Long-lasting alterations in 5-HT$_{2A}$ receptor after a binge regimen of methamphetamine in mice

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Abstract

The repeated administration of methamphetamine (MA) to animals in a single-day ‘binge’ dosing regimen produces damage to dopamine and serotonin terminals and psychosis-like behaviours similar to those observed in MA abusers. The present study aimed to examine the effects of MA binge exposure on 5-HT$_{2A}$ receptors, the subtype of serotonin receptors putatively involved in psychosis. ICR male mice were treated with MA (4 × 5 mg/kg) or saline at 2 h intervals. Recognition memory and social behaviours were sequentially evaluated by a novel location recognition test, a novel object recognition test, a social interaction and a nest-building test to confirm the persistent cognitive and behavioural impairments after this dosing regimen. Subsequently, a hallucinogenic 5-HT$_{2A/C}$ receptor agonist 2,5-dimethoxy-4-iodoamphetamine (DOI)-induced head-twitch, molecular and electrophysiological responses were monitored. Finally, the levels of 5-HT$_{2C}$, 5-HT$_{1A}$, 5-HT$_{2A}$ and mGlu2 receptors in the medial prefrontal cortex were determined. MA binge exposure produced recognition memory impairment, reduced social behaviours, and increased DOI-induced head-twitch response, c-Fos and Egr-2 expression and field potentials in the medial prefrontal cortex. Furthermore, MA binge exposure increased 5-HT$_{2A}$ and decreased mGlu2 receptor expression in the medial prefrontal cortex, whereas 5-HT$_{2C}$ and 5-HT$_{1A}$ receptors were unaffected. These data reveal that the increased behavioural, molecular and electrophysiological responses to DOI might be associated with an up-regulation of 5-HT$_{2A}$ receptors in the medial prefrontal cortex after MA binge exposure. Identifying the biochemical alterations that parallel the behavioural changes in a mouse model of MA binge exposure may facilitate targeting therapies for treatment of MA-related psychiatric disorders.

Keywords: 5-HT$_{2A}$ receptor, methamphetamine, psychosis.

Introduction

Methamphetamine (MA) is a globally popular and highly addictive drug. Prolonged MA use results in dependence and psychosis that is typically characterized by paranoid delusions and auditory hallucinations (Yui et al., 2000; Jacobs et al., 2008; Mahoney et al., 2008) and often associated with disturbances in mood and cognitive function (Bechara and Martin, 2004; Moon et al., 2007; Scott et al., 2007). Furthermore, the density of dopamine transporters (DAT) in the striatum and frontal cortex (Volkow et al., 2001; Sekine et al., 2003; McCann et al., 2008) and serotonin reuptake transporter (SERT) in the orbitofrontal and occipital cortices (Kish et al., 2009) are significantly decreased in chronic users of MA.

A single-day multiple-dose MA exposure produces long-lasting decreases in markers of dopaminergic and serotonergic terminals, such as tyrosine hydroxylase, DAT and SERT (O’Dell et al.; Schroder et al., 2003; Marshall et al., 2007; Belcher et al., 2008) as well as persistent behavioural deficits including cognitive impairment in several domains of learning and memory (O’Dell et al.; Bisagno et al., 2002; Schroder et al., 2003; Izquierdo et al., 2010) and decreased social interaction (Clemens et al., 2004). This binge-like regimen appears to induce behavioural manifestations and neurochemical changes like those observed in MA abusers although it does not fully mimic human abuse patterns of intake on the first occasion.

The alterations in the receptor density or function have been anticipated following damage to the dopaminergic and serotonergic terminals. It has been reported that...
animals exposed to a binge regimen of MA known to damage monoaminergic systems could enhance the non-selective dopamine agonist apomorphine-elicited stereotypic behavioural response (Wallace et al., 2001), while reducing the apomorphine-induced cortical and subcortical immediate early gene, c-fos and Jun-B, expression (Belcher et al., 2009), demonstrating profound and enduring effects of MA on dopaminergic function. However, little is known about the consequences of a binge regimen of MA exposure on serotoninergic receptor expression and function.

