Δ FosB: A Molecular Mechanism of MDMA Dependence

by

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Table of Contents

Abstract.....	3
Introduction	5
Pharmacology of MDMA	5
Effects of repeated administration of MDMA - Human studies	6
Effects of Repeated MDMA – Animal Studies.....	8
Self-Administration.....	10
Pharmacology of Self-Administration.....	13
MDMA Self-Administration.....	14
Neurobiological Substrates of Self-administration	15
Δ FosB.....	19
Current study.....	21
Method	23
Subjects	23
Surgery	23
Apparatus.....	24
Procedure	26
Perfusion.....	27
Slicing.....	27
Immunohistochemical Analysis: Δ FosB.....	27
Cell Counts and Analysis of Immunohistochemical Data:.....	29
Drugs	30
Data Analysis.....	30
Results.....	30
Part 1: Self-Administration.....	30
Part 2: Δ FosB expression within the striatum and mPFC	32
Part 3: Behavioural Correlates of Δ FosB	36
Discussion	36
Conclusions.....	44
References	46

Abstract

Rationale. 3,4-methylenedioxymethamphetamine (MDMA) is a widely used illicit substance and some users show signs of abuse and dependence. It has been suggested that addiction reflects persistent neuroplasticity and one proposed mechanism has been a change in the expression of the transcription factor, Δ FosB.

Objectives. This study determined whether Δ FosB expression in reward-relevant brain areas was altered as a function of MDMA self-administration.

Methods. Rats were separated into triads. One rat self-administered MDMA (master rat) and the other 2 rats received either MDMA (yoked MDMA) or saline (yoked saline) infusions contingent on the behaviour of the master rat. Testing continued until a total intake of 350 mg/kg of MDMA was delivered. Two days following the final self-administration session, rats were sacrificed and perfused transcardially. Brains were removed, and Δ FosB immunohistochemistry was conducted. Δ FosB expression in striatum and medial prefrontal cortex was compared across groups.

Results. Unfortunately the tissue from many of the yoked MDMA rats was compromised and therefore data from this group were not included in any analyses. MDMA self-administration produced a significantly greater expression of Δ FosB in the ventromedial and ventrolateral portions of the caudate putamen when compared to expression produced following yoked saline exposure. Within the infralimbic cortex, accumbens shell and dorsolateral caudate putamen differences approached significance. A significant correlation between Δ FosB expression in the ventromedial caudate putamen and cumulative active lever presses across the final 5 days of self-administration was also found.

Conclusions. These findings provide the first evidence of MDMA-induced expression of Δ FosB. Increased expression of Δ FosB was observed in regions associated with the development and maintenance of drug addiction. These data support the idea that induction of Δ FosB may present a mechanism by which MDMA can induce alterations in genetic transcription, which may underlie

the development of MDMA dependence.

Future studies should utilise antagonism of Δ FosB via region-selective administration of Δ c-jun in order to further elucidate the role of these transcriptional changes in the development and maintenance of self-administration.

Introduction

(±) 3,4-methylenedioxymethamphetamine (MDMA), or more commonly ‘ecstasy’, is one of the most widely used illicit substances in the world (World Drug Report, 2015). New Zealand has amongst the world’s highest prevalence of MDMA use with 2.6% of the population having used MDMA at least once in their lifetime (Ministry of Health, 2010). Early descriptions of the subjective effects of MDMA suggested that there was minimal abuse liability as “it’s most desirable effects diminish with frequency of use” (Shulgin, 1986, p. 300; Solowij, Hall & Lee, 1992), while the adverse effects become more pronounced. However, more recent evidence indicates that subsets of users engage in problematic use and meet diagnostic criteria for a substance use disorder (Degenhardt, Bruno & Topp, 2010; Parrott, 2013).

The proportion of users engaging in problematic use is also increasing (Degenhardt, Bruno & Topp, 2010). Additionally, MDMA has been suggested to be neurotoxic (McCann, Ridenour, Shaham, & Ricuarte, 1994; McCann, Szabo, Scheffel, Dannals & Ricaurte, 1998; McCann et al., 2005; McCann et al., 2008) and use has been associated with cognitive, and other, impairments (Rodgers, 2000; Stough et al., 2012; Wagner, Becker, Koester, Gouzoulis-Mayfrank & Daumann, 2013). This is a concern for people who use MDMA recreationally as well as for patients who are being treated for post-traumatic stress disorder with MDMA (Mithoefer et al., 2013; Mithoefer, Wagner, Mithoefer, Jerome & Doblin, 2011). As a result, many studies have investigated the consequences of MDMA exposure.

Pharmacology of MDMA

MDMA increases extracellular concentrations of the monoamines, serotonin (5-HT), dopamine (DA) and norepinephrine (Baumann, Wang & Rothman, 2007; Green et al., 2003). It preferentially stimulates 5-HT release via reversal of the transporter (Gu & Azmitia, 1993; Rudnick & Wall, 1992). Additionally, MDMA binds to the vesicular monoamine transporter (VMAT) and inhibits vesicular repackaging, while promoting vesicular release of 5-HT. Both of these

mechanisms increase cytosolic 5-HT available for release (Bogen, Haug, Myhre & Fonnum, 2003; Erickson, Schafer, Bonner, Eiden, & Weihe, 1996; Rudnick & Wall, 1992). MDMA also induces an inhibition of monoamine oxidase-A (MAO-A) which further increases cytosolic 5-HT (Leonardi & Azmitia, 1994).

Effects of repeated administration of MDMA - Human studies

Varied patterns of MDMA use have been reported. For example, a multi-part study assessed MDMA use in a sample of 109 new users over a 2-year period (Wagner, Becker, Koester, Gouzoulis-Mayfrank & Daumann, 2013; Wagner, Tkotz, Koester, Becker, Gouzoulis-Mayfrank & Daumann, 2015). In the year following their first exposure, 43 subjects did not use MDMA again, whereas 23 subjects reported 10 or more occasions of MDMA use. The remaining participants consumed MDMA between 1 and 10 times and were not included for further analyses. Of the 96 individuals examined 2 years following first exposure, 10 were regarded as heavy-users with consumption of over 50 pills. Subsequent tests on these heavy users revealed deficits in measures of cognitive performance such as the visual paired associates task. Although heavy users constituted only a minority in Wagner and colleagues' studies (2013; 2015), another study (Cottler, Leunge, Abdallah, 2009) that examined 593 MDMA users with a median lifetime use of 50 pills, found that 15% of this sample met DSM criteria for abuse and 59% met criteria for dependence. A meta-analysis conducted across 33 studies suggested that MDMA use is associated with attentional deficits, reduced verbal learning, memory, motor speed and executive system function (Kalechstein, De La Garza, Mahoney, Fantegrossi & Newton, 2007).

It has been suggested that these adverse consequences of MDMA use reflect drug-induced alterations in brain chemistry. Imaging studies in MDMA users have repeatedly reported evidence of compromised 5-HT function (McCann, Ridenour, Shaham, & Ricuarte, 1994; McCann, Szabo, Scheffel, Dannals & Ricaurte, 1998; McCann et al., 2005; McCann et al., 2008). Reductions in 5-HT transporter (SERT) binding are a consistently observed consequence of repeated MDMA

exposure (McCann et al., 1998; McCann et al., 2005; McCann et al., 2008) and the decrease in SERT binding was correlated with extent of MDMA use (McCann et al., 1998). Furthermore, reductions in SERT binding were correlated with impaired performance on memory tasks (McCann et al., 2008).

The primary metabolite of 5-HT, 5-hydroxyindoleacetic acid (5-HIAA) was reduced in cerebrospinal fluid (CSF) of MDMA users (McCann, Ridenour, Shaham & Ricaurte, 1994). Subsequent tests on this group showed reduced scores on measures of impulsivity and indirect hostility, two behaviours that have been suggested as being regulated via 5-HTergic mechanisms (Coccaro et al., 1989; McCann et al., 1994). Because prior-drug history was not reported, and because MDMA users are often polydrug users, the conclusion that these deficits were due to MDMA alone, another substance, or an interaction between the effects of multiple substances is equivocal. One study that examined a group that used only MDMA also found evidence for 5-HT deficits (Gerra, Zaimovic, Guicastro, Maestri, et al., 1998). However, they noted that premorbid conditions were not controlled.

