

Behavioural and neuroinflammatory effects of the combination of binge ethanol and MDMA in mice

Clara Ros-Simó · Jessica Ruiz-Medina · Olga Valverde

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Abstract

Rationale Binge drinking is a common pattern of alcohol consumption among young people. Binge drinkers are especially susceptible to brain damage when other substances are co-administered, in particular, 3,4-methylenedioxymethamphetamine (MDMA).

Objective To evaluate the behavioural consequences of voluntary binge ethanol consumption, alone and in combination to MDMA. Also, to elucidate the effects of the combined consumption of these two drugs on neuroinflammation.

Materials and methods Adolescent mice received MDMA (MDMA-treated mice), ethanol (ethanol-treated mice group) or both (ethanol plus MDMA-treated mice). Drinking in the dark (DID) procedure was used as a model of binge. Body temperature, locomotor activity, motor coordination, anxiety-like and despair behaviour in adolescent mice were evaluated 48 h, 72 h, and 7 days after the treatments. Also, neuroinflammatory response to these treatments was measured in the striatum.

Results The hyperthermia observed in MDMA-treated mice was abolished by pre-exposition to ethanol. Ethanol plus MDMA-treated mice showed lower locomotor activity. Ethanol-treated mice showed motor coordination impairment and increased despair behaviour. Anxiety-like behaviour

was only seen in animals that were treated with both drugs. Contrarily, neuroinflammation was mostly seen in animals treated only with MDMA.

Conclusions Ethanol and MDMA co-administration increases the neurobehavioural changes induced by the consumption of each one of these drugs. However, as ethanol consumption did not increase neuroinflammatory responses induced by MDMA, other mechanisms, mediated by ethanol, are likely to account for this effect and need to be evaluated.

Keywords Ethanol · MDMA · Behaviour · Neuroinflammation · Microglia · Astrocytes · Mice

Abbreviations

MDMA	3,4-Methylenedioxymethamphetamine
DID	Drinking in the dark
GFAP	Glial fibrillary acid protein
5-HT	5-Hydroxytryptamine
CRF	Corticotrophin-releasing factor

Introduction

Binge drinking is defined as drinking at least 5 units of alcohol (ethanol) on a single occasion (Wechsler and Nelson 2001). It is a common pattern of alcohol consumption among adolescents and young adults that may lead to brain damage due to inflammatory processes and causes long-lasting neurobehavioural consequences (Pascual et al. 2007) with implications for the development of alcohol dependence. Several studies have demonstrated that binge drinking itself presents negative behavioural consequences, affecting mood and cognitive performance (White 2003;

J. Ruiz-Medina and O. Valverde contributed equally to this work.

C. Ros-Simó · J. Ruiz-Medina · O. Valverde (✉)
Grup de Recerca en Neurobiologia del Comportament (GRNC).
Departament de Ciències Experimentals i de la Salut,
Universitat Pompeu Fabra,
Parc de Recerca Biomèdica de Barcelona,
Dr Aiguader 88,
08003 Barcelona, Spain
e-mail: olga.valverde@upf.edu

Grothues et al. 2008). Additionally, binge drinkers are particularly susceptible to eventual brain damage induced by other often co-administered drugs (Hunt 1993). Nowadays, ethanol and 3,4-methylenedioxymethamphetamine (MDMA or “ecstasy”) are two of the most common co-abused substances (Winstock et al. 2001; Barrett et al. 2006), and the behavioural and neurotoxic consequences of such association have not been yet elucidated.

MDMA is an amphetamine derivative compound that exhibits rewarding properties in humans (Camí et al. 2000) and in experimental animals, such as monkeys (Lamb and Griffiths 1987; Fantegrossi et al. 2008), rats (Bilsky et al. 1991), and mice (Trigo et al. 2006; Touriño et al. 2008; Ruiz-Medina et al. 2011). However, MDMA ingestion in humans can also induce neurochemical, behavioural, and endocrine alterations similar to those produced by exposure to acute stress, suggesting a potential role for MDMA as a “chemical stressor” (Pacifci et al. 1999). There are several MDMA toxic effects reported in humans and laboratory animals, with the induction of hyperthermia following its ingestion being the most predominant (Lyles and Cadet 2003; Cadet et al. 2007). Our team has previously demonstrated that MDMA-induced hyperthermia promotes glial activation in discrete brain areas such as striatum in mice (Touriño et al. 2010; Ruiz-Medina et al. 2011), which has been postulated to be involved in MDMA-induced neurotoxicity (Green et al. 2003; Gudelsky and Yamamoto 2003; O’Shea and Colado 2003; Sanchez et al. 2004).

Although the combined consumption of ethanol and MDMA in humans is not fully understood, some explanations have been proposed. Among them, is the fact that the combined intake of ethanol and MDMA produces a longer lasting euphoria and feelings of well being, than the consumption of either drug alone, suggesting an increased subjective perception of the positive effects induced by the drug (Hernández-López et al. 2002). Also, it has been proposed that ethanol attenuates the negative side effects of MDMA, in particular, regarding the hyperthermia associated to MDMA intake (Winstock et al. 2001).

In this study, a daily limited-access ethanol intake model named drinking in the dark (DID) test (Rhodes et al. 2005) was used as a model to assess the increased vulnerability to neural damage observed in binge drinkers. The work presented here evaluates, first, the negative consequences of voluntary binge ethanol intake on body temperature, locomotion and motor coordination, and emotional-like related responses in adolescent mice. Second, it measures the effects of acute co-administration of MDMA on behavioural deficits induced by binge ethanol consumption. Finally, it reveals the neuroinflammatory response induced by binge ethanol drinking and MDMA acute co-administration.

Materials and methods

Subjects

Adolescent naive male CD-1 mice (postnatal day 21) weighing 25–30 g at the beginning of the experiments were used in this study. Mice were purchased from Charles River (France) and housed four per cage during 7 days (quarantine period) until 1 week prior to the beginning of experiments when mice were individually housed (postnatal day 28). Experiments started 7 days after the individualization (postnatal day 36). Animal rooms were controlled for temperature ($22\pm1^{\circ}\text{C}$), humidity ($55\pm10\%$) and photo-period (12:12 L/D). Lights were turned on at 0800 hours and off at 2000 hours. One week prior to the experiment, mice were switched to a reverse light/dark schedule in which lights turned on at 1900 hours and off at 0700 hours. Food and water were available ad libitum except when water was substituted for ethanol for 2 or 4 h per day according to DID procedure, described below. All animal care and experimental procedures were conducted according to the guidelines of the European Communities Directive 86/609/EEC regulating animal research and were approved by the local ethical committee (CEEA-PRBB). All procedures were conducted by an experimenter blind to the treatment.

Drugs

Racemic MDMA hydrochloride was purchased from Lipomed, A.G. (Arlesheim, Switzerland), dissolved in 0.9% physiological saline to obtain a dose of 20 mg/kg (2 mg/ml) expressed as the salt, and injected in a volume of 0.1 ml/10 g body weight by intraperitoneal (i.p.) route of administration. Ethyl alcohol was purchased from Merck Chemicals (Darmstadt, Germany) and diluted in tap water in order to obtain a 20% (v/v) ethanol solution. Ketamine hydrochloride (100 mg/kg; Imalgène 1000®, Rhône Mérieux, Lyon, France) and xylazine hydrochloride (20 mg/kg; Sigma, Madrid, Spain) were mixed in ethanol and water (1:9). This anaesthetic mixture was injected in a volume of 0.2 ml/10 g body weight i.p. and used for intracardiac perfusion.

Behavioural testing

Drinking in the dark procedure

This procedure was conducted as previously reported (Rhodes et al. 2005). Briefly, food was removed and the water bottles were replaced with 10-ml graduated cylinders fitted with sipper tubes containing either 20% (v/v) ethanol

in tap water or only tap water (groups Ethanol and Water, respectively) 3 h after lights were turned off in the animal rooms. During this time, animals were maintained in home cages individually housed (see above). The ethanol or water cylinders remained in place for 2 h. After the 2-h period, individual intake was recorded and food and water bottles were replaced. This procedure was repeated on days 2 and 3 and fresh fluids were provided each day. On day 4, all subjects were injected with a single dose of saline (0.1 ml/10 g, i.p.) prior to the DID procedure. On the following week, the DID procedure was conducted again as described above. In addition, on day 4, 30 min before the ethanol or water exposure, rectal temperatures were measured in each mouse with a rectal probe (Panlab, Madrid, Spain). After that, subjects were injected by a single dose of MDMA (20 mg/kg) or saline (i.p.). In this day, the ethanol or water cylinders were left for 4 h and intakes were recorded after 2 and 4 h, respectively. After the first recording (2 h), rectal temperatures were registered again and animals received a second injection of MDMA (20 mg/kg) or saline (i.p.). The dose of MDMA was selected in accordance to previous studies performed by our team (Tourinho et al. 2010; Ruiz-Medina et al. 2011). Following the 4 h free access to fluid and immediately after recording fluid intake, ethanol and water cylinders were replaced with water bottles. Then, animals were separated in three groups: 48 h (postnatal day 40), 72 h (postnatal day 41) or 7 days (postnatal day 45) after the last MDMA or saline injection (under ethanol absence). Each group of animals was subjected to a battery of behavioural tests to evaluate

different alterations on behavioural responses: (1) locomotor activity, (2) motor coordination, (3) anxiety-like responses and (4) depressive-like responses (see schedule of the experimental procedure in Fig. 1), leaving at least 1 h between each test. Inflammatory responses were also evaluated as explained below.