Of all the serotonin receptors, the 5-HT2A receptor is the one most closely linked to complex behaviours and neuropsychiatric disorders. There has been extensive research performed to establish the role of the 5-HT2A receptor within the brain, where it has been shown to participate in processes such as cognition and working memory (Williams et al., 2002), mediate the primary effects of hallucinogenic drugs (Vollenweider et al., 1998; Gonzalez-Maeso et al., 2007) and be implicated in mechanisms underlying schizophrenia (Aghajanian and Marek, 2000). In fact, hallucinations and cognitive impairment are the typical clinical symptoms of MA psychosis. Therefore, the present study has focused on the changes in 5-HT2A receptors after a binge regimen of MA.

After the behavioural deficits in MA-exposed mice were confirmed by a novel location recognition test, a novel object recognition test, a social interaction test, and a nest-building test, the 5-HT2A agonist 2,5-dimethoxy-4-iodoamphetamine (DOI)-induced head-twitch behaviour was examined. The head-twitch response in mice provides a behavioural proxy of a hallucinogenic response (Willins and Meltzer, 1997; Gonzalez-Maeso et al., 2003) since the hallucinogenic drugs that induce hallucinations in humans produce a head-twitch response in mice. Furthermore, the 5-HT2A receptor-associated molecular and electrophysiological responses were measured by DOI-induced immediate early genes c-Fos and Egr-2 expression and DOI-evoked field potentials in the medial prefrontal cortex of brain slices.

The DOI-induced head-twitch response has been reported to be strongly modulated by 5-HT1A (Willins and Meltzer, 1997; Fox et al., 2010), 5-HT3C (Canal et al., 2010; Fantegrossi et al., 2010) and mGlu2 receptor activities (Moreno et al., 2011a). After finding the long-lasting enhancement of DOI-induced responses after MA binge exposure, the protein levels of 5-HT2A, 5-HT3C, 5-HT1A and mGlu2 receptors in the medial prefrontal cortex were examined. Finally, the dopaminergic neurotoxicity by MA binge exposure was verified.

**Materials and method**

**Animals and drugs**

Male ICR mice (8–9 weeks, 33–40 g) were supplied from the BioLasco Taiwan (under Charles River. Laboratories Technology License) and housed 4–5 per cage in a 12 h light/dark cycle with ad libitum access to water and food. The experimental protocol was approved by the Review Committee of the Tzu Chi University and the National Health Research Institute for the Use of Animals. (+)-Methamphetamine hydrochloride was obtained from the Food and Drug Administration, Department of Health, Executive Yuan, Taipei, Taiwan. DOI was from Sigma Chemical Company (USA). Mice received four 5 mg/kg injections (s.c.) of MA or saline at 2 h intervals in their home cage. The timeline of experimental procedures is shown in Fig. 1.

**Novel location/object recognition test**

The experimental apparatus consisted of a Plexiglas open field box (50×50×25 cm) located in a sound-attenuated room and illuminated with a 20-W light bulb. The novel location/object recognition procedure consisted of habituation, training and retention sessions. A video camera recorded behaviour during the sample and testing phases. Habituation was conducted in three consecutive daily sessions, during which each mouse was allowed to individually explore the box without objects for 10 min. During the sample phase, each animal was placed in the box, and after 5 min, two identical objects were simultaneously introduced in two corners. Each animal was allowed to explore the objects for 5 min. An animal was considered to explore the object when its head was facing the object at a distance of approximately 1 cm or less between the head and object or when it was touching or sniffing the object. After the sample phase, the mice were immediately returned to their home cages for a 30 min retention period and then reintroduced to the arena for the 5 min testing phase in which one object had been moved to a different location to perform novel location recognition test. The time spent exploring each object in both phases was recorded using stopwatches by an experimenter blind to the treatment condition.

The novel object recognition test was conducted 24 h after the sample phase. The animals were returned to the same box as during the sample phase and one of the two objects of the sample phase was replaced with a novel object. The animals were allowed to explore the box freely for 5 min, and the time spent exploring each object was recorded as described above. The preference index in the testing phase was defined as the ratio of

**Fig. 1.** Experimental timeline. NLRT: novel location recognition test; NORT: novel recognition test object.
the amount of time spent exploring the object in novel location or novel object and the total time spent exploring both objects was used to evaluate recognition memory. In the sample phase, the preference index was defined as the ratio of the time spent exploring the original object that was replaced in the retention session and the total exploration time.