Experimental research in humans can be limited by both ethical and experimental constraints. For instance, it would not be ethically acceptable to administer harmful substances or substances with high abuse potential to human subjects. Because polydrug use is common amongst MDMA users (Fox, Parrott & Turner, 2001; Gouzoulis-Mayfrank & Daumann, 2006) and pre-existing conditions and personal history differs, the ability to obtain meaningful findings can be compromised. Moreover, the bulk of research utilising human participants relies upon self-report data particularly to document past drug use. Such methods of data collection are often highly unreliable (Dunning, Heath & Suls, 2004); an issue that might be exacerbated when the topic is of an illicit nature. Circumvention of these issues of self-report can be achieved through methods such as hair analysis sampling (Ledgerwood, Goldberger, Risk, Lewis & Price, 2008), but do little to address other potential confounds.

Even if past use is accurate, impurities of illicitly purchased MDMA constitute an additional constraint when examining effects of exposure. Toxicological analyses have shown that the actual MDMA content in ecstasy pills varies considerably (Tanner-Smith, 2006), and pills often contain a number of other psychoactive compounds such as amphetamine, caffeine, ketamine MDEA and MDA (Parrott, 2004; Sherlock, Wolff, Hay & Conner, 1999; Tanner-Smith, 2006). Therefore, it is difficult to attribute effects of interest to MDMA. Due to the myriad ethical and experimental constraints, researchers often use animal models to gain greater experimental control.

Effects of Repeated MDMA – Animal Studies

Repeated exposure of MDMA to laboratory animals produces dose-dependent deficits in various indices of 5-HT neurotransmission (Ali et al., 1993; Battaglia et al., 1987; Baumann, Clark, Franken, Rutter & Rothman, 2008; Insel, Battaglia, Johannessen, Marra & De Souza, 1989; Ricaurte, Martello, Katz & Martello, 1992; Sabol et al., 1996; Stone, Merchant, Hanson & Gibb 1987). Oral administration of 1.25, 2.5 or 20 mg/kg MDMA to rhesus monkeys produced some indications of persistent neurotoxicity that was particularly apparent following repeated administration of the 20 mg/kg dosing regimen (Ali et al., 1993). Two weeks following twice daily MDMA (20 mg/kg, s.c) to rats produced reductions in 5-HIAA (Battaglia et al., 1987). Deficits in 5-HT transporter binding densities were also observed. Twice daily administration of MDMA (2.5 or 10 mg/kg) for four days reduced both 5-HT and 5-HIAA in rhesus monkeys (Insel et al., 1989). Reductions in the density of 5-HT uptake sites were also reported, which led to the suggestion that higher doses of MDMA may be neurotoxic.

In rats, even a single dose of MDMA produces neurochemical effects that were consistent with neuroadaptive responses in brain 5-HT (Stone et al., 1987). When measured 15-minutes following a single experimenter-administered dose of MDMA (10 mg/kg, s.c) reductions of basal 5-HT concentrations and the 5-HT rate-limiting enzyme, tryptophan hydroxylase, were found within the frontal cortex. Further analyses 1 hour following administration of MDMA revealed reduced

tryptophan hydroxylase in the neostriatum, hippocampus and hypothalamus. Indices of 5-HT deficits were still apparent 110 days following administration of 5 doses of MDMA within a 24-hour period (Stone et al., 1987).

Effects of a high dose of MDMA (20 mg/kg) administered twice daily for four days were measured 2, 8, 16, 32 and 52 weeks following administration (Sabol et al., 1996). When measured 2 weeks following exposure, concentrations of 5-HT were decreased in all brain regions analysed, aside from the septum. Recovery to baseline concentrations was observed within 52 weeks following administration. Similar effects were produced in squirrel monkeys following a regime of twice daily administration of 5 mg/kg (s.c) MDMA for four consecutive days (Ricaurte, et al., 1992). Two weeks following the final exposure, deficits in a range of 5-HT markers were observed. Partial recovery was reported in the caudate, hippocampus and frontal cortex 10 weeks following exposure. Even 18 months following MDMA treatment, however, some deficits were still present.

These findings clearly demonstrate that repeated exposure to MDMA produces a number of dose-dependent deficits in 5-HTergic neurotransmission. While there is recovery, the degree of recovery is species-, time- and region-dependent. A potential problem in terms of interpretation of these studies, however, is that MDMA doses were much greater than what might be expected to be consumed by most users. For example, the average MDMA user consumes 1.8 pills per use (Parrott & Lasky, 1998) and the average MDMA pill sold contains 80-150 mg of MDMA (Green et al., 2003). Accordingly, a 70 kg MDMA user would be consuming 1.0 to 4.0 mg/kg, far less than the doses administered to animals in the studies cited above.

The peak serum concentration of MDMA following a 2.0 mg/kg dose in humans was comparable to the concentration produced by 7 mg/kg administered to rats (Green et al., 2009). Through allometric scaling and consideration of different pharmacokinetic profiles, a human MDMA dose of roughly 1 mg/kg is similar to approximately 5 mg/kg in a rat (De La Torre et al., 2000; Fitzgerald, Blanke & Poklis, 1990). Thus, the relevance of effects produced by administration

of these high doses of MDMA in non-human animals is somewhat tenuous.

Self-Administration

A more relevant means of testing effects of repeated exposure to MDMA is to allow the subject to self-inject the drug. The development of the indwelling intravenous (i.v) catheter (Weeks, 1962) has led to a wealth of research concerning self-administration of drugs of abuse in non-human animals. In a typical self-administration procedure, an animal is surgically implanted with an i.v catheter and trained to perform an operant response (e.g. lever depression, nose poke), which is reinforced by drug delivery. Thus, an animal learns to perform a response in order to self-inject drugs, thereby permitting researchers to examine factors that impact voluntary drug-taking behaviour.

The external validity of the self-administration model can be found in the types of drugs self-administered by both humans and non-humans. With the exception of a handful of substances, drugs abused by humans are self-administered by animals (Johanson & Balster, 1978; O'Connor, Chapman, Butler & Mead, 2011; Schuster & Johanson, 1974). Further support for the external validity of the self-administration paradigm can be found in the similarities of patterns of drug-taking between humans and non-human animals. For instance self-administration of psychostimulants such as amphetamine or cocaine are marked by both alternating periods of consumption and abstinences (Lowinson, 2005), as is often observed in humans who use these substances. In a similar manner, ethanol self-administration is characterised by patterns of bingeing followed by periods of abstinence in both humans and non-humans (Lowinson, 2005). In rats that self-administer MDMA, responding during initial sessions tends to be low (De La Garza, Fabrizio & Gupta, 2007; Schenk, Colussi-Mas, Do & Bird, 2012), as is also observed in humans who consume ecstasy (Hansen, Maycock & Lower, 2001). With repeated testing, however, a different pattern of responding is produced. For some rats, there is an escalation of intake, which might reflect the pattern of use seen in users who consume substantial quantities of ecstasy (Schenk, Hely,

Lake, Daniela, Gittings & Mash, 2007; Schenk et al., 2012). For these rats it is not unusual for intake to exceed 20 mg/kg in a single session (Schenk, et al., 2012).

It has been argued that self-administration alone is not an adequate index of addiction. Everitt and colleagues (2005; 2008) have suggested that drug addiction is the final stage in a series of events that initiates with drug use and culminates in habitual and compulsive usage of drugs. These transitional stages of drug use can all be modelled within the self-administration paradigm (Haney & Spealman, 2008; Panlilio & Goldberg, 2008).

Firstly, initiation of drug-use can be measured by examining the acquisition of self-administration. During this stage, a subject is trained to perform an operant response, which is reinforced by drug-delivery. Profiles of acquisition of self-administration, measured as the latency to acquisition and the percentage of subjects that achieve acquisition criteria, can differ across substances. These differences can help to inform researchers as to the initial reinforcing value and the relative abuse-liability of a drug. Many experimental and genetic manipulations that impact the acquisition of self-administration have been identified (Caine et al., 2007; Carrol & Lac, 1997; Howell & Byrd, 1995; Piazza, Deminière, Le Moal, & Simon, 1989; Schenk et al., 1993).

