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Published in:
Pharmacology, Biochemistry and Behavior

DOI:
10.1016/j.pbb.2009.08.009

Publication date:
2009

Pharmacology, Biochemistry and Behavior 94 (2009) 227–233

Contents lists available at ScienceDirect

Pharmacology, Biochemistry and Behavior

journal homepage: www.elsevier.com/locate/pharmbiochembeh

Long-term neurobiological consequences of ecstasy: A role for pre-existing trait-like differences in brain monoaminergic functioning?

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ARTICLE INFO

Article history:
Received 13 January 2009
Received in revised form 31 July 2009
Accepted 13 August 2009
Available online 21 August 2009

Keywords:
3,4-Methylenedioxymethamphetamine
MDMA
Serotonin
Dopamine
SAL and LAL mice
Brain
Personality
Coping style
5-HT
DA

ABSTRACT

This study investigated whether trait-like differences in brain monoaminergic functioning relate to differential vulnerability for the long-term neurochemical depletion effects of MDMA. Genetically selected aggressive (SAL) and non-aggressive (LAL) house-mice differing in baseline serotonergic and dopaminergic neurotransmission were administered MDMA. An acute binge-like MDMA injection protocol (three times, using either of the dosages of 0, 5, 10 and 20 mg/kg i.p. with 3 h interval) was employed. Three and 28 days after treatment with MDMA induced a dose-dependent depletion of striatal dopamine and its metabolites that did not differ between SAL and LAL mice. Similarly, the dose-dependent MDMA-induced serotonergic depletion did not differ between lines 3 days after treatment. Interestingly, 28 days after MDMA in LAL mice, 5-HT and 5-HIAA levels were still significantly depleted after treatment with 3×10 mg/kg, while in SAL mice 5-HT depletion was only seen after the highest dosage. Surprisingly, LAL mice did not show any long-term 5-HT depletion after treatment with the highest dose. In conclusion, only LAL mice are able to restore initial severe loss of MDMA-evoked 5-HT and 5-HIAA levels. SAL and LAL mice are differentially susceptible for the long-term but not short-term MDMA-induced serotonergic depletion in the striatum. The differentiation between both lines in the long-term striatal serotonergic response to MDMA seems to depend on the capacity of the brain to adapt to the short-term depletion of monoaminergic levels and may somehow be related to individual, trait-like characteristics of brain monoaminergic systems.

1. Introduction

Ecstasy or 3,4-methylenedioxymethamphetamine (MDMA) is a serotonin releaser that is frequently used for its acute euphoric effects. However, the recreational use of this drug has recently given rise to concern since there is substantial evidence that MDMA users are at risk to develop persistent negative mood and personality disorders (Gerra et al., 2000, 2002; Karlsen et al., 2008; McCann and Ricaurte, 1991; Montoya et al., 2002; Reid et al., 2007). Since brain monoaminergic and in particular serotonergic neurotransmission is considered a major molecular orchestrator of emotion as well as the primary pharmacological target of MDMA, it is likely that these MDMA-induced behavioural disturbances are associated with long-term detrimental effects of MDMA on the serotonergic system. Indeed, several preclinical studies in a variety of animal species have shown that short-term MDMA treatment can cause long-lasting and perhaps even persistent loss of brain serotonergic neuron functioning as indicated by depletion of serotonin (5-hydroxytryptamine; 5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) levels, decrease in tryptophan hydroxylase (TPH) activity and a reduction in the density of the serotonin transporter (SERT) (Battaglia et al., 1987; Hewitt and Green, 1994; Schmidt and Taylor, 1987; Sharkey et al., 1991; Stone et al., 1986, 1987b; Xie et al., 2006). However, clinical studies on the long-term neurochemical/neurotoxic effects of current and former binge-like MDMA users are less clear (de Win et al., 2004; Grob 2002; McCann et al., 2000; Reneman et al., 2001, 2006; Turner and Parrott, 2000).

