Behavioural changes in rats treated with a neurotoxic dose regimen of dextrorotatory amphetamine derivatives

J. Timár[a], S. Gyarmati[a], A. Szabó[b] and S. Fürst[a]

Repeated administration of amphetamine derivatives is reported to induce neurotoxicity in rat brain. Methamphetamine (MA) impairs the function of both the dopaminergic and serotonergic systems, 3,4-methylenedioxymethamphetamine (MDMA, Ecstasy) affects primarily the latter system. The neurochemical deficits induced by these amphetamines have been described in detail, but relatively few data have been reported regarding the behavioural consequences of the neurotoxic treatment. The aim of the present study was to investigate short- and long-term behavioural consequences of treatment with neurotoxic doses of (+)MA or (+)MDMA. Spontaneous locomotor activity in novel surroundings and cognitive function (avoidance behaviour) were evaluated. Rats were treated with four s.c. injections of 10 mg/kg (+)MA or (+)MDMA with a 2-hour interval between each injection. Behavioural tests were then carried out 3 days, 1, 2 and 4 weeks after these treatments. A reduction of spontaneous locomotor activity in novel surroundings was detected 3 days after the treatment with the study drugs, but not after 1, 2 and 4 weeks. No change in passive and active avoidance learning was observed. The lack of marked behavioural changes after a neurotoxic dose regimen indicates that some compensatory mechanisms may develop and counterbalance the neurochemical changes induced by these amphetamine compounds. *Behavioural Pharmacology* 14:199–206 © 2003 Lippincott Williams & Wilkins.

Behavioural Pharmacology 2003, **14:**199–206

Keywords: neurotoxicity, (+)methamphetamine, (+)3,4-methylenedioxymethamphetamine, locomotor activity, learning and memory, rat

[a]Semmelweis University, Department of Pharmacology and Pharmacotherapy, Budapest, Hungary and [b]Sanofi-Synthelabo-Chinoin Ltd, Budapest, Hungary.

Sponsorship: This study was supported by Hungarian grants OTKA TO-32736 and ETT-156/2000.

Correspondence and requests for reprints to Julia Timár, Semmelweis University, Department of Pharmacology and Pharmacotherapy, Nagyvárad tér 4., Budapest, Hungary, H-1089.
E-mail: timjul@pharma.sote.hu

Received 7 October 2002 **Accepted as revised** 19 March 2003

Introduction

Amphetamine and its congeners are potent psychostimulants with marked abuse potential. The methylated derivative, methamphetamine (MA) and the methylenedioxy derivatives, such as methylenedioxyamphetamine (MDA) or 3,4-methylenedioxymethamphetamine (MDMA, Ecstasy), are widely used recreational drugs. The methyl substitution on the alkyl chain and the presence of the methylenedioxy group on the phenyl ring enhance the stimulant potency and result in much more complex pharmacological effects, both on serotonergic (5-HT) and dopaminergic (DA) transmission. The popularity of MDMA – as a rather 'safe' drug – has been growing continuously since the second half of the 1980s. Recently, however, these psychomotor stimulants have been reported to produce neurotoxicity in experimental animals.

Neurochemical consequences of administration of the two most frequently applied amphetamine-like abused drugs, MA and MDMA, are different. While MA has been reported to induce a long-term reduction in markers of both DA-ergic and 5-HT-ergic function (Ricaurte *et al.*, 1980; Robinson *et al.*, 1990; Walsh and Wagner, 1992; Wallace *et al.*, 1999), MDMA affects primarily 5-HT (Stone *et al.*, 1986; Ricaurte *et al.*, 1988; Marston *et al.*, 1999; Wallace *et al.*, 2001). The neurochemical changes produced by the so-called 'neurotoxic' doses of these compounds have been demonstrated to persist for a long period in certain parts of the brain, including striatum, hypothalamus and cortex (Lucot *et al.*, 1980; Ricaurte *et al.*, 1980; Battaglia *et al.*, 1988; Sabol *et al.*, 1996). Doses that produce neurotoxicity in experimental animals are rather close to doses used by abusers. Recently some results raised the possibility that persisting neurochemical changes and impairment of cognitive function may also develop in MDMA abusers (Parott and Lasky, 1998; McCann *et al.*, 1998, 1999).

In comparison to the number of papers published on the neurochemical deficit in experimental animals, relatively few studies can be found in the literature regarding long-term behavioural consequences of the MA- or MDMA-induced neurotoxicity. Walsh and Wagner (1992) measured a performance deficit in a beam-balance apparatus and an increase in response latency during active avoidance learning in animals treated with repeated doses of MA; however, they did not observe differences in the number of avoidance responses. Wallace *et al.* (1999) observed a significant attenuation of spontaneous

0955-8810 © 2003 Lippincott Williams & Wilkins DOI: 10.1097/01.fbp.0000074775.49104.47

activity 1 week after MA-induced neurotoxicity. Richards *et al.* (1993) observed a long-lasting deficit on a reaction–time task with a rather high dose of MA (3×50 mg/kg) and Friedmann *et al.* (1998) described a water-maze performance deficit more than 2 months after neurotoxic MA treatment. Recently Mechan *et al.* (2002) demonstrated a reduction in anxiety-related behaviour 2 months after administration of a single neurotoxic dose of MDMA.

Ricaurte *et al.* (1993) studied the long-term behavioural consequences of neurotoxic MDMA treatment and reported no change in memory performance measured in a T-maze procedure. Marston *et al.* (1999) did not observe any difference between vehicle and MDMA-treated groups with respect to locomotor activity, skilled paw-reaching ability and the level of performance measured in a delayed non-match to place procedure; however, they found a difference in the progressive improvement in performance.