**Social interaction test**

This protocol was adopted for evaluation of negative schizophrenic symptom-like behaviours, which was modified from the original social interaction test in that aggressive behaviours (biting, boxing) and passive contact (sitting or lying with bodies in contact) were not included in the social interaction score (Qiao et al., 2001). The social interaction between pairs of mice was examined in an open-field box (35 × 35 × 30 cm) under normal room lighting.

Every mouse was randomly assigned to an unfamiliar partner in the same treatment group. That is, a MA-treated mouse was paired with another unfamiliar MA-treated mouse, and a saline-treated mouse was paired with another unfamiliar saline-treated mouse. Each pair of unfamiliar mice was placed in the apparatus for 10 min and the time that a pair spent in social interaction (sniffing and grooming the partner, following, mounting and crawling under or over the partner) was recorded by an observer who was blind to the drug treatments.

**Nest-building experiment**

Approximately 1 h before the dark phase, the group-housed mice were transferred into individual cages. A standard piece of paper towel (23 cm × 23 cm) was placed in each cage overnight. The nests were assessed the next morning using a scoring system described previously (Keisala et al., 2007): 0 = no nest, 1 = primitive flat nest, 2 = more complex nest (wrapping and biting the paper), 3 = complex accurate cup-shaped nest (walls and shredded paper) and, 4 = complex hooded nest. The amount of paper damage was also assessed, using the following scale: 0 = intact paper or little damage (< 5% paper destroyed), 1 = some paper damage (5–20%), 2 = pronounced paper damage (20–40%) and, 3 = severe paper damage (> 40%).

**DOI-induced head twitch response**

Mice were transferred to the observation room and allowed to habituate for 30 min. Mice were administered with DOI (1 mg/kg, i.p.) or vehicle (saline), and then placed in a cylindrical glass container. The number of head-twitch responses (rapid movements of the head with little or no involvement of the trunk) was counted for 30 min following DOI administration by the observer (blind to the treatment).

**Immunohistochemical analysis for DOI-induced c-Fos and Egr-2 expression**

Sixty minutes after DOI administration, mice were deeply anesthetized with thiamylal sodium (150 mg/kg, i.p.) and then perfused transcardially with 0.1 M phosphate-buffered saline (PBS), and then with 0.1 M PBS containing 4% paraformaldehyde. The brains were removed and post-fixed for 24 h and then stored in 30% sucrose solution. The brains were frozen and cut into 25 μm thick coronal sections in a microtome. Free-floating sections were processed for c-Fos and Egr-2 immunohistochemistry. The sections were incubated for 30 min in 1% H2O2 to inactivate endogenous peroxidase and decrease nonspecific staining. Sections were washed three times (10 min per wash) in PBS containing 0.3% Triton X-100 and 2% BSA. Blocking was performed with 10% BSA for 1 h and then incubated on a rocking table with c-Fos (1:1000, Cat# Sc-52, Santa Cruz Biotechnology Inc., USA) or Egr-2 (1:200, Cat # ab90518, Abcam Inc., USA) polyclonal antibody overnight at 4 °C. Sections were washed again three times in PBS prior to being incubated with the biotinylated anti-rabbit IgG (Sigma, 1:200) overnight at 4 °C. The sections were then washed three times in PBS buffer, and incubated with 0.2% avidin-biotinylated horseradish peroxidase complex (ABC solution, Vector Laboratories, USA) for 1 h. After three 10 min washes in PBS, the sections were placed in the chromogen 3, 3′-diaminobenzidine tetrahydrochloride (DAB, Sigma) for 15 min. The sections were again washed three times in PBS, mounted on gelatinized slides, air-dried overnight and cover-slipped.

c-Fos and Egr-2 immunoreactivity was observed in the cell nuclei and brain sections showed brownish round dots under a light microscope. According to the atlas (Paxinos and Franklin, 2004), the coronal sections (+1.70 mm relative to bregma) including the infralimbic cortex (IL) and motor cortex area 2 (MC2) were defined and analysed. Bright-field images were captured with a digital camera mounted on a Nikon Eclipse 800 microscope at 200× magnification. An image of 0.12 mm² area was analysed for the number of c-Fos and Egr-2 immunoreactive positive cells. Cell counts were made with the help of Image J and manually counted by an observer who was blind to group arrangement. For each brain area, data were obtained from three slices per mice (n = 4).