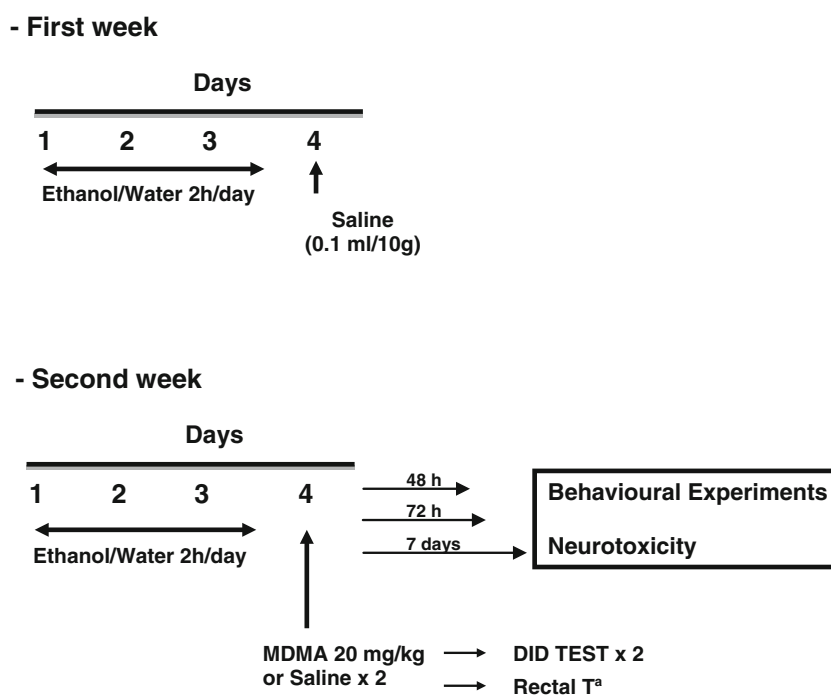
Body temperature

Changes in body temperature were evaluated using an electronic thermocoupled flexible rectal probe (Panlab) placed in the mice rectum for 10 s. Room temperature was maintained at $21 \pm 1^\circ\text{C}$. Basal temperature was measured 30 min before the first MDMA (20 mg/kg) or saline injection (i.p.). Temperature was also registered 120 min after MDMA or saline injection and changes in body temperature were calculated for each mouse as changes of baseline temperature for each mouse.

Locomotor responses

Locomotor activity was evaluated using locomotor activity boxes ($9 \times 20 \times 11$ cm) (Imetronic, Lyon, France) in a low luminosity room (5 lx) with white noise, as previously reported (Soria et al. 2006). At 48 h, 72 h or 7 days after last MDMA or saline injection, mice were placed in the locomotor activity boxes and their horizontal and vertical movements were recorded during 20 min.

Fig. 1 DID procedure (see Materials and methods section)



Motor coordination

Coordination was assessed using an accelerating rotarod apparatus (five-line accelerating rotarod; LE 8200, Panlab). The rotarod accelerated from 4 to 40 rev/min over 2 min. 48 h, 72 h or 7 days after last MDMA or saline injection, motor coordination was measured in a session consisting of ten consecutive trials interleaved by 30-s breaks. The three last values of the session were averaged as previously reported (Valverde et al. 2009).

Anxiety-like responses

To evaluate anxiety-like responses the dark–light box test was conducted at 48 h, 72 h and 7 days after last MDMA or saline injection as reported (Valverde et al. 2009). Briefly, the apparatus consisted of two plastic chambers, connected by a small tunnel. The dark measured chamber (20×15 cm) and the adjacent chamber, measuring 30×15 cm, was white and illuminated from above with 600 lx. Mice were placed into the dark compartment and number of entries and percentage of time in the lit compartment were recorded for 5 min.

Despair behaviour

Manifestations of despair behaviour were evaluated using the tail suspension test 48 h, 72 h and 7 days after last MDMA or saline injection. Briefly, mice were individually suspended by adhesive tape 1 cm from the tip of the tail 50 cm above a bench top for a 6-min period as described by Steru et al. (1985). The time of complete inactivity during this period was recorded.

Evaluation of neuroinflammatory responses in the striatum

Neuroinflammatory reactions were assessed in the striatum of mice 48 h, 72 h and 7 days after the MDMA or saline treatment (see Fig. 1) in order to assess the time-course of anti-glial fibrillary acid protein (GFAP) and microglial activation (anti-Iba1) in the striatum.

Immunofluorescence procedure

The presence of GFAP and Iba1 in astrocytes and microglia was evaluated by immunofluorescence by using anti-glial fibrillary acid protein GFAP (1:500; DakoCytomation, Glostrup, Denmark) and rabbit polyclonal anti Iba1 (1:500; Wako Pure Chemical Industries, Ltd., Japan) staining, respectively, as previously reported (Tourinho et al. 2010; Ruiz-Medina et al. 2011). Animals were deeply anesthetized with ketamine/xylazine mixture and transcardially perfused with 0.1 M phosphate buffer containing

4% paraformaldehyde. Brains were quickly removed and postfixed in the same solution for 4 h and cryoprotected in 20% v/v sucrose in 0.1 M in phosphate buffer, pH 7.4 (24 h at 4°C). After freezing in dry ice, brains were sliced into 30-μm coronal sections containing striatum. Free floating brains sections were washed three times in 0.1 M phosphate buffer and then sections were incubated in a solution of 3% v/v normal goat serum (Vector Laboratories, Inc., Burlingame, CA) and 0.3% triton X-100 (Sigma-Aldrich, Spain) for 2 h at room temperature. Sections were incubated overnight with the aforementioned primary antibodies (GFAP and Iba1 for astrocytes and microglia expression, respectively). After being washed three times for 10 min in 0.1 M PB, sections were incubated during 2 h with the following fluorescent secondary antibody, goat anti-rabbit IgG Alexa Fluor 488 (1:500; Invitrogen, Barcelona, Spain) for astrocyte and microglia expression, at room temperature. Finally, sections were mounted onto slide with a fluorescence mounting medium of Mowiol 40–88 (Sigma-Aldrich), glycerol 87%, water and 2,5% 1,4-diazabicyclo-[2.2.2]octane which is prepared by laboratory technicians and coverslipped for microscopic observation and photography. Astrocytes and microglial cells were counted from dorsal striatum and its ventral portion at three different rostro-caudal levels through the striatum (2–3 sample areas/level/mouse, bilaterally). Sample areas were visualized under a 20× or 40× objectives in a Leica DMR microscope and digitized through a digital camera Leica DFC 300 FX. Immunoreactive cells and stained area percentages were analyzed using the ImageJ software.

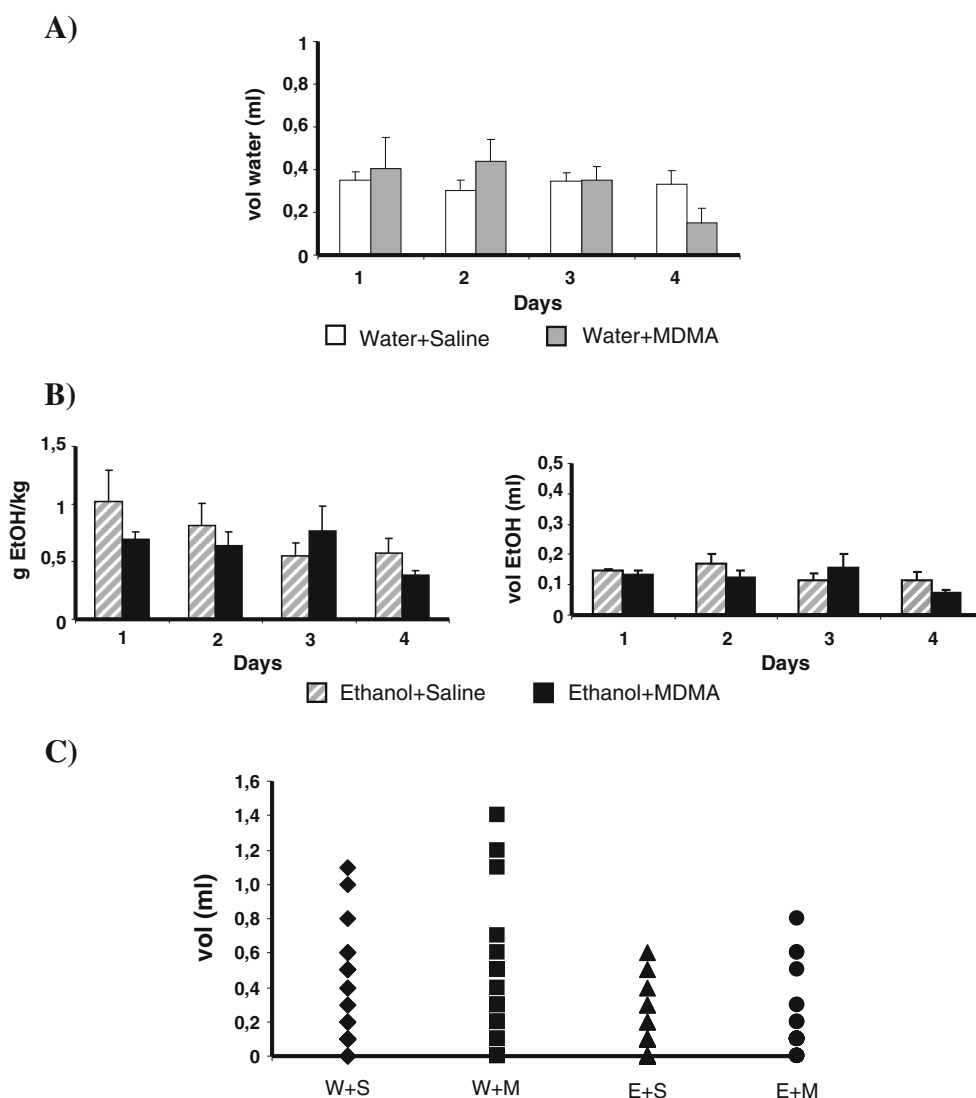
Statistical analysis

Results are expressed as the mean ± SEM. Statistical analysis was determined by a one-way ANOVA test and subsequent Tukey post-hoc test when required. In all experiments, differences were considered significant if the probability of error was less than 5%. SPSS statistical package was used.

