day 2.

The CPP pre-conditioning test was performed on the morning of day 1. Conditioning/MDMA exposure was performed from day 3 through day 8; all animals received saline (i.p.) in the morning and MDMA and/or DM (for group 2 to group 4) was injected in the afternoon. MDMA withdrawal was started on day 9. On day 9, place preference at the post-conditioning status was measured to determine whether MDMA could induce reward and assess the possible effects of co-administration of DM. The place preference was measured again after the withdrawal period on day 14, to determine whether the reward effect of MDMA was still sustained. The rats were sacrificed after all behavioral tests.

2.2.2.2. Experiment 2: postadministration of DM. The aim of experiment 2 was to determine whether posttreatment with DM could reduce the MDMA-induced reward effect and behavioral sensitization. As shown in Fig. 1B, the schedule of experiment 2 was similar to that of experiment 1. However, the animals were only conditioned with MDMA (5 mg/kg, s.c.), and DM (10 or 20 mg/kg, i.p.) was injected twice per day (at 9:00 and 16:30) during the withdrawal period from day 10 to day 13.

2.2.2.3. Experiment 3: PET imaging. The aim of experiment 3 was to determine the effects of DM on MDMA-induced SERT loss. A 49-day schedule with separate groups of animals exposed to the same treatment was used (Fig. 1C). This experiment was designed to rule out the effects of in vivo PET scanning on behavioral experiments, because we found that scanning may impose some stress that affects the behavioral results (data not shown). Animal PET was performed on days 14 and 42. Some of the rats were sacrificed on day 14 and the brain tissues were collected for immunohistochemical analysis.

2.3. CPP test

The CPP test was carried out to determine the reward effect induced by MDMA. A distinctive environment was paired repeatedly with administration of the drug and a different environment was associated with the non-drugged (saline) state, as

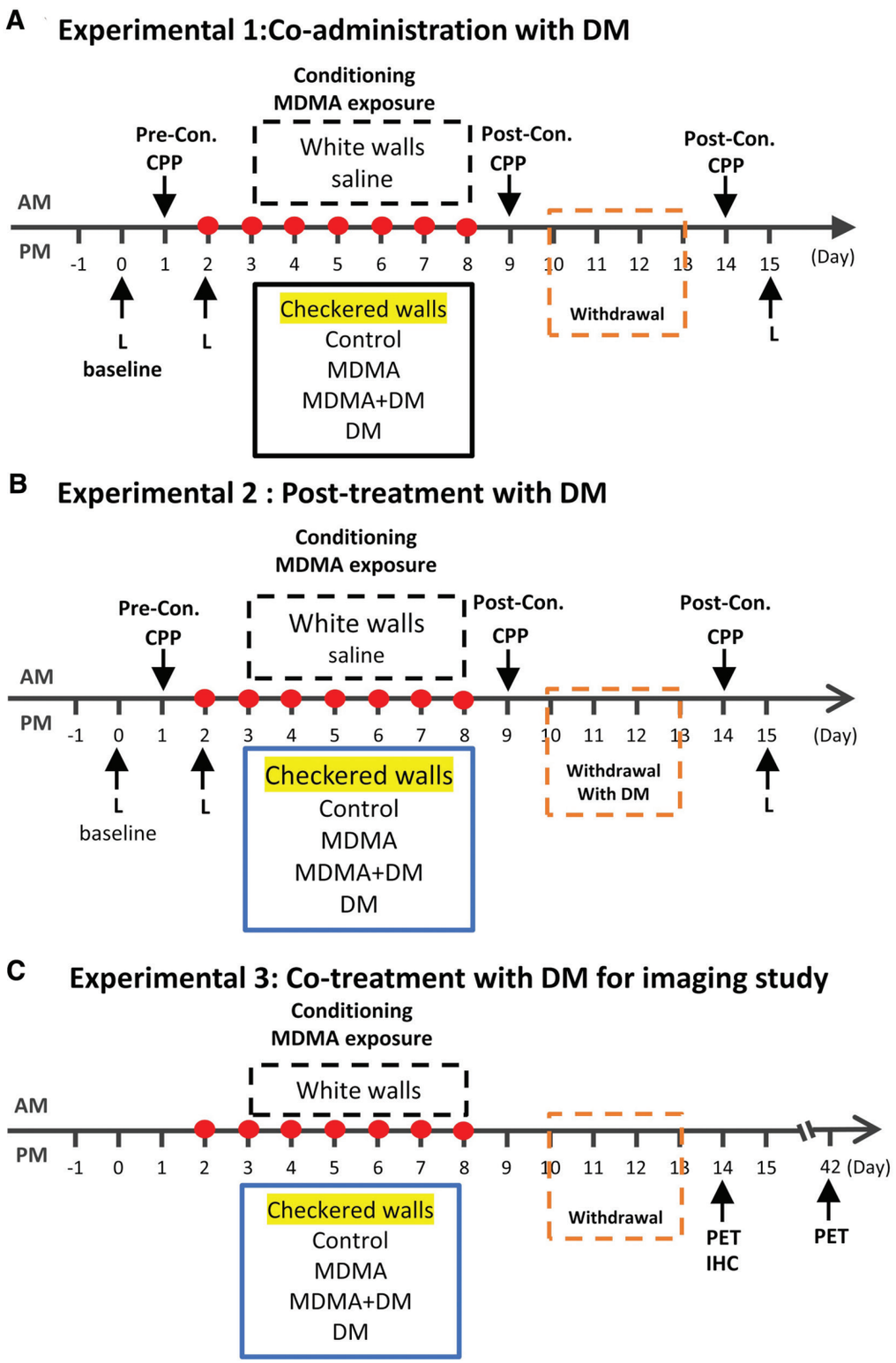

Fig. 1 Schematic of experimental design: (A) experiment 1: co-administration of DM with MDMA during conditioning, (B) posttreatment with DM during the withdrawal period after MDMA conditioning, and (C) animal PET and IHC study. CPP = conditioned place preference test; DM = dextromethorphan; L = locomotor activity test; MDMA = methylenedioxy-methamphetamine.

described in a previous report.[22,23] In brief, the CPP test apparatus is an acrylic plastic box divided into three compartments. Two identical compartments are separated by a narrow corridor and by guillotine doors at both ends of the corridor. One of the compartments was covered with mosaic paper on the walls and floor as a visual cue; the other large compartment was covered with white paper. To aid the recognition of visual cues, a blue light bulb and a red light bulb were hung separately above the two large compartments.

During the experiments, the CPP apparatus was placed in an isolated room and was lit only using the blue and red light bulbs. The entire box was cleaned thoroughly after each behavioral test or place conditioning to prevent interference due to the smell of feces or urine.

