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Effects of 3,4-methylenedioxymethamphetamine on locomotor activity and extracellular dopamine in the nucleus accumbens of Fischer 344 and Lewis rats

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Abstract

Previous studies have shown that Fischer 344 (F344) and Lewis (LEW) rats may differ with respect to their behavioural and neurochemical responses to several drugs of abuse, including amphetamines. Herein, we have examined whether such strain differences extend to a ring-substituted amphetamine, namely 3,4-methylenedioxymethamphetamine (MDMA, ecstasy), a recreationally-used drug endowed with euphoric, but also long-term neurotoxic effects. Beside strain differences in baseline locomotor activity (F344 > LEW), it was found that the subcutaneous administration of 10 mg/kg, but not 5 mg/kg, MDMA increased locomotor activity in F344 rats only. On the other hand, such a treatment increased to similar extents extracellular dopamine (DA) levels in the nucleus accumbens of F344 and LEW rats, thus suggesting that genetic differences in MDMA locomotor effects are not accounted for by accumbal DA release.

Keywords: Ecstasy; Extracellular dopamine; Locomotor activity; Shell of the nucleus accumbens; Microdialysis; Inbred rats

Numerous studies have compared the behavioural responses of inbred Fischer 344 (F344) and Lewis (LEW) rats to drugs of abuse as a means to elucidate how genetics may impact on the vulnerability to drug addiction [15]. Unfortunately, the results obtained so far do not permit any conclusion as the nature of the drug, the paradigm (route, frequency and dose of administration) of drug treatment, and the behavioural response (conditioned, unconditioned) may vary between studies. Indeed, this is also true when focusing on a behavioural response to a given drug. For example, F344 rats have been shown to display higher [12], equivalent [9,16] or lower [3] acute locomotor responses to cocaine, compared with LEW rats. A similar comment applies to amphetamine as its hyperlocomotor effect has been shown to be higher [9,22] or weaker [17] in F344 rats than in LEW rats. In keeping with such a heterogeneity of results, it is not a surprise that studies aimed at measuring the neurochemical effects of these drugs of abuse in F344 and LEW rats reached divergent results. For example, the amplitude of dopamine (DA) release from the nucleus accumbens has been shown to be equivalent [23] or lower [3] in cocaine-treated F344 rats, compared to their LEW counterparts.

The data mentioned above, albeit contradictory, illustrate the care that has been paid to differences in the behavioural and neurochemical impacts of cocaine and amphetamine (and to a lower extent, methamphetamine: [3]) in F344 and LEW rats. Surprisingly, no information is available as to the possibility that these two rat strains differ in their responses to ring-substituted amphetamine derivatives, a class of recreationally-used drugs whose subjective effects differ from those elicited by amphetamine [11]. Among these, 3,4-methylenedioxymethamphetamine (MDMA, ecstasy) has gained much attention as it is the most commonly abused of the ring-substituted amphetamine derivatives although animal studies strongly suggest that it possesses strong neurotoxic properties (e.g. on central serotonergic systems: see Ref. [11]). Taken with past evidence for accumbal DA transmission being involved in MDMA-elicited hyperlocomotion [10] (but see Ref. [2]) we have

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thus examined whether F344 and LEW rats differ with respect to the hyperlocomotor [2,10,19] and the accumbal DA-releasing [13,25,26] effects of MDMA, the latter being assessed by means of intracerebral microdialysis. Experiments were performed in freely-moving animals, an experimental condition permitting a simultaneous monitoring of biochemical and behavioural parameters.

Male F344 and LEW rats (IFFA CREDO, Les Oncins, France), aged 7–8 weeks on arrival (respective body weights: 200–250 and 250–300 g), were housed in individual cages with food and water ad libitum under constant temperature (22 ± 1°C) and a 12:12 h light/dark cycle of illumination (lights on at 07:00 h). The minimal number of rats was used and all efforts were made to avoid suffering of the animals (rules established by the French legislation on animal welfare: O.J. Nos. 87–848).