Results

Water and ethanol consumption was measured for each mouse everyday during the DID procedure (Fig. 1). One-way ANOVA for water consumption did not show differences in the total fluid (ml) consumed between water-treated groups (Water × MDMA vs. Water × Saline) (Fig. 2a). Regarding the amount of ethanol (g EtOH/kg and ml) consumed no differences were found between groups (Ethanol × MDMA vs. Ethanol × Saline) in none of the days of the DID procedure (Fig. 2b). Thus, consumption of water

Fig. 2 Ethanol and water intake during the second week of the DID procedure (we show the first 2 h during the fourth day). **a** Total water intake (ml/day). **b** Amount of ethanol consumption as g/kg/day and as ml/day. Data are presented as mean $\pm$ SEM of the total consumption of water or ethanol. **c** Intake (ml) of water and ethanol for each animal on the third day of the second week of the DID procedure ($n=26$ –30 mice per group)



and ethanol remained consistent across days in each group. Water and ethanol consumption for each animal at the third day of the second week of the DID procedure is represented in Fig. 2c.

Ethanol binge drinking and MDMA effects on body temperature

Rectal temperature was evaluated in animals housed at room temperature ($22 \pm 1^\circ\text{C}$). Mice baseline body temperature was $38.2 \pm 1^\circ\text{C}$. Once baseline temperature had been recorded, animals were injected with MDMA (20 mg/kg) or saline (0.1 ml/10 g, i.p.). Then, body temperature was measured 120 min after MDMA or saline injection. One-way ANOVA showed significant effect of treatment ($F_{3, 114} = 10.13$, $p < 0.001$). Post-hoc analysis showed that MDMA induced a significant hyperthermia in mice pre-

exposed to water ($p < 0.001$ vs. all groups). Surprisingly, the hyperthermia induced by MDMA was not observed in mice pre-exposed to binge ethanol and treated with MDMA (Fig. 3). The number of animals per group was 26–30.

Ethanol binge drinking and MDMA effects on locomotor activity

Forty-eight hours after last MDMA or saline injection One-way ANOVA for vertical activity revealed a significant effect of the treatment ($F_{3, 33} = 5.4$, $p < 0.01$). Post-hoc analysis revealed a decreased vertical activity in ethanol + MDMA-treated mice when compared with control group (water + saline) group ($p < 0.01$) and ethanol-treated group ($p < 0.05$), respectively (Fig. 4a).

One-way ANOVA for horizontal movements revealed a significant effect of the treatment ($F_{3, 33} = 6.5$, $p < 0.01$).

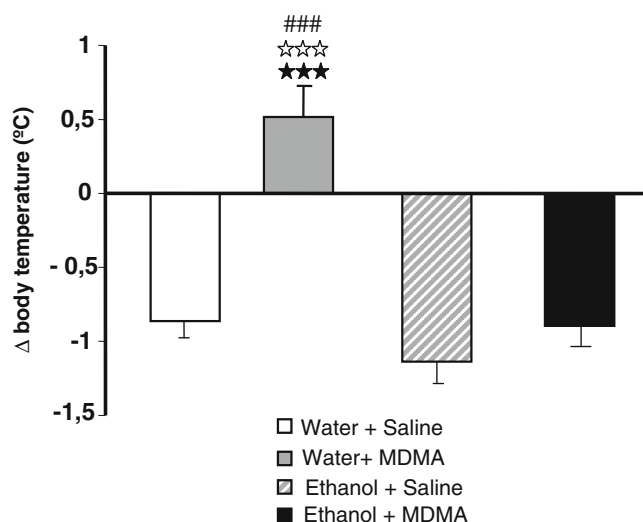


Fig. 3 Ethanol prevents MDMA-induced hyperthermia. After baseline temperature recordings, animals were injected with MDMA (20 mg/kg, i.p.) or saline (0.1 ml/mg, i.p.). Body temperature was measured 120 min after MDMA or saline injection. Data are expressed as mean $\pm$ SEM of the body temperature increase ($n=26$ –30 mice per group). Three filled stars, $p<0.001$ vs. water + saline group; three empty stars, $p<0.001$ vs. water + MDMA; ### $p<0.001$ vs. ethanol + saline group in the Tukey test

Post-hoc analysis showed a lower horizontal activity in ethanol + MDMA-treated mice compared to control group ($p<0.01$) and ethanol-treated group ($p<0.01$), respectively (Fig. 4b). The number of animals per group was 8–10.

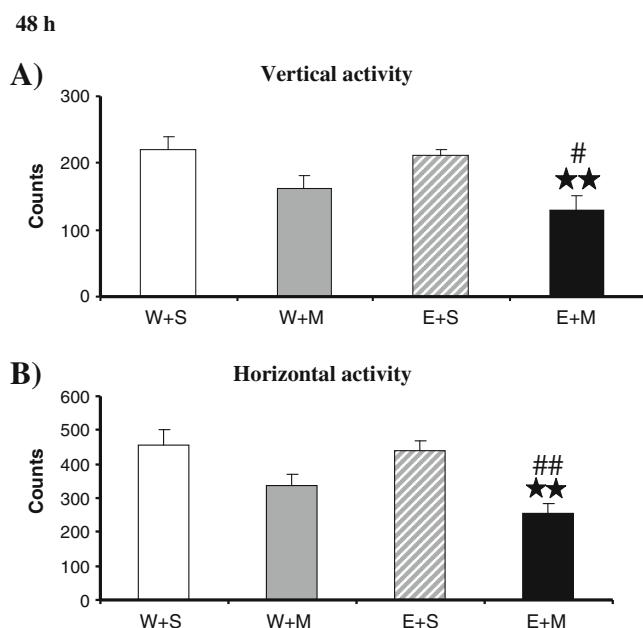


Fig. 4 Vertical (a) and horizontal (b) locomotor activity were assessed 48 h after saline or MDMA administration. Data are expressed as mean $\pm$ SEM of photocell counts during a 20-min period in ethanol and water pre-exposed mice ($n=8$ –10 mice per group). Two filled stars, $p<0.01$ vs. water + saline group; # $p<0.05$ vs. ethanol + saline group; ## $p<0.01$ vs. ethanol + saline group in the Tukey test

No differences were found on locomotor activity 72 h or 7 days after last MDMA or saline injection (data not shown).

Ethanol binge drinking and MDMA effects on motor coordination

Forty-eight hours after last MDMA or saline injection At that time, no differences on motor coordination were found (Fig. 5a).

Seventy-two hours after last MDMA or saline injection One-way ANOVA in the rotarod test revealed a significant effect of the treatment ($F_{3, 54}=4.2$, $p<0.05$). Post-hoc analysis

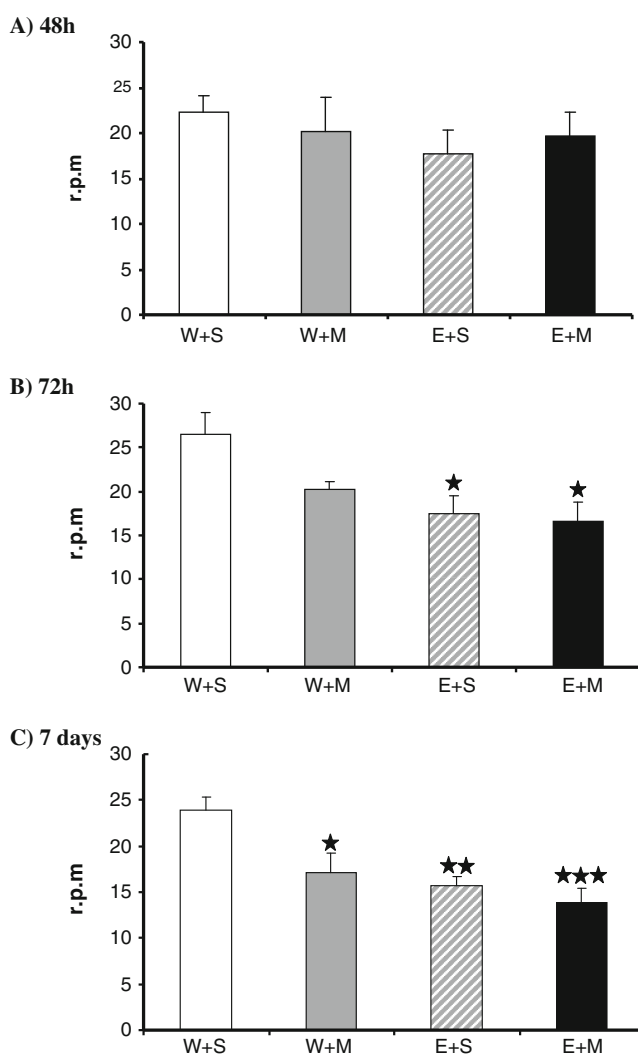


Fig. 5 Ethanol impairs motor coordination assessed using an accelerating rotarod apparatus. Motor coordination was assessed a 48 h, b 72 h or c 7 days after saline or MDMA administration. Data are expressed as mean $\pm$ SEM of the rpm achieved in each test in ethanol and water pre-exposed mice ($n=9$ –15 mice per group). Filled star, $p<0.05$ vs. water + saline mice; two filled stars, $p<0.01$ vs. water + saline group; three filled stars, $p<0.001$ vs. water + saline group in the Tukey test

revealed motor coordination impairment in ethanol + MDMA group when compared with control ($p<0.05$). In the same way, ethanol-treated mice exhibited impaired motor coordination when compared with control group ($p<0.05$) (Fig. 5b). The number of animals per group was 9–15.