The CPP apparatus had an unbiased design, which was confirmed by the rats' lack of place preference when placed into the central compartment of the apparatus. For CPP conditioning, the rats were administered saline in the morning and then kept in the compartment with white walls and the red light bulb above with the doors closed for 40 minutes. In the afternoon, the rats were given drug(s) and then kept in the compartment with mosaic-type walls and the blue light bulb above for 40 minutes. CPP tests were carried out before and after conditioning by placing the rats into the central compartment of the apparatus with the doors opened for 15 minutes. The time that the rats stayed in each compartment was recorded to determine the place preference. The reward effect of the drug was quantified as the increase in the time spent in the compartment previously paired with drug injection compared to the time spent in the saline-paired compartment. The place preference for the drug-paired compartment was calculated by subtracting the time spent in the saline-paired compartment from the time spent in the drug-paired compartment.

2.4. Locomotor activity test

The ambulatory activity and total activity of the rats were measured in transparent standard polypropylene animal cages (38 cm × 22 cm × 15 cm) using a four-photobeam activity system (San Diego Instruments, San Diego, CA) in an isolated, noise-free room. A computer control unit recorded the interruptions of the photobeams in the four individual cages. Ambulatory activity was measured by counting the number of breaks in two consecutive beams, while total activity was measured by counting the number of breaks in any single photobeam. The activities were recorded in 5-minute intervals for 2 hours immediately after administration of MDMA or saline.

2.5. Synthesis of 4-[^{18}F]-ADAM

The 4-[^{18}F]-ADAM was prepared as described previously.[24–26] Briefly, the dinitro precursor was first reacted with K[^{18}F]/K2.2.2, followed by reduction with $NaBH_4$/$Cu(OAc)_2$ for nucleophilic fluorination. Next, purification with HPLC produced the desired compound at ~5% radiochemical yield end of bombardment (EOB) in a synthesis time of 120 minutes from EOB. The radiochemical yield of 4-[^{18}F]-ADAM increased to ~15% when using a different precursor.[27] The chemical and radiochemical purities were >95% and the specific activity was >3 Ci/μmol.

2.6. Animal-PET imaging

The imaging protocol, data acquisition, and data analyses were performed as described previously[28,29] with minor modifications. Briefly, rats were anesthetized by inhalation of isoflurane/oxygen (5% isoflurane for induction and 2% for maintenance). Static images were reconstructed from three-dimensional list-mode data acquired 60 to 90 minutes after injection of 4-[^{18}F]-ADAM (14.8-18.5 MBq; 0.4-0.5 mCi) via the tail vein. The data were collected for 30 minutes using an animal-PET R4 scanner (Concorde MicroSystems, Knoxville, TN) with the energy window set to 350 to 650 keV and timing set to 6 nanoseconds. The images were reconstructed from raw data by Fourier rebinning and two-dimensional filtered back projection with a ramp filter using a cutoff at the Nyquist frequency.

The reconstructed images were analyzed with PMOD (PMOD Technologies, Switzerland) to measure the standardized uptake values (SUVs) in various brain regions. The volumes of interest for the accumbens, amygdala, auditory cortex, cingulate cortex, medial prefrontal cortex, motor cortex, orbital frontal cortex, visual cortex, midbrain, hypothalamus, thalamus, striatum, and cerebellum were drawn manually on the reconstructed PET images, using an MRI-based rat brain atlas with PMOD (PMOD Technologies). The regional radioactivity concentrations (KBq/mL) of 4-[^{18}F]-ADAM PET were estimated from the maximum pixel values within each region of interest (ROI) and expressed as SUVs. The final data were expressed as specific uptake ratios (SURs), calculated as $(SUV_{target\ region} - SUV_{cerebellum})/SUV_{cerebellum}$. The SERT recovery rate was calculated as $(SUVR_{day\text{-}x} - SUV_{day\text{-}0}) \times 100\%$.[30]

2.7. Immunohistochemistry

Rats were anesthetized with 7% chloral hydrate solution (0.4 mL/100 g body weight) and perfused with 0.9% normal saline (100 mL/100 g) followed by 4% paraformaldehyde (pH = 7.2-7.4) via the ascending aorta. The brains were removed, immersed in 0.1 M phosphate buffered saline (PBS) containing 30% sucrose overnight, sectioned at 30 μm on a cryostat (Leica CM 3050, Frankfort, Germany), treated with 1% H_2O_2 in PBS for 30 minutes, washed four times in PBS, transferred to blocking medium (1% normal goat serum in 0.1 M PBS plus 1% Triton X-100), incubated with primary antibody (1:1000, rabbit anti-serotonin transporter; Merck KGaA, Germany) overnight at 4°C, washed, incubated with secondary antibody (1:200, biotinylated anti-rabbit IgG; Vector Laboratories, Burlingame, CA) for 1 hour at room temperature, washed, treated with avidin-biotin-peroxidase complex (1:200, Vector) for 1 hour, washed, treated (3-5 minutes) with 0.05% 3,3'-diaminobenzidine tetrahydrochloride (in 0.05 M Tris buffer), washed, and mounted on gelatin-coated slides.

The optical density (OD) of SERT fibers in each brain region was measured as previously reported[31–33] with minor modifications. For each brain region, three sections were selected at one-section intervals from six consecutive sections and imaged using a color CCD camera coupled with a microscope (MICROPHOT-FXA; Nikon, Tokyo, Japan). The images were converted to 8-bit gray scale (0-255 gray levels) and Image-Pro Plus v. 6.0 (Media Cybernetics, Inc., Bethesda, MD) image analysis software was used to determine the OD of SERT immunoreactivity. The OD ratio of the target region was expressed relative to the reference region (corpus callosum, which is devoid of SERT) as (OD of target region – OD of corpus callosum)/OD of corpus callosum.

2.8. Data analysis and statistical assessment

All data are expressed as mean ± SEM. Two-way analysis of variance (ANOVA) and the post-hoc Bonferroni multiple comparisons test were used to analyze the results for CPP and locomotor activity. The SURs were analyzed by three-way ANOVA with the post-hoc Bonferroni test. Two-way ANOVA and the post-hoc Bonferroni test were used to analyze the OD ratios of SERT immunoreactivity. Differences were considered to be significant at $p < 0.05$.

3. RESULTS

3.1. CPP test

3.1.1. MDMA significantly increases CPP

The place preference for the drug-paired compartment was calculated by subtracting the time spent in the saline-paired compartment from the time spent in the drug-paired compartment. As shown in Fig. 2A, the control group exhibited no difference in place preference for the drug-paired compartment before conditioning on day 1, after conditioning on day 9, or after the withdrawal period on day 14. After the conditioning period of administration of MDMA for 6 days, CPP markedly increased for the MDMA-paired compartment on day 9 compared to day 1 (***$p < 0.001$ vs control group). After 4 days of withdrawal from MDMA, the rats still exhibited significant place preference for the MDMA-paired compartment on day 14 (***$p < 0.001$ vs control group). We also observed a time-dependent increase in place preference in the MDMA group (from day 1 to day 15, $p < 0.001$).