Similarly to other amphetamines, MA and MDMA exist in two stereoisomer forms. In the majority of experiments the racemic form of MA or MDMA was used. The dextrorotatory forms of amphetamines are, however, more potent releasers of DA and/or 5-HT, and more potent stimulators of locomotor activity, than the levorotatory forms (Schechter, 1987; Schmidt *et al.*, 1987b; Hiramatsu *et al.*, 1989; Timár *et al.*, 1996). According to Hiramatsu and Cho (1990) only (+)MDMA causes DA release in the striatum. In good agreement with the animal data, dextrorotatory MDMA has also been demonstrated to be more potent in humans (Anderson *et al.*, 1978). These results raise the possibility that the stimulant effects of raceme MDMA reside primarily in the (+) enantiomer.

The aim of the present study was to examine whether treatment with neurotoxic dose regimens of (+) enantiomers results in long-term changes of behaviour. For this purpose the spontaneous activity (in novel surroundings) and the cognitive function of animals (in both active and passive avoidance procedures) were examined, following treatment with (+)MA or (+)MDMA. In order to follow both the short-term and long-term changes, the experiments were carried out at different time intervals after treatment.

Methods

Subjects

Male Wistar rats (Charles River, Budapest), weighing 140–160 g at the beginning of the experiments, were housed in groups of five at constant temperature (20–21°C) and humidity ($55 \pm 5\%$) under a standard 12/12 h light/dark cycle (light on at 06.00 hours). Water and food were freely available, except during the tests. All the observations were made at comparable times of day during the light period. Each animal was used for only one behavioural test.

All experiments were performed according to the Semmelweis University guidelines on the use of experimental animals under the licence of the Ethical Committee.

Drug treatment

Single treatment

Altogether 80 animals were used to study the acute effect of the compounds. The animals were divided into eight groups (10 in each) and were treated with single subcutaneous (s.c.) injections of (+)MA or (+)MDMA. Control animals received physiological saline. Two doses of the test compounds were applied: 3 mg/kg, which is a stimulant dose of amphetamines, and 10 mg/kg, which was used to induce neurotoxicity following repeated administration in the second series of experiments. Spontaneous motor activity was measured 30 min after the treatment.

Repeated injections

The animals were treated with four s.c. injections of either 10 mg/kg (+)MA or 10 mg/kg (+)MDMA, with 2-hour intervals between injections, starting at 08.00 hours. Body weight and body temperature were measured immediately before the first and 30 min after the last injection. The animals of the control groups were injected with physiological saline according to the same treatment schedule. One day prior to treatment the animals were placed individually in small wire-mesh cages ($20 \times 16 \times 16$ cm), and remained there during the treatment and for three consecutive days. Afterwards they were placed back in the usual Macrolon cages in groups of five.

Altogether 280 animals were used for this series of experiments, divided into 14 groups (20 in each). Animals in each group were treated on the same day, 12 with one of the test drugs and 8 with saline.

Apparatus and procedure

Simultaneous measurement of horizontal and vertical activity

Horizontal locomotor activity (HA) and vertical locomotor activity (VA, rearing) were measured simultaneously by an 'Animal Activity Measurement System' (Research Institute of the Electrical Industry, Budapest, Hungary). The apparatus consists of two testing boxes ($50 \times 50 \times 30$ cm), each with a TV camera. The testing boxes are black-painted open-field areas, set in an isolated dark room, illuminated by two standard laboratory lamps. The experimental animals were placed into the boxes individually and their movement was followed by the TV cameras, using a mirror inclined in an angle of 45°. The computer separated the above-mentioned elements

of the activity; the time passed in each activity during the whole observation period being recorded. All data were registered, processed and analysed statistically by a PC.

Thirty minutes after the single treatment, and 3 days, 1 week, 2 weeks and 4 weeks after the repeated (neurotoxic) treatment, the animals were transported to the activity testing room and put into the testing boxes individually. Observation started immediately without any habituation period, and lasted 20 min.

Passive avoidance

Rats were trained to perform an avoidance response in a step-through-type passive avoidance apparatus. The apparatus consists of two compartments, a smaller, illuminated one (8×17×17 cm) and a bigger, dark one (22×25×25 cm), separated by a guillotine door, placed in an isolated dark room. One day prior to acquisition training the animals were allowed to habituate to the boxes by having free access to both sides for a 5 min period. On the day of the acquisition trial (training session) the animals were placed into the light compartment with the guillotine door open, and the latency time to step through into the dark compartment with all four feet was measured. When the animal stepped through, the door was closed and a footshock (1.2 mA) was delivered to the grid floor of the dark compartment for 10 s. The animal was then removed from the box. Rats that did not step through during the maximum latency time (180 s) were dropped from the experiment. In the test trial, 48 h after training (retention session), the animals were placed again into the light compartment and the response latency to step through into the dark compartment was measured, with a maximum latency of 180 s.

Passive avoidance experiments were initiated 3 days and 4 weeks after the repeated (neurotoxic) treatment with amphetamines.

Active avoidance procedure (shuttle-box behaviour)

Two-way avoidance and escape behaviour was tested in a shuttle-box apparatus, consisting of two identical compartments (30 × 30 × 30 cm each) separated by a barrier with a gate in the middle, placed in an isolated room. Animals were trained to cross the barrier on presentation of a light flash (conditioned stimulus, CS). If they failed to do so, they were punished with a footshock (unconditioned stimulus, US) of 0.3 mA delivered via the grid floor of the shuttle-box. They were given 100 trials in daily sessions for five consecutive days.