Seven days after last MDMA or saline injection One-way ANOVA for the rotarod test revealed a significant effect of the treatment ($F_{1, 43}=7.13$, $p<0.01$). Post-hoc analysis showed motor coordination impairment in MDMA-treated mice pre-exposed to ethanol when compared with control group ($p<0.001$). In the same way, ethanol-treated mice showed less motor coordination than control group ($p<0.01$) and MDMA-treated group vs. control group ($p<0.05$) (Fig. 5c). The number of animals per group was 9–15.

Ethanol binge drinking- and MDMA-induced anxiety-like responses in the lit and dark box

Forty-eight hours after last MDMA or saline injection No differences were found in the lit and dark box at this timing (Fig. 6a).

Seventy-two hours after last MDMA or saline injection One-way ANOVA for the percentage of time in the lit box revealed effect of the treatment ($F_{3, 56}=6.5$, $p<0.01$). Post-hoc analysis indicated an anxiety-like effect in ethanol-treated animals that received MDMA (ethanol + MDMA), revealed as a decrease in the time spent in the lit compartment compared to ethanol-treated group ($p<0.01$) and MDMA-treated group ($p<0.01$) (Fig. 6b). No differences were found in the number of entries in the lit compartment (data not shown). The number of animals per group was 9–15.

Seven days after last MDMA or saline injection One-way ANOVA for the percentage of time in the lit box revealed effect of the treatment ($F_{3, 55}=4.3$, $p<0.01$). Post-hoc analysis indicated that ethanol and MDMA-treated animals (ethanol + MDMA) spent less time in the lit compartment than ethanol-treated group ($p<0.05$), MDMA-treated group ($p<0.05$) and control group ($p<0.05$), respectively (Fig. 6c). One-way ANOVA for the number of entries in the lit compartment did not show significant effects of treatment (data not shown). The number of animals per group was 9–15.

Ethanol binge drinking- and MDMA-induced despair behaviour

Forty-eight hours after last MDMA or saline injection One-way ANOVA for the total time of immobility in the tail suspension test did not reveal any significant effect (Fig. 7a).

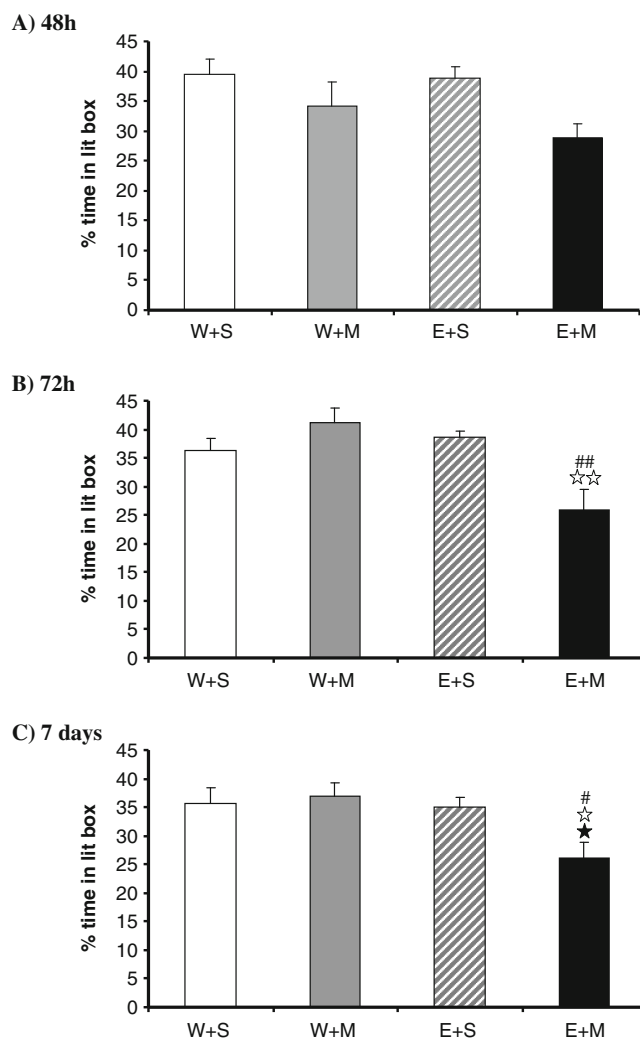


Fig. 6 Anxiety-like effects induced by binge ethanol administration are enhanced by acute MDMA co-administration. The time spent in the lit compartment of the dark–light box test was measured **a** 48 h, **b** 72 h or **c** 7 days after saline or MDMA administration. Data are expressed as mean $\pm$ SEM of the percentage of time spent in the lit compartment in ethanol and water pre-exposed mice ($n=9$ –15 mice per group). Filled star, $p<0.05$ vs. water + saline treated; empty star, $p<0.05$ vs. water + MDMA treated; two empty stars, $p<0.01$ vs. water + MDMA treated; # $p<0.05$ vs. ethanol + saline treated; ## $p<0.01$ vs. ethanol + saline treated in the Tukey test

Seventy-two hours after last MDMA or saline injection One-way ANOVA for the total time of immobility in the tail suspension test showed a significant effect of the treatment ($F_{3, 73}=3.17$, $p<0.05$). Post-hoc analysis revealed a higher immobility in the ethanol + MDMA group when compared to control group ($p<0.05$) (Fig. 7b). The number of animals per group was 15–20.

Seven days after last MDMA or saline injection One-way ANOVA for the total time of immobility in the tail suspension test showed a significant effect of the treatment ($F_{3, 48}=5.1$, $p<0.01$). Post-hoc analysis revealed higher

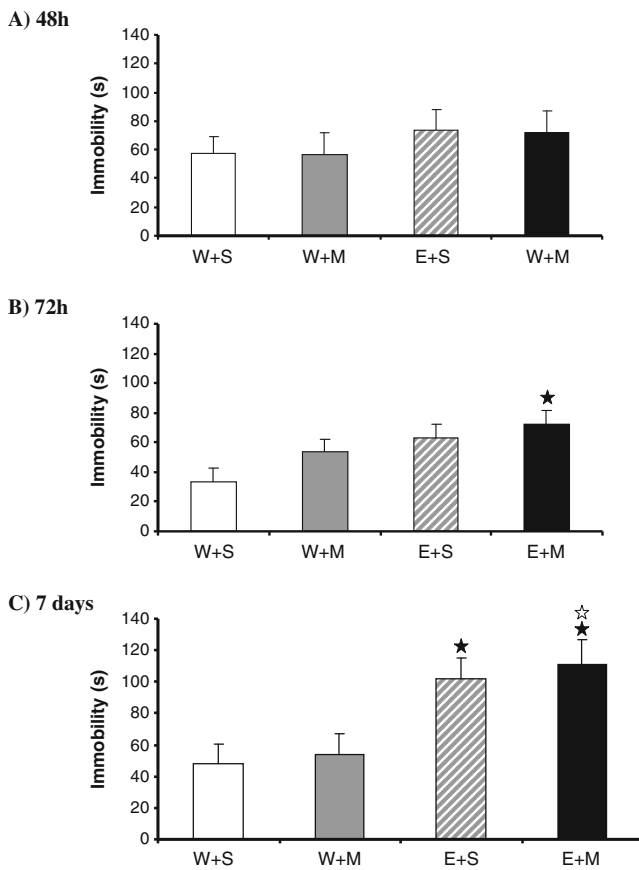


Fig. 7 Depressive-like effects induced by binge ethanol administration. The behavioural responses in the tail suspension test were measured **a** 48 h, **b** 72 h or **c** 7 days after saline or MDMA administration. Data are expressed as mean $\pm$ SEM of the percentage of immobility in ethanol and water pre-exposed mice ($n=9$ –15 mice per group). *Filled star*, $p<0.05$ vs. water + saline group; *empty star*, $p<0.05$ water + MDMA group in the Tukey test

immobility in ethanol + MDMA group when compared with MDMA-treated animals ($p<0.05$) and control animals ($p<0.05$). In the same way, mice pre-exposed to ethanol spent more time than control group ($p<0.05$) (Fig. 7c). The number of animals per group was 9–15.

Ethanol binge drinking- and MDMA-induced neuroinflammation

Astrocytes and microglia immunostaining (Figs. 8 and 9) were evaluated in the striatum of animals exposed to DID procedure and sacrificed 48 h, 72 h or 7 days after last MDMA or saline injection (see Fig. 1).

GFAP immunostaining

Forty-eight hours after last MDMA or saline injection One-way ANOVA for the number of GFAP positive cells

revealed a significant effect of the treatment ($F_{13, 11}=17.09$, $p<0.01$). Post-hoc analysis showed a significant higher GFAP expression in the MDMA-treated mice compared with ethanol-treated group ($p<0.001$) and control group ($p<0.001$). Surprisingly, GFAP reactivity induced by MDMA treatment was attenuated in those mice pre-exposed to ethanol (ethanol + MDMA) ($p<0.01$) (Fig. 8a). The number of animals per group was 3–4.