3.1.2. Co-administration of DM suppresses MDMA-induced CPP

As shown in Fig. 2A, the place preference was significantly lower in the MDMA + DM group on day 9 and day 14 compared the MDMA group ($p < 0.001$). DM alone did not significantly affect place preference at any timepoint.

3.1.3. Posttreatment with 20 mg/kg DM (but not 10 mg/kg DM) decreases MDMA-induced CPP

After MDMA conditioning, posttreatment with 10 mg/kg DM continuously for 4 days (from day 9 to day 13) during the withdrawal period had no effect on place preference; however, 20 mg/kg DM significantly reduced place preference on day 14 compared to day 9 (Fig. 2B).

3.2. Locomotor activity test

3.2.1. DM suppresses MDMA-induced behavioral sensitization

As shown in Fig. 3A, acute administration of MDMA on day 2 significantly increased total and locomotor activities compared to day 0, while conditioning with MDMA for 6 days enhanced MDMA-induced time-dependent behavioral sensitization on day 15. Co-administration of MDMA with DM in the conditioning and withdrawal periods markedly reduced locomotor activities on day 15 compared to the MDMA group (Fig. 3A, B, $p < 0.05$-0.001). DM alone did not significantly affect locomotor activities (Fig. 3A–D). Moreover, postadministration of 20 mg/kg DM, but not 10 mg/kg DM, significantly reduced locomotor activities on day 15 ($p < 0.01$-0.001; Fig. 3B, D).

3.3. Animal PET imaging

3.3.1. Region-specific and time-dependent SERT recovery

Representative 4-[^{18}F]-ADAM PET images of the rat brain are shown in Fig. 4A (day 14) and Fig. 4B (day 42). In the control group, the hypothalamus showed the highest SERT uptake and other regions exhibited similarly high SERT accumulation on day 14 or day 42 (Fig. 4A, B left panel and Fig. 5 control group [white bar]). However, in rats pretreated with MDMA, brain uptake of 4-[^{18}F]-ADAM was significantly lower in all regions, except for the auditory cortex, compared to the control rats on day 14 (Figs. 4A and 5 MDMA group [red bar]; MDMA vs control $p < 0.05$-0.001). On day 42, after long-term abstinence from MDM4, the striatum and thalamus still exhibited significantly lower uptake of 4-[^{18}F]-ADAM; other brain regions showed similar trends, although these differences were not significant (Figs. 4B and 5 MDMA group [red bar]).

The SERT recovery rate at each time point was calculated after normalization to the control group. Fig. 6 shows that the MDMA group (red bar) appeared to reach self-recovery on day 14 (71.71% ± 5.92%) and that SERT recovery increased further by day 42 (87.64% ± 6.05%).

3.3.2. DM accelerates SERT recovery in a region-specific manner after MDMA-induced toxicity

Co-administration of DM with MDMA resulted in higher 4-[^{18}F]-ADAM uptake in the accumbens, cingulate cortex, medial prefrontal cortex, motor cortex, orbito-frontal cortex, midbrain, striatum, thalamus, and hypothalamus on day 14 compared to the MDMA group (Figs. 4A and 5 DM + MDMA group [blue bar]; DM + MDMA vs MDMA, $p < 0.05$-0.01). As shown in Fig. 6, DM (blue bar) enhanced the average recovery rate by ~23% at day 14 (MDMA 71.671% ± 5.92% vs MDMA + DM 94.83% ± 6.20%) and by ~17% at day 42 (MDMA 87.64% ± 6.05% vs MDMA + DM 104.44% ± 5.35%) compared with the

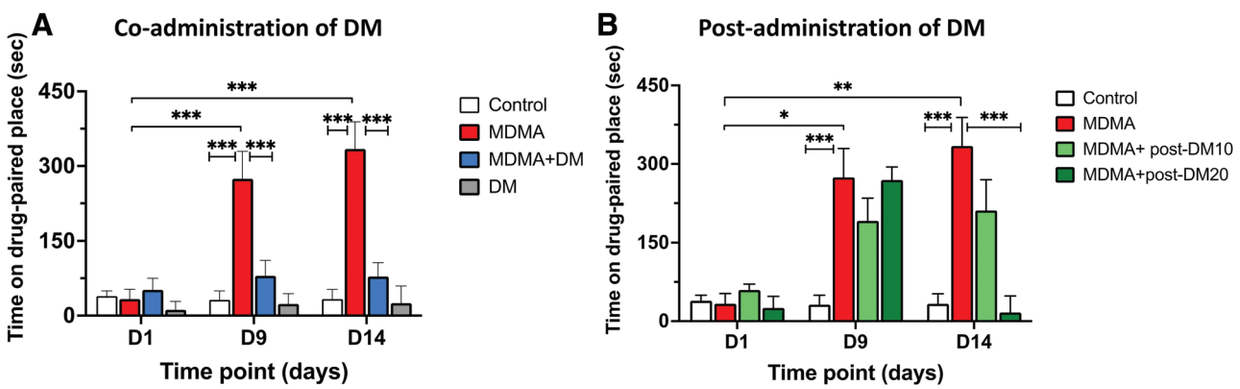

Fig. 2 Effects of co-administration or postadministration of DM on MDMA-induced reward behavior measured by the CPP test. The MDMA group exhibited a significant increase in the CPP index on day 9 (after conditioning) and day 14 (after withdrawal period) compared to the control group; co-administration of DM dramatically reduced the CPP index (A). Similar protective effects were observed in the group post-administered 20 mg/kg DM (but not 10 mg/kg DM) (B). Two-way ANOVA and the Bonferroni multiple comparisons test were used to analyze the data. Data are mean ± SD; *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$. ANOVA = analysis of variance; CPP = conditioned place preference; DM = dextromethorphan; MDMA = methylenedioxy-methamphetamine.