Each trial started with a resting period (intertrial interval) of 15 s. Intertrial crossings (IC) were not punished. After the intertrial interval, the light (CS) was switched on, which continued for a maximum of 10 s, signalling the impending footshock. If the rat responded within this 10 s period, the CS was terminated and the trial was ended without delivery of the US. If the animal failed to respond, the footshock (US) was applied together with the light until the rat responded, or for a maximum of 5 s. If the rat failed to escape the US within 5 s, an escape failure (EF) was counted. The number of conditioned avoidance responses (CAR), EF and IC were recorded during the five daily sessions.

Shuttle-box experiments were initiated 4 days after the repeated (neurotoxic) treatment with (+)MA or (+)MDMA and the first series (5 days) were followed by a second series (5 days again) with a 1 week break between them.

Drugs

(+)Methamphetamine hydrochloride (MA) and (+)methylenedioxymethamphetamine hydrochloride (MDMA) were generously provided by Sanofi–Synthelabo–Chinoin (Budapest, Hungary). Both compounds were dissolved in physiological saline, and given in a volume of 1 ml/kg body weight. The doses refer to free base.

Statistical analysis

The data are presented as mean values, with standard errors of the mean (mean ± SEM). Statistical significance of difference between mean values was evaluated by paired Student's *t*-test for body weight and body temperature, one-way ANOVA for locomotor activity following single treatment, by unpaired Student's *t*-test for locomotor activity following repeated treatment and for passive avoidance. Shuttle-box behaviour was analysed by two-way ANOVA. A P value of less than 0.05 was considered to be significant.

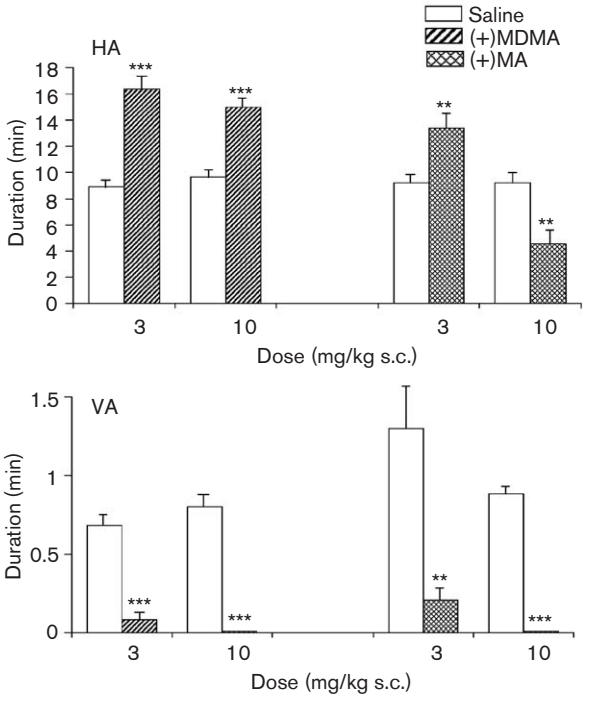
Results

Locomotor activity after single treatment

The results obtained following single treatments are summarized in Fig. 1. (+)MDMA-treated animals displayed significantly higher time in HA and less time in VA [$F(3,27) = 21.22$, $F(3,27) = 30.27$, $P < 0.001$, respectively]. Newman–Keuls *post hoc* analysis confirmed a statistically significant difference between the MDMA- and saline-treated animals for both doses (3 and 10 mg/kg) of (+)MDMA.

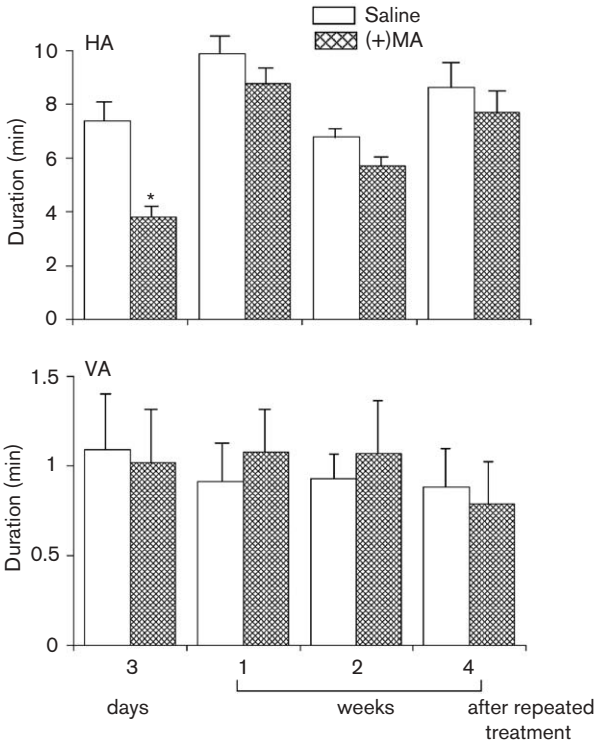
One-way ANOVA analysis of the results following (+)MA treatment also confirmed a significant difference in both HA and VA [$F(3,27) = 15.68$, $F(3,27) = 11.96$, $P < 0.001$, respectively]. Newman–Keuls *post hoc* analysis confirmed a statistically significant decrease in time spent in vertical activity for both doses (3 and 10 mg/kg) of (+)MA, but demonstrated different directions of changes in the case of horizontal activity. Relative to saline-treated animals, those treated with 3 mg/kg (+)MA displayed a signifi-

Fig. 1



Effects of single doses of methamphetamine [(+)MA] and 3,4-methylenedioxymethamphetamine [(+)MDMA] on horizontal (HA) and vertical (VA) locomotor activity. Vertical axis: duration (min) of HA or VA. The observation period (20 min) started 30 min after the s.c. drug or saline treatment. ** $P<0.01$; *** $P<0.001$ compared to the saline-treated group (unpaired Student's *t*-test).