Considering the behavioural consequences of MDMA consumption more in detail, a large inter-individual variation is observed in the increase of depressive symptoms, impulsive and aggressive behaviour after MDMA (ab)use (de Win et al., 2004; Reid et al., 2007). While some individuals show pronounced behavioural changes after MDMA, others seem to be only marginally affected or totally resilient. Interestingly, in a recent human study, it has been demonstrated that the magnitude of change in aggressive/impulsive behaviour after ecstasy consumption is dependent on their expressed personality trait characteristics. The study revealed that individuals with high self-control are more vulnerable for increase in aggressive behaviour (Reid et al., 2007). In this study it was not assessed whether differences in the vulnerability for the MDMA-evoked changes in aggressive behaviour were related with differences in MDMA-induced serotonergic

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doi:10.1016/j.pbb.2009.08.009

neurotoxicity. Converging evidence from rodent, non-human primate and human research has implicated variability in 5-HT neurotransmission as a key predictor of individual differences in affect, temperament and risk for developing mood disorders (Lesch and Merschdorf, 2000; Lucki, 1998). Therefore, it can be hypothesized that individual variation in behavioural changes after MDMA abuse might result from a different vulnerability for the MDMA-induced serotonergic depletion.

To investigate this, we made use of feral (wild-derived) house-mice genetically selected for high (Short-Attack Latency; SAL) and low (Long-Attack Latency; LAL) aggressiveness that are known to differ not only in several other behavioural traits or coping style but also in the homeostatic regulation of monoaminergic neurotransmission. Indeed, our research in these mice has shown that the wide individual differences in offensive aggression (van Oortmerssen and Bakker, 1981) is more generally related to their behavioural coping style with environmental challenges (Benus et al., 1989; Sluyter et al., 1996; Veenema et al., 2003a,b, 2005). Furthermore, extensive neurochemical research has shown that these two selection lines differ considerably in their (re)activity of monoaminergic systems. As to the indolaminergic system, the aggressive SAL mice have lower baseline brain levels of 5-HT than the non-aggressive LAL mice (Caramaschi et al., 2007; Olivier et al., 1990; Veenema et al., 2005). In addition, SAL mice show enhanced structural (Korte et al., 1996; Veenema et al., 2005) and functional (Caramaschi et al., 2007; van der Vegt et al., 2001) 5-HT_{1A} receptor properties than LAL mice and recent experiments in our lab showed that SAL mice have decreased functional SERT capacity (Natarajan et al., unpublished results). Concerning the catecholaminergic system, SAL mice are more sensitive to a dopaminergic D_1/D_2 receptor agonist (apomorphine), which suggests that SAL mice have a lower neostriatal dopaminergic activity than LAL mice (Benus et al., 1991).

Compared to rats, there are relatively few MDMA studies in mice. One of the reasons for this might be that mice are considered to be more susceptible to MDMA-induced dopaminergic rather than serotonergic depletion (Green et al., 2003; Logan et al., 1988; Stone et al., 1987a). More specifically, it has been found that MDMA induces severe and long-lasting reductions in dopamine, 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) levels and a reduction in the density and expression of the dopamine transporter (DAT) in mice (Kindlundh-Hogberg et al., 2007; Logan et al., 1988; Mann et al., 1997; O'Callaghan and Miller, 1994; O'Shea et al., 2001; Reveron et al., 2005; Zhang et al., 2006). The mechanism underlying this species difference is unknown so far. In both rats and mice, SERT is shown to be an essential molecular target for the 5-HT depleting effects of MDMA (Malberg et al., 1996; O'Shea et al., 2001; Renoir et al., 2008; Sanchez et al., 2001; Schmidt, 1987; Shankaran et al., 1999).

To investigate whether SAL and LAL mice are differentially vulnerable for depleting effects of MDMA, we measured the short-term and long-term depleting effects of MDMA treatment on brain monoamine concentrations in the striatum of both lines.

2. Methods

2.1. Animals and housing

This study has been approved by the animal experiments committee of the University of Groningen (DEC protocol # 4501A). Forty eight male SAL (Short-Attack Latency) and 48 male LAL (Long-Attack Latency) mice were used (mice were 140 ± 14 days of age at the moment of injections) for the short-term and long-term experiments. Both experiments were performed in two separate cohorts. The mice, offspring of parents that were selected for differences in attack latency time, originated from a colony of wild house-mice (*Mus musculus domesticus*), maintained at the University of Groningen, the Netherlands, since 1971. Until decapitation, mice were housed as male–female pairs in Perspex cages (17 × 11 × 13 cm) with sawdust bedding in a room with 12:12 light–dark cycle (lights on at 08:00h). Food (chow) and water was available ad libitum. Ambient temperature was 21 ± 0.5 °C. Mice in the long-term experiment underwent an attack latency time test according to standard procedures in our lab (van Oortmerssen and Bakker, 1981).