A range of experimental and genetic factors can also influence the maintenance of self-administration (Bradbury, Colussi-Mas, Mueller, Ricaurte & Schenk, 2014; Caine, Thomsen, Gabriel, Berkowitz, Gold & Koob, 2007; Roberts, Corcoran & Fibiger, 1977; Lyness, Friedle & Moore, 1980; Roberts & Koob, 1982; Dianna et al., 1986; Smith, Schultz, Co, Goeders & Dworkin, 1987; Yokel & Wise, 1976). Importantly, following extensive exposure to self-administered drugs there is the emergence of behaviours that are consistent with addiction for some subjects (Ahmed & Koob, 1998; Ahmed, Walker & Koob, 2000; Deroche-Gamonet, Belin & Piazza, 2004; Panlilio & Goldberg, 2008; Lynch, Nicholson, Dance, Morgan & Foley, 2010).

Pharmacology of Self-Administration

All drugs of abuse increase synaptic DA (Di Chiara et al., 2004; Di Chiara & Imperato, 1988; Nestler, 2005) and a wealth of studies has shown that pharmacological manipulations of DA alter self-administration (Caine and Koob, 1993; Dianna, Smith, Smith & Lyness, 1986; Howell & Byrd, 1991; Self, Belluzi, Kossuth & Stein, 1996; Wee & Woolverton, 2006; Yokel & Wise, 1976; Yokel & Wise, 1978). Accordingly it has been suggested that neuroadaptations in central DAergic mechanisms underlie the shift from drug use to abuse (Gardner, 1997; Everitt & Robbins, 2013; Wise, 2004).

An inhibitory role of 5-HT on self-administration has also been demonstrated. Roberts and colleagues (1999) assessed the reinforcing efficacy of a range of substances with varying affinities for DA and 5-HT. A higher DA:5-HT affinity ratio was a more effective predictor of reliable self-administration than affinity for DA alone. A similar study (Wee et al., 2005) assessed a range of amphetamine analogues which possessed similar affinities for DA, but varied affinities for 5-HT. Self-administration was negatively correlated with greater 5-HT release. Further evidence of an inhibitory role of 5-HT on self-administration has been derived by examining effects of neurotoxic lesions of 5-HTergic neurons. These lesions facilitated morphine (Smith, Schultz, Co, Goeders & Dworkin, 1987), amphetamine (Lyness, Friedle & Moore, 1980) and MDMA (Bradbury, Bird, Colussi-Mas, Mueller, Ricaurte & Schenk, 2014) self-administration. A genetic deletion of the SERT also rendered rats more susceptible to MDMA (Oakly, Brox, Schenk & Ellenbroek, 2014) and cocaine (Homberg et al., 2007) self-administration.

Overall, the available evidence suggests that DAergic mechanisms underlie the reinforcing effects of drugs of abuse and that 5-HT is inhibitory to self-administration of drugs of abuse. This begs the question; why is MDMA, a preferential indirect 5-HTergic agonist, self-administered by animals and abused by humans?

MDMA Self-Administration

MDMA is self-administered by monkeys (Beardsley, Balster & Harris, 1986; Lamb & Griffiths, 1987), rats (Ratzenboeck, Saria, Kriechbaum & Zernig, 2001; Schenk, Gittings & Colussi-Mas, 2011; Schenk, Gittings, Johnstone & Daniela, 2003) and mice (Trigo, Panayi, Soria, Maldonado & Robledo, 2006). The profile of acquisition of MDMA self-administration is, however, quite different from the profile of self-administration of other drugs of abuse. Nearly 100% of rats acquired reliable cocaine and amphetamine self-administration (Carroll & Lac, 1997), but acquisition of MDMA self-administration occurs in only approximately 50% of rats (Bradbury, Bird, Colussi-Mas, Mueller, Ricaurte & Schenk, 2014; Schenk, Colussi-Mas, Do & Bird, 2012). Similarly, the latency to acquisition of reliable self-administration of MDMA tends to require approximately 15 daily sessions, whereas acquisition of cocaine or amphetamine self-administration requires only a few test sessions (Carroll & Lac, 1997). One hypothesis is that MDMA self-administration becomes dependent on DAergic mechanisms following repeated exposure for some rats (Schenk, 2011; Schenk, Gittings & Colussi-Mas, 2011).

The initial DAergic response to MDMA is relatively small, but with repeated exposure, this response became sensitized (Kalivas, Duffy & White, 1998; Schenk, Gittings & Colussi-Mas, 2011). Conversely, repeated exposure attenuated the magnitude of the 5-HTergic effect of MDMA as measured by *in vivo* microdialysis (Reveron, Maier & Duvauchelle, 2010). A similar sensitization effect was produced by prior exposure to cocaine. The latency to acquire MDMA self-administration was reduced when rats were previously trained to self-administer cocaine (Schenk, Gittings, Johnstone & Daniela, 2003). Because cocaine self-administration has been attributed to sensitization of cocaine-produced DA (Kalivas & Duffy, 1993), these findings support the idea that a sensitized DA response also mediated the acquisition of MDMA self-administration. In support of this idea, microdialysis revealed a greater MDMA-induced DA overflow for rats that acquired MDMA self-administration (Colussi-Mas, Wise, Howard & Schenk, 2010). Repeated MDMA

exposure produced cross-sensitization to the behavioural effects of DA agonists (Bradbury, Gittings & Schenk, 2012) and also facilitated the acquisition of MDMA self-administration (Van de Wetering and Schenk, 2017).

The maintenance of MDMA self-administration also becomes dependent on DAergic mechanisms. MDMA self-administration was attenuated by pre-treatment with dopamine antagonists (Brennan, Carati, Lea, Fitzmaurice & Schenk, 2009; Daniela, Brennan, Gittings, Hely & Schenk, 2004; Shin, Qin, Liu & Ikemoto, 2008). Drug-seeking following extensive self-administration was also modified by pharmacological manipulations of DA (Colussi-Mas, Wise, Howard & Schenk, 2010; Nawata, Kitaichi, Yamamoto, 2016; Schenk et al., 2016; Schenk, Gittings & Colussi-Mas, 2011) and was positively correlated with MDMA-induced DA overflow in the dorsal striatum (Colussi-Mas et al., 2010).

Neurobiological Substrates of Self-administration

A number of brain regions have been implicated in the transition from drug use to compulsive drug use. For the purpose of this thesis, the focus will be on striatum and medial prefrontal cortex. The striatum can be separated into the ventral and dorsal subdivisions. The ventral striatum consists of the nucleus accumbens, which can be further subdivided into the shell and core. The nucleus accumbens has often been implicated as an essential substrate for the initiation of drug-taking because of the role in positive reinforcement (Di Chiara et al., 2004; Koob, 1992; Malenka, Nestler, & Hyman, 2009) and different roles of the core and shell have been suggested (Rodd-Henricks, McKinzie, Li, Murphy & McBride, 2002). Rats readily self-administered cocaine into the shell, but not the core of the nucleus accumbens (Rodd-Henricks et al., 2002). Moreover, experimenter-administered MDMA in dosages that sustain self-administration increase DA overflow in the NAcc, with a preference for the shell, relative to the core (Cadoni et al., 2005).

Habit formation might be a critical component of the development of compulsive drug-taking (Di Chiara et al., 1999). The dorsal portion of the striatum (or caudate putamen) has

traditionally been associated with motor movements due to the importance in Parkinson's disease (Malenka, Nestler & Hyman, 2009). It has also been suggested that the dorsal striatum may mediate procedural and associative learning and the inhibition of action control, which contributes to its proposed role in habit formation (Gerdeman, Partridge, Lupica & Lovinger, 2003). During the development of addiction, a switch from goal-directed action to stimulus-response habit is associated with a shift from ventral to dorsal striatal DA activation (Everitt et al., 2008; Everitt & Robbins, 2005; Gerdeman et al., 2003).

Both dorsal and ventral portions of the striatum have been shown to play a role in mediating self-administration behaviours. *In vivo* microdialysis demonstrate that increases in extracellular DA in the NAcc underlie the reinforcing value of cocaine (Pettit & Justice, 1989) and amphetamine (Ranaldi, Pocock, Zereik & Wise, 1999). Bilateral electrolytic lesions to either the NAcc or the ventral or dorsal portion of the caudate putamen reduced the rewarding value of cocaine and morphine in a dose-dependent manner (Suto, Wise & Vezina, 2011). Similarly, neurotoxic lesions that reduced DAergic neurons by 90% in the NAcc produced a persistent and significant reduction in cocaine self-administration (Roberts, Corcoran & Fibiger, 1977).