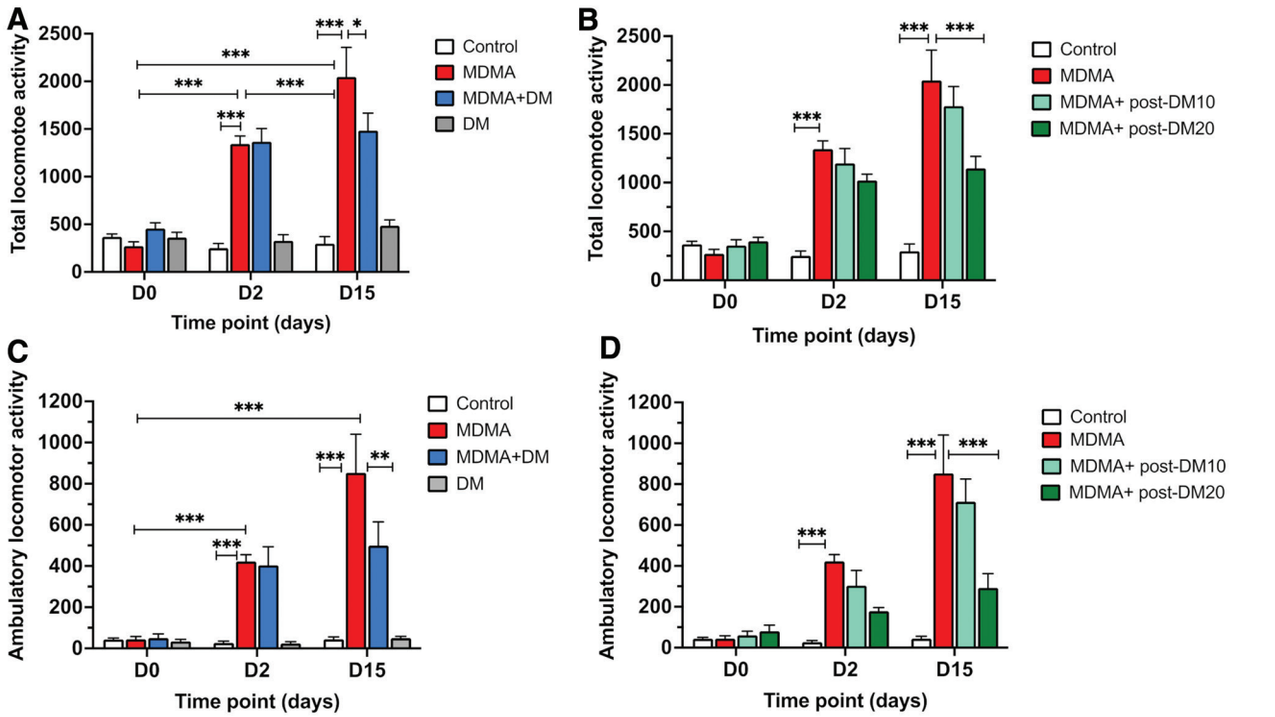

Fig. 3 Effects of co-administration or postadministration of DM on MDMA-induced behavioral sensitization measured by total locomotor activity (A, B) and ambulatory locomotor activity (C, D). Acute effects of MDMA were observed on day 2, as indicated by significant increases in total and locomotor activities compared to day 0. Chronic exposure of MDMA for 6 d enhanced MDMA-induced time-dependent behavioral sensitization at day 15. Co-administration of DM with MDMA markedly reduced locomotor activities at day 15 compared to the MDMA group. Similar protective effects were observed in the group post-administered 20 mg/kg DM with MDMA (but not 10 mg/kg DM). Two-way ANOVA and the Bonferroni multiple comparisons test were used to analyze the data. Data are mean ± SD; $^{**}p < 0.01$ and $^{***}p < 0.001$. ANOVA = analysis of variance; DM = dextromethorphan; MDMA = methylenedioxy-methamphetamine.

MDMA group ($p < 0.05$-0.01). The acceleration of the SERT recovery rate by DM appeared to region-specific.

3.3.3. DM does not affect SERT levels in the brain of animals not treated with MDMA

Pretreatment with DM alone slightly decreased 4-[^{18}F]-ADAM uptake in all brain regions. However, no significant changes in the SUR curves or recovery rate were observed in the DM group compared to the control group (Fig. 4A, B, right panel and Figs. 5 and 6 DM group [gray bar]).

3.3.4. DM attenuates MDMA-induced reward and SERT deficiency but does not affect locomotor activity

Compared to the controls (white circles), MDMA induction increased behavior conditioning place preference and locomotor activity but decreased PET SERT availability in all brain regions (red circles). In animals co-administered DM with MDMA (blue circles), the PET SERT availability values tended to increase (*y*-axis) and conditioning place preference reduced (*x*-axis, Fig. 7A); however, DM had no effect on the total or ambulatory locomotor activity in animals administered MDMA (*x*-axis; Fig. 7B, C).

3.4. Immunohistochemistry

3.4.1. Co-administration of DM with MDMA markedly increases the density of serotonergic fibers after MDMA induction

SERT was also localized using immunohistochemistry to validate the in vivo PET images. A dense meshwork of fibers and high densities of SERT labeling were observed in all brain regions (ie, dorsal raphe nucleus and median raphe nucleus) in the control group (Fig. 8, left panel). Strong, widespread heterogeneous distributions of SERT immunostaining were observed in the dorsal raphe (midbrain), hypothalamus, thalamus, striatum, and frontal cortex. In contrast to the control group, MDMA induction reduced the density of the SERT immunosignals in all brain regions (Fig. 8, second panel). However, co-administration of DM with MDMA attenuated these changes in SERT immunoactivity (Fig. 8, middle panel). Moreover, all brain regions of the DM group exhibited slight reductions in SERT immunoreactivity compared with the controls (Fig. 8, right panel).

In confirmation of the PET imaging results, quantitative analysis of SERT immunoreactivity revealed a significant decrease in SERT immunosignals in all brain regions in the MDMA-induced group compared to the control rats ($p < 0.01$-0.0001; Fig. 9). However, co-administration of DM significantly increased the SERT immunosignals in animals exposed to MDMA ($p < 0.01$-0.005; Fig. 9). Treatment with DM alone did not significantly affect SERT expression (Fig. 9).

4. DISCUSSION

We evaluated the neuroprotective effects of DM on MDMA induction by combining conditional place preference and locomotor activity behavioral tests with PET imaging using a selective SERT PET radiotracer. Acute administration of MDMA at day 1 significantly increased conditional place preference (CPP)-reward effect and locomotor sensitization. Chronic/conditioning administration of MDMA from day 3 to day 8 enhanced the reward effect, locomotor sensitization, and reduced SERT availability, in robust support of previous studies that reported MDMA altered behavior[34,35] or SERT binding.[30,36]