**Preparation of prefrontal cortex slices for electrophysiological recordings**

The brains of control or MA-exposed mice were removed and immersed in an ice-cold artificial cerebrospinal fluid (ACSF) of the following composition (in molar): NaCl (127), KCl (1.9), CaCl2 (2.4), MgCl2 (1.3), NaHCO3 (26), KH2PO4 (1.2) and D-glucose, bubbled with a mixture of 95% O2/5% CO2, pH 7.4. Coronal slices (300 μm) were cut from the frontal cortex (3–5.16 mm anterior to bregma) using a vibrating tissue slicer. After recovery for at least
1 h at room temperature, a single slice was transferred to the centre area of the coated MED probe and positioned to cover the 8×8 micro-electrode array by a paint brush. The positioned slice was superfused at 2.0 ml/min with ACSF saturated with O2.

**Electrophysiological recordings**

For electrophysiological recordings, the MED probe containing the brain slice was placed in a small incubator for 8 h at room temperature. The preparation of the multi-electrode dish has been described previously (Chen et al., 2011; Lee et al., 2014). Briefly, the MED probe is an array of 64 planar micro-electrodes where each micro-electrode has a size of 50×50 μm and is arranged in an 8×8 pattern. The interporal distance in this type of probe (MED-P515A) is 150 μm. For sufficient adhesion of the brain slice to the MED probe, the surface of probe was treated with 0.1% polyethylenimine in 25 mM borate buffer for 8 h at room temperature. The probe surface was then rinsed three times with distilled water for future experiments.

The field potentials at 8 sites in the 64 multi-electrode probe were recorded simultaneously with the multi-channel recording system at a 20 kHz sampling rate. The electrodes in the medial prefrontal cortex were selected as the recording electrodes. In order to prevent the sodium-channel-mediated spontaneous components, all the experiments were performed with 0.3 μM TTX. The recording of baseline field potentials was first carried out in the absence of any chemical stimulation for 10 min to establish a baseline. To examine the DOI-evoked field potentials, DOI (1 μM) was applied for 7 min. The field potentials were quantified by averaging over the last 5 min of baseline and DOI-evoked recording.

**Tissue extraction and Western blot**

Mice were rapidly decapitated and brains removed 1 h after DOI treatment. The medial prefrontal cortex from each mice were collected immediately and stored at −80 °C until use. The tissues were homogenized and the concentration of protein in the homogenate was measured using bovine albumin as standard. Equal amounts of protein (20 or 30 μg) were resolved using SDS-PAGE and transferred to nitrocellulose (NC) membrane (BioRad, USA). The membrane was blocked with 5% w/v powdered milk/Tris-buffered saline/Tween-20 and probed with antibodies for 5-HT1A (1:1000, Cat # ab85615, Abcam), 5-HT2A (1:400, Cat # ab66049, Abcam.), 5-HT2C (1:500, Cat # sc-15081, Santa Cruz), mGluR2 (1:1000, Cat # ab15672, Abcam) and tyrosine hydroxylase (1:500, Cat # ab137721, Abcam), overnight at 4 °C. After washing, the membranes were incubated with secondary anti-rabbit (or mouse) antibody (1:3000–1:5000, Santa Cruz Biotechnology Inc.) conjugated to horseradish peroxidase, which was measured with Western Lightning Plus-ECL reagent (PerkinElmer Inc., USA). Protein signals were captured and the intensity of immunoreactive bands of interest was quantified by BioSpectrum 500 Imaging System (UVP, UK). After detecting, the NC membranes were washed three times for 10 min each in TBS-T. NC membranes were stripped with stripping buffer (20.8 mM Tris-HCl, pH 6.8, 32.6 mM 2-mercaptoethanol, 22.9 mM SDS); the stripped membranes were then re-probed with anti-actin.