Inactivation of the dorsolateral caudate putamen via local infusions of GABA agonists, attenuated drug-seeking by cocaine experienced rats (Epstein, Preston, Stewart & Shaham, 2006). Similarly, lesions of the dorsolateral caudate putamen inhibited cocaine-seeking (Gabriele & See, 2011). Moreover, DAergic antagonists administered directly into the dorsal striatum attenuated cocaine-seeking (Vanderschuren, Di Ciano & Everitt, 2005). Overall, the available evidence suggests that both the ventral and dorsal portions of the striatum are critical substrates in the mediating various aspects of self-administration.

The prefrontal cortex has also been suggested as relevant to self-administration. The prefrontal cortex is involved in higher cognitive function such as working memory, attention, information processing and judgement (Roberts, Robbins & Weiskrantz, 1998). Imaging studies

have identified structural deficits in the frontal cortex in dependent cocaine (Franklin et al., 2002; Liu, Matochik, Cadet & London, 1998), alcohol (Jernigan, Schafer, Butters & Cermak, 1991; Pfefferbaum, Sullivan, Mathalon & Lim, 1997) and heroin users (Liu et al., 1998). These structural deficits are thought to underlie functional impairment reported following extensive exposure to drugs of abuse (Goldstein & Volkow, 2002; Mandyam, Wee, Eisch, Richardson & Koob, 2007). Drug-seeking responses are understood to rely upon activity within the PFC, as cocaine-induced drug-seeking is blocked by inactivation of the PFC (McFarland & Kalivas, 2001).

The medial prefrontal cortex (PFC) consists of the anterior cingulate, infralimbic cortex and prelimbic cortex. All of these subdivisions have been implicated in self-administration behaviours. The anterior cingulate mediates goal-directed behaviours, motivation and action initiation (Devinsky, Morrell & Vogt, 1995). Relative to drug-naïve controls, rats trained to self-administer cocaine showed an increase in c-fos expression following cocaine challenge (Neisewander et al., 2000). As c-fos is used as a marker of neuronal activation, this study indicates greater activation of anterior cingulate cells in cocaine experienced subjects, which may suggest a role of the anterior cingulate in the motivational processes associated with extended cocaine self-administration.

The prelimbic cortex is also understood to mediate drug-seeking responses. Inhibition of the prelimbic cortex impeded cocaine- (Stefanik et al., 2012) and heroin- (Rogers, Ghee & See, 2008) seeking. Administration of cocaine directly into the mPFC is sufficient to reinstate cocaine-seeking (Park et al., 2002), an effect that is blocked by AMPA administration in the NAcc, which suggests the importance of an mPFC-NAcc circuit in mediating cocaine-seeking.

The infralimbic cortex has also been implicated in self-administration behaviours (LaLumiere, Niehoff & Kalivas, 2010; Peters, LaLumiere & Kalivas, 2008). Inhibition of the infralimbic cortex via selective intracranial administration of GABA agonists, increased drug-seeking (Peters, LaLumiere & Kalivas, 2008). While, drug-seeking was potentiated by infusions of the glutamate agonist AMPA directly into the infralimbic cortex (Peters, LaLumiere & Kalivas,

2008). Infralimbic inactivation via GABA agonists prevented the consolidation of extinction learning following cocaine self-administration (LaLumiere, Niehoff & Kalivas, 2010). These data suggest a role of the infralimbic cortex in mediating drug-seeking responses and extinction learning.

As can be inferred from the evidence above, both the striatum and PFC are implicated in mediating various aspects of self-administration behaviours. However, while experimental research continues making headway in understanding the neurological substrates of self-administration of commonly abused drugs, much still remains unknown. Moreover, relative to typical drugs of abuse, the neurological mechanisms that contribute to the initiation and maintenance of MDMA self-administration are not as well documented.

Δ FosB

One hypothesised mechanism for the persistent neuroplastic changes that underlie addiction posits that repeated drug exposure alters genetic transcription (Nestler, 2008). A potential target for the induction of these genetic alterations is Δ FosB, a transcription factor. Δ FosB is a member of the Fos family of proteins, but unlike the remainder of the Fos family of proteins, which are induced rapidly and transiently, Δ FosB is far more stable. It accumulates within certain regions even weeks after the cessation of drug exposure (Nestler, 2008). Δ FosB dimerizes with the jun family of proteins to form the early response transcription factor AP-1. Once formed, AP-1 initiates a number of molecular pathways, providing the capacity to alter genetic, molecular and structural makeup (Nestler, 2008; Ruffle, 2014).

In the nucleus accumbens, increases in Δ FosB inhibited transcription of the opioid peptide, dynorphin (McClung et al., 2004), and increased transcription of the glutamate AMPA receptor subunit, GluR2 (Kelz & Nestler, 2000; Nestler, Barrot & Self, 2001) and nuclear factor kappa B (NF- κ B)(Ang et al., 2001). These products of Δ FosB over-expression have been shown to be involved in the behavioural and neurochemical response to drugs of abuse. For instance, overexpression of GluR2 increased sensitivity to the rewarding value of cocaine (Kelz et al. 1999).

Dynorphin inhibits DAergic transmission (Shippenberg & Rea, 1997), and so the increase in Δ FosB might contribute to persistent effects of drugs of abuse via this disinhibition of DA. NF- κ B was selectively induced in the NAcc following chronic cocaine exposure (Ang et al., 2001), but the relevance of this increase is not yet understood.

Expression and accumulation of Δ FosB within the NAcc has been recorded following exposure to drugs of abuse such as cocaine (Nye, Hope, Kelz, Iadarola & Nestler, 1995; Pich et al., 1997), methamphetamine (Cornish, Hunt, Robins & McGregor, 2012), morphine (Nye & Nestler, 1996), nicotine (Pich et al., 1997) and phencyclidine (Robbins, Everitt & Nutt, 2010). Mice with selective induction of Δ FosB in the NAcc were more sensitive to both the rewarding and locomotor-activating effects of cocaine (Kelz et al., 1999) and the rewarding value of morphine was increased in mice bred to overexpress Δ FosB (Zachariou et al., 2006).

Within the caudate putamen, chronic exposure to cocaine, morphine, ethanol and Δ 9-THC all induce Δ FosB expression (Perrotti et al., 2008). Induction of Δ FosB remained similar across rats self-administering cocaine and those receiving yoked-infusions (Larson et al., 2010), indicating that induction in this region is a direct pharmacological action of cocaine exposure.

Expression of Δ FosB within the prefrontal cortex following exposure to cocaine, morphine, Δ 9-THC and ethanol (Perrotti et al., 2008) was increased, albeit to a lesser extent than what was found in the NAcc. FosB/ Δ FosB was reliably expressed in all three structures of the mPFC as well as the NAcc, and caudate putamen following repeated exposure to morphine (Kaplan, Leite-Morris, Fan, Young & Guy, 2011). Furthermore, increases in AP-1 binding following cocaine and nicotine self-administration were found across the mPFC (Pich et al., 1997), which the authors suggested was a product of increased Δ FosB expression.

There was preferential induction of Δ FosB within the NAcc following cocaine administration (Larson et al., 2010; Perrotti et al., 2008) but in this study the induction was not specific to self-administered cocaine, as increased expression was similarly produced in rats that

received non-contingent cocaine administration. Differences in expression in the orbitofrontal cortex but not the prefrontal cortex, was observed following cocaine exposure delivered contingently and non-contingently (Winstanley et al., 2007). Thus, Δ FosB induction within the NAcc appears to be a direct pharmacological action of cocaine as no specific requirement for contingency was reported. In the orbitofrontal cortex, Δ FosB induction appears to be related to the volitional intake of cocaine. This implies a role of Δ FosB expression within the orbitofrontal cortex in the motivational states associated with cocaine self-administration.

Currently, a scarcity of literature on the effects of MDMA and Δ FosB expression exists. MDMA as well as other psychostimulants invoked FosB/ Δ FosB within the tail of the ventral tegmental area (Kaufling et al., 2010). These transcription factors were not, however, induced following administration of other drugs that were not psychostimulants. This suggests that MDMA produces FosB/ Δ FosB expression in patterns consistent with other psychostimulants such as cocaine and amphetamine.