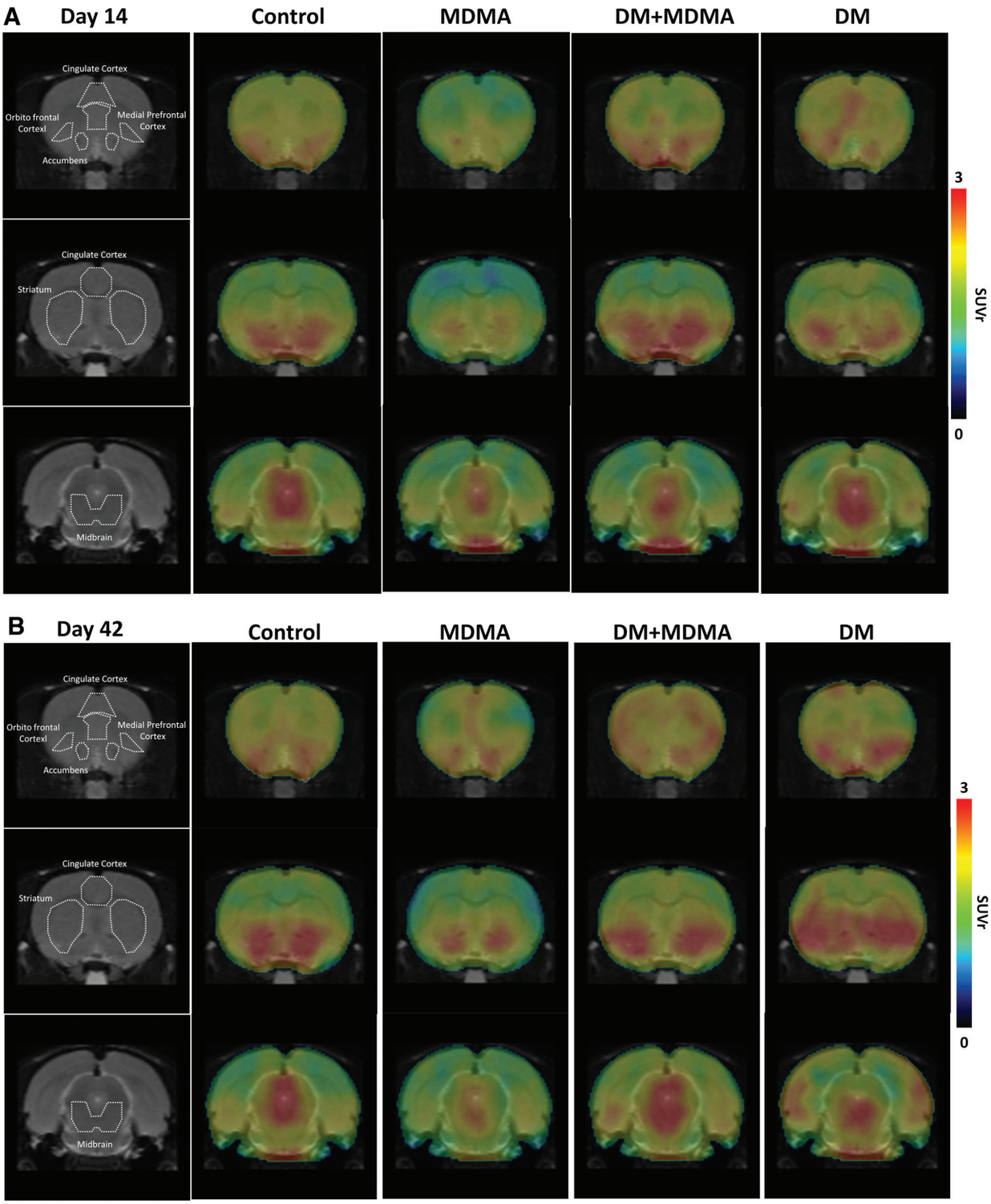

Fig. 4 Representative 4-[18F]-ADAM PET images illustration of brain areas of interest (ROIs) used to estimate SERT binding of 4-[^{18}F]-ADAM on (A) day 14 and (B) day 42 after MDMA induction. In control group, 4-[^{18}F]-ADAM binding to SERT in the motor cortex, cingulate cortex, auditory cortex, visual cortex, and thalamus, striatum, hippocampus, and anterodorsal hippocampus. The accumulations of 4-[^{18}F]-ADAM in above regions were significantly reduced in MDMA group as compared to the controls and gradually increased from day 14 to day 42 (MDMA group). Co-administration of DM with MDMA demonstrated significant increase in the accumulations of 4-[^{18}F]-ADAM (DM + MDMA) whereas DM alone had no effect on the uptake of 4-[^{18}F]-ADAM (DM group). DM = dextromethorphan; MDMA = methylenedioxy-methamphetamine; ROI = region of interest.

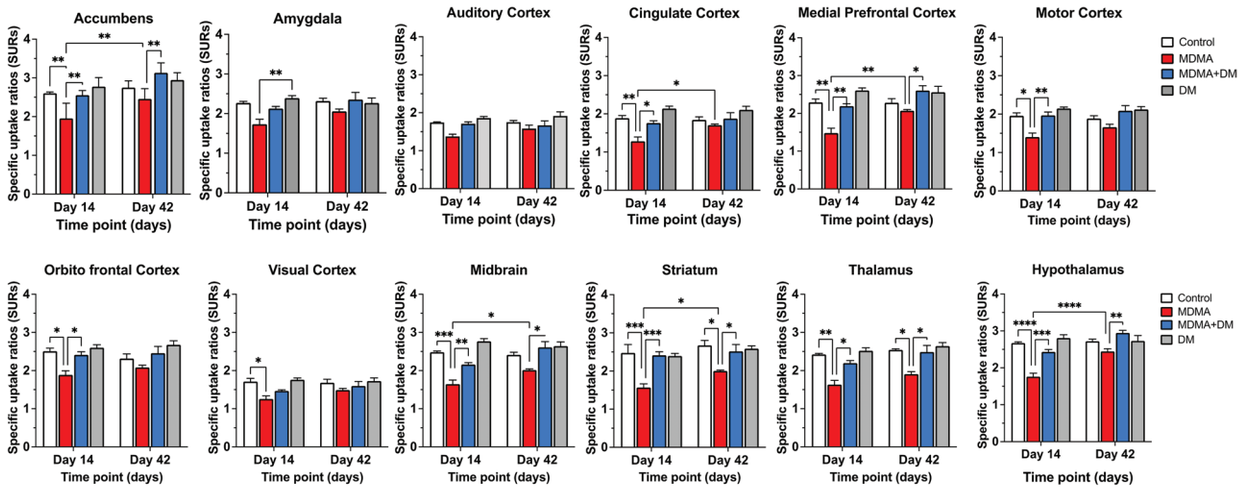

Fig. 5 SURs of 4-[^{18}F]-ADAM on day 14 and day 42 after MDMA induction. 4-[^{18}F]-ADAM distribution in different brain regions after administration of different drugs according to the study design. The MDMA group exhibited a significant reduction in 4-[^{18}F]-ADAM binding to SERT on day 14, which progressively increased by day 42 (red bar). Co-administration of DM with MDMA (blue bar) exerted neuroprotective effects on day 14 in most brain regions, expect the amygdala, auditory cortex, and visual cortex, compared to the MDMA group. After the withdrawal period, the therapeutic effects of DM were also observed in the accumbens, midbrain, striatum, thalamus, and hypothalamus. The DM alone group (gray bars) exhibited a slight increase in 4-[^{18}F]-ADAM binding to SERT on day 14 and day 42 compared to the control group. Data are mean ± SD; $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$. DM = dextromethorphan; MDMA = methylenedioxy-methamphetamine; SERT = serotonin transporter; SUR = specific uptake ratio.

