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Repeated weekly exposure to MDMA, methamphetamine or their combination: Long-term behavioural and neurochemical effects in rats

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Abstract

In recent work we have documented lasting adverse neurochemical and behavioural effects in rats given short-term 'binge' dosing with methylenedioxymethamphetamine (MDMA, Ecstasy), methamphetamine (METH) or their combination. Here we investigated whether similar effects persist in rats given 16 weekly injections followed by a 10 week period of abstinence. Female rats received MDMA (8 mg/kg, IP), METH (8 mg/kg), or a MDMA/METH combination (4 mg/kg MDMA + 4 mg/kg METH), once a week for 16 weeks, with locomotor activity and body temperature measured on weeks 1, 8 and 16. The MDMA and MDMA/METH groups showed acute drug-induced hyperthermia on week 1 only. MDMA-treated rats demonstrated an acute hyperactivity while METH and MDMA/METH treated rats showed pronounced stereotypy. Seven weeks after drug-treatment concluded, a decrease in social interaction was observed in all chronically drug-treated rats. No group differences were evident on the emergence, object recognition or forced swim tests. Neurochemical analysis revealed modest noradrenaline and serotonin depletion in chronically treated rats that was not evident following a single equivalent administration. These results indicate that although chronic, intermittent exposure to MDMA, METH or their combination, may not lead to significant long-term monoamine depletion, lasting adverse behavioural effects may persist, especially those related to social behaviour.

Keywords: MDMA; Ecstasy; Methamphetamine; Neurotoxicity; Anxiety; Polydrug use

1. Introduction

The recreational use of substituted amphetamines such as 'ecstasy' (3,4-methylenedioxymethamphetamine; MDMA) and methamphetamine (METH, 'speed', 'ice') is common across many cultures (United Nations Office on Drugs and Crime, 2003), and co-administration of MDMA and METH is frequently reported (Barrett et al., 2005; Degenhardt et al., 2005). In addition to intentional combined use, many ecstasy users may inadvertently consume MDMA and METH simultaneously through ingesting 'ecstasy' tablets that contain little or no MDMA, but rather a cheaper substitute such as METH (Kalasinsky et al., 2004; Quinn et al., 2004). These findings support the notion that polydrug use is the norm in party drug users, and that MDMA and METH are often used together.

Particular concern surrounds the possible adverse effects of mixing MDMA with METH. Both drugs have been independently linked with long-term behavioural and cognitive deficits in humans (Parrott, 2001; Meredith et al., 2005), and when regularly used together, combining MDMA with METH has been associated with more severe long-term cognitive, behavioural and neurological changes (Brecht and von Mayrhauser, 2002; Fox et al., 2002; Reneman et al., 2002). This notion has been supported through investigation of the long-term effects of combined MDMA/METH administration in laboratory animals. Results from our laboratory indicate that exposure to MDMA and METH in combination results in a more severe pattern of long-term behavioural and neurochemical alteration in rats than administering either drug alone (Clemens et al., 2004, 2005).

Administration of moderate to high doses of MDMA or METH to rats produces long-term social anxiety, as well

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as marked serotonin (5-HT) and dopamine (DA) depletion, respectively (Clemens et al., 2004, 2005). Administration of a MDMA/METH cocktail resulted in similar long-term decreases in social interaction, and this was also accompanied by increased anxiety on the emergence test and altered behaviour on the forced swim test of depression (Clemens et al., 2005). Lasting effects on the forced swim test are usually only seen with higher doses of MDMA when given alone (McGregor et al., 2003b; Thompson et al., 2004). Notably, the behavioural changes evident in the MDMA/METH group were paralleled by unique depletions of noradrenaline (NA), and significant DA and 5-HT depletion in MDMA/METH treated rats, in some brain regions beyond that in rats given MDMA or METH alone (Clemens et al., 2005).

While these results represent the effects of a single day of dosing, in the current study we wished to extend these findings to encompass the long-term behavioural and neurochemical effects of intermittent MDMA, METH or MDMA/METH administration once a week, for 16 weeks. The majority of MDMA or METH neurotoxicity studies examine depletion following a brief, 1- or 2-day exposure to relatively high levels of MDMA or METH, thus attempting to model the effects of 'binge' episodes of drug taking in humans (Green et al., 2003). While such studies are highly informative and valid, they may be restricted in their applicability to humans as the majority of party-drug takers report 'infrequent' consumption, using once every 1–3 months (Degenhardt et al., 2004; McCambridge et al., 2005). For this reason it is important to examine whether the robust effects obtained following a single, high dose administration of MDMA and METH are also obtained with a less rigorous, intermittent pattern of administration maintained over a much longer period of time.

