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Increased anxiety and impaired spatial memory in young adult rats following adolescent exposure to methylone

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ABSTRACT

This study investigated the possibility that treatment of adolescent rats with the substituted cathinone, 3,4-methylenedioxymethcathinone (methylone), might result in heightened anxiety and/or impaired memory during early adulthood, as has been shown for other designer drugs. For 10 consecutive days from 35 days after birth (PND35-44, early adolescence) or 45 days after birth (PND45-54, late adolescence), male and female PVG/c rats were administered saline or 8.0 mg/kg methylone via intraperitoneal injection. When 90 days old (early adulthood), their anxiety-related behavior was recorded in an open field and a light/dark box. Acoustic startle amplitude was also measured as well as their spatial memory which was determined by their ability to detect which arm of a Y maze had changed in brightness between an acquisition and a retention trial. Previously methylone-treated rats showed increased anxiety-related behavior only in the open field as reflected in decreased ambulation, and increased corner occupancy and defecation. In the latter two cases, the increases depended on the age of treatment. Also, for defecation, only male rats were affected. In addition, methylone-treated rats displayed signs of impaired spatial memory, independent of anxiety, through their reduced ability to detect a novel changed Y-maze arm. The results of the study suggested some possible consequences in adulthood of methylone use during adolescence. There were also several examples of female rats exhibiting higher overall frequencies of activity and anxiety-related responding than males that were consistent with them being the more active and less anxious of the two sexes.

1. Introduction

In recent years there has been a major world-wide increase in the production and abuse of a range of synthetic compounds designed to mimic the psychological effects of psychostimulant drugs such as methamphetamine, cocaine and methylene dioxymethylamphetamine (MDMA). A group of such compounds that has become particularly popular is the substituted cathinones often referred to as "bath salts" to disguise their psychoactive properties (Lindsay and White, 2012). These substances are derivatives of cathinone found naturally in the leaves of the khat plant (*Catha edulis*) which has been chewed for centuries in some Middle Eastern and African countries for their amphetamine-like euphoric effects (Kalix and Braenden, 1985). Methylone is a notable example that has relatively recently become popular in this respect (Bossong et al., 2005) after having been originally synthesized in the 1930s for possible use as an antidepressant (Lindsay and White, 2012).

The behavioral effects of methylone are similar to those of MDMA (Bossong et al., 2005) which it also resembles chemically (Gibbons, 2012). As with all cathinones, users report that it produces euphoria and increases energy and libido, amongst other effects (Prosser and Nelson, 2012). Centrally, methylone inhibits the reuptake of dopamine, serotonin and norepinephrine (Cozzi et al., 1999) and has recently been shown to facilitate intracranial self-stimulation and intravenous self-administration in rats thereby suggesting that it has abuse potential (Bonano et al., 2014; Creehan et al., 2015; Watterson et al., 2012). Contrary to effects of the related cathinone, mephedrone, there is currently little evidence of long-term behavioral or neurochemical changes in adult rodents following treatment with methylone (Baumann et al., 2012; Gregg and Rawls, 2014). However, binge exposure of adolescent rats to the substance has recently been shown to cause serotonergic impairment accompanied by impaired spatial memory (but not acquisition) measured in a Morris water maze (López-Arnau et al., 2014).

Investigation of methylone's effects on adolescent animals is highly relevant to humans since experimentation with psychoactive substances typically begins during this period of development when the immature brain is especially vulnerable to chemical insults (Spear, 2000). In this respect, it is known that a number of substances that are abused during adolescence for their psychological effects can modify subsequent behavioral development. For example, the designer drugs benzylpiperazine and MDMA have been shown to lead to long-lasting increased anxiety in adult rats when experienced during adolescence (Aitchison and Hughes, 2006; Kolyaduke and Hughes, 2013). As both

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of these drugs and methylone affect dopaminergic and serotonergic activity to varying extents (Baumann et al., 2005; Cozzi et al., 1999), it seemed desirable to determine whether or not a similar behavioral outcome might follow rats' exposure to the substance during adolescence.

In a previous investigation, MDMA was administered to rats for 10 consecutive days during either early or late adolescence i.e., from 35 or 45 days after birth (Kolyaduke and Hughes, 2013). When tested in early adulthood 90 days after birth, there were increases in several anxiety-related responses, especially after treatment during late adolescence. Given its neurochemical and apparent behavioral similarities with MDMA, the present study aimed to determine if a similar outcome might follow methylone treatment during early or late adolescence, and if there was any evidence of subsequent effects on a measure of short-term spatial memory. In these respects, it is important to note that methylone can substitute for MDMA when rats are trained to discriminate between MDMA and saline (Dal Cason et al., 1997).

Following saline or methylone treatment, all rats were tested during early adulthood for levels of anxiety-related behavior in an open field, a light/dark box and an acoustic startle chamber. Heightened open-field anxiety is often seen as reflected in lower levels of ambulation, rearing and occupancy of the center of the apparatus, as well as higher frequencies of defecation and occupancy of corners (Archer, 1973; Prut and Belzung, 2003). In the light/dark box, decreased occupation of and entries into the light side of the apparatus is related to higher anxiety (Belzung, 1999; Hascoët et al., 2001), as is increased acoustic startle responsiveness (Rosen and Schulkin, 1998).