Current study

The current study aims to examine the regional specificity of MDMA induced Δ FosB expression. By comparing expression in tissue from rats that self-administered MDMA with tissue from rats that received saline infusions, it is expected that MDMA will induce similar patterns of Δ FosB expression induced by other psychostimulants such as cocaine.

It is hypothesised that MDMA self-administration will increase expression of Δ FosB within the nucleus accumbens. After repeated exposure, MDMA becomes reliant upon DAergic mechanisms (Schenk, Gittings & Colussi-Mas, 2011; Schenk, 2011). DAergic transmission within the NAcc is understood to underlie the reinforcing value of drugs (Di Chiara et al., 2004; Di Chiara & Imperato, 1988; Nestler, 2005). Thus, neuroadaptations within the NAcc may contribute to the abuse liability of MDMA. DAergic transmission in the NAcc in response to MDMA occurs preferentially in the shell, relative to the core (Cadoni et al., 2005), therefore, it is expected MDMA

induced Δ FosB expression will similarly show a preference for the NAcc shell.

Previous research has reported induction of Δ FosB in the caudate putamen following exposure to drugs of abuse (Kaplan, Leite-Morris, Fan, Young & Guy, 2011; Larson et al., 2010; Perrotti et al., 2008). The caudate putamen has been implicated in mediating drug-seeking behaviours (Epstein, Preston, Stewart & Shaham, 2006; Gabriele & See, 2011; Vanderschuren, Di Ciano & Everitt, 2005), which is controlled similarly by DAergic mechanisms for both MDMA (Colussi-Mas, Wise, Howard & Schenk, 2010; Nawata, Kitaichi, Yamamoto, 2016; Schenk et al., 2016; Schenk, Gittings & Colussi-Mas, 2011) and cocaine (Phillips et al., 2003). Thus, as MDMA and cocaine self-administration behaviours are under control of similar mechanisms within the caudate putamen, it is expected that MDMA will show a similar increased expression of Δ FosB here. Previous studies that have reported Δ FosB expression have not separated the caudate putamen into sub-regions as the current study has done. Therefore, local specific predictions of MDMA-induced Δ FosB expression within the caudate putamen cannot be made without resort to speculation.

Expression of Δ FosB in the PFC has been reported following chronic exposure to drugs of abuse (Perrotti et al., 2008). Within the mPFC chronic cocaine and nicotine exposure increases AP-1 binding (Pich et al., 1997), which is thought to be representative of increased Δ FosB expression. Structures of the mPFC have been implicated in cocaine-induced drug-seeking (LaLumiere, Niehoff & Kalivas, 2010; Park et al., 2002; Peters, LaLumiere & Kalivas, 2008; Stefanik et al., 2012). Considering other similarities between neurological control of self-administration behaviours between MDMA and cocaine, it is expected that neuroadaptations will be similar. Thus, it is expected that MDMA self-administration will increase Δ FosB expression across all three structures of the mPFC.

Method

Subjects

Male Sprague-Dawley rats ($n = 36$) bred in the vivarium at Victoria University of Wellington, New Zealand were used. Rats were housed in hanging polycarbonate boxes in groups of four until reaching weights of 280-300g. They were then separated and housed individually. The animal colony was temperature- (19-21°) and humidity- (55%) controlled and maintained on a 12h hour light/dark cycle (lights on at 0700). All experimental procedures were conducted during the light cycle. Outside of testing hours food and water was available *ad libitum*. The Animal Ethics Committee of Victoria University of Wellington approved all experiments.

Surgery

Deep anaesthesia was produced via administration (i.p) of ketamine (90 mg/kg, PhoenixPharm) and xylazine (9 mg/kg, ProVet) solution. This was followed by an injection (s.c) of an anti-inflammatory analgesic agent (Carprofen® 5.0 mg/kg, Pfizer Animal Health). The scalp and right side of the upper torso were shaved using electric clippers and swabbed with ethanol (70%) and vetadine successively. An eye lubricant (Lacrilube) was applied to prevent corneal desiccation.

An incision along the midline of the scalp was made and surrounding tissue was cleared to expose the skull. The pulse of the right external jugular vein was located and an incision was made. The jugular vein was isolated and surrounding tissue cleared. The anterior end of the jugular was then tied off with a length of surgical suture. Posterior to this knot, an incision into the jugular was made, and a silastic catheter was inserted and secured with knots of sterile surgical suture. The distal end of the catheter was attached to a length of 22 gauge stainless steel tubing and threaded s.c past the scapula and through to the incision made on the scalp. Four jeweller's screws were set into the skull and embedded with dental cement in which the steel tubing was set. Electrolyte solutions (5 mL, s.c, Hartmann's solution) were administered following the conclusion of surgical procedures. Two days post-surgery, rats were administered carprofen and the catheters were flushed

with 0.2 mL of saline, heparin (30 IU/mL) and penicillin (250,000 IU/mL) solution. Rats were weighed each day and were subject to testing procedures once pre-surgery weight was reached (between 5-7 days). Weekly catheter patency tests were conducted via drawing blood, or if this failed, immediate observance of a loss of the righting reflex following administration of sodium pentobarbital (20 mg/kg, i.v).

Apparatus

The room in which self-administration testing was conducted was both temperature (19-21°C) and humidity (55%) controlled. Testing was conducted in operant chambers (Medical Associates, ENV-001) consisting of 2 levers and a stimulus light. Tests were conducted on triads of rats consisting of a master rat and 2 yoked rats, one receiving yoked MDMA infusions and one receiving yoked saline infusions.

Figure 1. Organisation of yoked self-administration paradigm in the current study. The master rat (left) self-administers MDMA via operant responding. The yoked rat (centre) receives MDMA infusions contingent to the master rat's drug infusions. The yoked control (right) receives saline infusions contingent to the master rat's infusions. A computer controls drug delivery and allows the simultaneous delivery of respective infusions via 20 mL syringes housed in mechanical pumps (Med Associates Inc, USA; model – PHM-100A). Image adapted from Haracz, Mash &

Sircar (1999).

For some rats, depression of the active, right lever resulted in a 12 second infusion (0.1 mL) of MDMA and concurrent activation of the stimulus light. Depression of the inactive left lever produced no response. The other rats were yoked to the master rat and received infusions of either MDMA or saline with the associated light stimulus contingent on the behaviour of the master rat. Drug delivery data were controlled via Med Associates software (USA).

Microscopy work was conducted with an Olympus BX-51 microscope and images were captured at x10 magnification using an MBF Biosciences camera (CX 9000) controlled remotely via a joystick and viewed using Picture Frame software (version 2.3), installed on a computer running Windows XP. In order to contain all regions of interest within a single image, individual images were stitched together using FIJI stitching tool (Preibisch, Saalfeld, & Tomancak, 2009) to acquire high-resolution images. With reference to Paxinos and Watson's (2005) rat brain atlas, templates for each region of interest were overlaid over each image. The regions selected were three regions in the mPFC; cingulate, prelimbic and infralimbic, the two subdivisions of the NAcc; shell and core and four subdivisions of the caudate putamen; ventral lateral, ventral medial, dorsal lateral and dorsal medial.

Procedure

Each day prior to testing, rats were weighed and catheters were flushed with 0.2 mL of heparin/penicillin solution. They were then placed into operant chambers and the 22-gauge stainless steel end of the catheter was connected to the 20 mL syringe housed in the infusion pump via a length of tubing protected by a spring. Self-administration testing was conducted in daily 2 hour sessions, 6 days per week. Every session commenced with an experimenter-delivered infusion in order to clear the catheter of the penicillin/heparin solution. Thereafter, depression of the active lever by the master rat was reinforced by an infusion of MDMA (1.0 mg/kg) to both the master and yoked rats and an infusion of heparinised saline to the control rat. Testing continued until a total

intake of 90.0 mg/kg had been administered or upon reaching 25 total days of testing. On average, 14.5 ($SD = 7.15$) days were required to reach this criterion. Five rats failed to self-administer 90.0 mg/kg within this 25 day period and were omitted from further testing along with their yoked-mates ($n = 15$). The dosage of MDMA was then reduced to 0.5 mg/kg and testing continued until a further 150 infusions were self-administered.