Seventy-two hours after last MDMA or saline injection One-way ANOVA for the number of GFAP positive cells revealed a significant effect of the treatment ($F_{3, 11}=8.48$, $p<0.01$). Post-hoc analysis revealed a major GFAP immunoreactivity in those ethanol + MDMA-treated mice compared with ethanol-treated group ($p<0.05$) and vs. control group ($p<0.05$). In the same way, MDMA-treated group showed higher GFAP immunoreactivity compared to control group ($p<0.05$) (Fig. 8b). The number of animals per group was 3–4.

Seven days after last MDMA or saline injection One-way ANOVA for the number of GFAP positive cells (Fig. 8c) indicated no significant effects.

Iba1 immunostaining

Forty-eight hours after last MDMA or saline injection One-way ANOVA showed no differences for the number of microglial cells, but a hypertrophy of these cells was observed and was measured by an increase on the percentage of stained area in MDMA-treated animals ($F_{3, 11}=7.16$, $p<0.01$). Post-hoc analysis showed differences between both groups: MDMA- and saline-treated mice ($p<0.05$) (Fig. 9a). The number of animals per group was 3–4.

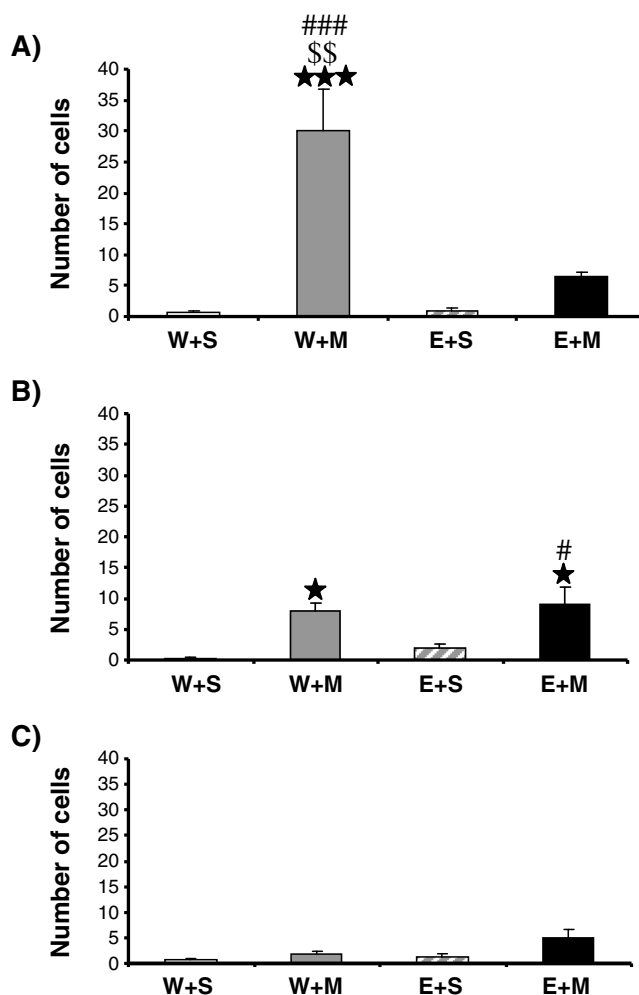
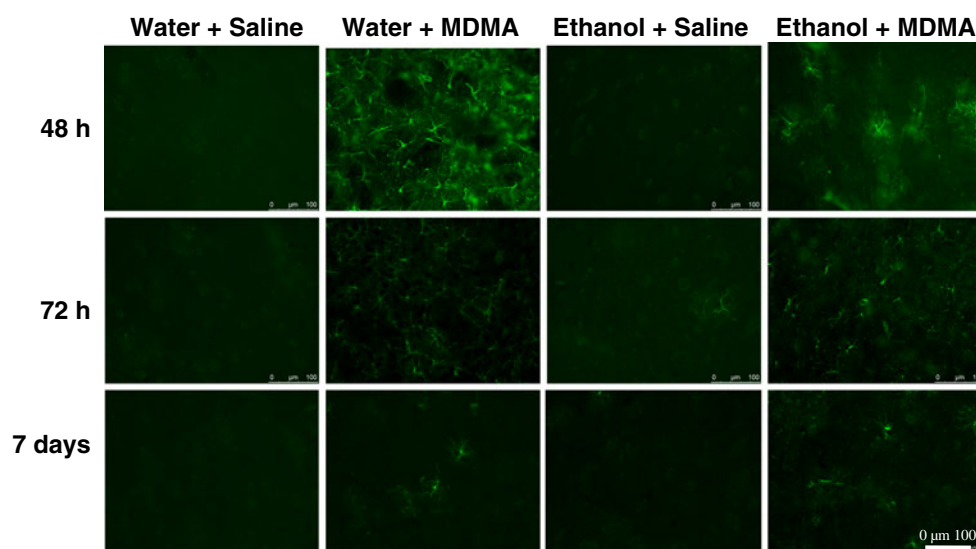
Seventy-two hours after last MDMA or saline injection One-way ANOVA for the number of microglial cells showed a significant effect of the treatment ($F_{3, 11}=5.02$, $p<0.05$). Post-hoc analysis revealed greater number of microglial cells in MDMA-treated mice and ethanol + MDMA-treated animals compared to the control group ($p<0.05$ in both cases). As in the previous point, a hypertrophy of microglial cells was observed in MDMA-treated mice revealed as an increase in the percentage of stained area ($F_{3, 11}=4.07$, $p<0.05$). Post-hoc analysis showed significant differences when compared MDMA-treated mice with water + saline group ($p<0.05$) (Fig. 9b). The number of animals per group was 3–4.

Seven days after last MDMA or saline injection One-way ANOVA for the number of microglial cells showed significant effect of the treatment ($F_{3, 11}=4.7$, $p<0.05$). Post-hoc

Fig. 8 *Upper panel:* GFAP staining in the striatum of animals treated with saline or MDMA (20 mg/kg, i.p., $\times 2$) and sacrificed 48 h, 72 h or 7 days after last injection of MDMA neurotoxic regimen or saline.

Lower panel: Data on astrocytes staining quantification are expressed as mean $\pm$ SEM of number of cells 48 h (a), 72 h (b) and 7 days (c) after treatment ($n=3-4$ mice per group).

Filled star, $p<0.05$ vs. water + saline group; *three filled stars*, $p<0.001$ vs. water + saline group; $$$p<0.01$ vs. ethanol + MDMA group; $\#p<0.05$ vs. ethanol + saline; $###p<0.001$ vs. ethanol + saline group in the Tukey test



analysis revealed greater number of microglial cells in MDMA-treated mice compared to the control group ($p<0.05$). As in the previous experiments, significant effect was found for the percentage of stained area ($F_{3, 11}=27.82$,

$p<0.001$). Post-hoc analysis revealed greater stained area in the MDMA-treated group vs. water + saline ($p<0.001$), ethanol-treated group ($p<0.01$) and ethanol + MDMA ($p<0.01$) (Fig. 9c). The number of animals per group was 3–4.