2.2. Experimental design

Two experiments were performed. In both experiments, SAL and LAL mice were each divided into 4 different treatment groups ($N = 6$ for SAL and LAL mice in each treatment group, except for the first experiment where LAL 3 × 5 mg/kg and 3 × 10 mg/kg consisted of $N = 5$ and the 3 × 20 mg/kg treatment group consisted of $N = 8$). In the first experiment monoamine levels of the mice were measured 3 days after MDMA/saline injections ('short-term experiment') and in the second experiment mice monoamine levels of mice were measured 28 days after MDMA/saline administration ('long-term experiment').

2.3. MDMA injections

3,4-Methylenedioxymethamphetamine (± MDMA-HCl, 99.6% obtained from the Dutch Forensic Institute, The Hague, The Netherlands) was dissolved in ultra purified water and injected intraperitoneally (i.p.). Prior to injections animals underwent light anaesthesia (O_2–isoflurane). Both lines were administered four different doses of MDMA; saline, 5, 10 or 20 mg/kg. MDMA was dissolved in 10 ml ultra purified water. MDMA or saline was injected three times with 3 h interval (binge-like administration) during the light phase. The first injection was given between 9:45 and 10:15h (1:45–2:15h after lights went on). In total, 7 mice died after being treated with the higher dosages (3 × 10 and 3 × 20 mg/kg MDMA).

2.4. Brain analysis

Between 1 and 3 h before lights went off, all animals were rapidly decapitated under brief CO_2 anaesthesia in their home cage. For determination of 5-HT, 5-HIAA, DA, DOPAC, HVA, and noradrenalin (NA),

Table 1
The monoamine tissue concentrations (±s.e.m.) for SAL and LAL mice administered three times saline are presented.

	DA (ng/g tissue)	DOPAC (ng/g tissue)	HVA (ng/g tissue)	5-HT (ng/g tissue)	5-HIAA (ng/g tissue)
Short-term					
SAL	8886.60 ± 1144.59	1382.61 ± 201.02	884.34 ± 62.72	587.76 ± 38.74	232.03 ± 14.46
LAL	11,564.33 ± 1924.12*	1799.19 ± 556.03	1010.07 ± 72.13	786.11 ± 60.53*	286.58 ± 30.22*
Long-term					
SAL	9510.9 ± 1243.9	780.5 ± 72.7	824.2 ± 108.2	582.0 ± 53.9	217.5 ± 15.3
LAL	13,157.6 ± 880.1*	929.9 ± 42.6	1029.7 ± 68.0*	856.1 ± 71.8*	346.4 ± 28.4*

* $p < 0.05$; SAL versus LAL for each monoamine.