Fig. 2



Horizontal (HA) and vertical (VA) locomotor activity of rats pretreated with a neurotoxic dose regimen of methamphetamine [(+)MA; 4 × 10 mg/kg s.c. at 2-hour intervals]. Vertical axis: duration (min) of HA or VA; horizontal axis: post-treatment time. * $P<0.05$ compared to the saline-pretreated group (unpaired Student's *t*-test).

cantly longer time in HA, while animals treated with 10 mg/kg (+)MA displayed significantly less time in HA.

Repeated (neurotoxic) treatment

Body weight and body temperature

The body weight of the (+)MA-treated animals decreased significantly (-23.9 ± 2.7 g, $t = 10.43$, $P < 0.001$) and their body temperature increased significantly (2.37 ± 0.32°C, $t = 7.36$, $P < 0.001$) 30 min after the fourth injection, compared to the baseline values measured immediately before the first treatment. Similar results were obtained after the fourth injection of (+)MDMA: body weight was lower (-27.9 ± 2.6 g, $t = 10.83$, $P < 0.001$) and body temperature was higher (2.26 ± 0.28°C, $t = 7.93$, $P < 0.001$). No statistically significant difference was observed either in the body weight or in the body temperature during the repeated saline treatment (-1.5 ± 1.6 g; $t = 0.93$ and -0.04 ± 0.11°C, $t = 0.35$, respectively).

Spontaneous locomotor activity (simultaneous measurement of horizontal and vertical activity)

The changes in spontaneous locomotor activity induced by repeated doses of (+)MA and (+)MDMA were examined 3 days, 1, 2 and 4 weeks after drug administration. The results are summarized in Figs. 2 and 3.

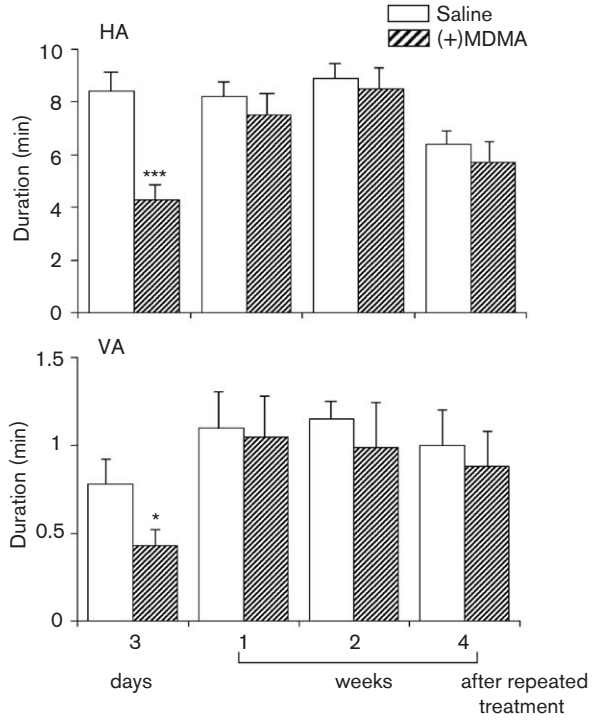
Spontaneous locomotor activity was found to be reduced 3 days after repeated (+)MA and (+)MDMA pretreatment. For HA, the difference was significant for both compounds ($t = 2.691$, $P < 0.05$ and $t = 4.868$, $P < 0.001$, respectively). VA was significantly reduced after repeated (+)MDMA pretreatment ($t = 2.194$, $P < 0.05$) but not after the (+)MA pretreatment.

No difference was observed in the spontaneous locomotor activity of animals 1, 2 and 4 weeks after repeated pretreatment with amphetamines, compared to that of the controls.

Passive avoidance

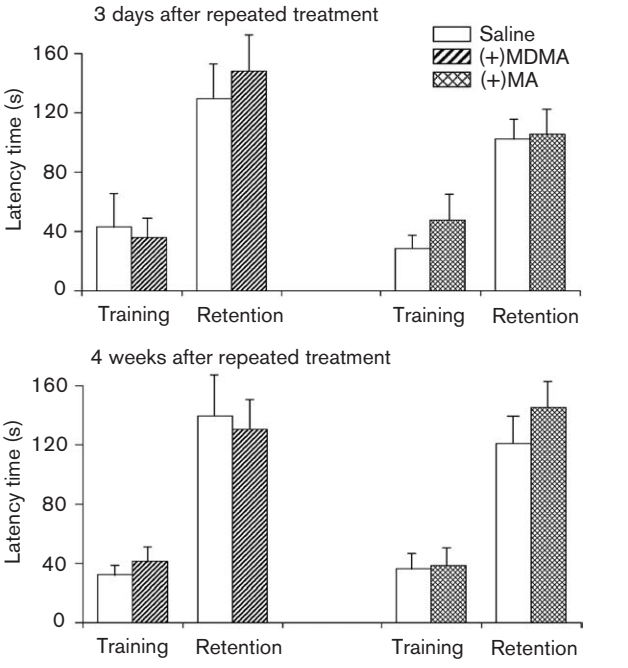
Passive avoidance behaviour was evaluated 3 days and 4 weeks after repeated administration of neurotoxic doses of (+)MA or (+)MDMA. On the training session, the majority of animals entered the dark compartment within 40 s. On the retention session, the average latency to step-through was over 100 s. There was no significant

Fig. 3



Horizontal (HA) and vertical (VA) locomotor activity of rats pretreated with a neurotoxic dose regimen of 3,4-methylenedioxymethamphetamine [(+)MDMA; 4 × 10 mg/kg s.c. at 2-hour intervals]. Vertical axis: duration (min) of HA or VA; horizontal axis: post-treatment time. *** $P<0.001$ compared to the saline-pretreated group (unpaired Student's *t*-test).