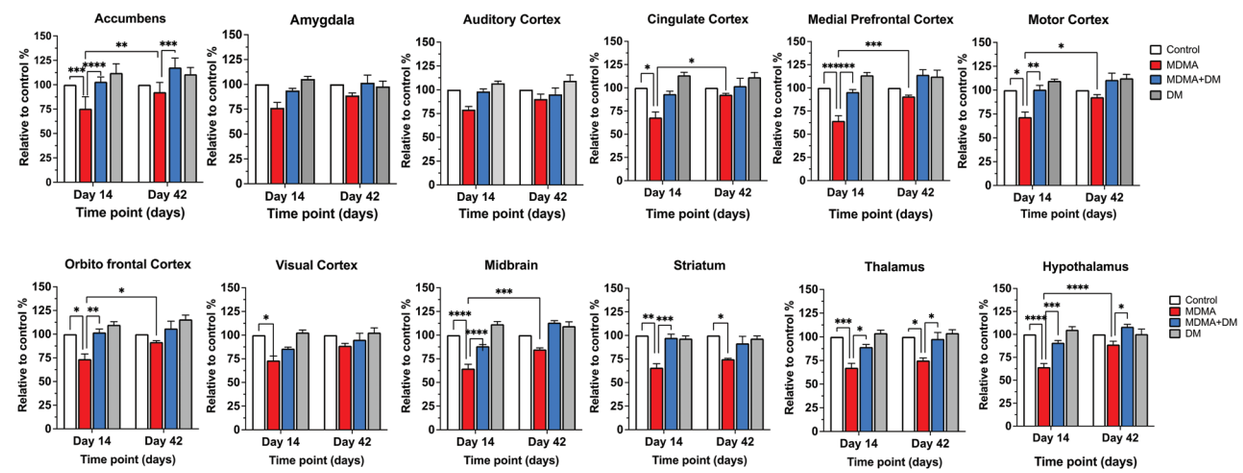

Fig. 6 Comparison of the recovery rate of 4-[^{18}F]-ADAM SERT binding before and after administration of DM. After normalization to the control group, the MDMA group (red bar) exhibited the lowest recovery rate at day 14, which slightly increased to ~88% by day 42. Administration of DM with MDMA (blue bar) significantly enhanced the recovery rate on day 14 (by ~23%) and day 42 (by ~17%). The trends in the curves of the control and DM alone (gray bar) groups were not significantly different. Data are mean ± SD; $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$. MDMA = methylenedioxy-methamphetamine.

We investigated the effects of MDMA on the reward effect and locomotor sensitization by examining the alterations in CPP and locomotor activity before and after MDMA administration (conditioning) and abstinence (withdrawal). Three-dimensional (3D) PET revealed the 4-[^{18}F]-ADAM PET uptake ratio varied in different brain regions involved in the reward system, including the accumbens, prefrontal cortex, cingulate, striatum, and orbito-frontal cortex. Analysis of the SERT recovery rates and SERT availability revealed MDMA-induced region-specific neurotoxicity in the rat brain that was directly proportional to the self-recovery rate. MDMA induction decreased the 4-[^{18}F]-ADAM PET uptake ratio in the regions associated with the reward system compared to the control group, in agreement with previous reports of MDMA-induced reward-related decision-making deficits.[37,38]

DM also attenuated MDMA-induced reward and drug-seeking. Co-administration of DM with MDMA significantly reduced MDMA-induced reward in the CPP test, consistent with our previous results for MA.[39] MDMA is an analog of MA, thus these drugs exert many of the same pharmacological effects in the CNS. This study confirmed that DM reduced MDMA-induced reward and drug-seeking, as previously observed for MA.[39] However, treatment for drug dependence, including MA and MDMA, is normally required after addiction is established by repeated use. Thus, DM could potentially be useful in the treatment of addiction. In this study, posttreatment with DM also reduced MDMA-induced drug-seeking behavior, although only the higher dose of 20 mg/kg (i.p.) was effective. We previously reported that posttreatment DM blocked morphine- and MA-induced drug-seeking behavior in rats in the CPP test.[19]