Microdialysis experiments and detection of DA concentrations in dialysates were performed according to procedures previously described [18]. Thus, at least 2 weeks after their arrival, rats were anaesthetised with a 40 mg/kg dose of pentobarbital sodium and placed in a stereotaxic frame. A siliconised stainless steel guide cannula (Carnegie Medecin, Phymep, Paris, France) was implanted in the shell of the right nucleus accumbens (coordinates from the interaural point: anteroposterior = 10.6, lateral = 0.9 and ventral = 4, according to Ref. [20]), and fixed to the skull by means of stainless steel screws and methylacrylic cement. Five to 7 days later, a microdialysis probe (CMA/11, 2 mm in length, 240-μm in outer diameter; Carnegie Medecin, Phymep) filled with artificial cerebrospinal fluid containing 154.1 mM Cl^-, 147 mM Na^+, 2.7 mM K^+, 1 mM Mg^{2+} and 1.2 mM Ca^{2+} (adjusted to pH 7.4 with 2 mM sodium phosphate buffer) was lowered through the guide-cannula so that the tip of the probe reached a depth value of 2 mm above the interaural point. After an 18-h stabilisation period, rats were individually housed within a sound-attenuating enclosure in clear Plexiglas experimental chamber (40 × 45 × 25 cm) that allowed the monitoring of horizontal locomotor activity (by means of two sets of infra-red lights and photocell detectors connected to a computer) during repeated dialysis sampling. Note that for technical reasons, only some rats in each of the six groups could be monitored for their locomotion scores.

The probe (in vitro recovery ~10% for DA) was then perfused at a constant flow rate of 2 μl/min by means of a microperfusion pump (CMA 111; Carnegie Medecin, Phymep). Dialysates (40 μl) were collected on ice every 20 min and immediately analysed by reverse-phase high performance liquid chromatography coupled with electrochemical detection. The mobile phase (70 mM NaH_2PO_4, 0.1 mM Na_2EDTA, 0.7 mM triethylamine, 0.1 mM octylsulfonic acid, and 10% methanol, adjusted to pH 4.8 with orthophosphoric acid) was delivered with a 1-ml/min flow rate (system LC-10AD-*VP*; Shimadzu, Touzard and Matignon, Paris, France) through a Hypersyl column (C18, 4.6 × 150 mm, particle size 5 μm, Touzard et Matignon). Detection of DA was carried out with a coulometric detector (Coulochem II; ESA, Paris, France) coupled to a dual electrode analytic cell (model 5014). The potential of the electrodes was set at −175 and +175 mV. Under these conditions, the sensitivity for DA was 0.5 pg/30 μl with a signal/noise ratio of 3:1. Saline or MDMA (5 or 10 mg/kg; National Institute of Drug Abuse, Bethesda, USA) s.c. injections were performed when stability of perfusate DA levels was reached, stability being defined as three consecutive samples in which DA contents varied by less than 10% (this was reached at maximum 120 min after the beginning of the perfusion). Three stable 20-min dialysis samples were collected prior to s.c. treatments, dialysis sampling being performed thereafter for the following 220 min (11 samples). At the end of each experiment, the rat was anaesthetised, the brain removed and fixed in a saline/paraformaldehyde (0.9/10%) solution. The probe location was determined histologically on serial coronal sections (60 μm) stained with cresyl violet and only data obtained from rats with cannulae within the NAc shell were included for statistical analysis.

All data are given as mean ± SEM. In keeping with the high heterogeneity of variances (partly due to low numbers of animals in some groups), all data were first log-transformed before comparison by means of multiple analyses of variance which included the treatment and the strain as between-factors, and time as a within-factor.

Fig. 1 shows the respective patterns of locomotion (non-cumulated values) in F344 and LEW rats treated with saline or MDMA (5 or 10 mg/kg). The strain ($F(1,16) = 65.37$; $P < 0.001$), the treatment ($F(2,16) = 18.96$; $P < 0.001$), and the strain × treatment and the treatment × time interactions ($F(20,160) = 2.85$; $P < 0.001$) all had major influences. Thus, the 10 mg/kg, but not the 5 mg/kg, dose of MDMA increased locomotion but did so in F344 rats only (Fig. 1). The analysis of the locomotion scores cumulated over the 220 min that followed the respective treatments confirmed the above statement: actually, the comparison between F344 rats (39 ± 4, 46 ± 10, and 194 ± 25 beam breaks/220 min in rats treated respectively with saline, 5 mg/kg MDMA, and 10 mg/kg MDMA) and LEW rats (14 ± 6, 27 ± 4, and 24 ± 10 beam breaks/220 min in rats treated respectively with saline, 5 mg/kg MDMA, and 10 mg/kg MDMA) revealed again major influences of the strain ($F(1,16) = 30.17$; $P < 0.001$), the treatment ($F(2,16) = 5.16$; $P = 0.019$), and a strain × treatment interaction ($F(2,16) = 4.96$; $P < 0.021$). Post hoc differences between saline and 10 mg/kg MDMA in F344 rats (MDMA > saline; $P < 0.001$), between saline-treated F344 and LEW rats (F344 > LEW; $P < 0.01$), and between F344 and LEW rats treated with the highest dose of MDMA (F344 > LEW; $P < 0.001$) were observed.