Fig. 4



Passive avoidance behaviour 3 days and 4 weeks after pretreatment with neurotoxic dose regimens of methamphetamine [(+)MA] or 3,4-methylenedioxymethamphetamine [(+)MDMA] (4 × 10 mg/kg s.c. at 2-hour intervals). Vertical axis: step-through latency time (s).

difference in the latency time between the drug-treated and the saline-treated animals, either on the training or the retention session. The results are presented in Fig. 4.

Active avoidance

Shuttle-box behaviour was assessed 4 days after pretreatment with repeated doses of (+)MA or (+)MDMA. Animals were observed for five consecutive days and, after a one-week interval, for another 5 days, that is until the 20th post-treatment day. The results are summarized in Fig. 5. Neither the (+)MA-pretreated, nor the (+)MDMA-pretreated animals showed any significant difference compared to saline-pretreated animals. This was true for all the parameters measured: active avoidance responses (CAR), escape failures (EF) and number of intertrial crossings (IC).

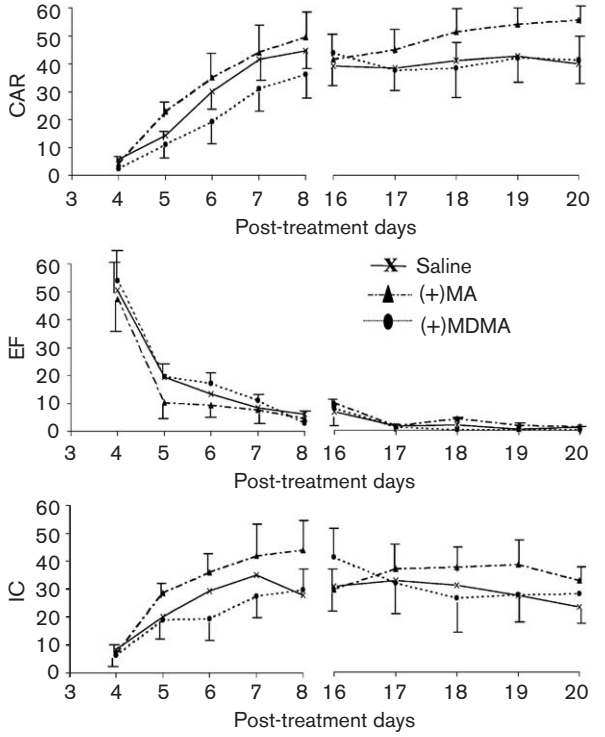
Discussion

Amphetamine derivatives, administered in high repeated doses, have been reported to induce long-term depletion of monoamines. While (±)MDMA-treated animals develop long-term reduction in various 5-HT axonal markers (Stone *et al.*, 1986; Battaglia *et al.*, 1987; Schmidt and Taylor, 1987; Obradovic *et al.*, 1998), high doses of (±)MA affect both the 5-HT-ergic and DA-ergic neurones, producing reductions not only in 5-HT, but also in DA axonal markers (Hotchkiss and Gibb, 1980; Lucot *et al.*, 1980; Ricaurte *et al.*, 1980, 1982).

However, MDMA enantiomers affect monoamine concentrations in different ways: the (+) enantiomer was demonstrated to result in a significant decrease in both DA and 5-HT concentrations in the striatum, while the same dose of the (–) enantiomer influenced only 5-HT (Schmidt *et al.*, 1987). At the same time, the dextrorotatory enantiomers of amphetamine derivatives have been reported to be more potent stimulators of behavioural activity, as discussed in the Introduction. That is why in the present experiments the (+) forms of the two amphetamine derivatives, MA and MDMA, were used.

In the early neurotoxic experiments, rather high single or repeated doses of amphetamine derivatives were applied (Lucot *et al.*, 1980; Ricaurte *et al.*, 1980, 1982; Battaglia *et al.*, 1988); later, however, smaller doses, given at close intervals, were also reported to induce marked neurochemical changes (Scanzello *et al.*, 1993; Fischer *et al.*, 1995; Friedmann *et al.*, 1998; Wallace *et al.*, 1999, 2001). In addition, the dose – 150–1250 mg/day – reported to be

Fig. 5



Active avoidance (shuttle-box) behaviour after pretreatment with neurotoxic dose regimens of methamphetamine [(+)MA] or 3,4-methylenedioxymethamphetamine [(+)MDMA] (4 × 10 mg/kg s.c. at 2-hour intervals). Vertical axis: number of active conditioned response (CAR), escape failure (EF) and intertrial crossings (IC).

used by MDMA abusers (McCann *et al.*, 1998) is closer to the smaller doses. Therefore, we chose a treatment schedule with a relatively small dose (10 mg/kg) administered every 2 h, four doses in all. This dose regimen has been reported to induce a marked reduction of striatal DA and 5-HT concentrations measured 15–17 days after treatment with (+)MA, and that of 5-HT after (±)MDMA treatment (Wallace *et al.*, 2001).

