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CHRONIC COCAINE ADMINISTRATION REDUCES STRIATAL DOPAMINE TERMINAL DENSITY AND STRIATAL DOPAMINE RELEASE WHICH LEADS TO DRUG-SEEKING BEHAVIOUR

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Abstract—Drug addiction is associated with altered dopamine (DA) neurotransmission in the basal ganglia. We have previously shown that chronic stimulation of the dopamine D2 receptor (D_2R) with cocaine results in reduced striatal DA terminal density. The aims of this study were to establish whether this reduction in DA terminal density results in reduced striatal DA release and increased cocaine-seeking behaviour and whether D_2R antagonism can restore the cocaine-induced alterations in DA neurotransmission and drug-seeking behaviour. Rats were housed individually and either control, cocaine, haloperidol (D_2R antagonist), or cocaine and haloperidol was administered in the drinking water for 16 weeks. Chronic cocaine treatment, which reduced striatal DA terminal density by 20%, resulted in a reduction in basal (−34%) and cocaine-evoked (−33%) striatal DA release and increased cocaine-seeking behaviour. These cocaine-mediated effects on striatal DA terminal density, DA release and drug-seeking could be prevented by co-administration with haloperidol. Basal and cocaine-evoked DA release in the striatum directly correlated with DA terminal density and with preference for cocaine. We conclude that striatal DA terminal density and DA release is an important factor in maintaining drug preference and should be considered as a factor in drug-seeking behaviour and relapse.

Key words: cocaine, catecholamine, dopamine transporter, addiction, anti-psychotic, *in vivo* microdialysis.

The development of addictive behaviours depends on activation of the mesolimbic DA system. Cocaine administration markedly elevates extracellular dopamine (DA) levels in the nucleus accumbens (NAcc) and dorsal striatum (Di Chiara and Imperato, 1988; Kalivas and Duffy, 1990) by binding to the DA transporter (DAT) and blocking the reuptake of DA into pre-synaptic terminals (Kuhar et al., 1988, 1991). Elevation of DA in these regions results in prolonged activation of post- and pre-synaptic DA receptors (Rouge-Pont et al., 2002; Nader et al., 2006; Martinez et al., 2009), which is important in establishing the addiction to cocaine (Martinez et al., 2004, 2009; Nader et al., 2006; Di Chiara and Bassareo, 2007).

Prolonged use of cocaine is associated with a decrease in basal DA levels (Rossetti et al., 1992; Weiss et al., 1992b; Wu et al., 1997) in the absence of cocaine, implying reduced DA neurotransmission, or "tone." Human PET and post-mortem studies demonstrate impaired presynaptic DA terminal activity and levels in the striatum of chronic cocaine users (Wilson et al., 1996; Volkow et al., 1997; Wu et al., 1997; Martinez et al., 2007). Similarly, *in vivo* microdialysis studies in animals indicate that baseline NAcc or striatal DA levels are lower in chronic cocaine-treated rats (Weiss et al., 1992a). This reduction in DA release that follows chronic drug use persists beyond the acute withdrawal phase and is present long after cessation of the drug (Rossetti et al., 1992; Diana et al., 1993). Although long-lasting post-synaptic molecular changes in the medium spiny neurons of the NAcc and dorsal striatum are important in drug addiction (Thomas and Malenka, 2003; Nestler, 2005; Hyman et al., 2006), it is also likely that this persistent hypo-dopaminergic state (Volkow et al., 1997) is required for both the persistence of these molecular changes and drug seeking behaviour (Diana et al., 1993; Volkow et al., 1993; Koob, 2006; Koob and Le Moal, 2008; Volkow et al., 2009).