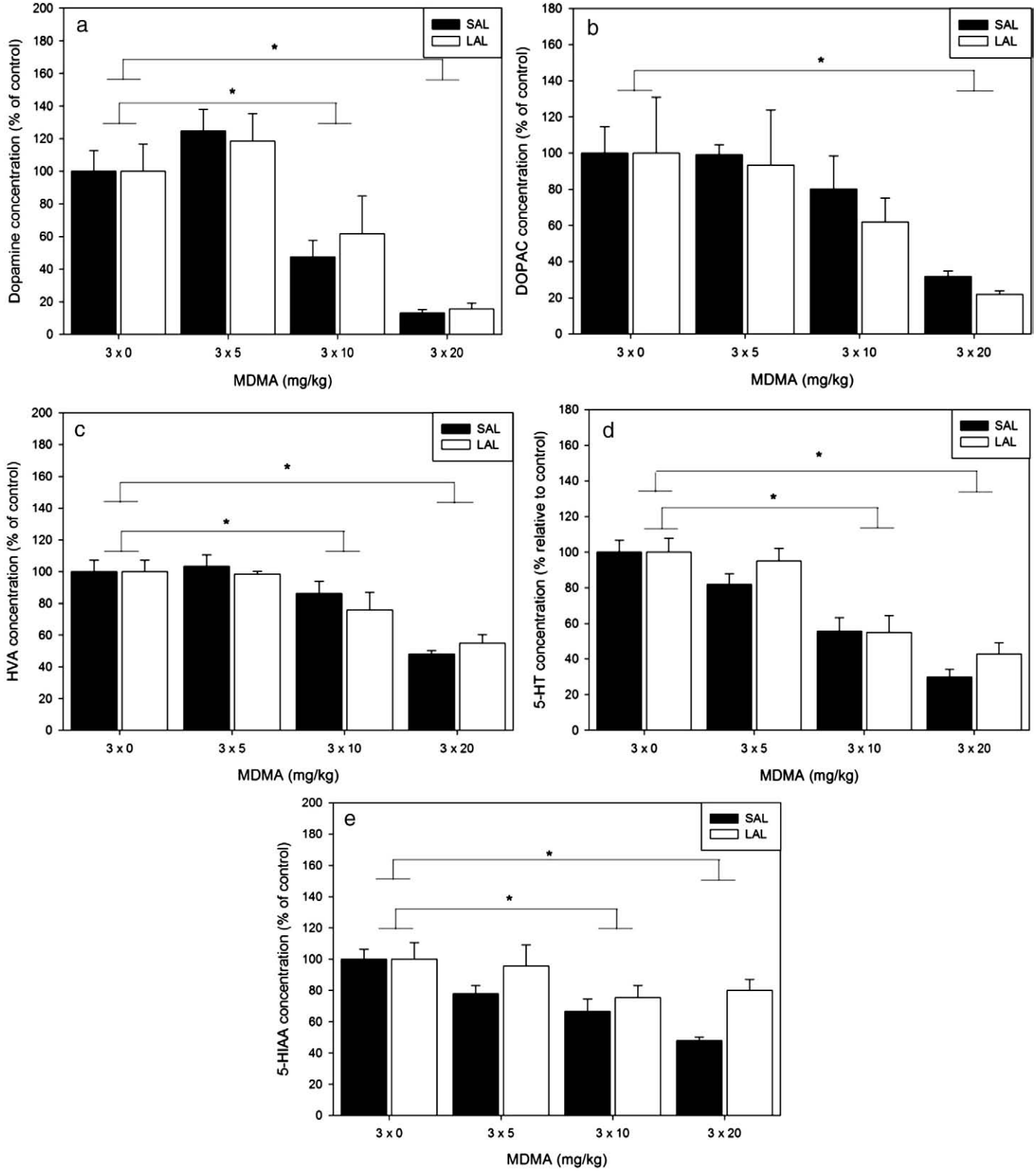

Fig. 1. Short-term effect of MDMA (3×0, 5, 10 and 20 mg/kg) on DA (a), DOPAC b), HVA (c), 5-HT (d) and 5-HIAA (e) concentrations in the striatum. Data are presented as percentage of control (3×0 mg/kg MDMA) SAL and LAL mice ± S.E.M. for SAL and LAL mice. Control SAL and LAL mice are set at 100%. "*" represents a significant difference ($p < 0.05$) between different doses of MDMA, independent of the lines.

brains were immediately dissected on a chilled plate. Striatum was removed and snap frozen in Eppendorf vials in liquid nitrogen. All samples were stored at −80 °C until further analysis. Monoamine levels were determined in all dissected brain areas using HPLC method with electrochemical detection. For this, samples were homogenized in 0.5 ml 0.1 M perchloric acid and centrifuged at 14,000 RPM for 10 min at 4 °C. Supernatant was removed and assayed for 5-HT, 5-HIAA, DA, DOPAC and NA by injecting 100 µl onto a reversed phase Gemini C18 column (150×4.6 mm, 5 µm particle size), connected to an electrochemical detector (ESA coulechem model 5100A) with a 5011A detector

cell. A difference in potential of 340 mV was set (the potential of one electrode being 0 mV and the other 340 mV). The mobile phase consisted of 62.7 nM Na_2HPO_4, 40.0 nM citric acid, 0.27 mM EDTA, 4.94 mM HSA, 10% methanol at pH 4.1 with a flow of 0.5 ml/min. Known amounts of 5-HT, 5-HIAA, DA, DOPAC, HVA (Sigma Chemicals), and NA (Research Biochemicals International) were run throughout the whole procedure for standardization. Monoamine levels were calculated as ng/g wet tissue.