Single doses of amphetamine derivatives result in hyperthermia, increase motor activity and can induce the 5-HT syndrome in rats (Green *et al.*, 1995). In our experiments (+)MDMA in doses of 3 and 10 mg/kg – in good agreement with the literature (Gold *et al.*, 1988; Scearce-Levie *et al.*, 1999) – significantly enhanced HA and reduced VA. However, (+)MA displayed the characteristic biphasic effect of amphetamines: the low dose significantly increased, while the higher dose significantly decreased, HA. Both the low and the high doses decreased VA.

Three days after repeated administration of the high dose (10 mg/kg), a decrease of HA was measured in novel surroundings. VA was also found to be reduced in animals pretreated with the neurotoxic dose regimen of (+)MDMA, but no change was observed after (+)MA pretreatment. However, 1 week, 2 weeks and 4 weeks after this pretreatment, there was no difference in the spontaneous activity of animals between drug and saline groups.

Our results are in good agreement with those of Lucot *et al.* (1980) and Marston *et al.* (1999). Both observed no difference in the locomotor activity of animals treated 2 weeks earlier with neurotoxic dose regimens of MA (Lucot *et al.*, 1980) or (±) MDMA (Marston *et al.*, 1999).

Wallace *et al.* (2001) applied the same neurotoxic regimens for (+)MA and (±)MDMA as we did, and reported a decrease in spontaneous locomotor activity 7 days after treatment. In contrast, Kita *et al.* (1998) demonstrated an increase in ambulatory activity during the dark cycle of the day following neurotoxic MA treatment. The main difference between these and our studies lies in the behavioural procedure. Both earlier studies measured the spontaneous motor activity in a residential activity chamber during the whole day, while in our experiment the changes in the investigatory behaviour in novel surroundings were measured for 20 min.

Novel surroundings might mean a relatively strong stimulus and might produce a stronger activation. It can be speculated that the reduction of dopaminergic transmission induced by the dose regimen of (+)MA and (+)MDMA applied both by Wallace *et al.* (2001) and in our experiments is marked enough to decrease the investigatory behaviour 3 days after the treatment. During the following days, however, some compensatory mechanisms might partly counterbalance the stimulus without affecting the spontaneous locomotion in a residential situation. In addition, the strain of rat that Wallace *et al.* (2001) used was different (Sprague–Dawley) and it is conceivable that compensatory mechanisms work with different intensity in different strains.

While measuring some temporary decreases in the spontaneous activity in novel surroundings, we could not detect any change either in active or in passive avoidance performance following neurotoxic treatment with (+)MA or (+)MDMA. These data are consistent with the results of Ricaurte *et al.* (1993), who observed no effect on memory assessed in a spatial alternation task in a T-maze, following neurotoxic (+)MDMA treatment. Marston *et al.* (1999) also reported no difference between saline and the (±)MDMA-treated groups in the level of performance measured in a delayed non-match to place

procedure task; however, the (±)MDMA-treated animals did not exhibit progressive improvement in further performances.

Walsh and Wagner (1992) applied a similar neurotoxic dose schedule of MA as in the present study and did not observe any difference in inhibitory avoidance or rotarod performance 1 week after MA-induced neurotoxicity. However, they reported the appearance of significant deficits in active avoidance performance and balance beam performance. This change in active avoidance was an increase in latency to respond which – as the authors also emphasized – was attributable to motor disturbances rather than to drug-induced cognitive impairment.

Richards *et al.* (1993) observed a persistent deficit on a reaction-time task in rats, which lasted 3 months after MA treatment. Mechan *et al.* (2002) reported a reduction in anxiety-related behaviour tested in the elevated-plus maze and open field on days 71–73 (open field) and on day 80 (plus maze) – but not earlier – following a single neurotoxic dose of MDMA. Therefore, it is possible that the time points after the treatment we used in our experiments were not long enough to detect any changes. It must be mentioned, however, that the neurotoxic dose schedule applied by Richards *et al.* (1993) was rather high and Mechan *et al.* (2002) used the Dark Agouti strain of rats, which seemed to be more sensitive to MDMA-produced degeneration than the Wistar rats.

Based on our results, we may state that apart from a short-term decrease in the spontaneous locomotor activity in novel surroundings, no long-term behavioural changes or impairment of the learning and memory processes were observed in animals treated with neurotoxic dose regimens of (+)MA or (+)MDMA. The results indicate a marked discrepancy between the behavioural and biochemical data. An explanation for the discrepancy might be that a decrease in monoamine levels is not enough to provoke marked changes in spontaneous behaviour. Following the administration of a similar dose schedule to that applied in the present study, a depletion of serotonin (in the case of MDMA) or of serotonin and dopamine (in the case of MA) to 30–50% of the control value was demonstrated (Schmidt *et al.*, 1987; Walsh and Wagner, 1992; Scanzello *et al.*, 1993; Wallace *et al.*, 2001). If the fact that more than an 80% depletion of tissue catecholamine is required to provoke a functional deficit (Castaneda *et al.*, 1990) is also valid for the 5-HT system, the incomplete decrease in monoaminergic concentration induced by MA or MDMA may explain the lack of development of marked long-term behavioural deficits.

Another explanation may reside in the activation of the previously hypothesized compensatory mechanisms. As the neurochemical changes are demonstrated to persist for a long period, the development of some adaptive mechanisms, and their role in compensation of long-term behavioural consequences, has to be considered.

Gyarmati *et al.* (2001) reported no change in the sensitivity of postsynaptic receptors to the DA receptor agonist apomorphine and the 5-HT receptor agonist 5-methoxy-desmethyltryptamine, 1 week and 4 weeks following pretreatment with a neurotoxic dose regimen of (+)MA, which indicates that basal transmitter function is not affected too strongly.