We previously showed that prolonged stimulation of dopamine D_2 receptors (D_2R) with D_2R agonists result in reduced density and number of dopaminergic (DAergic) terminals in the dorsal or ventral striatum, through pruning of the axonal arbour (Parish et al., 2002). Similarly, chronic cocaine treatment prolonged stimulation of D_2R results in pruning of striatal DAergic axon terminals in the striatum, while D_2R antagonists (i.e. haloperidol, EEDQ or sulpiride) caused sprouting of striatal DA axon terminals (Parish et al., 2005, 2002). Thus, the main regulator of DA terminal density is the D_2R, which is located post-synaptically on striatal medium spiny neurons, as well as pre-synaptically on nigrostriatal DA terminals (Missale et al., 1998). The D_2R regulates DA levels in the striatum across several timescales: short-term (millisecs to mins) by controlling DA nerve terminal excitability (Tepper et al., 1984) and release and reuptake (Bowyer and Weiner, 1987; Tepper et al.,

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Abbreviations: AUC [DA], area under the curve of DA concentration; DA, dopamine; DAT, dopamine transporter; D_2R, dopamine D2 receptor; HPLC, high performance liquid chromatography; ir, immunoreactive; NAcc, nucleus accumbens; PBS, phosphate buffered saline; TH, tyrosine hydroxylase; TT, terminal tree.

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1987, 1997); medium-term (hours to days) by regulating DA synthesis (Lindgren et al., 2001); and long-term (days to weeks) by regulating DA terminal density (Parish et al., 2001; Stanic et al., 2003a). Thus, cocaine-induced pruning of striatal DA terminals is a homeostatic mechanism to maintain normal DA "tone." Yet when DAT functions normally during cocaine abstinence, DA tone will be reduced because of the reduced number of DA terminals, which may underlie craving. If this were the case, then re-establishing normal DA terminal density in the striatum and NAcc would restore DA tone and should reduce drug-seeking behaviour.

In the current study, we assessed whether there was a relationship between striatal DA terminal density, striatal DA release, and cocaine-seeking behaviour and whether restoration of striatal DA terminal density, with a D_2R antagonist, would restore DA tone and reduce drug-seeking behaviour.

EXPERIMENTAL PROCEDURES

Animals

All experiments were performed in accordance with the Prevention of Cruelty to Animals Act, 1986 under the guidelines of the National Health and Medical Research Council Code of Practice for the Care and Use of Animals for Experimental Purposes in Australia, and were approved by the Howard Florey Institute Animal Ethics Committee. Thirty-two male Wistar O/B rats weighing 250–350 g were used, with eight animals in each treatment group.

Study design

The study was designed to establish whether the density of DA terminals in the striatum correlated with striatal DA release and cocaine-seeking behaviour. Animals were tested for cocaine preference prior to treatment, and subsequently treated for 16 weeks under one of four conditions: control, cocaine, haloperidol, or cocaine and haloperidol. At the end of the treatment the animals were assessed again for their cocaine preference (Fig. 1A). One day following the second cocaine preference test, basal and cocaine-evoked striatal DA release was measured in these animals by *in vivo* microdialysis. At cessation of the microdialysis, the animals were intracardially perfused and the brains were processed for immunohistochemistry to assess DA terminal density.

Assessment of cocaine-seeking behaviour

Cocaine-seeking behaviour was assessed by the two-bottle preference test (Parish et al., 2005). Animals were deprived of fluids for 8 h and then provided a choice of two drinking spigots for 3 h. One spigot supplied 0.15% sucrose solution (10 ml) and the other supplied cocaine dissolved in 0.15% sucrose solution (2.5 mg/ml in 10 ml). At the end of 3 h, the volume consumed from each spigot was determined. If there was no preference for either bottle then the consumption from each should be ~50%. The location of the bottles was changed sporadically approximately every 3–4 days to avoid bias related to place preference. Cocaine seeking behaviour was assessed before (i.e. week 0) and after the 16-week drug treatment (i.e. week 16) (see Fig. 1A). Importantly, the total volume of solution consumed in the cocaine preference test by the haloperidol group (7.4±0.9 ml) was not significantly different from those of the control (7.6±0.9 ml), cocaine (8.2±0.8 ml) or cocaine/haloperidol (7.4±0.5 ml) group, indicating that haloperidol pre-treatment had no effect on motor function and thereby ability to drug seek.