2.5. Statistics

SPSS 14.0 for Windows was employed to analyse the data statistically. Lethality scores between SAL and LAL were tested statistically with the Pearson chi-square test. Each monoamine and metabolite was analysed by a two-factor ANOVA with treatment (4 levels) and line (2 levels) as between-subject factors. In case of significant main effects Dunnett post hoc testing with vehicle as control category was used. In case of significant interaction effects, post hoc analyses were performed using a oneway ANOVA or *t*-test.

3. Results

3.1. Lethality

In the short-term experiment three out of eight LAL mice died after treatment with 3×20 mg/kg MDMA. No SAL mice died. In the long-term experiment two out of six SAL mice and one out of six LAL mice died after treatment with 3×20 mg/kg MDMA. Furthermore, one out of six LAL mice died after administration with 3×10 mg/kg MDMA. When tested statistically, lethality rate did not differ between SAL and LAL mice for each dose.

3.2. Short-term effect of MDMA on monoamine and metabolite concentrations

As demonstrated before in other studies, LAL mice have higher tissue levels of DA ($F(1,37)=4.600$, $p<0.05$), 5-HT ($F(1,37)=27.976$, $p<0.001$) and 5-HIAA ($F(1,37)=28.238$, $p<0.001$) than SAL mice (Table 1). As can be seen in Fig. 1a–e, 3 days after treatment MDMA induced a depletion of 5-HT ($F(3,37)=35.937$, $p<0.001$), 5-HIAA ($F(3,37)=8.359$, $p<0.001$), DA ($F(3,37)=24.223$, $p<0.001$), DOPAC ($F(3,37)=6.820$, $p<0.01$) and HVA ($F(3,37)=22.605$, $p<0.001$), but not of NA ($F(3,37)=1.990$, $p=0.132$) (Table 2). However, MDMA treatment did not result in a differential monoamine depletion in SAL and LAL mice (no significant Line×Dose interaction effects $F(3,37)<1.487$, $p>0.234$).

3.3. Long-term effect of MDMA on monoamine and metabolite concentrations

Also in this experiment, LAL mice had higher concentrations of DA, HVA, 5-HT and 5-HIAA ($F(1,36)=5.638$, $p<0.05$; $F(1,36)=6.059$, $p<0.05$; $F(1,36)=26.886$, $p<0.0001$; $F(1,36)=76.731$, $p<0.0001$, respectively) than SAL mice, confirming line differences in monoamine levels (Table 1). MDMA induced a long-term depletion of dopamine ($F(3,36)=13.236$, $p<0.001$), DOPAC ($F(3,36)=6.241$, $p<0.01$) and HVA ($F(3,36)=3.790$, $p<0.05$), 5-HT ($F(3,36)=9.326$, $p<0.001$), 5-HIAA ($F(3,36)=10.437$, $p<0.001$), but not in NA ($F(3,35)=1.002$, $p=0.403$) (Table 2). As can be seen in Fig. 2a–c, MDMA treatment did not result in a differential depletion of dopamine, DOPAC and HVA in SAL and LAL mice (no significant Line×Dose interaction effect $F(3,36)<2.459$, $p>0.079$). However, as can be seen in Fig. 2d–e, SAL and LAL mice differed in their MDMA induced 5-HT and 5-HIAA depletion (Line×Dose interaction effect, resp. $F(3,36)=3.544$, $p<0.05$; $F(3,36)=10.437$, $p<0.001$). MDMA induced a depletion of 5-HT ($F(3,18)=13.220$, $p<0.001$) and 5-HIAA ($F(3,18)=8.714$, $p<0.001$) in LAL mice already at a dose of 10 mg/kg ($F(3,18)=13.220$, $p<0.001$). Strikingly, MDMA treatment with 20 mg/kg MDMA did not induce long-term 5-HT and 5-HIAA depletion in LAL mice. SAL mice showed significant 5-HT ($F(3,18)=4.048$, $p<0.05$) and 5-HIAA ($F(3,18)=3.476$, $p<0.05$) depletion only after the highest dose of MDMA (20 mg/kg).