Robinson *et al.* (1990) reported that, in contrast to marked depletion of striatal DA in animals treated with neurotoxic doses of MA, there was no difference in the resting extracellular concentration of DA in the striatum, compared to that of saline-pretreated controls. They suggested that (at least in part) these adaptive responses might be responsible for the absence of obvious behavioural deficits in animals exposed the neurotoxic dose of MA. The significance of the compensatory presynaptic changes was also emphasized by Wallace *et al.* (1999), who observed no difference in the DA release stimulated by a small dose of MA, in spite of significant decreases in DA and 5-HT contents in animals treated with a neurotoxic dose regimen of (+)MA. Series *et al.* (1994) demonstrated no difference in basal levels of 5-HT in frontal cortex, measured by microdialysis procedure, between control animals and animals treated with a neurotoxic dose regimen of (±)MDMA 2 weeks earlier. They concluded that 5-HT output from spared axons increases to offset or prevent loss of function.

Whether these compensatory mechanisms also occur for monoamine depletion induced by (+)MDMA and for the 5-HT depletion induced by (+)MA remains to be demonstrated. The data mentioned above, however, make it rather likely. If this is the case, it offers potential for speculating about the role of these compensatory responses in the lack of obvious behavioural deficits in animals exposed to neurotoxic doses of (+)MA or (+)MDMA.

In summary, treatment of rats with neurotoxic dose regimens of (+)MA or (+)MDMA resulted in a short-term reduction of spontaneous locomotor activity in novel surroundings, which could not be detected after 1 week. At the same time, no changes in passive or active avoidance performance were detected. Whether the lack of marked behavioural changes, as a consequence of neurotoxic treatment, can be explained by the development of compensatory mechanisms remains to be demonstrated.

References

Anderson GM, Braun G, Braun U, Nichols DE, Shulgin AT (1978). Absolute configuration and psychotomimetic activity. *NIDA Res Monograph* **22**:8–15.

Battaglia G, Yeh SY, O'Hearn E, Molliver ME, Kuhar MJ, De Souza EB (1987). 3,4-methylenedioxymethamphetamine and 3,4-methylenedioxyamphetamine destroy serotonin terminals in rat brain: quantification of neurodegeneration by measurement of [^{3}H]paroxetine-labeled serotonin uptake sites. *J Pharm Exp Ther* **242**:911–916.

Battaglia G, Yeh SY, De Souza EB (1988). MDMA-induced neurotoxicity: parameters of degeneration and recovery of brain serotonin neurons. *Pharmacol Biochem Behav* **29**:269–274.

Castaneda E, Whishaw IQ, Robinson TE (1990). Changes in striatal dopamine neurotransmission assessed with microdialysis following recovery from a bilateral 6-OHDA lesion: variation as a function of lesion size. *J Neurosci* **10**:1847–1854.

Fischer C, Hatzidimitriou G, Wlos J, Katz J, Ricaurte G (1995). Reorganization of ascending 5-HT axon projections in animals previously exposed to the recreational drug (±)3,4-methylenedioxymethamphetamine (MDMA, 'Ecstasy'). *J Neurosci* **15**:5476–5485.

Friedmann SD, Castaneda E, Hodge GK (1998). Long-term monoamine depletion, differential recovery, and subtle behavioral impairment following methamphetamine-induced neurotoxicity. *Pharmacol Biochem Behav* **61**:35–44.

Gold LH, Koob GF, Geyer MA (1988). Stimulant and hallucinogenic behavioral profiles of 3,4-methylenedioxymethamphetamine and *N*-ethyl-3,4-methylenedioxyamphetamine in rats. *J Pharmacol Exp Ther* **247**:547–555.

Green AR, Cross AJ, Goodwin GM (1995). Review of the pharmacology and clinical pharmacology of 3,4-methylenedioxymethamphetamine (MDMA or 'Ecstasy'). *Psychopharmacology* **119**:247–260.

Gyarmati Zs, Timár J, Fürst Zs (2001). Behavioural consequences of methamphetamine-induced neurotoxicity in rats. *Neurobiology* **9**:37–39.

Hiramatsu M, Cho AK (1990). Enantiomeric differences in the effects of 3,4-methylenedioxymethamphetamine on extracellular monoamines and metabolites in the striatum of freely-moving rats: an *in vivo* microdialysis study. *Neuropharmacology* **29**:269–275.

Hiramatsu M, Nabeshima T, Kameyama T, Maeda Y, Cho AK (1989). The effect of optical isomers of 3,4-methylenedioxymethamphetamine (MDMA) on stereotyped behavior in rats. *Pharmacol Biochem Behav* **33**:343–347.

Hotchkiss AJ, Gibb JW (1980). Long-term effects of multiple doses of methamphetamine on tryptophan hydroxylase and tyrosine hydroxylase activity in rat brain. *J Pharmacol Exp Ther* **214**:257–262.

Kita T, Takahashi M, Wagner GC, Kubo K, Nakashima T (1998). Methamphetamine-induced changes in activity and water intake during light and dark cycles in rats. *Prog Neuropsychopharmacol Biol Psychiatry* **22**:1185–1196.

Lucot JB, Wagner GC, Schuster CR, Seiden LS (1980). The effects of dopaminergic agents on the locomotor activity of rats after high doses of methylamphetamine. *Pharmacol Biochem Behav* **13**:409–413.

Marston HM, Reid ME, Lawrence JA, Olverman HJ, Butcher SP (1999). Behavioural analysis of the acute and chronic effects of MDMA treatment in the rat. *Psychopharmacology* **144**:67–76.