In order to determine whether or not the rats' memory had been affected by their adolescent methylone exposure, their ability to detect the more novel of two Y-maze arms was assessed. Novelty was provided by changing the brightness of one of the arms between an acquisition and a retention trial, similar to the procedure originally devised by Dember (1956). Such curiosity-motivated ability has since been shown to rely on spatial memory (Hughes, 2001; Hughes and Maginnity, 2007).

2. Methods

2.1. Subjects

The subjects were 40 male and 40 female PVG/c hooded rats bred in the Animal Facility of the Department of Psychology, weaned when 30 days old (PND30) and then housed in same-sexed groups of 3 or 4 in 475 mm × 280 mm × 320 mm (l × w × h) plastic cages in a humidity-controlled (48 ± 10%) and temperature-regulated (22 ± 2 °C) holding room with 12 h light: 12 h dark (lights on at 08.00). Food and water was freely available at all times.

The maintenance and experimental treatment of all rats conformed to requirements of Part 5 (Codes of Welfare) and Part 6 (Use of Animals in Research, Testing and Teaching) of the New Zealand Animal Welfare Act (1999), and had been approved by the Animal Ethics Committee of the University of Canterbury.

2.2. Drug treatment

Methylone (3,4-methylenedioxymethcathinone) was synthesized by and purchased from BDH Synthesis, Lower Hutt, New Zealand. It was mixed with isotonic saline to produce a dose of 8 mg/kg. This dose was within the range shown to exert stimulant effects in male rats (Baumann et al., 2012; López-Arnau et al., 2012). When half of the rats reached PND35 (early adolescence) and the other half reached PND45 (late adolescence), equal numbers of each sex were given a daily i.p. injection of either methylone or saline for 10 consecutive days. The volume of each injection was 1 ml/kg. Body weights were recorded each day, and injection volumes adjusted accordingly to maintain the dose level. When the rats reached PND90 (early adulthood), behavioral testing began after 36 (late adolescent treatment) or 46 (early adolescent treatment) drug-free days.

2.3. General procedure

All rats experienced a single trial in each of four types of apparatus – an open field, a light/dark box a Y maze and an acoustic startle chamber – over 4 consecutive days. The experimental room in which all tests were conducted was kept at 22 °C ± 2 °C with humidity of 48% ± 10%, and dim overhead lighting (44 lx) approximately 120 cm above each type of apparatus. All tests were conducted between 10.00 and 16.00 h, in the light phase of the rats' light/dark cycle. To avoid order effects, the order in which individual rats completed the tests was randomized. After a trial in each type of apparatus, it was thoroughly cleaned with a disinfectant solution of 20% Paraquat Blue, and wiped dry.

2.3.1. Open field — apparatus and testing

The open field was constructed from Perspex and measured 60 × 60 × 30.5 cm high. Its walls were clear, its floor was divided into 16 numbered 15 × 15 cm squares, and it sat on a 70 cm-high table.

Each rat was placed in the center of a Perspex open field and, for 5 min, its location and the type of behavior it was engaged in were recorded every 3 s in terms of the following response categories:

1. occupation of a corner of the apparatus (corner occupancy);
2. occupation of one of the 4 central squares of the apparatus (center occupancy);
3. rearing up on hind legs either unsupported or against a wall (rearing);
4. licking any part of its body (grooming).

In accordance with an earlier procedure (Hughes and Beveridge, 1987; Kolyaduke and Hughes, 2013), the number of times the rat was located in a different square from where it had been 3 s beforehand enabled estimation of its ambulatory activity or distance traveled (ambulation). On completion of the trial, the number of fecal boluses eliminated in the apparatus by the rat was also counted. Higher numbers of fecal boluses are often viewed as indicative of heightened anxiety (Archer, 1973; Prut and Belzung, 2003).

2.3.2. Light/dark box — apparatus and testing

The light/dark box was constructed from Perspex and comprised two compartments. Apart from the roof, one compartment was entirely black and measured 20 × 15 × 20 (l × w × h) cm. The other was entirely white and measured 50 × 15 × 20 (l × w × h) cm. The two compartments were covered by clear lids and separated by a partition that contained a guillotine door which enabled access from one to the other.

Each rat was placed in the dark compartment and, 30 s later, the guillotine was withdrawn. The time it took to fully emerge (all four paws) into the light side was recorded, after which, for 5 min, its total entries of and time spent in the light compartment were also recorded.

2.3.3. Y maze — apparatus and testing

The Y maze was constructed from wood subsequently painted with clear varnish, and consisted of a 30 cm-long stem and two 45 cm-long arms set at an angle of 120° to each other. All parts of the maze were 10 cm wide and 14 cm high and, apart from the first 15 cm start area of the stem, were covered by a clear Perspex hinged lid. Both arms were provided with removable painted metal inserts that occupied all of their floor and walls, except for the first 5 cm.

Each rat was placed in the south end of the stem and, for 6 min, was allowed to freely explore the arms one of which contained a black insert, and the other a white insert. During this acquisition trial, half of the rats in each cell of the design explored the maze with the black insert in the left arm, while the other half explored it with the black insert in the right. The rat was then removed while both inserts were replaced with clean black ones. (Replacement of both inserts was necessary to