Table 2
Short-term and long-term effects of MDMA (3×0, 5, 10 and 20 mg/kg) on noradrenaline (NA) tissue concentrations in the striatum.

	NA (ng/g tissue) ± s.e.m.			
	Short-term		Long-term	
	SAL	LAL	SAL	LAL
3×0	196.85 ± 59.89	222.78 ± 50.33	160.35 ± 22.14	182.85 ± 11.62
3×5	154.53 ± 26.58	112.14 ± 9.98	169.57 ± 35.36	241.96 ± 42.41
3×10	154.79 ± 33.08	149.77 ± 56.67	142.33 ± 17.42	172.15 ± 25.33
3×20	120.78 ± 20.71	115.37 ± 29.09	181.62 ± 31.97	173.74 ± 34.72

Data are presented as averages ± S.E.M. for SAL and LAL mice.

4. Discussion

The present study was conducted to investigate whether SAL and LAL mice, known to represent a more general difference in coping style and known to differ in their monoaminergic signalling, differ in their short- and long-term susceptibility for MDMA-induced DA and 5-HT depletion.

On the short term, SAL and LAL mice did not differ in the MDMA-evoked dose-dependent depletion of 5-HT and DA and their metabolites. Four weeks after MDMA treatment this dose-dependent decrease in DA was still present despite a slight increase in absolute DA and HVA levels. At this time point also no individual differences in MDMA-evoked decrease in DA, DOPAC and HVA levels were found.

However, a difference was observed when comparing DOPAC levels in the short-term and long-term experiments. The absolute concentrations of DOPAC in the striatum in the long-term experiment are half of the DOPAC levels measured in the short-term experiment. This unexpected difference was not seen for the other monoamines and metabolites when comparing both experiments. Altogether it can be concluded that MDMA treatment induced a long-lasting persistent dose-dependent depletion of dopamine levels/metabolites, which does not differ between SAL and LAL mice.

When considering the long-term depleting effects of MDMA on the 5-HT system, a striking difference was found between SAL and LAL mice. Twenty eight days after MDMA treatment, only SAL mice showed a decrease in 5-HT and 5-HIAA levels after treatment with 3×20 mg/kg MDMA. At this time point, LAL mice similarly had a significant depletion of 5-HT and 5-HIAA levels after the 3×10 mg/kg dose, but, interestingly, did not show a depletion of 5-HT and 5-HIAA levels after 3×20 mg/kg. Combining the short-term and the long-term data for 5-HT and 5-HIAA for both lines, it seems that SAL mice are able to partly restore their 5-HT and 5-HIAA levels over time, but they are still vulnerable for long-lasting 5-HT and 5-HIAA depletion after the highest dose of MDMA administered. In contrast to SAL mice, LAL mice did not restore 5-HT and 5-HIAA levels after MDMA treatment with 3×10 mg/kg. Interestingly, LAL mice do seem to compensate for the initial loss of MDMA-evoked 5-HT and 5-HIAA levels after the highest dosage. From this we may conclude that SAL and LAL mice are differently vulnerable for the long-term 5-HT and 5-HIAA depleting effects of MDMA.

