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Long-term cognitive, emotional and neurogenic alterations induced by alcohol and methamphetamine exposure in adolescent rats

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ABSTRACT

A high proportion of young methamphetamine (MA) users simultaneously consume alcohol. However, the potential neurological and behavioural alterations induced by such a drug combination have not been systematically examined. We studied in adolescent rats the long-term effects of alcohol, MA, and alcohol and MA combined on anxiety-like behaviour, memory, and neurogenesis in the adult hippocampus. Rats received saline, ethanol (ETOH, 1.5 g/kg), MA (MA, 2 mg/kg), or ethanol and MA combined (ETHOH-MA, 1.5 g/kg ethanol plus 2 mg/kg MA) via oral gavage, once daily for 5 consecutive days. Open field (OF), elevated plus maze (EPM) and radial arm maze (RAM) tests were conducted following a 15-day withdrawal period. The results showed alterations in exploratory behaviour in the OF in the MA and ETOH-MA groups, and anxiety-like effects in the EPM in all three drug treatment groups. All three drug groups exhibited reference memory deficits in the RAM, but only the combination treatment group displayed alterations in working memory. Both MA and ETOH-MA treatments increased the length of doublecortin (DCX)-void gaps in the dentate gyrus but only ETOH-MA treatment increased the number of such gaps. An increased number and length of DCX-void gaps correlated with decreased exploratory activity in the OF, and impaired working memory in the RAM was associated with an augmented number of gaps. These findings suggest that alterations in adult hippocampal neurogenesis are linked to the persistent cognitive and behavioural deficits produced by alcohol and MA exposure.

1. Introduction

The powerful stimulant, methamphetamine (MA), is the second most abused illicit substance worldwide, while alcohol is the most commonly abused substance, illegal or otherwise, particularly in countries that adopt a binge drinking culture (UNODC, 2015). Considerable evidence suggests that stimulant intoxication with MA is associated with excessive drinking in adolescents and young adults (McKetin et al., 2014; Olthuis et al., 2013). Such pattern of poly-substance abuse is not only linked to increased risk of negative health outcomes, including neurotoxicity, substance abuse progression, cognitive impairment and psychiatric vulnerability, but also complicates the implementation of effective intervention programmes (Barrett et al., 2006; Grov et al., 2009). Albeit the mechanisms mediating the physiological interactions between alcohol and MA are poorly understood, the combined use of both substances induces heightened peripheral physiological effects, reduces MA-induced sleep disturbances and increases perceived global intoxication ratings (Kirkpatrick et al., 2012; Mendelson et al., 1995), which may partly account for the popularity of their simultaneous use.

Neurogenesis in the adult hippocampus is a key physiological process with wide implications for understanding neural plasticity, functional modifiability, normal behaviour and cognition, and psychopathology (Cameron and Glover, 2015; Canales, 2010; Drew et al., 2013, Opendak and Gould, 2015). Addictive drugs, including opiates, alcohol and psychomotor stimulants, such as MA, impair adult neurogenesis in the hippocampus, disrupting the proliferation of neural progenitors, neuronal survival rates and maturation and differentiation in a drug- and regimen-specific fashion (Canales, 2013, Mandyam and Koob, 2012). Converging evidence revealed that alcohol exposure produces severe alterations of neurogenic processes in the adult hippocampus. Semi-chronic binge exposure to gavaged alcohol decreased proliferation and survival of newborn cells in the dentate gyrus (DG) of rats (Nixon and Crews, 2002), with alcohol self-administration producing similar effects, including inhibition of proliferating nuclear antigen (PCNA) expression, decreased survival of 2,5-bromodeoxyuridine labelling, and reduced size of the dendritic arbours of doublecortin-labelled, immature differentiating neurons (He et al., 2005). Similarly, short and long daily access to MA self-administration decreased proliferation, early phase maturation and survival of adult-born hippocampal neurons (Mandyam et al., 2008). Impairments in adult hippocampal neurogenesis, especially those evoked by psychoactive substances, have been consistently linked to deficits in learning and memory function and dysregulation of

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affective behaviour (Canales, 2010), craving and perpetuation of addictive behaviours (Mandyam and Koob, 2012), and neuropsychiatric vulnerability (DeCarolis and Eisch, 2010). Previous research showed that co-administration of alcohol and the psychedelic stimulant, 3,4-methylenedioxy-methamphetamine (MDMA), at doses that did not produce apparent cognitive impairments when given separately, produced persistent memory deficits as well as granule cell depletion and alterations in hippocampal neurogenesis in adolescent rats (Hernandez-Rabaza et al., 2010). Similar mutually potentiating effects of alcohol and MDMA have been reported in rats exposed prenatally, with neurogenic and behavioural deficits induced by the combination extending into late adolescence (Canales and Ferrer-Donato, 2014). Despite the prevalent use of both alcohol and MA at a young age in humans, the potential deleterious effects of such drug combination on adult hippocampal neurogenesis and related behaviours have not been previously investigated.

In the present experiments, to model the long-term neurological and behavioural effects of multi-drug use in young human adults, we examined in adolescent rats the effects of alcohol, MA, and combinations thereof, on reference memory and working memory performance, anxiety-like behaviour and adult hippocampal neurogenesis. To examine the persistence of the predicted behavioural deficits, rats underwent a period of extended withdrawal following semi-chronic exposure to the drug treatments and were subsequently tested in a drug-free state.