**Statistical analysis**

Data were obtained and expressed as mean±S.E.M. The significant difference between control and MA-exposed groups was determined using a Student’s t-test. Where appropriate, for example with scaled data from the primary behavioural observations, non-parametric analyses were conducted using a Mann-Whitney test. The head-twitch response and immunoreactive cells of c-Fos and Egr-2 in response to DOI were analysed by two-way ANOVA. The data for the recording of field potentials evoked by DOI were analysed by a mixed designed ANOVA. The Newman-Keuls test was used for post-hoc comparisons. The level of statistical significance was set as p<0.05.

**Results**

**Novel location/object recognition test**

Seven days after a binge regimen of MA, mice were tested by a novel location recognition test and a novel object recognition test. As shown in Fig. 2a, there was no difference in the preference index in the sample phase between MA-treated and control mice, whereas MA treatment produced a significant reduction in preference index in the testing phase of the novel location recognition test (p<0.01) and novel object recognition test (p<0.01).

**Social interaction test**

Two days after the novel object recognition test, social interaction was assessed between treatment-matched pairs derived from different cages. Control mice exposed to a novel conspecific exhibited typical behaviours of sniffing, following and climbing, however, the counts of following were profoundly decreased in MA-treated mice (Fig. 2b). In addition, the total contact time in 5 min was significantly reduced after MA exposure (Fig. 2c).

**Nest-building test**

Nest building was examined three days after the social interaction test. MA-exposed mice showed significantly lower nest scores (p<0.01) and a modest reduction of paper damage scores compared to control mice (Fig. 2d).
DOI-induced head twitch response

DOI-induced head-twitch response was conducted three days after the nest-building test. As shown in Fig. 3a, DOI remarkably elicited a head-twitch response, whereas saline scarcely produced a head-twitch. A two-way ANOVA revealed that there are significant main effects for MA and DOI (MA: $F_{1,28} = 20.9, p < 0.001$; DOI: $F_{1,28} = 462.9, p < 0.001$) and MA × DOI interaction ($F_{1,28} = 20.5, p < 0.001$). Post-hoc analysis indicated that DOI-induced head-twitch response was significantly increased in MA-exposed mice compared with control mice.

DOI-induced c-Fos and Egr-2 expression

Our previous study demonstrated that DOI could induce c-Fos and Egr-2 expression in several brain regions including the infralimbic cortex (IL), a cortical region in the ventromedial prefrontal cortex, and the motor cortex (Lee et al., 2014). Two-way ANOVAs revealed that there were significant effects of MA and DOI and MA × DOI interaction on the expression levels of c-Fos (motor cortex: MA: $F_{1,12} = 15.66, p < 0.01$, DOI: $F_{1,12} = 363.33, p < 0.001$, MA × DOI: $F_{1,12} = 5.46, p < 0.05$; infralimbic cortex c-Fos: MA: $F_{1,12} = 16.63, p < 0.01$, DOI: $F_{1,12} = 694.44, p < 0.001$, MA × DOI: $F_{1,12} = 5.94, p < 0.05$) and Egr-2 (motor cortex: MA: $F_{1,12} = 266.76, p < 0.001$, DOI: $F_{1,12} = 1143.49, p < 0.001$, MA × DOI: $F_{1,12} = 112.03, p < 0.001$; infralimbic cortex: MA: $F_{1,12} = 813.4, p < 0.001$, DOI: $F_{1,12} = 3989.8, p < 0.001$, MA × DOI: $F_{1,12} = 351.43, p < 0.05$) in the motor and infralimbic cortices. MA exposure profoundly enhanced egr-2 expression after vehicle treatment and the DOI-induced c-Fos and Egr-2 expression in these two brain regions (Fig. 3b–g).

DOI-induced field potentials in the medial prefrontal cortex

As shown in Fig. 4, excitatory and inhibitory field potentials were recorded. The excitatory and inhibitory field potentials were inhibited by ketamine and bicuculline, respectively (Lee et al., 2014). MA-exposed mice had a higher frequency and amplitude of the excitatory baseline field potentials compared to control mice (Fig. 4b, d). After application of DOI the frequency of the excitatory field potentials significantly increased in both control and MA-exposed mice. Moreover, the extent of increasing frequency of field potentials in response to DOI in MA-exposed mice was significantly greater than control mice.