Fig. 2 reports the respective patterns of extracellular DA levels in F344 and LEW rats treated with saline or MDMA (5 or 10 mg/kg). The treatment ($F(2,22) = 12.48$; $P < 0.001$), the sampling period ($F(10,220) = 16.81$;

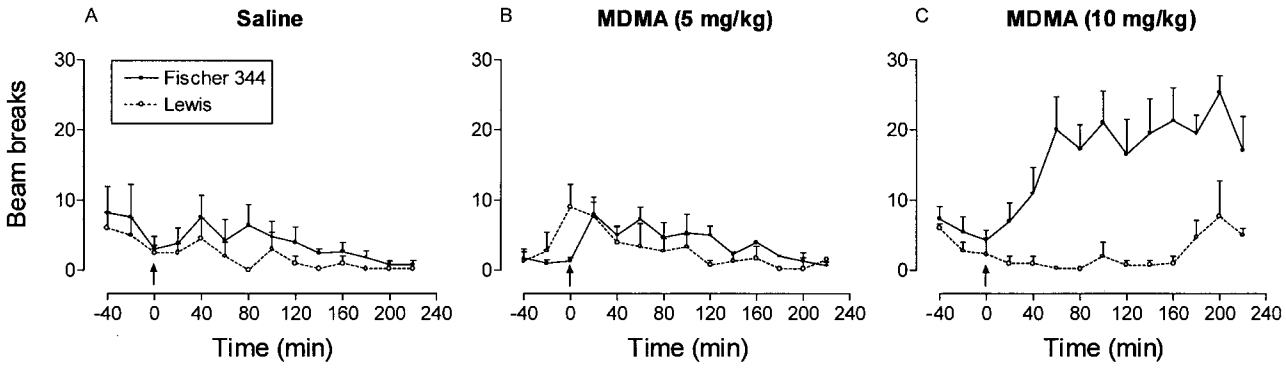

Fig. 1. Locomotor effects of saline (A); or MDMA (B and C) injection (at time 0) in F344 and LEW rats. Data, presented as the number of beam breaks in each 20-min time interval, are the mean ± SEM of $n = 3$–5 F344 rats/group and $n = 3$–4 LEW rats/group. See text for statistics.

$P < 0.001$), and the interaction between both variables ($F(20, 220) = 9.21$; $P < 0.001$) all affected extracellular DA concentrations. Thus, the 10 mg/kg dose of MDMA was found to increase in a strain-independent manner extracellular DA levels, the plateau level being reached 100–120 min after MDMA administration (Fig. 2). Such a strain-independent effect of MDMA was confirmed when DA concentrations were examined as percent changes from baseline values (relative concentrations): thus, neither the strain ($F(1, 22) = 0.01$; NS) nor the strain × treatment ($F(2, 22) = 0.99$; NS) and strain × treatment × time ($F(20, 220) = 0.48$; NS) interactions affected relative DA concentrations.

The present study reports that a dose of MDMA endowed with stimulatory influences on extracellular DA levels in the nucleus accumbens of F344 and LEW rats did promote hyperactivity in the former, but not in the latter, strain. Interestingly, strain differences in baseline locomotion (F344 > LEW), but not in baseline extracellular DA levels, were also observed, thus raising the hypothesis of a link between baseline locomotion and the amplitude of the hyperlocomotor effect of MDMA. With regard to baseline locomotor activity, our finding of a strain difference in favor of the F344 strain confirms previous findings [4] but contrast with others [3,16,17], a discrepancy that may be accounted for by differences in the evaluation of locomotion [22]. As concerns our observation of a greater locomotor impact of MDMA in F344 rats, compared to LEW rats (and which extends to the (+)-MDMA isomer: unpublished observations), one possibility would be that drug pharmacokinetics differ between strains. Although such a possibility merits consideration, it is noteworthy that Camp et al. [3] have shown that the acute injection of methamphetamine leads to higher plasma and brain levels of methamphetamine and amphetamine in LEW rats, compared to F344 rats. Then, the strain differences found in the latter pharmacokinetic study as well as our observation of strain differences in the locomotor, but not in the neurochemical, response to MDMA could speak against the pharmacokinetic hypothesis. Another possibility that may be considered is linked to the stereotypies elicited by MDMA (the so-called '5-hydroxytryptamine (5-HT) syndrome': [21]), which could have masked its hyperlocomotor effect in LEW rats. This is however unlikely as administration of *p*-chloroamphetamine, an amphetamine derivative that also promotes locomotion and the 5-HT syndrome, suggested that F344, rather than LEW, rats may