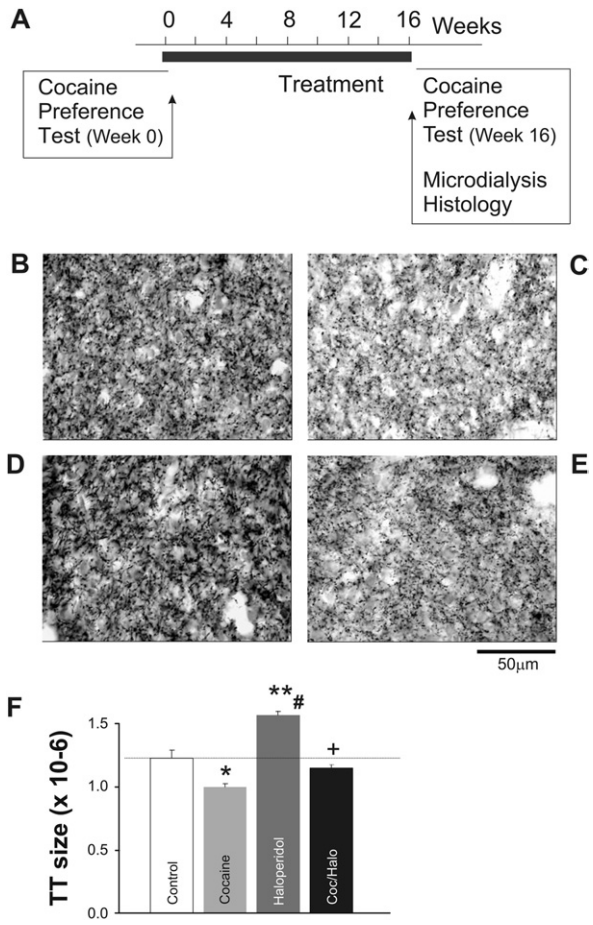

Fig. 1. (A) Experimental design: Two-bottle preference test was performed prior to chronic treatments. Treatments were given for 16 wks and included control, cocaine, haloperidol or haloperidol plus cocaine. After 16 wks, treatment was ceased for 24 h and the two-bottle preference test repeated. One day following the two bottle preference test, the animals were anesthetized and microdialysis probes were placed into the striatum to measure striatal DA levels. Subsequently, the brains were processed for immunohistochemistry. Photomicrographs showing DAT-ir terminals in the dorsal striatum (63×, scale bar=50 μm) in (B) control, (C) cocaine, (D) haloperidol, and (E) cocaine plus haloperidol treated animals. (F) Histogram showing striatal dopamine terminal tree (TT) size of different treatment groups (see also Table 1). * $P<0.05$, significantly different from control; ** $P<0.01$, significantly different from control or cocaine; # $P<0.01$, significantly different from cocaine/haloperidol; + $P<0.05$, significantly different from cocaine.

Chronic administration of cocaine and/or haloperidol

Rats were housed individually and drug treatments were delivered in the drinking water for 16 weeks. Animals were administered one of four treatments, each consisting of daily access to two bottles: (A) CONTROL: a bottle containing 0.15% sucrose solution (10 ml) and the other containing water (600 ml), (B) COCAINE: a bottle containing cocaine in 0.15% sucrose solution (2.5 mg/ml in 10 ml) and the other containing water (600 ml), (C) HALOPERIDOL: a bottle containing 0.15% sucrose solution (10 ml) and the other containing haloperidol (0.02 mg/ml in water; average volume consumed each day approximately 32 ml) or (D) COC/HALO: a bottle containing cocaine in 0.15% sucrose solution (15 mg/kg in 10 ml) and the other containing haloperidol (0.02 mg/ml in water; volume consumed each day approximately 32 ml). Daily intake from bot-