MA-exposed mice had a lower frequency of inhibitory baseline field potentials compared to control mice. DOI significantly increased the frequency of inhibitory field potentials in MA-treated mice, but did not produce the same effect in control mice (Fig. 4c). The amplitude of inhibitory field potentials was not affected by MA exposure and DOI application (Fig. 4d).

Fig. 2. Effects of a binge regimen of MA on cognitive memory and social behaviours. Retention session for novel location and novel object recognition tests (a) was carried out 30 min and 24 h after the sample phase, respectively. The preference index was calculated as described in materials and method section. The counts of social behaviours (b) and total interaction time (c) were recorded in a social interaction test. The nesting scores and paper damage scores (d) were analysed by a Mann-Whitney test. Values are mean±S.E.M (n=12). **p<0.01, ***p<0.001 compared with saline group.
Protein levels of 5-HT$_{1A}$, 5-HT$_{2A}$, 5-HT$_{2C}$ and mGluR2 receptors in the medial prefrontal cortex

The levels of 5-HT$_{1A}$, 5-HT$_{2A}$, 5-HT$_{2C}$ and mGlu2 receptor proteins in the medial prefrontal cortex were measured. As shown in Fig. 5, the levels of 5-HT$_{2A}$ receptor expression were significantly increased, whereas the expression levels of mGlu2 receptor were reduced after a MA binge. However, MA did not alter the expression of 5-HT$_{1A}$ and 5-HT$_{2C}$ receptors.

Fig. 3. For legend see next page.
MA-induced dopaminergic neurotoxicity

The dopaminergic neurotoxicity was confirmed by measuring the tyrosine hydroxylase (TH) levels in the striatum using Western blotting and immunohistochemical analyses. As shown in Fig. 6, the levels of TH in the striatum were significantly reduced after a binge regimen of MA.

Discussion

The present study demonstrated that a neurotoxic/binge regimen of MA, which produced damage to dopaminergic terminals, resulted in significant and enduring impairments in recognition memory and social behaviours in mice. In addition, the hallucinogenic 5-HT2A agonist DOI-induced head-twitch response, c-Fos and Egr-2 expression and electrophysiological response in the medial prefrontal cortex were remarkably increased. MA binge exposure also caused up-regulation of the 5-HT2A receptors and down-regulation of the mGlu2 receptors in the medial prefrontal cortex. Apparently, the increased 5-HT2A receptor levels produce functional consequences as illustrated by the enhancement of DOI-induced responses. The changes in 5-HT2A and mGlu2 receptors might contribute to the psychosis-like behavioural deficits observed after binge-like MA exposure.

Although a previous study demonstrated that a single-day binge regimen of MA did not produce significant effects on the novel object recognition test in C57BL/6 mice (Grace et al., 2010), the results of the present study demonstrate that after MA-binge exposure, ICR mice exhibited persistent impairment in recognition memory, as observed in Swiss Webster mice (Semineri et al., 2013) and rats (O’Dell et al.; Bisagno et al., 2002; Schroder et al., 2003; Clemens et al., 2004). Our results further showed that our treatment regimen resulted in impaired performance in the novel location recognition test in ICR mice. In fact, it has been reported that repeated exposure to a low dose of MA (1 mg/kg) could impair novel object recognition memory in ICR mice (Kamei et al., 2006). It is likely that ICR mice are more susceptible to MA-induced recognition dysfunction than C57BL/6 mice.

It has been reported that MA-binge exposure results in a long-lasting decrease in social interaction in rats (Clemens et al., 2004, 2005). The present report, for the first time, showed that MA-binge exposure reduced social interaction and nestling behaviour in mice. It has been suggested that mechanisms other than or additional to the decrement in striatal DA are responsible for the deficit in recognition memory associated with an acute MA binge (Belcher et al., 2006; Clark et al., 2007). Additional work is needed to determine whether the alterations in 5-HT2A and/or mGlu2 receptors are involved in recognition impairment and social withdrawal associated with MA-binge exposure.