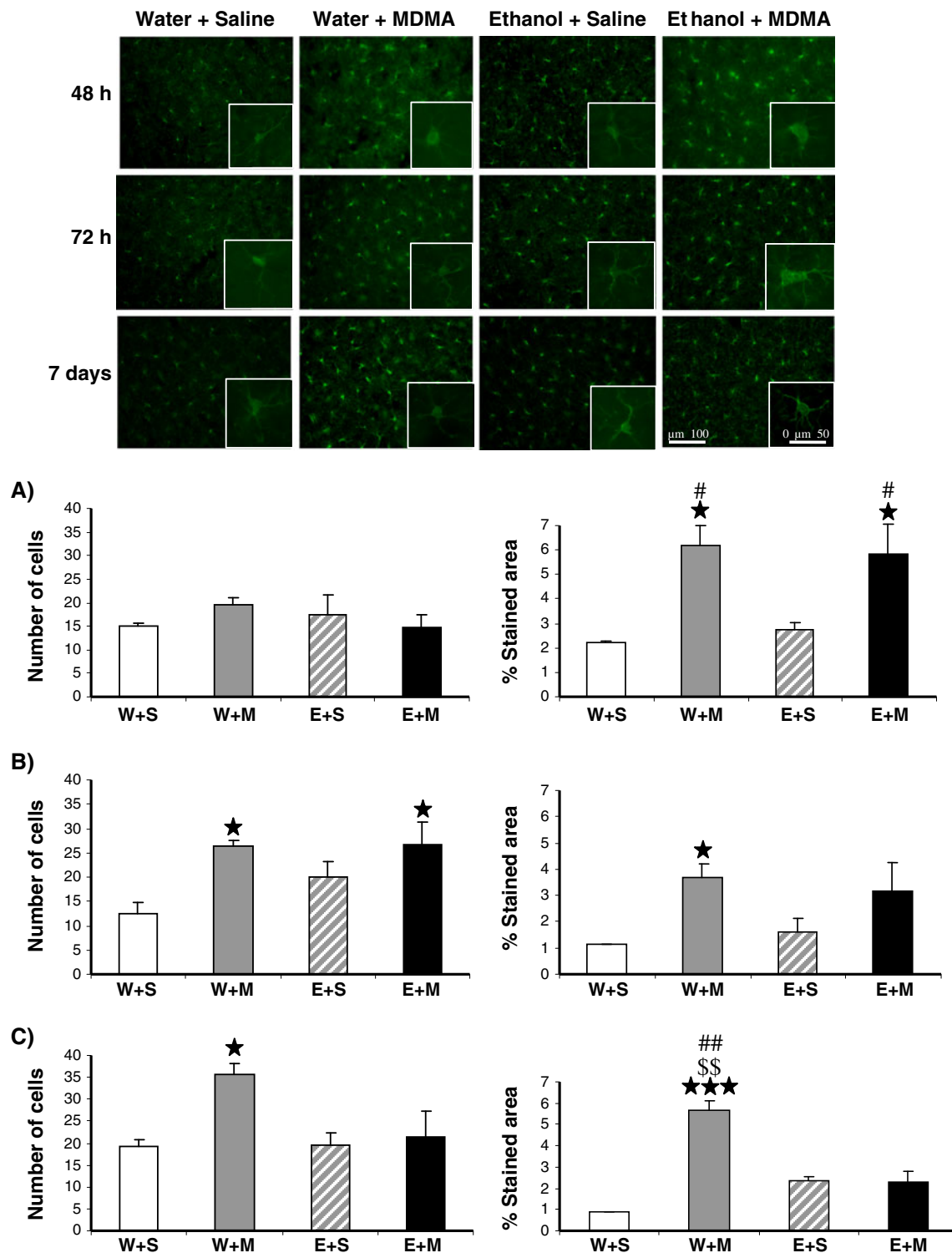


Fig. 9 Upper panel: Iba1 staining in the striatum of animals treated with saline or MDMA (20 mg/kg, i.p., $\times 2$) and sacrificed 48 h, 72 h or 7 days after last injection of MDMA neurotoxic regimen or saline. Lower panel: Data on microglia staining quantification are expressed as mean $\pm$ SEM of number of cells and % of stained area 48 h (a), 72 h

(b) and 7 days (c) after treatment ($n=3-4$ mice per group). Filled star, $p<0.05$ vs. water + saline group; three filled stars, $p<0.001$ vs. water + saline group; \$\$ $p<0.01$ vs. ethanol + MDMA group; # $p<0.05$ vs. ethanol + saline group; ## $p<0.01$ vs. ethanol + saline group in the Tukey test

Discussion

The present study attempts to elucidate the pharmacological, behavioural and neuroinflammatory effects of a binge pattern of ethanol consumption, and the influence of MDMA co-administration on these alterations. Our findings revealed that ethanol binge drinking induced changes in some of the parameters evaluated, such as motor coordination and despair behaviour. MDMA per se did not induce relevant behavioural alterations, but the combination of both drugs provoked changes in all of the behavioural parameters evaluated. These deleterious effects seem to be more relevant 7 days rather than 48 h after the treatment, suggesting possible long-term effects of the treatment which may favour future compulsive use of the drugs (Do Couto et al. 2011). The observed neuroinflammatory effects do not seem to explain the behavioural alterations since major neuroinflammatory responses are observed in MDMA-alone treated mice, whereas behavioural changes are preferentially present in ethanol-treated animals.

CD1 mice were selected for our experiments due to the fact that this strain is considered to have low preference for alcohol (Ryabinin et al. 2003; Rhodes et al. 2005). The ethanol preference differences between CD1 and other strains such as C57BL/6 J, have been attributed to changes in ethanol metabolism and palatability (Rhodes et al. 2007). In any case, CD1 animals reach a Blood Ethanol Concentration of about 50–70 mg%, which is high enough to cause behavioural effects. Indeed, the National Institute on Alcohol Abuse and Alcoholism considers a Blood Ethanol Concentration of 80 mg% as intoxication in humans (Crabbe et al. 2011).

Ethanol alone did not induce significant changes on basal body temperature. However, MDMA produced hyperthermia ($0.52 \pm 0.2^\circ\text{C}$) which has been reported to produce fatal consequences in rodents, primates and humans (Lyles and Cadet 2003; Cadet et al. 2007). Ethanol abolished MDMA-induced hyperthermia, thus confirming its hypothermic capacity. Our findings agree well with those found in previous studies (Cassel et al. 2004) showing that the acute combination of ethanol prevents the MDMA-induced hyperthermia. As in the study performed by Cassel et al. (2004), we found no differences between saline and ethanol-treated groups. Indeed, ethanol may exert protective effects against MDMA administration in different ways: (1) by reducing psychostimulant-induced hyperthermia (Cassel et al. 2004); (2) by causing peripheral vasodilatation, especially in the tail, where heat dissipation occurs (Green et al. 2005), and thus counterbalancing the MDMA-induced vasoconstriction; (3) by modulating the sensitivity of the thermosensitive hypothalamic neurons (Zoeller and Rudeen 1992), which may result in changes of the hyperpyretic effects of MDMA; and (4) by affecting

the distribution, biotransformation and elimination of MDMA (Hamida et al. 2007). In the same line, previous data also showed an increase in MDMA plasma or brain levels when MDMA was co-administered with ethanol (Hernández-López et al. 2002; Johnson et al. 2004).

Both ethanol (at low doses) and MDMA produce increases in spontaneous activity in humans and rodents (Kamens and Phillips 2008; Hamida et al. 2008; Rodsiri et al. 2011). In this sense, different studies have found that ethanol potentiated the hyperlocomotion effect of MDMA in rats (Hamida et al. 2007, 2008; Cassel et al. 2004, 2007). The stimulant effects of ethanol are known to be mediated by the release of dopamine in the mesocorticolimbic system (Di Chiara and Imperato 1985; Nestby et al. 1997), whereas hyperlocomotion induced by MDMA is due to a rapid release of dopamine in the mesocorticolimbic system (Tourinho et al. 2008), but also a release of serotonin (5-HT) at the cortical level (Green et al. 2003). Frequently, the stimulant effects of ethanol and MDMA are observed a few minutes after the co-administration of both drugs (Riepert et al. 2008). In these previous experiments, only acute, short term effects of the combination were investigated. However, in our study, the effects of drug co-administration on locomotor activity were assessed at long term, that was 48 h, 72 h or 7 days after the MDMA (or saline) treatment, and during ethanol absence, characterised by an irritability and dysphoria (Cole et al. 1999). Thus, our findings revealed lower horizontal and vertical activities 48 h after treatment in MDMA-injected mice pre-exposed to ethanol when compared with control groups. However, this change was not observed 72 h and 7 days after treatment. This hypoactive phenotype in ethanol plus MDMA group could be explained by behavioural alterations produced by the combination of both drugs rather than a specific acute pharmacological effect of these drugs. Indeed, when ethanol and MDMA were administered alone, no effects on locomotion were observed. However, the co-administration of MDMA and ethanol induced other behavioural changes including motor coordination impairment, anxiety-like behaviour and despair behaviour.

Repeated ethanol treatment induces impairment in motor coordination due to a long-lasting dysfunction of cerebellar neurons (Gruol et al. 1997; Wang et al. 1999) that alters the necessary integration of motor commands and sensory information to produce coordinated movements. Our results showed lower motor coordination skills 72 h and 7 days after ethanol treatment in mice, but not 48 h after. Additionally, 7 days after treatment, MDMA-treated animals showed this impairment but the combination of both drugs did not increase the motor coordination affectation. In mice, MDMA produced neurotoxicity in dopaminergic terminals which has been shown to produce motor impairment consequences. This effect is

attributed to the loss of nigrostriatal dopaminergic neurons (Touriño et al. 2010). A previous study by Cassel and colleagues found that co-administration of these drugs induced a severe impairment of the sensory–motor coordination compared with ethanol or MDMA alone in rats exposed to the beam-walking test (Cassel et al. 2005). Our results are not in agreement with this previous study as we do not find a major motor coordination impairment in animals treated with both drugs when compared to MDMA-treated or ethanol-treated animals. The apparent discrepancy could be due to different animal species (rat vs. mice) and the different methodology used to assess motor coordination test (rotarod apparatus vs. beam-walking test).

Previous studies have reported signs of anxiety and despair behaviour during withdrawal to long-term ethanol treatment in animals (File et al. 1993; Huang et al. 2010). These behavioural alterations could be explained by the important hormonal and neurotransmitter changes produced by ethanol, such as elevated levels of corticotrophin releasing factor (CRF) in the central nucleus of the amygdala (Merlo Pich et al. 1995), and decreased levels of GABA, dopamine and 5-HT in different limbic areas (Díaz et al. 2011; Weiss et al. 1996; Diana et al. 1993). Consistent with these data, the administration of CRF antagonists or serotonin 5-HT_{1A} receptor stimulation ameliorated the anxiety-like effects of ethanol absence, as measured on the elevated plus-maze (Lal et al. 1991; Rassnick et al. 1993). Although our study does not reveal that mice pre-exposed to ethanol spent less time in the lit compartment of the dark–light box, it does show that these mice spent more time immobile in the tail suspension test than the non ethanol-treated animals. This despair behaviour was observed 72 h and 7 days after the treatment, but not 48 h after. Conversely, different studies have shown that acute MDMA intoxication produces anxiety effects (Sumnall et al. 2004) that can be evidenced weeks, and even months after repeated MDMA injections (Bull et al. 2004; Clemens et al. 2004). On the contrary, our findings did not reveal a residual anxiety-like effect when MDMA was given alone (2×20 mg/kg, i.p., at 2 h interval) at any of the times evaluated after the treatment. Anxiety-like responses were only observed in animals that received both ethanol and MDMA. In agreement, Daza-Losada et al. (2009) demonstrated that acute MDMA given alone did not exert anxiety-like effects in the social interaction test in adolescent mice, whereas MDMA and cocaine co-administration induced anxiety-like responses in the same experimental conditions (Daza-Losada et al. 2009). A possible explanation might be that acute MDMA (2×20 mg/kg, i.p., at 2-h intervals) per se was not enough to induce significant levels of anxiety whereas it may be able to enhance anxiety-like effects of ethanol absence. On the other hand, experimental studies

have found that binge drinking of ethanol is an additional risk factor to develop depressive symptoms (Bazargan-Hejazi et al. 2008; Paljärvi et al. 2009) and that the brain of adolescent mice exhibited a higher sensitivity to ethanol and MDMA, thus effects of these drugs can be manifested after long-term (White and Swartzwelder 2005; Rodríguez-Arias et al. 2011). Moreover, this pattern of drinking during adolescence increases the long-lasting effects of MDMA on the loss of brain DA levels in adulthood (Rodríguez-Arias et al. 2011). In our study, mice pre-exposed to ethanol showed higher levels of inactivity in the tail suspension test (a behaviour of despair) compared with control group. This response was observed 72 h and 7 days after the treatment. MDMA given alone did not induce despair behaviour, but, importantly, its administration to ethanol pre-exposed mice did not enhance but accelerated the presentation of this depressive symptom. Anxiety and despair behaviours observed could be explained, as we stated above, by the fact that hormonal and monoamine levels are altered by ethanol, MDMA or both, and that adolescent brain is more sensitive to the combined effects of these drugs.