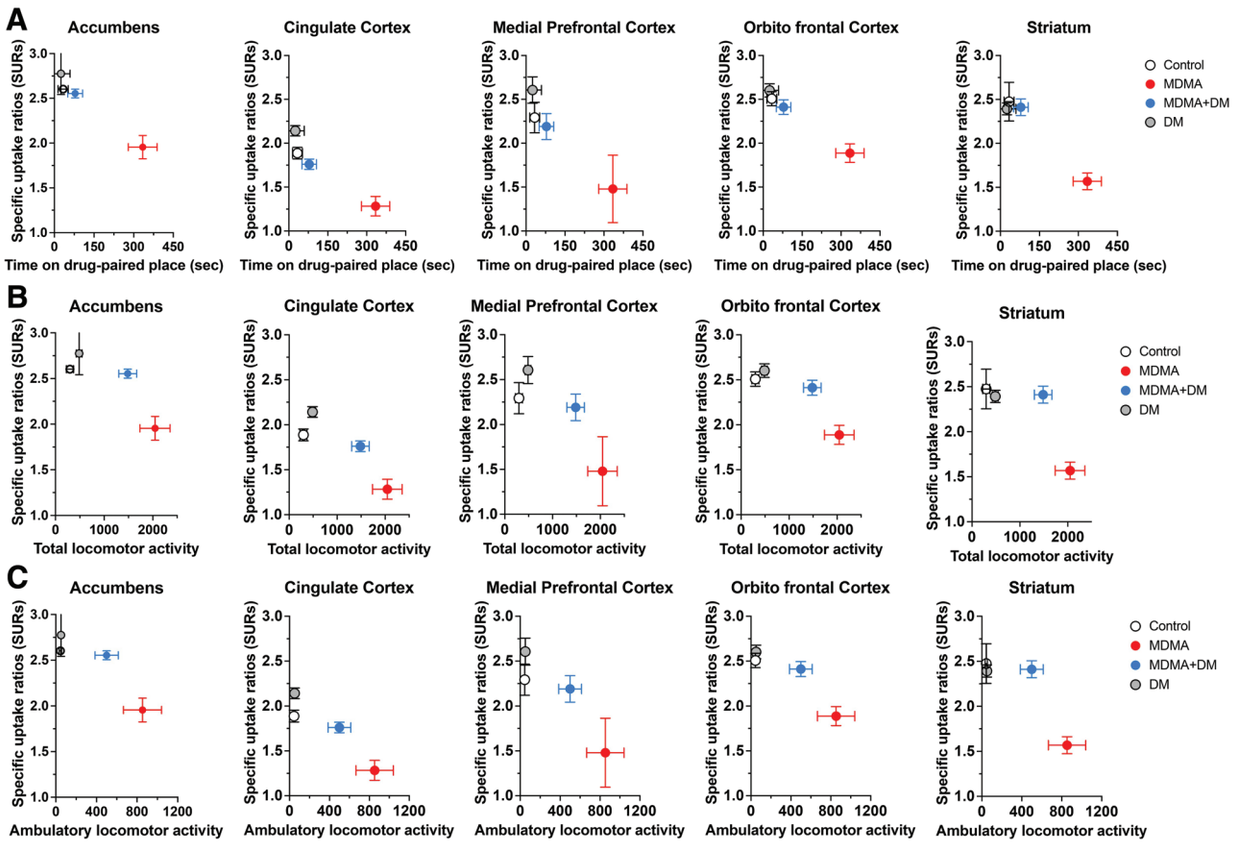

Fig. 7 Relationship between the SUVr determined by [^{18}F]-4-ADAM SERT PET (*y*-axis) and CPP or locomotor activity (*x*-axis). The reduction in [^{18}F]-4-ADAM SERT activity negatively correlated with the increases in the CPP index (A) or locomotor hyperactivity (B, C) after administration of MDMA (red cycle). Co-administration of DM with MDMA shifted this correlation left, as indicated by increased [^{18}F]-4-ADAM SERT activity and reductions in the CPP index (A) and locomotor activity (B, C). The distribution trends for the control and DM alone (gray bar) groups were not significantly different. CPP = conditioned place preference; DM = dextromethorphan; MDMA = methylenedioxy-methamphetamine; SERT = serotonin transporter; SUV = standardized uptake value.

Moreover, the findings of our MA experiments suggest a possible common mechanism by which administration of DM during withdrawal reduces seeking behavior for addictive drugs.[40]

The results of our behavioral experiments led to similar conclusions. Posttreatment with 20 mg/kg DM (but not 10 mg/kg DM) blocked behavioral sensitization of locomotor hyperactivity. Behavioral sensitization was initially presumed to be mediated by the mesocorticolimbic dopaminergic system,[41] the same neuronal system that is thought to mediate reward effects.[42,43] However, the noradrenergic and serotoninergic systems can also be sensitized by repeated administration of amphetamine or psychostimulants.[44,45] In terms of activation of the monoamine systems, behavioral sensitization may serve as another model to identify and quantify drug dependence, especially psychostimulant dependence. The ability of both co-administration and posttreatment with DM to reduce MDMA-induced behavioral sensitization demonstrate the potentially beneficial effects of DM on MDMA dependence. Nevertheless, posttreatment DM attenuated MDMA-induced behavioral sensitization, but did not affect morphine-induced behavioral sensitization in a previous study.[19] Behavioral sensitization is recognized to be related to sensitization of the mesolimbic dopaminergic pathway or other monoamine neural pathways; however, morphine initiates sensitization via disinhibition of GABAergic neurons at the ventral tegmental area (VTA), whereas psychostimulants (MA and MDMA) mainly act at the dopaminergic terminals, for example, nucleus accumbens (NAc). This may be one factor that possibly explains the different actions of DM; however, the precise details of the mechanisms underlying these differences require further study.

In addition to the targets mentioned above, DM could also act as a SERT blocker,[46] which could also explain the effects of DM on MDMA-induced drug seeking and behavioral sensitization. MDMA significantly impacts the serotoninergic system; thus, it is possible that DM could increase serotonin release by blocking its reuptake and thereby reverse the behavioral changes induced by MDMA. In support of this suggestion, Trigo et al[46] reported an absence of MDMA self-administration by SERT knock-out mice. Acute administration of MDMA could significantly increase locomotor activity, which could be blocked by 5-HT$_{2A}$ and 5-HT$_{1B}$ antagonists or 5-HT$_{2B}$/$_{2CR}$ antagonists.[47–49] Interestingly, Ramos et al[50] demonstrated that blockade of the expression (not the induction) of MDMA-induced behavioral sensitization by SCH 23390 (a D1 antagonist and 5-HT$_{2C}$ agonist) was mediated by 5-HT$_{2C}$ receptor activation in the medial prefrontal cortex (mPFC) and not by blockade of mPFC D1 receptors. This implies DM may possibly block mPFC SERT by increasing activation of the 5-HT$_{2C}$ receptor to reduce behavioral sensitization.

We previously used a different dose regimen of MDMA (5 mg/kg subcutaneously, twice a day for 4 consecutive days) to study the protective effects of DM in a non-human primate model. In vivo SERT imaging showed the MDMA-induced decrease in