Our results demonstrated that MA-binge exposure evidently leads to a greater responsiveness to DOI. Although DOI acts on 5-HT2A and 5-HT2C receptors, DOI-induced head-twitch response (Dursun and Handley, 1996; Willins and Meltzer, 1997; Fantegrossi et al., 2010), c-Fos (Scruoggs et al., 2000) and Egr-2 (Gonzalez-Maeso et al., 2007) expression have been proven to be mediated via activation of 5-HT2A receptors, but not 5-HT2C receptors. We found that MA-exposed mice have greater responsiveness to DOI and higher levels of 5-HT2A receptors compared with controls. However, MA exposure did not produce a significant effect on the levels of 5-HT2C receptors. These findings further support that 5-HT2A receptors are mainly involved in DOI-induced responses. DOI-induced head-twitch response is dependent on 5-HT2A receptor-mediated glutamate release in the prefrontal cortex (Egashira et al., 2011). A dysfunctional

Fig. 3. Effects of a binge regimen of MA on DOI-induced head-twitch response and expression of immediate early genes. After administration of DOI (1 mg/kg) or vehicle, head-twitch response (n=8) was observed for 30 min (a). Animals (n=4) were sacrificed 1 h after DOI or vehicle treatment for immunohistochemical analysis of the expression of c-Fos (b, c) and Egr-2 (d, e). The c-Fos and Egr-2 immunoreactive cells were counted per 0.12 mm² area in the motor and infralimbic cortices (f, g). Scale bar: 10 μm. Values are mean±SEM. ***p<0.001 saline vs. MA, **p<0.01 vehicle vs. DOI.
enhancement of cortical excitatory transmission may be the synaptic effect of DOI and other hallucinogens that induced schizophrenic-like symptoms. Our electrophysiological data revealed that MA-binge exposure enhanced the frequency of baseline and DOI-evoked excitatory field potentials in the medial prefrontal cortex, suggesting MA-binge exposure may not only cause hypersensitivity to DOI-induced glutamatergic transmission, but also result in glutamatergic hyperactivity in the medial prefrontal cortex. Actually, hyperactive glutamatergic neurons in several brain regions, including the medial prefrontal cortex, have been proposed to underlie the psychotic, cognitive and emotional manifestations in schizophrenia (Krystal et al., 2003; Moghaddam, 2003). Our electrophysiological
findings, therefore, further support that this binge regimen of MA can be a suitable mouse model for MA psychosis.

Enhancement of DOI-evoked electrophysiological response might not occur in the medial prefrontal cortex only. A previous study has shown that treatment with MA (2.5 kg/mg/day) for five days augmented ventral pallidal cell-firing in response to DOI (Napier and Istre, 2008). Although it remains unknown whether enhanced responsiveness to DOI occurred in the same brain regions by different MA exposure regimens, these findings at least revealed that functional alteration in 5-HT2A receptors might be critical for behavioural abnormality and psychiatric disorders associated with MA exposure. The main characteristics of MA psychosis are the presence of prominent hallucinations and delusions. It has been reported that sensitization to MA has been observed in patients with MA dependence and psychosis (Ujike and Sato, 2004). The present findings raise the possibility that MA abusers might be also more sensitive to the hallucinogenic drugs of abuse acting on 5-HT2A receptors, including mescaline, psilocybin and lysergic acid diethylamide (LSD).

![Diagram of protein levels](image1)

**Fig. 5.** Effects of a binge regimen of MA on the protein levels of 5-HT1A, 5-HT2A, 5-HT2C and mGluR2 receptors in the medial prefrontal cortex. Brain samples were collected immediately after DOI-induced head-twitch experiment. 5-HT1A, 5-HT2A, 5-HT2C and mGluR2 receptors and actin were analysed by Western blot (a). Graphical representation of the degree of change in protein levels (b). Data are expressed as percentage of control group, mean±S.E.M (n=3). **p<0.01, ***p<0.001 compared with control group.

![Diagram of TH levels](image2)

**Fig. 6.** Dopaminergic neurotoxicity of a binge regimen of MA. Brain samples were collected from the animals treated with vehicle in the head-twitch experiment. Tyrosine hydroxylase (TH) was analysed by Western blot (n=3) (a) and immunohistochemistry (n=4) (c) Graphical representation of the degree of change in TH protein levels and optical densities of TH-IR (b, d). Optical density measurements were the ratio of the striatum and a control region (corpus callosum). Scale bar: 100 μm. Data are expressed as percentage of control group, mean±S.E.M. ***p<0.001 compared with control group.