McCann UD, Szabo Z, Scheffel U, Dannals RF, Ricaurte GA (1998). Positron emission tomographic evidence of toxic effect of MDMA ('Ecstasy') on brain serotonin neurons in human beings. *Lancet* **352**:1433–1437.

McCann UD, Eligulashvili MMV, Ricaurte GA (1999). Cognitive performance in (±) 3,4-methylenedioxymethamphetamine (MDMA, 'ecstasy') users: a controlled study. *Psychopharmacology* **143**:417–425.

Mechan AO, Moran PM, Elliott JM, Young AMJ, Joseph MH, Green AR (2002). A study of the effect of a single neurotoxic dose of 3,4-methylenedioxymethamphetamine (MDMA; 'ecstasy') on the subsequent long-term behaviour of rats in the plus maze and open field. *Psychopharmacology* **159**:167–175.

Obradovic T, Imel KM, White SR (1998). Repeated exposure to methylenedioxymethamphetamine (MDMA) alters nucleus accumbens neuronal responses to dopamine and serotonin. *Brain Res* **785**:1–9.

Parott AC, Lasky J (1998). Ecstasy (MDMA) effects upon mood and cognition: before, during and after a Saturday night dance. *Psychopharmacology* **139**:261–268.

Ricaurte GA, Schuster CR, Seiden LS (1980). Long-term effects of repeated methylamphetamine administration on dopamine and serotonin neurons in the rat brain: a regional study. *Brain Res* **193**:153–163.

Ricaurte GA, Guillery RW, Seiden LS, Schuster CR, Moore RY (1982). Dopamine nerve terminal degeneration produced by high doses of methylamphetamine in the rat brain. *Brain Res* **235**:93–103.

Ricaurte GA, Forno LS, Wilson MA, DeLanney LE, Irwin I, Molliver ME, Langston JW (1988). MDMA selectively damages central serotonergic neurons in the primate. *JAMA* **260**:51–55.

Ricaurte GA, Markowska AL, Wenk GL, Hatzidimitriou G, Wlos J, Olton DS (1993). 3,4-methylenedioxymethamphetamine, serotonin and memory. *J Pharmacol Exp Ther* **266**:1097–1105.

Richards JB, Bagott MJ, Sabol KE, Seiden LS (1993). A high-dose methamphetamine regimen results in long-lasting deficits on performance of a reaction-time task. *Brain Res* **627**:254–260.

Robinson TE, Yew J, Paulson PE, Camp DM (1990). The long-term effects of neurotoxic doses of methamphetamine on the extracellular concentration of dopamine measured with microdialysis in striatum. *Neurosci Lett* **110**:193–198.

Sabol KE, Lew R, Richards JB, Vosmer GL, Seiden LS (1996). Methylenedioxymethamphetamine-induced serotonin deficits are followed by partial recovery over a 52-week period. Part I: synaptosomal uptake and tissue concentrations. *J Pharmacol Exp Ther* **276**:846–854.

Scanzello CR, Hatzidimitriou G, Martello AL, Katz JL, Ricaurte GA (1993). Serotonergic recovery after (±)3,4-(methylenedioxy) methamphetamine injury: observations in rats. *J Pharmacol Exp Ther* **264**: 1484–1491.

Scearce-Levie K, Viswanathan SS, Hen R (1999). Locomotor response to MDMA is attenuated in knockout mice lacking the 5-HT1B receptor. *Psychopharmacology* **141**:154–161.

Schechter MD (1987). MDMA as a discriminative stimulus: isomeric comparisons. *Phamacol Biochem Behav* **27**:41–44.

Schmidt CJ, Taylor VL (1987). Depression of rat brain tryptophan hydroxylase activity following the acute administration of methylenedioxymethamphetamine. *Biochem Pharmacol* **36**:4095–4102.

Schmidt CJ, Levin JA, Lovenberg W (1987). *In vitro* and *in vivo* neurochemical effects of 3,4-methylenedioxymethamphetamine on striatal monoaminergic systems in the rat brain. *Biochem Pharmacol* **36**:747–755.

Series HG, Cowen PJ, Sharp T (1994). p-Chloroamphetamine (PCA), 3,4-methylenedioxy-methamphetamine (MDMA) and D-fenfluramine pretreatment attenuates D-fenfluramine-evoked release of 5-HT *in vivo*. *Psychopharmacology* **116**:508–514.

Stone DM, Stahl DC, Hanson GR, Gibb JW (1986). The effects of 3,4-methylenedioxymethamphetamine (MDMA) and 3,4-methylenedioxyamphetamine (MDA) on monoaminergic systems in the rat brain. *Eur J Pharmacol* **128**:41–48.

Timár J, Gyarmati Zs, Barna L, Knoll B (1996). Differences in some behavioural effects of deprenyl and amphetamine enantiomers in rats. *Physiol Behav* **60**:581–587.

Wallace TL, Gudelsky GA, Vorhees CV (1999). Methamphetamine-induced neurotoxicity alters locomotor activity, stereotypic behavior, and stimulated dopamine release in the rat. *J Neurosci* **19**:9141–9148.

Wallace TL, Gudelsky GA, Vorhees CV (2001). Alterations in diurnal and nocturnal locomotor activity in rats treated with a monoamine-depleting regimen of methamphetamine or 3,4-methylenedioxymethamphetamine. *Psychopharmacology* **153**:321–326.

Walsh SL, Wagner GC (1992). Motor impairments after methamphetamine-induced neurotoxicity in the rat. *J Pharmacol Exp Ther* **263**:617–626.