When operant responding was stable and showed less than 20% variability over a 3-day period, the schedule of reinforcement was changed to FR2 (fixed-ratio 2), meaning two depressions of the active lever were required to receive an infusion. Upon the FR2 schedule, responses were monitored until stable responding occurred (less than 20% variability for 3 consecutive days), and then the FR schedule was increased to FR5 until a total of 350 mg/kg of MDMA had been self-administered. Of the 7 master rats that reached the 90 mg/kg criterion for acquisition, one did not reach the 350 mg/kg criterion due to poor responding. The remaining 6 subjects took on average 20.83 ($SD = 8.59$) days to meet the 350 mg/kg criterion and complete testing. *Perfusion.*

Two days following the final self-administration session, deep anaesthesia was produced by administration (i.v) of sodium pentobarbital (50 mg/kg, i.p). Rats were then perfused transcardially with 0.1% heparinised saline followed by 200 mL of paraformaldehyde solution (PFA) in 0.1M phosphate buffer (PB, pH 7.2). All perfusions were conducted via perfusion pump (EYLA microtube pump MP-3, Tokyo Rikakikai Co., LTD Tokyo, Japan) at a speed of 1450 mL/hr (or 24.16 mL/min). Brains were extracted and immersed in baths of 4% PFA overnight. The following day, brains were then cryoprotected in 20% glycerol containing 0.1 M PB and 0.05% sodium azide at 4°C, before being frozen over a 4-minute period in isopentane at -40°C and then stored at -80°C. *Slicing.*

A sliding microtome (Microm HM 450) was used to slice 35 μ m thick coronal sections. Slices were made upon a stage connected to a freezing unit (Microm KS 34, Microm International GmbH part of Thermo Fisher Scientific, Auckland, New Zealand) set at -40°C. Sliced tissue was

immediately stored in 0.1 M phosphate buffered saline (PBS) with 0.05% of sodium azide at 4°C until immunohistochemical analysis began.

Immunohistochemical Analysis: Δ FosB.

All washes and incubations during immunohistochemical analyses were conducted at room temperature (20-22°C) on a gently rocking platform to produce mild agitation. For the revelation of Δ FosB positive cells, free-floating sections were first washed in three consecutive PBST (10 mM PB pH 7.4 + 0.9% NaCl + 0.3% Triton X-100) baths, each for 5 minutes. PBST was stored at room temperature (20-20°C). Slices were then incubated in 3% H₂O₂ solution (100 uL 30% H₂O₂ + 900 uL dH₂O per mL of solution) for 10 minutes in order to quench endogenous peroxidase that may have been present in the tissue. Another triple (3 x 5 minute) wash in PBST occurred before slices were incubated for 1 hour in bovine serum albumin (BSA) solution (1% BSA per 1 mL PBST) as a blocking step to reduce background staining. Again, a triple was in PBST occurred before slices were incubated overnight with the primary rabbit antibody (diluted 1/2000 in PBST + 1% BSA: 0.5 uL anti- Δ FosB antibody, stored at 4°C)(Santa Cruz Biotechnology Inc, Dallas, Texas, USA). The following morning, the slices were removed from the primary antibody and washed 3 x 5 minutes in PBST solution before being incubated for 120 minutes in the secondary biotinylated goat anti-rabbit antibody (stored at 4°C, diluted 1/1000 in PBST: 1uL secondary Ab per 1 mL of PBST)(Vector Laboratories, Burlingame, California, USA). Whilst incubating in the secondary antibody, the avidin-biotin complex (ABC) peroxidase (Vector Laboratories, Burlingame, California, USA) was made in order to ensure that compounds were properly dissolved before use. Following incubation in the secondary antibody, slices were washed 3 x 5 minutes in PBST before being incubated for 60 minutes in the preformed ABC peroxidase complex. Another 3 x 5 minute wash in PBST occurred before slices were incubated for 10 minutes in diaminobenzidine (DAB) + nickel (NiCl₂)(For 2 mL of solution: 100uL DAB (4 mg/mL, -20C), 200uL NiCl₂ (8 mg/mL, -20°C), 20 uL H₂O₂ 0.3% (10 uL H₂O₂, in 900 uL Tris HCl buffer 50 mM), 1680 uL Tris HCL buffer 50 mM pH 7.4). Finally,

slices were washed 2 x 5 minutes in PBST before being stored in PBST at 4°C until sections were mounted on gelatinised slides. After allowing mounted slices to dry, counter staining commenced in which slices were stained with neutral red, rinsed in dH₂O and then dehydrated in successive baths of EtOH (70%, 95% x 2, 100% x 2) and then rinsed in consecutive baths of histoclear before D-PX was applied and a coverslip added. A negative control was included in which the primary antibody was omitted to ensure that any non-specific staining was not present.

Cell Counts and Analysis of Immunohistochemical Data.

Cells with dark spherical nuclei were regarded as Δ FosB positive cells and were counted manually within FIJI, using the cell counter plugin. Individual counts for each unilateral region were made and recorded. Densities for each region were then calculated as positive Δ FosB cells/mm². Averages for each region were then calculated for each rat.

Figure 2. A schematic representation of the template used to quantify Δ FosB positive cells.

Adapted from Paxinos and Watson (2005). 1. Cingulate; 2. Prelimbic; 3. Infralimbic; 4.

Dorsolateral caudate putamen; 5. Dorsomedial caudate putamen; 6. Ventrolateral caudate putamen;

7. Ventromedial caudate putamen; 8. Nucleus accumbens core; 9. Nucleus accumbens shell. The B

value refers to the distance of the given slice from the bregma of the skull.

Drugs

MDMA-HCl (ESR, Porirua, New Zealand) used for self-administration testing was dissolved into a sterile heparinised saline solution (3 IU heparin, 0.9% NaCl). All drug doses were calculated based upon salt weights.

Data analysis

Data were analysed using SPSS (SPSS Inc.; version 22). An $\alpha = .05$ was utilised for all analyses. To examine the effect of condition of lever responding a 2 (group) x 2 (lever) repeated measures analysis of variance (ANOVA) was used. Condition (MDMA, saline) was the between subjects factor and lever (active, inactive) was the within subjects factor.

To determine whether or not differences in Δ FosB expression were significantly different between MDMA and saline subjects, individual t-tests were conducted to compare Δ FosB expression between MDMA and saline subjects for each region.

Finally, correlations between Δ FosB densities and average active lever responses across the final 5 days of testing were computed using Pearson's correlation. For each subject, densities of Δ FosB positive cells were plotted against each subject's average active lever responses in the final 5 days of testing.

Results

Part 1: Self-Administration

Unfortunately, the tissue quality of many sections from the yoked-MDMA rats was compromised and therefore the data were omitted from further analysis. Additionally two control subjects and one control subject were omitted from analysis in the PFC and striatum, respectively, due to poor tissue quality. Four data points were omitted on the basis of significant identification via Grubbs test for statistical outliers (Grubbs, 1979) at $\alpha = .05$. These were data from the ventrolateral region of the caudate putamen of 1 MDMA rat and the ventrolateral, ventromedial and

dorsolateral regions of the caudate putamen of 1 saline rat.

The effect of self-administration condition (MDMA or saline) on lever responding (active, inactive) in the final 5 days of testing is shown in Figure 3. An ANOVA showed a significant interaction between level and condition ($F(1,9) = 21.38, p < .05$). Furthermore, main effects of both lever ($F(1,9) = 24.57, p < .05$) and self-administration condition ($F(1,9) = 21.42, p < .05$) were revealed. Post-hoc analyses demonstrated a significant preference for the active lever in the MDMA condition ($p < 0.05$), but not in the control condition (*ns*).

Figure 3. The effect of self-administration condition on lever responding. Each bar represents the average number of lever presses across the final 5 days of testing. (+SEM). *= $p < 0.05$.

Part 2: Δ FosB expression within the striatum and mPFC

Figure 4 shows the effect of MDMA self-administration on Δ FosB expression within the subregions of the medial prefrontal cortex; the cingulate, prelimbic and infralimbic cortices.

Figure 4. Quantification of Δ FosB density in subregions of the medial prefrontal cortex across self-administration group. Data are expressed as the mean number of Δ FosB positive cells per mm² of each structure (+SEM).

Differences in Δ FosB expression in the cingulate ($t(7) = 1.23$, *ns*) and prelimbic cortex ($t(7) = 1.30$, *ns*) were not significant. Differences in Δ FosB expression in the infralimbic cortex were also not significant ($t(7) = 1.95$, $p = .093$), but they approached significance.