We found that MA-binge exposure not only enhanced function, but also the protein expression of 5-HT2A receptors. Actually, up-regulation of 5-HT2A receptors has been suggested to be involved in the pathophysiology of psychosis in schizophrenia patients.
5-HT2A and mGlu2/3 receptors has been described in after chronic MA self-administration (Schwendt et al., 2011b), prenatal stress (Holloway et al., 2013) and models of schizophrenia, whereas chronic PCP treatment, a most commonly used model of schizophrenia, only augmented the DOI-induced responses (Santini et al., 2013), but not protein levels of 5-HT2A receptors. Together with our findings in binge MA-exposed mice, it appears that greater 5-HT2A receptor responsiveness might be a common feature of different types of psychosis. In fact, repeated administration of clozapine, the atypical antipsychotic agent with a high 5-HT2A receptor affinity, has been shown to decrease 5-HT2A receptor mRNA (Huang et al., 2007) and reverse repeated MA-induced memory impairment (Nagai et al., 2007). Further studies to reveal whether clozapine or selective 5-HT2A receptor antagonists can reverse the behavioural, molecular and electrophysiological changes after MA binge are required for delineating the role of 5-HT2A receptor in MA psychosis.

In addition to up-regulation of 5-HT2A receptors, our results demonstrated that MA binge reduced the protein levels of mGlu2 receptors in the medial prefrontal cortex. Similar changes in mGlu2 receptors were observed after chronic MA self-administration (Schwendt et al., 2012). In fact, a reciprocal functional inhibition of 5-HT2A and mGlu2/3 receptors has been described in the prefrontal cortex of rats (Aghajanian and Marek, 1999; Marek et al., 2000). The studies in mGlu2 and mGlu3 knockout mice revealed that mGlu2, but not mGlu3 receptors, are responsible for the functional 5-HT2A antagonism (Fribourg et al., 2011; Moreno et al., 2011a). Recently, down-regulation of mGlu2 receptors together with up-regulation of 5-HT2A receptors has been proposed as a pattern that could predispose to psychosis (Gonzalez-Maeso et al., 2008). Thus, up-regulation of 5-HT2A receptors and down-regulation of mGlu2 receptors after acute MA-binge exposure may explain the phenomenon that repeated abuse of MA frequently predisposes to psychotic conditions (Sato, 1992) and might be one of the shared pathological mechanisms in MA psychosis and schizophrenia.

Although MA is more toxic to 5-HT terminals than MDMA (‘Ecstasy’) in forebrain regions (Armstrong and Noguchi, 2004), the enhancement of 5-HT2A receptor-mediated functionality is also observed after MDMA exposure. Recent clinical studies demonstrated that the use of MDMA is associated with long-lasting increases in 5-HT2A receptor density (Di Iorio et al., 2012; Urban et al., 2012; Benningfield and Cowan, 2013). Furthermore, repeated MDMA exposure in rats also increases behavioural and neuroendocrine responses to DOI (Biezonski et al., 2009). Prolonged psychosis has been reported after MDMA use (Schifano, 1991; Winstock, 1991; Milas, 2000; Potash et al., 2009). Psychosis presenting subsequent to MA and MDMA use share many common features and perhaps share a common neural mechanism. Despite that the up-regulation of 5-HT2A receptors has been asserted after MDMA use, it remains to be determined whether MDMA can similarly affect mGlu2 receptor expression in the medial prefrontal cortex as observed after MA exposure.

In summary, the results of present study demonstrate that a neurotoxic/binge regimen of MA could result in behavioural changes including cognition impairments and social deficits, and increased sensitivity to the hallucinogenic compound DOI-induced behavioural, molecular and electrophysiological responses in mice. Furthermore, MA-binge exposure results in schizophrenic-like alterations in 5-HT2A and mGlu2 receptors in the medial prefrontal cortex, a brain region involved in perception, cognition and mood. These findings provide a rodent model suitable for research into the pathophysiology of neurological and psychiatric consequences of MA-binge exposure, which might facilitate the discovery of novel therapeutic targets for MA psychosis and other types of psychotic disorders.

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Conflict of Interest Statement
None

References


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