Previous research addressing chronic exposure to MDMA has suggested that long-term sporadic administration may be less likely to produce neurochemical depletion than a single higher dose episode of exposure (O'Shea et al., 1998). It has been hypothesised that this difference is reliant on the additional time between administrations to allow sufficient recovery of the neurochemical processes affected by MDMA (O'Shea et al., 1998). While this spacing between drug administrations may well allow recovery of neurochemical levels, it is not clear how it will impact on the long-term adverse behavioural effects typically associated with this drug. This is especially pertinent when we consider that significant persistent MDMA-induced behavioural changes may sometimes occur in the absence of any detectable 5-HT depletion (Fone et al., 2002; McGregor et al., 2003a).

Similarly, the long-term neurochemical and behavioural consequences of intermittent METH administration have yet to be fully investigated. Results of shorter term studies indicate that deficits appear to be largely dependent on the route of administration, the dose and frequency of administration, as well as the amount of time in 'withdrawal' before neurochemical measures are taken (Suzuki et al., 1997; Segal et al., 2003; Broom and Yamamoto, 2005). Such neurochemical alterations detected in the next few days after withdrawal may no longer be evident weeks later (Segal et al., 2005), indicating a transient nature of neurochemical alteration, that may or may not be reflected in behaviour.

Therefore the aims of the current experiment were to explore the lasting effects of repeated, weekly administration of MDMA, METH and a MDMA/METH combination. We aimed to detect if long-term behavioural changes were evident after an extended period of abstinence following chronic administration and also to measure if this was reflected in long-term modification of NA, DA and 5-HT levels. Additional control groups were included to verify the neurochemical changes produced by a single dose of the drugs used.

2. Methods

2.1. Experiment 1: repeated intermittent administration

2.1.1. Subjects. The subjects were 32 experimentally naïve female Albino Wistar rats weighing 238 ± 4 g at the beginning of the experiment. Our past research has indicated both male and female Albino rats respond similarly with regard to MDMA or METH effects (Clemens et al., 2004, 2005), therefore it is unlikely that the results achieved here will be affected by sex differences.

Rats were housed in large plastic tubs, eight rats per tub, with food and water freely available. The colony room was maintained at 22 °C with lighting on a 12 h reverse light cycle, and all experimentation was carried out during the dark phase. This study was approved by the University of Sydney Animal Ethics Committee in accordance with the *Australian Code of Practice for the Care and Use of Animal for Scientific Purposes, 7th Edition* (*2004*).

2.1.2. Drug administration. (±)3,4-Methylenedioxymethamphetamine HCl (MDMA) and (±)methamphetamine HCl (METH) were purchased from the Australian Government Analytical Laboratories (Pymble, NSW).

Treatment injections comprised of saline, MDMA (8 mg/kg), METH (8 mg/kg) or a MDMA/METH cocktail (4 mg/kg MDMA + 4 mg/kg METH). Drugs were dissolved in 0.9% saline and injected into the intraperitoneal cavity (IP) at 1 ml/kg. These doses were selected to represent low to moderate administration whereby a single exposure would be unlikely to cause substantial depletion of monoamines (McGregor et al., 2003a; Clemens et al., 2004).

2.1.3. Experimental design and general procedure. Rats were assigned to one of four treatment groups (vehicle, MDMA, METH or MDMA/METH), with each group evenly represented within home-cages and controlled for bodyweight. Rats received one injection per week for a total of 16 weeks (4 months) with locomotor activity and body temperature recorded in test chambers on weeks 1, 8 and 16.

Seven weeks after the last drug treatment, rats were tested on the emergence test of generalised anxiety, social interaction test of social anxiety, object recognition test for non-spatial working memory and the forced swim test of depression. The order of testing was determined by the degree of anxiety-inducing properties of each test, with the least stressful test (emergence) conducted first, and the most potentially distressing (forced swim) test last.

Ten weeks later after the final drug treatment all rats were killed on the same day.

2.1.4. Body temperature and locomotor activity. Body temperature and locomotor activity were recorded only on weeks 1, 8 and 16. All other injections were administered in the home cages that had been moved from the colony room into the hot test room (see below).

All drug administration was conducted in a 'hot' environment, with room temperature maintained at 28 °C for every injection procedure. Hot environmental temperatures mimic the environment where such drugs are often administered by humans (i.e. nightclubs and dance parties), and may exacerbate neurotoxic effects (Haughey et al., 2000; Pubill et al., 2003; Green et al., 2004).

All rats received 30 min of habituation to the hot environment prior to drug treatment, and then a further 3 h of monitoring in the hot environment. After all test sessions, rats remained in a 'warm' room (approximately 25 °C) overnight