Figure 5 shows the effect of self-administration on Δ FosB expression within the core and shell of the nucleus accumbens.

Figure 5. Quantification of Δ FosB density in subregions of the nucleus accumbens across self-administration group. Data are expressed as the mean number of Δ FosB positive cells per mm² of each structure (+SEM).

MDMA self-administration did not alter Δ FosB expression in either the shell ($t(9)=1.90$, $p = .089$) or the core ($t(9)=1.57$, ns). However, differences in Δ FosB expression in the NAcc shell approached significance.

Figure 6 shows the effect of self-administration on Δ FosB expression within the dorsomedial, dorsolateral, ventromedial and ventrolateral partitions of the dorsal striatum.

Figure 6. Quantification of Δ FosB density in subregions of the caudate putamen across self-administration group. Data are expressed as the mean number of Δ FosB positive cells per mm² of each structure (+SEM).

MDMA self-administration failed to alter expression in the dorsomedial portion ($t(9) = 1.23$, *ns*). There was also a failure to alter expression in the dorsolateral portion, ($t(5.14) = 2.47$, $p = .055$), but the increased expression approached significance. MDMA self-administration increased expression in the ventromedial ($t(8) = 3.355$, $p < .05$) and ventrolateral ($t(7) = 2.45$, $p < .05$) subdivisions of the caudate putamen.

Figure 7. Effect of self-administration condition on Δ FosB expression in the prelimbic cortex. (A) Microscopy image of a coronal section of a rat brain after immunohistological staining. (B-C) 20x magnification of boxed area depicted in (A). (B) MDMA, (C) saline.

Part 3: Behavioural Correlates of Δ FosB

Figure 8 shows the significant positive correlation between Δ FosB density in the ventromedial caudate nucleus and average active lever presses across the final 5 days of testing ($r = .720, n = 10, p < .05$).

Figure 8. Correlation between Δ FosB densities in the ventromedial caudate nucleus and average active lever presses across the final 5 days of testing. Got to put in the actual data, probably onto the graph.

There were no other significant correlations between Δ FosB density and average lever presses across the final 5 days of testing were found in any of the other regions analysed.

Discussion

The primary objective of the current study was to assess whether Δ FosB expression in the mPFC, caudate putamen and nucleus accumbens differed between rats self-administering MDMA and those receiving non-contingent saline infusions. The secondary objective was to assess the behavioural correlates of this Δ FosB induction, by correlating the final 5 days of self-administration with Δ FosB expression.

There was no change in Δ FosB expression in the cingulate, prelimbic or infralimbic cortices as a function of MDMA self-administration. The MDMA-produced increase in expression in the infralimbic cortex, however, was approaching significance. Previously, other studies have reported induction of Δ FosB within the PFC in response to cocaine and ethanol self-administration and morphine and THC exposure (Perrotti, et al., 2008; Pich et al., 1997). Pich and colleagues (1997) reported increased AP-1 binding in the mPFC following cocaine and nicotine exposure, which may be reflective of increased Δ FosB expression within these subregions. Following repeated experimenter-administered morphine there was a robust induction of FosB/ Δ FosB expression in all three subregions of the mPFC (Kaplan, Leite-Morris, Fan, Young & Guy, 2011). This might reflect effects of experiment- versus self-administered morphine, but it is also possible that exposure to MDMA produces a different profile of FosB/ Δ FosB expression.

There were no significant increase in Δ FosB expression in either component of the nucleus accumbens following MDMA self-administration but the increase in expression in the shell approached significance. Previous research (Rodd-Henricks, McKinzie, Li, Murphy & McBride, 2002) has indicated opposing roles of the shell and core in cocaine self-administration. This might reflect the finding that MDMA-induced DA release occurs preferentially in the shell of the accumbens (Cadoni, Solinas, Pisanu, Zernig, Acquas & Di Chiara, 2005). Previous studies have consistently reported increased expression of Δ FosB in the NAcc following exposure to cocaine (Nye, Hope, Kelz, Iadarola & Nestler, 1995; Perrotti et al., 2008; Pich et al., 1997;), methamphetamine (Cornish, Hunt, Robins & McGregor, 2012), morphine (Kaplan et al., 2011; Nye & Nestler, 1996), nicotine (Pich et al., 1997) and phencyclidine (Robbins, Everitt & Nutt, 2010). These increases were produced to a similar degree in both subregions of the NAcc for cocaine and morphine (Perrotti et al., 2008), whereas, Δ^9 -THC and ethanol both showed a preference for induction in the core, relative to the shell. The findings following MDMA self-administration might reflect a unique pattern of induction.

Finally, within the caudate putamen, expression of Δ FosB differed between MDMA and saline exposed subjects, with a greater expression found for the MDMA group in both the ventromedial and ventrolateral subdivisions. Increased expression in the dorsolateral portion of the caudate putamen approached significance. Few studies have subdivided the caudate putamen as the current study has done, however, previous research has reported increased Δ FosB expression in the dorsal striatum following exposure to amphetamine, cocaine, ethanol, Δ^9 -THC and morphine (Ehrlich, Sommer, Canas & Unterwald, 2002; Perrotti et al., 2008). Therefore, within the caudate putamen, Δ FosB expression in response to MDMA self-administration appears to bear some resemblance to induction by other drugs of abuse.

Taken together, these data suggest that; similar to other widely researched drugs of abuse, chronic MDMA exposure induces expression of Δ FosB in striatal regions. As important as these similarities are, other studies have noted that drug-specific patterns of Δ FosB expression are found depending on the drug (Perrotti, 2008). While the current study is the first of its kind to divide structures into their various subregions, Δ FosB expression in response to MDMA exposure also appears to demonstrate a unique pattern of brain activation.

A significant positive correlation was found between Δ FosB expression within the ventromedial caudate putamen and cumulative self-administration responding across the final 5 days of testing. Despite other increases that were significant, or approached significance, in other brain regions, no other correlations were found. Thus, it may be inferred from this data that the amount of MDMA self-administered is reflected in Δ FosB expression within the ventromedial division of the caudate putamen.

Previous studies have reported the caudate putamen as an important locus in mediating drug-seeking (Epstein, Preston, Stewart & Shaham, 2006; Gabriele & See, 2011; Vanderschuren, Di Ciano & Everitt, 2005). Rats in the current study underwent similar regimes of MDMA self-administration that produced drug-seeking responses in previous literature (Schenk, Gittings &

Colussi-Mas, 2011). Schenk and colleagues (2011) noted that this drug-seeking response was mediated by DAergic mechanisms. After repeated exposure to MDMA, the DAergic component became sensitized (Schenk, Gittings & Colussi-Mas, 2011). Alterations in DAergic transmission have been related to addicted states and have been suggested as mediated as least in part via alterations in genetic transcription (Nestler, 2008). Thus, it may be that transcriptional changes invoked by MDMA-induced Δ FosB expression within the ventromedial portion of the caudate putamen may contribute to the development of drug-seeking responses. To test this hypothesis, administration of Δ c-Jun the dominant negative antagonist of Δ FosB (Peakman et al., 2003) directly into the ventromedial caudate putamen could be conducted in order to block the products of Δ FosB expression within this region. If the proposed hypothesis were supported, it would be expected that Δ c-Jun treatment would attenuate the magnitude of an MDMA-seeking response.

In mice, overexpression of Δ FosB expression in striatal neurons increased sensitivity to cocaine self-administration (Colby, Whisler, Steffen, Nestler & Self, 2003). Mice overexpressing Δ FosB will administer and maintain self-administration of lower doses of cocaine, relative to controls. However, at higher doses, no differences are found. Operant responding reinforced by food was not altered between groups, indicating this effect was not simply due to changes in instrumental learning. Furthermore, breakpoints under progressive ratio schedules were increased, indicating an effect of increased motivation to obtain cocaine reinforcement. Therefore, the authors concluded that their results appear to indicate that Δ FosB overexpression sensitizes mice to the incentive properties of cocaine self-administration.