Although the mechanism behind the remarkable 5-HT and 5-HIAA compensation in the LAL mice has not been investigated in the present study, it can be hypothesized that the demonstrated recovery in 5-HT and 5-HIAA levels after the highest dose of MDMA (3×20 mg/kg) is the result of 5-HT sprouting. 5-HT sprouting, i.e. the increase in

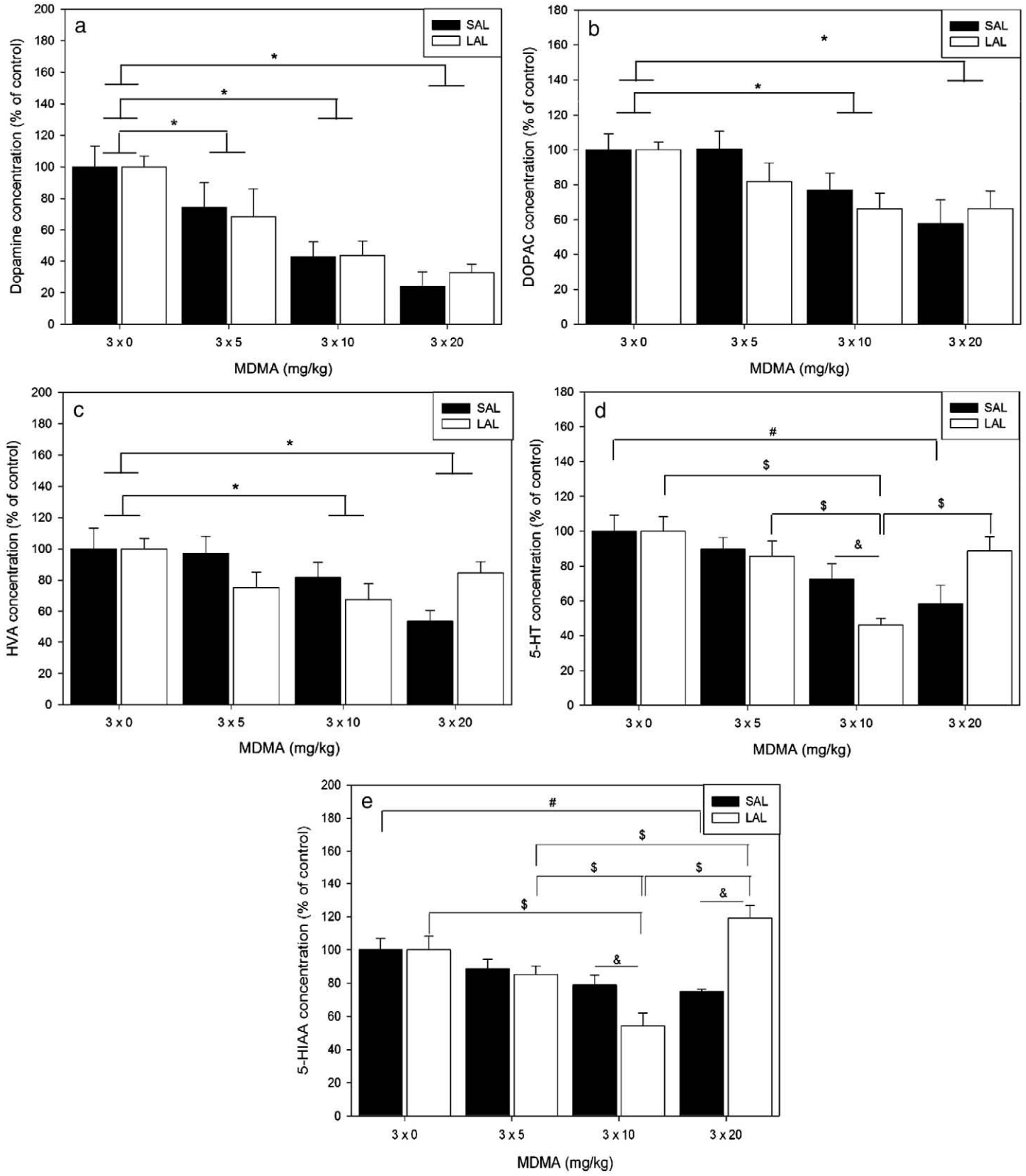

Fig. 2. Long-term effect of MDMA (3×0, 5, 10 and 20 mg/kg) on dopamine (a), DOPAC (b), HVA (c), 5-HT (d) and 5-HIAA (e) concentrations in the striatum. Data are presented as percentage of control (3×0 mg/kg MDMA) SAL and LAL mice ± S.E.M. for SAL and LAL mice. Control SAL and LAL mice are set at 100%. "*" represents a significant difference ($p<0.05$) between the different doses of MDMA for SAL and LAL mice. "&" represents significant differences ($p<0.05$) between SAL and LAL mice. "$" represents significant differences ($p<0.05$) between LAL mice that received different doses. "#" represents significant differences ($p<0.05$) between SAL mice receiving different doses.