Data in the literature regarding ethanol plus MDMA neurotoxicity are inconsistent. Thus, some authors have observed an increase of MDMA-induced serotonin depletion when co-administered with ethanol (Izco et al. 2007), whereas others have not seen this effect (Cassel et al. 2005). Some cases report a protective effect of ethanol on MDMA-induced neurotoxicity (Johnson et al. 2004) whereas others did not find such a protective effect of ethanol (Rodríguez-Arias et al. 2011). In our study, MDMA-treated animals exhibited GFAP positive cells in striatum. Microglial expression was enhanced in MDMA-treated animals. The GFAP immunoreactivity response in MDMA group was mostly observed 48 h after the last MDMA injection, and could be attributed to MDMA-induced hyperthermia that exacerbates neuroinflammation (Colado et al. 2001; Touriño et al. 2010). This GFAP expression was decreased 72 h after treatment and even disappeared 7 days after. In contrast, the lower GFAP expression in MDMA-injected mice pre-exposed to ethanol (ethanol plus MDMA-treated mice) could be explained by the body temperature lowering effects. This different GFAP expression between MDMA-treated and ethanol plus MDMA-treated mice was neither observed at 72 h nor at 7 days after the treatment. In addition, ethanol alone did not induce neuroinflammation in our experimental procedure. As stated above, microglial expression was enhanced in animals injected with MDMA. This increase was observed 48 h after the treatment as a hypertrophy of microglial cells (revealed as an increase in the percentage of stained area) without increasing the number of cells. These changes in microglial cells morphology are denominated “activated microglia” and consist in processes

of retraction and hypertrophy of the cell body (Stoll and Jander 1999). Such activation was also observed after 72 h after the treatment when an increase in the number of these cells was also observed. After 7 days of treatment, this activated microglia only persisted in the MDMA-treated group, whereas this activation disappeared in ethanol plus MDMA-treated mice. Activated glial cells release different proinflammatory cytokines and reactive oxygen species that promote neurobehavioural alterations and neurodegeneration (Leonard 2007; Block et al. 2007). So, even though we did not observe a significant difference in the number of microglial cells after 48 h, it was evident the existence of an activated morphology of microglial cells, 72 h and even 7 days after the treatment with MDMA. However, these neuroinflammation does not totally correlate with the behavioural alterations exhibited by animals, since major neuroinflammation was observed in MDMA-treated animals which exhibited a low behavioural affectation. In addition, MDMA-induced hyperthermia is one of the factors most closely related with MDMA-induced neurotoxicity; but considering the behavioural alterations observed in ethanol plus MDMA group, we hypothesize that other neurochemical mechanisms might be involved.

In conclusion, our study demonstrates that binge ethanol drinking induces important physiological and behavioural alterations in adolescent mice. These negative behavioural effects are enhanced and maintained as long-lasting effects after the acute co-administration of high-doses of MDMA. Contrarily, neuroinflammatory response is majorly observed in MDMA-treated animals, and therefore, it is unlikely to underlie the deleterious effects of the combination of ethanol and MDMA on neurotoxicity, whose mechanism needs to be elucidated. Finally, our findings demonstrate the potential risk of the consumption of ethanol and MDMA in combination by adolescents, since behavioural effects can persist during a long period after concomitant exposure to these drugs.

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References

- Barrett SP, Darredeau C, Pihl RO (2006) Patterns of simultaneous polysubstance use in drug using university students. *Hum Psychopharmacol* 21:255–263
- Bazargan-Hejazi S, Bazargan M, Gaines T, Jemanez M (2008) Alcohol misuse and report of recent depressive symptoms among ED patients. *Am J Emerg Med* 26:537–544
- Bilsky EJ, Hubbell CL, Delconte JD, Reid LD (1991) MDMA produces a conditioned place preference and elicits ejaculation in male rats: a modulatory role for the endogenous opioids. *Pharmacol Biochem Behav* 40:443–447
- Block ML, Zecca L, Hong JS (2007) Microglia-mediated neurotoxicity: uncovering the molecular mechanisms. *Nat Rev Neurosci* 8:57–69
- Bull EJ, Hutson PH, Fone KC (2004) Decreased social behaviour following 3,4-methylenedioxymethamphetamine (MDMA) is accompanied by changes in 5-HT_{2A} receptor responsivity. *Neuropharmacology* 46:202–210
- Cadet JL, Krasnova IN, Jayanthi S, Lyles J (2007) Neurotoxicity of substituted amphetamines: molecular and cellular mechanisms. *Neurotox Res* 11:183–202
- Camí J, Farré M, Mas M et al (2000) Human pharmacology of 3,4-methylenedioxymethamphetamine (“ecstasy”): psychomotor performance and subjective effects. *J Clin Psychopharmacol* 20:455–466
- Cassel JC, Jeltsch H, Koenig J, Jones BC (2004) Locomotor and pyretic effects of MDMA-ethanol associations in rats. *Alcohol* 34:285–289
- Cassel JC, Riegert C, Rutz S et al (2005) Ethanol, 3,4-methylenedioxymethamphetamine (ecstasy) and their combination: long-term behavioral, neurochemical and neuropharmacological effects in the rat. *Neuropsychopharmacology* 30:1870–1882
- Cassel JC, Ben Hamida S, Jones BC (2007) Attenuation of MDMA-induced hyperthermia by ethanol in rats depends on ambient temperature. *Eur J Pharmacol* 571(2–3):152–155
- Clemens KJ, Van Nieuwenhuyzen PS, Li KM et al (2004) MDMA (“ecstasy”), methamphetamine and their combination: long-term changes in social interaction and neurochemistry in the rat. *Psychopharmacology (Berl)* 173:318–325
- Colado MI, Camarero J, Mechan AO et al (2001) A study of the mechanisms involved in the neurotoxic action of 3,4-methylenedioxymethamphetamine (MDMA, ‘ecstasy’) on dopamine neurones in mouse brain. *Br J Pharmacol* 134:1711–1723
- Cole JC, Littleton JM, Little HJ (1999) Effects of repeated ethanol administration in the plus maze; a simple model for conditioned abstinence behaviour. *Psychopharmacology (Berl)* 142:270–279
- Crabbe JC, Harris RA, Koob GF (2011) Preclinical studies of alcohol binge drinking. *Ann N Y Acad Sci* 1216:24–40. doi:10.1111/j.1749-6632.2010.05895.x
- Daza-Losada M, Rodríguez-Arias M, Maldonado C et al (2009) Acute behavioural and neurotoxic effects of MDMA plus cocaine in adolescent mice. *Neurotoxicol Teratol* 31:49–59
- Di Chiara G, Imperato A (1985) Ethanol preferentially stimulates dopamine release in the nucleus accumbens of freely moving rats. *Eur J Pharmacol* 115:131–132
- Diana M, Pistis M, Carboni S et al (1993) Profound decrement of mesolimbic dopaminergic neuronal activity during ethanol withdrawal syndrome in rats: electrophysiological and biochemical evidence. *Proc Natl Acad Sci USA* 90:7966–7969
- Díaz MR, Christian DT, Anderson NJ, McCool BA (2011) Chronic ethanol and withdrawal differentially modulate lateral/basolateral amygdala paracapsular and local GABAergic synapses. *J Pharmacol Exp Ther* 337:162–170
- Do Couto BR, Rodríguez-Arias M, Fuentes S et al (2011) Adolescent pre-exposure to ethanol or MDMA prolongs the conditioned rewarding effects of MDMA. *Physiol Behav* 103:585–593
- Fantegrossi WE, Ciullo JR, Wakabayashi KT et al (2008) A comparison of the physiological, behavioral, neurochemical and microglial effects of methamphetamine and 3,4-methylenedioxymethamphetamine in the mouse. *Neuroscience* 151:533–543
- File SE, Andrews N, al-Farhan M (1993) Anxiogenic responses of rats on withdrawal from chronic ethanol treatment: effects of tianeptine. *Alcohol Alcohol* 28:281–286