As the current study has demonstrated, chronic MDMA self-administration produces increases in Δ FosB expression within subregions of the caudate putamen. Thus it may be that changes induced by Δ FosB expression facilitate the acquisition and maintenance of MDMA self-administration through potentiating the sensitivity to the incentive value of MDMA. A replication of Colby and colleagues (2003) study, replacing MDMA for cocaine could test the hypothesis of

whether or not Δ FosB potentiates the incentive value of MDMA. An additional way to test whether or not Δ FosB expression alters MDMA self-administration is through blocking the effect. Pre-treating subjects with Δ c-Jun, the effects of drug-induced Δ FosB could be diminished. Comparing MDMA self-administration dose-response curves between mice pre-treated with Δ c-Jun, mutant mice that overexpress Δ FosB and controls it could be determined if Δ FosB expression influences the reinforcing value of MDMA.

The induction and accumulation of Δ FosB induced by chronic exposure to drugs of abuse is thought to involve a sequence of neural events which is initiated by elevations in DA in the nucleus accumbens, producing activation of DA D¹ receptor - cAMP signalling pathways (Nye, Hope, Kelz, Iadarola & Nestler, 1995). The expression of Δ FosB in these DA neurons alters transcription of a number of genetic products that contain AP-1 binding sites. One of these products of AP-1 transcription, Cyclin-dependent kinase (CDK-5) is induced via Δ FosB accumulation, chronic cocaine exposure (Bibb, Chen, Taylor, Svenningsson, Nishi et al., 2001; McClung, Uler, Perrotti, Zachariou, Berton & Nestler, 2004) and high-dose experimenter administered MDMA (Busceti, 2008). CDK-5 converts dopamine-regulated phosphoprotein 32 (DARPP-32) into an inhibitor of cAMP-dependent protein kinase, thus Δ FosB-induced CDK-5 may act as a negative feedback pathway as both cocaine and morphine increase cAMP-dependent protein kinase in accumbal regions (Terwilliger, Beitner-Johnson, Sevarino, Crain & Nestler, 1991)

Previous research has identified that inhibition of accumbal cAMP-dependent protein kinase potentiates cocaine-seeking (Self, Genova, Hope, Barnhart, Spencer & Nestler, 1998). Therefore, chronic exposure to drugs and the subsequent induction of Δ FosB, producing CDK-5 expression, resulting in an inhibition of cAMP signalling presents a mechanism which Δ FosB can potentiate the sensitivity towards drugs of abuse. Considering that MDMA induces CDK-5 (Busceti et al., 2008) and produces elevations in both NAcc DAergic transmission (Cadoni et al., 2005) and Δ FosB

expression, it may be that CDK-5 presents a mechanism, which potentiates sensitivity to the reinforcing effects of MDMA.

Another potential mechanism through which Δ FosB sensitises responses to cocaine is through alterations in GluR2 (Kelz et al., 1999). These glutamate subunits contain AP-1 binding sites (Brené, Messer, Okado, Heninmann, Nestler, 1997) and provide an important function in mediating activity of neurons in the NAcc (Pennartz, Groenewege, & Lopes da Silva, 1994). Augmented AMPA function has been suggested as a mechanism that underlies sensitisation towards drugs of abuse (Nagayasu, Kitaichi, Shirakawa, Nakagawa & Kaneko, 2010; Wolf & Ferrario, 2010). In the accumbens core, AMPA antagonism reduces operant responding for cocaine, while antagonism of NDMA receptors produces no effect (Di Ciano & Everitt, 2001; Suto, Ecke & Wise, 2009). AMPA receptor antagonists administered concurrently with either cocaine or amphetamine prevents the development of sensitisation (Karler, Calder & Turkanis, 1991; Li et al., 1997). Similarly, AMPA antagonism prevents D1 supersensitivity development in the NAcc by cocaine (Li et al., 1999). Overexpression of Δ FosB in mutant mice, significantly increased concentrations of GluR2 in the NAcc (Kelz et al., 1999). Therefore, increases in GluR2 transcription provide a direct mechanism by which the sensitivity to drugs of abuse can be altered by Δ FosB expression.

Considering that the current study found differences in Δ FosB expression in the NAcc shell that were approaching significance, GluR2 transcription may play a role in sensitising responses to MDMA. Additionally, MDMA preferentially increases DAergic transmission in the shell, relative to the core (Cadoni et al., 2005) and this effect is thought to underlie the reinforcing value of a substance (Di Chiara, 1999). Therefore, it may be that Δ FosB increases the sensitivity to the reinforcing value of MDMA through alterations in transcription of glutamate receptors within the NAcc shell.

Another member of the fos family of proteins, c-fos, can be utilised as a marker to map neuronal activation (Hoffman, Smith & Verbalis, 1993). While acute exposure to MDMA increases expression of c-fos across a range of neural structures (Colussi-Mas & Schenk, 2008; Dragunow, Logan & Lavery, 1991; Hargreaves, Hunt, Cornish & McGregor, 2007; Hashimoto, Tomitaka, Narita, Minabe & Iyo 1997; Ross, Herin, Frankel, Thomas & Cunningham, 2006), repeated exposure attenuates this effect (Colussi-Mas & Schenk, 2008). A recent study by Renthal and colleagues (2008) found evidence to indicate that Δ FosB down-regulates transcriptional activity of c-fos. However, as no study concerning MDMA-induced Δ FosB expression has been conducted to date, the mechanism underlying attenuation of MDMA induced c-fos expression has been unknown. As the current study reported evidence of MDMA-induced Δ FosB expression, this presents a possible mechanism that underlies the attenuation of MDMA-induced c-fos expression.

A potential pharmacodynamic explanation for the lack of significant differences found within some of the regions analysed could be an effect of Δ FosB tolerance. Larson and colleagues (2010) gave evidence of tolerance to cocaine-induced Δ FosB expression in both subregions of the NAcc as well as the dorsal striatum in rats trained to self-administer cocaine. Similarly, De Pauli and colleagues (2014) hypothesized that their lack of differences in Δ FosB concentrations between subjects given experimenter-administered injections of ethanol and control subjects was due to an effect of Δ FosB tolerance.

De Pauli and colleagues' (2014) hypothesis was formulated on the basis that their period of drug-administration was a total of 21 days, while Larson and colleague's study which evidence of tolerance was only a period of 18 days. Within the current study, the average period of MDMA self-administration was 38 days, more than twice that of Larson and colleague's study (2010). Given the evidence that shows tolerance to drug-induced Δ FosB (De Pauli et al., 2014; Larson et al., 2010), it would be entirely reasonable to suggest that tolerance may have played a role in some of the lack of significant differences in Δ FosB concentrations found in the current study. Furthermore, Δ FosB

concentrations have been found to be at their highest observable baseline concentrations within the NAcc and caudate putamen (Nye, Hope, Kelz, Iadarola & Nestler, 1995). Thus, tolerance to MDMA-induced Δ FosB expression may be responsible for the lack of significant differences found within the NAcc and the dorsolateral and dorsomedial subdivisions of the caudate putamen.

An obvious limitation of the current study was the small sample sizes across each group. With the length of time required to conduct behavioural testing and immunohistochemistry procedures, considered alongside the high rates of attrition during MDMA self-administration, it was unfortunately not possible to increase the sample sizes any further. Furthermore, poor quality of tissue resulted in an omission of the yoked-MDMA group, which prevented testing of whether or not MDMA induced Δ FosB was a direct pharmacological action of MDMA or a product of the motivational states induced by self-administration.

Conclusions

The primary aim of the study was to determine the regional specificity of MDMA induced Δ FosB expression and whether this induction differed relative to a rat receiving passive saline infusions. Self-administration of MDMA was found to induce Δ FosB in the ventrolateral and ventromedial subregions of the caudate putamen, when compared with rats receiving non-contingent saline infusions. Similarly, differences were approaching significance in the infralimbic cortex, NAcc shell and the dorsolateral portion of the caudate putamen. Considering the small sample sizes and high variability between subjects, these findings must be regarded as important. A significant correlation between self-administration responses and Δ FosB expression was found in the ventromedial caudate putamen, indicating this as a critical neural substrate in MDMA produced Δ FosB expression.

The current study is the first experimental research to systematically investigate the regional selectivity of MDMA-induced Δ FosB expression. These results add to the growing body of literature implicating Δ FosB in addictive behaviours. Through a more thorough understanding of

the molecular mechanisms that underlie addictive states, researchers can aid in the development of treatment options for those suffering from compulsive drug use.

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