2. Experimental procedures

2.1. Subjects and treatments

2.1.1. Subjects

Subjects were male Long Evans rats bred in the Animal Facility of the Department of Psychology, University of Canterbury. The animals were housed in pairs in polycarbonate cages (48 cm × 28 cm × 22 cm) on a reverse 12 h light-dark cycle (lights on at 8.00 pm), with standard humidity (ca. 50%), and temperature conditions (ca. 20–22 °C). The animals had food available ad libitum until PND 48 and kept 90% of their free feeding thereafter. Water was made available ad libitum throughout. All procedures were carried out in accordance with the NIH Guide for the Care and Use of Laboratory Animals, and approved by the Animal Ethics Committee of the University of Canterbury (protocol 2013/25R). Rats were allowed one week to acclimatise to their environment before any manipulations were introduced and were extensively handled during this period.

2.1.2. Pharmacological treatments

Ethanol was obtained from the Chemistry Department of the University of Canterbury and MA HCl from the National Institute of Drug Abuse, NIH, USA. Both substances were dissolved in physiological saline (0.9%). The animals were assigned to four separate groups, namely Control ($n = 9$), ETOH ($n = 9$), MA ($n = 10$) and ETOH-MA ($n = 10$) receiving saline, ethanol (1.5 g/kg, 20%), MA (2 mg/kg) or ethanol and MA combined (1.5 g/kg of 20% ethanol plus 2 mg/kg MA), respectively, via oral gavage. Dose selection for the drugs was based on studies of dose translation from animal to human (Reagan-Shaw et al., 2008) and on data from rat self-administration assays (Cotter et al., 2015, Pei et al., 2016). The volume injected remained constant for all groups [ml injected = (weight of rat in g / 160) × 1.5 for each rat], as described previously (Canales, 2004). All rats were treated with a single daily dose over a 5-day period. The first treatment was administered in PND 36. Behavioural tests began after a 15 day withdrawal period during which rats were left undisturbed in their home cages (Fig. 1). Therefore, behavioural testing occurred at a time when immature hippocampal neurons typically exhibit unique plasticity features that contribute to relevant physiological, cognitive behavioural processes (Doetsch and Hen, 2005, Ge et al., 2007).

2.2. Behavioural tests

2.2.1. Open field (OF) test

The emergence/OF apparatus consisted of two joined compartments. The first area where the rats were initially placed for the emergence latency phase of the test was a small dark compartment (24 cm × 13 cm × 45 cm) with a sliding door to allow access into the larger area (90 cm × 90 cm × 45 cm) where the OF test was conducted. The first small chamber's floor, walls and door were painted black and featured a Perspex roof to allow observation. The floor of the main chamber was divided into nine zones defined by 30 cm × 30 cm squares. The emergence portion of this test will be used to assess anxiety/fearfulness, whereas the OF portion enabled assessment of activity and emotionality. Each rat was placed in the small, darkened start box dark side for 1 min. A sliding door separating the box from the OF was then withdrawn. The time taken for the rat to emerge into the OF was recorded (with an upper limit of 10 min). While in the OF the position where the rat was located was recorded every 3 s, for 5 min, with the slide between the field and the start box closed. Additional measures of locomotor activity (i.e. transitions between squares), immobility (i.e. discrete event during which the rat remained still for 3 s or more), rearing, defecation (as a measure of emotionality) and time spent in the four central squares were recorded.

2.2.2. Elevated plus maze (EPM)

To assess anxiety-like behaviours, a 4-armed plus maze was used. The maze consisted of two arms that were open (clear Perspex walls), and two other arms that were closed (wooden walls). The maze was elevated 1 m above the floor. Rats were placed individually in the centre of the EPM and allowed 5 min to explore it. The rat's entries into open and closed arms and the time spent in each set of arms were recorded.

2.2.3. Radial arm maze (RAM)

Training in the RAM was conducted as described previously (Hernandez-Rabaza et al., 2010). The apparatus consisted of a central area that gave access to eight equally-sized arms. The arms were 70 cm long and 10 cm wide and the central area was 30 cm in diameter. In the distal extreme of each arm, a recessed cup was installed for positioning of food rewards (20 mg Noyes chocolate pellets). The rats were first habituated to the maze for 10 min on two consecutive days in the presence of allocentric cues that remained in place throughout training. Training in the RAM consisted of 4 blocks of six trials each performed on 4 consecutive days. The task involved locating four pellets, each placed at the end of a different arm according to a random configuration. Configurations were specific for each rat and were kept constant throughout training. The number of reference memory errors (RME) (visits to unbaited arms) and working memory errors (WME) (visits to arms already visited in the same trial) were recorded.

2.3. Immunohistochemistry and histological analysis

Doublecortin expression was used as a sensitive marker of neurogenesis in the hippocampus (Dominguez-Escriba et al., 2006, Ferragud et al., 2010, Hernandez-Rabaza et al., 2006). On completion of the experiments rats were perfused transcardially under pentobarbital anaesthesia (125 mg/kg) with 4% paraformaldehyde in 0.1 M phosphate buffer (PB). Brains were removed and post-fixed overnight at 4 °C in the same fixative and subsequently transferred to graded saccharose solutions in 0.1 M PB with 0.1% sodium azide for cryoprotection. Serial free-floating 30-μm sections were cut on a sliding microtome. Every third section was stained with antiserum raised against doublecortin (DCX, goat polyclonal anti-DCX, 1:200; Santa Cruz Biotechnology, CA, USA), a marker for immature neurons. Immunocytochemistry was performed as previously described (Hernandez-Rabaza et al., 2006). Briefly,