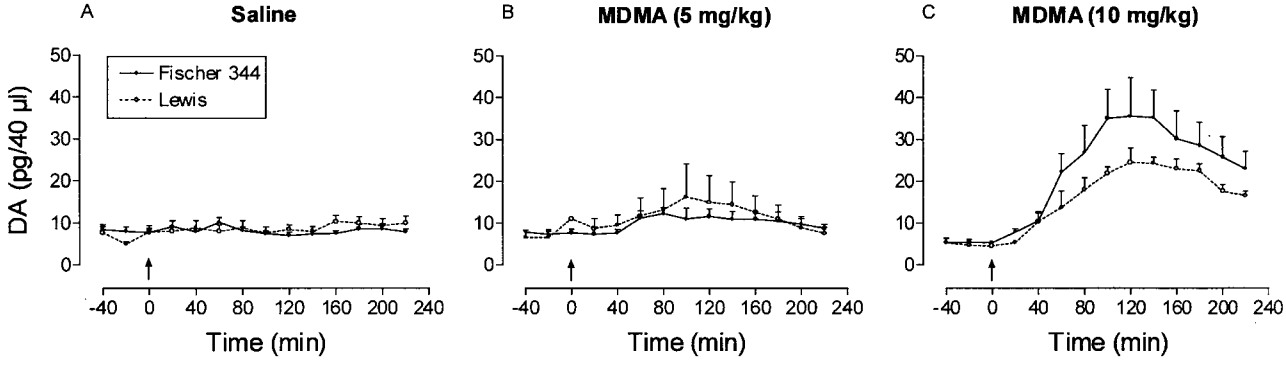

Fig. 2. Time courses of the effects of saline (A); or MDMA (B and C) injection (at time 0) on extracellular DA levels in the nucleus accumbens of F344 and LEW rats. Data are the mean ± SEM of $n = 3$–6 F344 rats/group and $n = 4$–5 LEW rats/group. See text for statistics.

be sensitive to the stereotypies elicited by high doses of that amphetamine [7]. The most likely explanation for an increased locomotor effect of MDMA in F344 rats, compared to LEW rats, may rather involve strain differences within the mechanisms thought to be responsible for MDMA-induced hyperlocomotion. Although in vitro studies indicate that MDMA may directly inhibit DA reuptake and increase DA release [24], in vivo studies have shown that MDMA-induced hyperlocomotion requires 5-HT release, and in turn 5-HT_{1B} receptor (and possibly 5-HT_{2A} receptor) stimulation [2,14,19]. Because accumbal DA release is positively controlled by 5-HT_{1B} [1] and 5-HT_{2A} receptors [6], it has been suggested that MDMA-elicited hyperlocomotion is accounted for by a 5-HT-mediated increase in DA transmission in the nucleus accumbens [19]. In keeping with the observation that our microdialysis experiments (which confirmed past evidence for MDMA increasing extracellular DA levels: [13,25,26]) failed to reveal significant differences between F344 and LEW rats, any explanation based on strain differences regarding the extent to which MDMA increase extracellular DA levels may however be rejected. Alternatively, because the hyperlocomotor effect of amphetamine has been shown to be related to only a small 'functional' fraction of its DA-releasing effect [5], it may be that the two strains differ with regard to that 'functional' fraction. Although this possibility cannot be dismissed, it however remains to be established that the pattern of DA responses to amphetamine into 'functional' and 'non-functional' pools extends to MDMA. If that was not the case, then an hypothesis based on postsynaptic events should be privileged. As an illustration, a strain difference related to genetic differences in the DA receptors controlling locomotor activity (e.g. D_2 receptors) is worthy of mention as accumbal D_2 receptor density is higher in F344 rats, compared to LEW rats [8]; it remains however to ensure that such a difference extends to receptor sensitivity. The last point of consideration is the extent to which accumbal DA release is a prerequisite for the expression of MDMA locomotor effects. Thus, the complete lesion of the nucleus accumbens attenuates but does not prevent MDMA-induced hyperlocomotion [10] (but see Ref. [2]). This could indicate that a significant part of the 5-HT-mediated hyperlocomotor influence of MDMA finds its origins in other sites than the nucleus accumbens.

Taken together, our results bring evidence for a strain-dependent effect of MDMA on locomotion in F344 and LEW rats that cannot be accounted for by the stimulatory influence of the amphetamine on extracellular DA levels in the nucleus accumbens. In turn, this observation raises two possibilities that merit future consideration: (i) the two strains provide a model of inter-individual variations in MDMA pharmacokinetics, and/or (ii) MDMA-treated F344 and LEW rats differ in accumbal DA transmission through genetic differences in the portion of extracellular DA required to elicit locomotion, in D_2 receptor sensitivity or in the relative contribution of accumbal DA transmission to the expression of locomotor hyperactivity.

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