- Green AR, Mehan AO, Elliott JM, O'Shea E, Colado MI (2003) The pharmacology and clinical pharmacology of 3,4-methylenedioxymethamphetamine (MDMA, "ecstasy"). *Pharmacol Rev* 55:463–508
- Green AR, O'Shea E, Saadat KS, Elliott JM, Colado MI (2005) Studies on the effect of MDMA ('ecstasy') on the body temperature of rats housed at different ambient room temperatures. *Br J Pharmacol* 146:306–312
- Grothues JM, Bischof G, Reinhardt S et al (2008) Effectiveness of brief alcohol interventions for general practice patients with problematic drinking behavior and comorbid anxiety or depressive disorders. *Drug Alcohol Depend* 94:214–220
- Gruol DL, Parsons KL, DiJulio N (1997) Acute ethanol alters calcium signals elicited by glutamate receptor agonists and K⁺ depolarization in cultured cerebellar Purkinje neurons. *Brain Res* 773:82–89
- Gudelsky GA, Yamamoto BK (2003) Neuropharmacology and neurotoxicity of 3,4-methylenedioxymethamphetamine. *Methods Mol Med* 79:55–73
- Hamida SB, Plute E, Bach S et al (2007) Ethanol-MDMA interactions in rats: the importance of interval between repeated treatments in biobehavioral tolerance and sensitization to the combination. *Psychopharmacology (Berl)* 192:555–569
- Hamida SB, Plute E, Cosquer B et al (2008) Interactions between ethanol and cocaine, amphetamine, or MDMA in the rat: thermoregulatory and locomotor effects. *Psychopharmacology (Berl)* 197:67–82
- Hernández-López C, Farré M, Roset PN et al (2002) 3,4-Methylenedioxymethamphetamine (ecstasy) and alcohol interactions in humans: psychomotor performance, subjective effects, and pharmacokinetics. *J Pharmacol Exp Ther* 300:236–244
- Huang MM, Overstreet DH, Knapp DJ (2010) Corticotropin-releasing factor (CRF) sensitization of ethanol withdrawal-induced anxiety-like behavior is brain site specific and mediated by CRF-1 receptors: relation to stress-induced sensitization. *J Pharmacol Exp Ther* 332:298–307
- Hunt WA (1993) Are binge drinkers more at risk of developing brain damage? *Alcohol* 10:559–561
- Izco M, Orio L, O'Shea E, Colado MI (2007) Binge ethanol administration enhances the MDMA-induced long-term 5-HT neurotoxicity in rat brain. *Psychopharmacology (Berl)* 189:459–470
- Johnson EA, O'Callaghan JP, Miller DB (2004) Brain concentrations of d-MDMA are increased after stress. *Psychopharmacology (Berl)* 173:278–286
- Kamens HM, Phillips TJ (2008) A role for neuronal nicotinic acetylcholine receptors in ethanol-induced stimulation, but not cocaine- or methamphetamine-induced stimulation. *Psychopharmacology (Berl)* 196:377–387
- Lal H, Prather PL, Rezazadeh SM (1991) Anxiogenic behavior in rats during acute and protracted ethanol withdrawal: reversal by buspirone. *Alcohol* 8:467–471
- Lamb RJ, Griffiths RR (1987) Self-injection of d,l-3,4 methylenedioxymethamphetamine (MDMA) in the baboon. *Psychopharmacology (Berl)* 91:268–272
- Leonard BE (2007) Inflammation, depression and dementia: are they connected? *Neurochem Res* 32:1749–1756
- Lyles J, Cadet JL (2003) Methylenedioxymethamphetamine (MDMA, Ecstasy) neurotoxicity: cellular and molecular mechanisms. *Brain Res Brain Res Rev* 42:155–168
- Merlo Pich E, Lorang M, Yeganeh M et al (1995) Increase of extracellular corticotropin-releasing factor-like immunoreactivity levels in the amygdala of awake rats during restraint stress and ethanol withdrawal as measured by microdialysis. *J Neurosci* 15:5439–5447
- Nestby P, Vanderschuren LJ, De Vries TJ et al (1997) Ethanol, like psychostimulants and morphine, causes long-lasting hyperactivity of dopamine and acetylcholine neurons of rat nucleus accumbens: possible role in behavioural sensitization. *Psychopharmacology (Berl)* 133:69–76
- O'Shea E, Colado MI (2003) Is frequent dosing with ecstasy a risky business for dopamine-containing neurons? *Trends Pharmacol Sci* 24:272–274
- Pacifici R, Zuccaro P, Farré M et al (1999) Immunomodulating properties of MDMA alone and in combination with alcohol: a pilot study. *Life Sci* 65:PL309–PL316
- Paljärvi T, Koskenvuo M, Poikolainen K et al (2009) Binge drinking and depressive symptoms: a 5-year population-based cohort study. *Addiction* 104:1168–1178
- Pascual M, Blanco AM, Cauli O et al (2007) Intermittent ethanol exposure induces inflammatory brain damage and causes long-term behavioural alterations in adolescent rats. *Eur J Neurosci* 25:541–550
- Rassnick S, D'Amico E, Riley E, Koob GF (1993) GABA antagonist and benzodiazepine partial inverse agonist reduce motivated responding for ethanol. *Alcohol Clin Exp Res* 17:124–130
- Rhodes JS, Best K, Belknap JK, Finn DA, Crabbe JC (2005) Evaluation of a simple model of ethanol drinking to intoxication in C57BL/6 J mice. *Physiol Behav* 84:53–63
- Rhodes JS, Ford MM, Yu CH et al (2007) Mouse inbred strain differences in ethanol drinking to intoxication. *Genes Brain Behav* 6:1–18
- Riegt C, Wedekind F, Hamida SB (2008) Effects of ethanol and 3,4-methylenedioxymethamphetamine (MDMA) alone or in combination on spontaneous and evoked overflow of dopamine, serotonin and acetylcholine in striatal slices of the rat brain. *Int J Neuropsychopharmacol* 11:743–763
- Rodríguez-Arias M, Maldonado C, Vidal-Infer A et al (2011) Intermittent ethanol exposure increases long-lasting behavioral and neurochemical effects of MDMA in adolescent mice. *Psychopharmacology (Berl)* 2011 [Epub ahead of print]
- Rodsiri R, Spicer C, Green AR, Marsden CA, Fone KC (2011) Acute concomitant effects of MDMA binge dosing on extracellular 5-HT, locomotion and body temperature and the long-term effect on novel object discrimination in rats. *Psychopharmacology (Berl)* 213:365–376
- Ruiz-Medina J, Ledent C, Carreton O, Valverde O (2011) The A2a adenosine receptor modulates the reinforcement efficacy and neurotoxicity of MDMA. *J Psychopharmacol* 25:550–560
- Ryabinin AE, Galvan-Rosas A, Bachtell RK, Risinger FO (2003) High alcohol/sucrose consumption during dark circadian phase in C57BL/6 J mice: involvement of hippocampus, lateral septum and urocortin-positive cells of the Edinger–Westphal nucleus. *Psychopharmacology (Berl)* 165:296–305
- Sanchez V, O'shea E, Saadat KS et al (2004) Effect of repeated ('binge') dosing of MDMA to rats housed at normal and high temperature on neurotoxic damage to cerebral 5-HT and dopamine neurones. *J Psychopharmacol* 18:412–416
- Soria G, Castañé A, Ledent C, Parmentier M, Maldonado R, Valverde O (2006) The lack of A2A adenosine receptors diminishes the reinforcing efficacy of cocaine. *Neuropsychopharmacology* 31:978–987
- Steru L, Chermat R, Thierry B, Simon P (1985) The tail suspension test: a new method for screening antidepressants in mice. *Psychopharmacology (Berl)* 85:367–370
- Stoll G, Jander S (1999) The role of microglia and macrophages in the pathophysiology of the CNS. *Prog Neurobiol* 58:233–247
- Sumnall HR, O'Shea E, Marsden CA, Cole JC (2004) The effects of MDMA pretreatment on the behavioural effects of other drugs of abuse in the rat elevated plus-maze test. *Pharmacol Biochem Behav* 77:805–814

- Touriño C, Ledent C, Maldonado R, Valverde O (2008) CB1 cannabinoid receptor modulates 3,4-methylenedioxymethamphetamine acute responses and reinforcement. *Biol Psychiatry* 63:1030–1038
- Touriño C, Zimmer A, Valverde O (2010) THC prevents MDMA neurotoxicity in mice. *PLoS One* 5:e9143
- Trigo JM, Panayi F, Soria G, Maldonado R, Robledo P (2006) A reliable model of intravenous MDMA self-administration in naïve mice. *Psychopharmacology (Berl)* 184:212–220
- Valverde O, Célérier E, Baranyi M et al (2009) GPR3 receptor, a novel actor in the emotional-like responses. *PLoS One* 4:e4704
- Wang Y, Freund RK, Palmer MR (1999) Potentiation of ethanol effects in cerebellum by activation of endogenous noradrenergic inputs. *J Pharmacol Exp Ther* 288:211–220
- Wechsler H, Nelson TF (2001) Binge drinking and the American college student: what's five/four drinks? *Psychol Addict Behav* 15:287–291
- Weiss F, Parsons LH, Schulteis G et al (1996) Ethanol self-administration restores withdrawal-associated deficiencies in accumbal dopamine and 5-hydroxytryptamine release in dependent rats. *J Neurosci* 16:3474–3485
- White AM (2003) What happened? Alcohol, memory blackouts, and the brain. *Alcohol Res Health* 27:186–196
- White AM, Swartzwelder HS (2005) Age-related effects of alcohol on memory and memory-related brain function in adolescents and adults. *Recent Dev Alcohol* 17:161–176
- Winstock AR, Griffiths P, Stewart D (2001) Drugs and the dance music scene: a survey of current drug use patterns among a sample of dance music enthusiasts in the UK. *Drug Alcohol Depend* 64:9–17
- Zoeller RT, Rudeen PK (1992) Ethanol blocks the cold-induced increase in thyrotropin-releasing hormone mRNA in paraventricular nuclei but not the cold-induced increase in thyrotropin. *Brain Res Mol Brain Res* 13:321–330

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