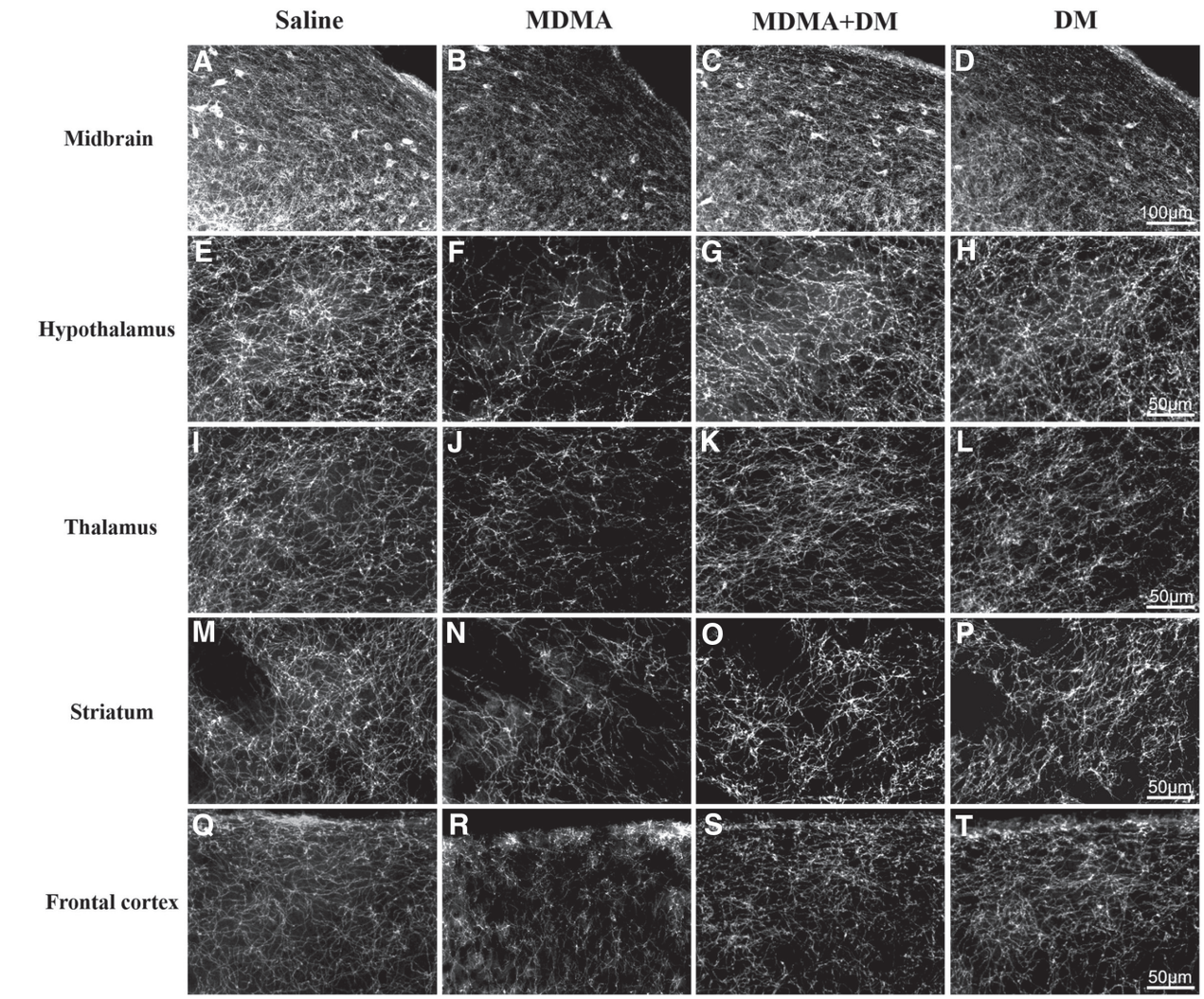

Fig. 8 Dark-field photomicrograph of SERT-positive fibers in different regions of the brain after drug treatment. SERT immunoreactivity was significantly lower in the MDMA group and almost equal in the control, MDMA + DM, and DM alone groups. DM = dextromethorphan; MDMA = methylenedioxy-methamphetamine; SERT = serotonin transporter.

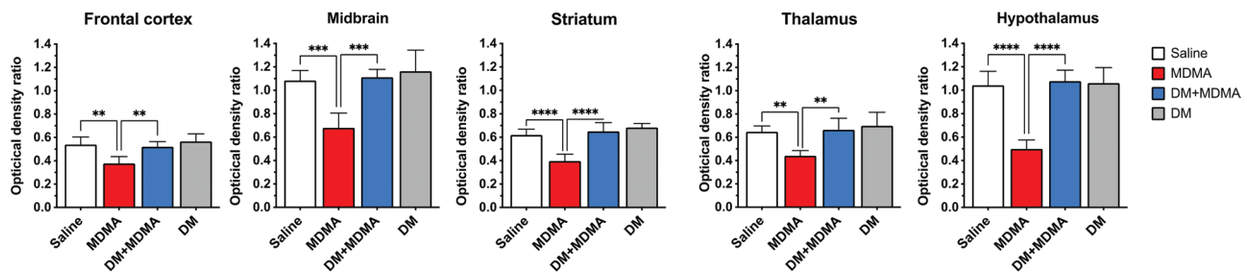

Fig. 9 Quantification of the optical density ratios of SERT immunoreactivity. Data are mean ± SD. Superscript letters indicate significant differences; $^{**}p < 0.01$, $^{***}p < 0.005$, and $^{****}p < 0.001$. SERT = serotonin transporter.

central SERT levels persisted for over 48 months. [^{123}I]ADAM single-photon emission computed tomography (SPECT) demonstrated MDMA-treated monkeys had lower brain SERT levels and that DM protected against these MDMA-induced serotonergic aberrations.[51] We further investigated MDMA-induced serotonergic deficiency in primates at 60 and 66 months after drug administration. 3D 4-[^{18}F]-ADAM PET/CT/MRI revealed MDMA-induced serotonergic deficiency may be region-specific, as indicated by significantly lower 4-[^{18}F]-ADAM binding ratios and recovery rates in most brain regions. DM exerted a significant neuroprotective effect in terms of SERT activity in the hippocampus and amygdala at 66 months after MDMA exposure but did not protect against MDMA-induced changes in brain volume.[18]

In this study, the dose regimen was 5 mg/kg MDMA subcutaneously, once a day for 6 successive days. Co-administration of DM (10 mg/kg, i.p.) with MDMA significantly increased SERT levels in the brain regions associated with the reward system and decreased MDMA-induced reward. In confirmation of the PET imaging, immunochemical staining revealed lower densities of serotonergic fibers and cell bodies on day 14 post-MDMA. Thus, the findings of the current rat model are similar to those of our previous primate study. The orbito- and middle-frontal cortex and striatum, important neural structures involved in decision-making, are associated with the reward system and play a role in addictive disorders.[52,53]

Additionally, in this study, the indexes of CPP and locomotor activities of the reward system correlated negatively with PET 4-[^{18}F]ADAM SERT activity in the same regions. These findings suggest that MDMA induces functional abnormalities in a network of brain regions that are important in decision-making processes and motive circuits. Moreover, MDMA is believed to activate 5-HT pathways and co-activate dopaminergic pathways.[54,55] In addition, co-administration of MDMA with DM rapidly blocked MDMA-induced global serotonin release and neurotoxicity and increased SERT levels (94.83% ± 6.20%) at day 14; and progressively increased the SERT recovery rate to ~95% of controls at day 42. In our previous studies, the SSRI fluoxetine restored the SERT binding rate to ~79.6% of control levels at day 31 post-MDMA (10 mg/kg, twice per day for 4 days, i.p.),[56] whereas the SERT inhibitor amitriptyline increased the recovery rate by 84.38% ± 2.05% at day 28 (5 mg/kg MDMA, twice per day × 4 days, i.p.).[30] Thus, in contrast to the SSRI or SERT inhibitor, the NMDA receptor antagonist DM completely or partially reduced the reward associated with addictive drugs; however, the mechanisms underlying the ability of DM to act as an antidote to MDMA require further research.

In conclusion, SERT recovery positively correlated with the duration of MDMA abstinence, implying that the lower SERT densities in MDMA-induced rats reflect neurotoxic effects, which are region-specific and reversible. Moreover, DM may exert neuroprotective effects on behavior indexes and globally accelerate the SERT recovery rate. The mechanisms underlying the ability of DM to act as an antidote for MDMA require further investigation.

ACKNOWLEDGMENTS

This work was supported by grants from the National Science Council (NSC97-3112-B-010-016, NSC99-2314-B-016-030-MY3, NSC99-2314-B-016-027-MY3), Tri-Service General Hospital, National Defense Medical Center (TSGH-C105-067, TSGH-D-112090, and TSGH-E-113240), Medical Affairs Bureau of the Ministry of National Defense, Taiwan (MND-MAB-112157) and the National Health Research Institutes (NHRI-EX98-9401NP and 02A1-NPPP01-021), Taiwan, ROC.

We are grateful to Mr. Ta-Kai Chou for his excellent technical assistance. We also acknowledge the technical support provided by the Molecular and Genetic Imaging Core.

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