number of 5-HT axons, has been described for adult rats and mice in the striatum after severe dopamine depletion (~90%) (Guerra et al., 1997; Maeda et al., 2003; Rozas et al., 1998a; Zhou et al., 1991). It has to be mentioned, however, that depletion in these papers was caused by different compounds than MDMA. Either 6-OHDA was used (Guerra et al., 1997; Maeda et al., 2003; Zhou et al., 1991) or MPTP (Rozas et al.,

1998a). The study of Maeda et al. showed that serotonergic hyper-innervation can already occur two weeks after massive dopaminergic denervation (Maeda et al., 2003). Only one study has investigated the functional relevance of 5-HT hyper-innervation. This study showed that mice that showed striatal 5-HT sprouting after severe dopamine depletion had a better motoric capacity than mice that did not show this increase in number of 5-HT axons after severe dopamine depletion (Rozas et al., 1998b). Consistent with the hypothesis of 5-HT sprouting is the severity of the dopamine depletion in the striatum after the highest dose of MDMA in the current experiment (~90%). According to literature, this depletion would be sufficient to induce the 5-HT sprouting process in our mice. Surprisingly, the long-term up-regulation of 5-HT is only observed in LAL mice and not in SAL mice. It is tempting to consider the possibility that only LAL mice have the capacity to use this sprouting mechanism.

Regarding the general question of neurotoxicity, one may question whether persistent 5-HT depletion in mice truly represents long-lasting serotonergic neurotoxicity (Renoir et al., 2008). However, this discussion does not undermine the possibility that the demonstrated long-lasting monoaminergic depletion might have severe negative behavioural consequences. Indeed, it has been demonstrated that individual variation in the vulnerability to the behavioural consequences of MDMA consumption depends on personality traits (Reid et al., 2007).

Evidence suggests that dopamine neurotoxicity in mice may result from free radical formation which leads to oxidative stress (Cadet et al., 1994, 1995; Camarero et al., 2002). Recently a study showed that SAL and LAL mice also differ in their serum antioxidant capacity (Costantini et al., 2008). According to this study LAL mice would be more resistant to free oxygen radical induced damage than SAL mice and therefore it could be expected that they would suffer less from MDMA-induced 5-HT depletion. However, this is in contrast to the result found in the present study. Of course it is possible that the antioxidant capacity in the brain is different than in the periphery.

It has been known for many years that the depleting effects of MDMA are different in mice compared to other rodents and primates including humans. Mice are particularly known for their MDMA-induced depletion of dopamine levels in the striatum (O'Shea et al., 2001). However, evidence indicates that MDMA can also induce 5-HT depletion in mice (Logan et al., 1988; Renoir et al., 2008; Zhang et al., 2006). As pointed out by Green et al. (2003) different mouse strains respond differently to MDMA, i.e. some strains show a decrease in 5-HT levels whereas others fail to do so. This supports the importance of a broader screening of the vulnerability of these monoaminergic systems for the depleting effects of MDMA. Furthermore, the differences within mice strains as to serotonergic and dopaminergic depletion in the striatum indicate that the species difference concerning the depleting effect of MDMA may be less pronounced.

In summary, this study shows a clear reduction in DA as well as in 5-HT levels in striatal tissue of SAL and LAL mice 3 days and 28 days following MDMA. The differentiation between the two lines in the long-term striatal serotonergic response to high MDMA dosages seems to be dependent on trait-like differences in serotonergic functioning. The trait-like differences in baseline monoamine levels in the striatum of these two mouse lines are clearly not reflected in a differential vulnerability to the dopaminergic depleting effects of MDMA. Our findings emphasize the importance of investigating MDMA-induced serotonergic changes in addition to MDMA-induced dopaminergic changes in mice.

Acknowledgements

The research of A.E. Wallinga was financed by ZonMw, project number 31000069. The authors want to thank Arjen Boender and Rudy Dupree for their excellent contribution to a part of the practical work.

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