Methylenedioxymethamphetamine (MDMA "Ecstasy"): A stressor on the immune system

Thomas J. Connor

Department of Physiology, Trinity College Institute of Neuroscience,

Trinity College, Dublin 2, Ireland

Correspondence to:

Dr. Thomas Connor

Department of Physiology,

Trinity College Institute of Neuroscience,

Trinity College,

Dublin 2,

Ireland.

Phone: +353-1-608 3095

Fax: +353-1-6793545

E-mail: connort@tcd.ie

Abstract

Drug abuse is a global problem of considerable concern to health. One such health concern stems from the fact that many drugs of abuse have immunosuppressive actions, and consequently have the potential to increase susceptibility to infectious disease. This article is focused on the impact of the amphetamine derivative methylenedioxymethamphetamine (MDMA; "Ecstasy") on immunity. Research conducted over the last five years has demonstrated that MDMA has immunosuppressive actions, in both laboratory animals and humans. Specifically, MDMA suppress neutrophil phagocytosis, suppresses production of the pro-inflammatory cytokines TNF-α and IL-1β, and increases production of an endogenous immunosuppressive cytokine IL-10, thereby promoting an immunosuppressive cytokine phenotype. MDMA also suppresses circulating lymphocyte numbers, with CD4$^+$ T-cells being particularly affected, and alters T-cell function as indicated by reduced mitogen stimulated T-cell proliferation, and a skewing of T-cell cytokine production in a Th$_2$ direction. For the most part the aforementioned effects of MDMA are not due to a direct action of the drug on immune cells, but rather due to the release of endogenous immunomodulatory substances. Consequently, the physiological mechanisms that are thought to underlie the immunosuppressive effects of MDMA will be discussed. As many of the physiological changes elicited by MDMA closely resemble those induced by acute stress, it is suggested that exposure to MDMA could be regarded as a "chemical stressor" on the immune system. Finally, the potential of MDMA-induced immunosuppression to translate into significant health risks for abusers of the drug will be discussed.

Keywords: MDMA, Drug abuse, Immunity, Cytokine, T-Cell, Macrophage, NK-cell.

Introduction

Drug abuse is a worldwide problem that has major social, health and economic implications. Over the last two decades investigators have documented the ability of a number of drugs of abuse such as cocaine, opioids, cannabinoids and amphetamines to impair many aspects of immune function, either directly, or via neuroimmune pathways [1-6]. In addition, there is substantial evidence that the stress associated with morphine and cocaine withdrawal also induces an immunosuppressive state [7,8]. Moreover, numerous reports indicate that the immunosuppression elicited by drugs of abuse can result in diminished host resistance to disease in a number of experimental models [9-12]. Indeed some investigators have implicated drug abuse as a co-factor in susceptibility to infection with HIV or other viruses [10,11,13,14]. This review focuses on the immunomodulatory capacity of methylenedioxymethamphetamine (MDMA; "Ecstasy"), an amphetamine-derivative that has become a popular drug of abuse over the last two decades [15,16].

MDMA – History and biological actions

MDMA is a ring-substituted phenylisopropylamine that is structurally related to both amphetamines and hallucinogens. The compound was initially developed as an appetite suppressant in 1914 by Merck Pharmaceuticals, but was never marketed for this purpose. It was later discovered that MDMA had psychoactive properties, and it was reported to confer benefit as an adjunct to psychotherapy, where it was shown to enhance communication and intimacy [16]. In the 1980's MDMA became a popular drug of abuse, particularly at raves and all night dance clubs. However, following an increase in the prevalence of it's abuse, and

evidence of adverse health effect's MDMA was classified as a schedule I controlled substance by the FDA in 1985.

In terms of its neurochemical mechanism of action, MDMA is a potent releaser of the indolamine neurotransmitter serotonin, and to a lesser extent the catecholamine neurotransmitter dopamine in the central nervous system (CNS) [17,18]. These neurochemical actions of MDMA result in psychoactive properties that have been studied both in animals and humans [19-21]. In humans, MDMA produces a relaxed, euphoric state, including emotional openness, increased empathy and a decrease in inhibitions. These subjective behavioural effects elicited by MDMA have led to its widespread abuse over the last 15-20 years [see 15].
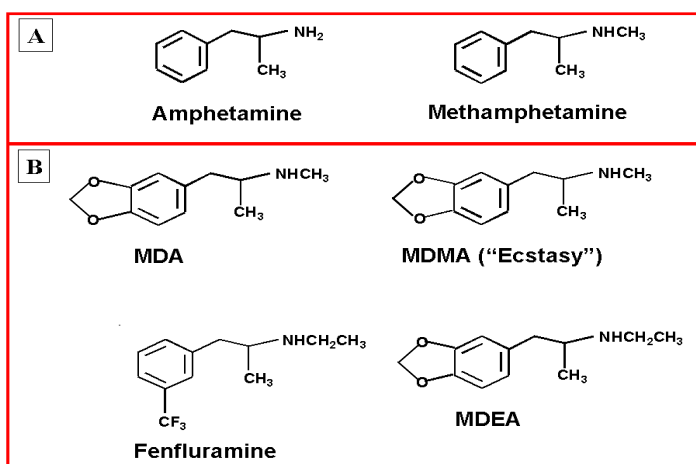
Figure 1: An illustration of the chemical structures of (A) Amphetamine and Methamphetamine, both of which stimulate catecholamine release in the CNS, and (B) MDA, MDMA, MDEA and fenfluramine; substituted amphetamines all of which are potent releasers of serotonin

In addition to the psychoactive properties of MDMA, it produces an array of physiological actions such as hyperthermia, acute sympathomimetic effects, such as increased heart rate and

blood pressure, a transient increase in anxiety, and increased activation of the hypothalamic pituitary adrenal axis as indicated by increased circulating glucocorticoid concentrations [see 15]. Experimental studies conducted in laboratory animals [22,23] and humans [21] have concluded that many of these actions of MDMA occur secondary to central release of either serotonin or dopamine.

MDMA use has been associated with a number of serious side effects, such as cardiac arrhythmias, hyperthermia, renal failure, seizures and intracranial hemorrhage [see 15,16]. In addition, to these acute toxic effects there is substantial evidence that MDMA can result in long-term neurotoxic effects on central serotonergic neurons [24,25], and that this may represent a predisposing factor to psychological disturbances/psychiatric disease [see 26].

In the last number of years it has become evident that, like other drugs of abuse, MDMA has immunosuppressive properties. A number of studies indicate that MDMA suppresses aspects of both innate and adaptive immunity in laboratory animals [27-34] and in humans [35-39]. In the remainder of this review I will discuss the evidence that MDMA impacts upon immune function, discuss the potential mechanisms by which MDMA elicits it's immunomodulatory actions, and also discuss the potential of MDMA-induced immunosuppression to translate into significant health risks for abusers.
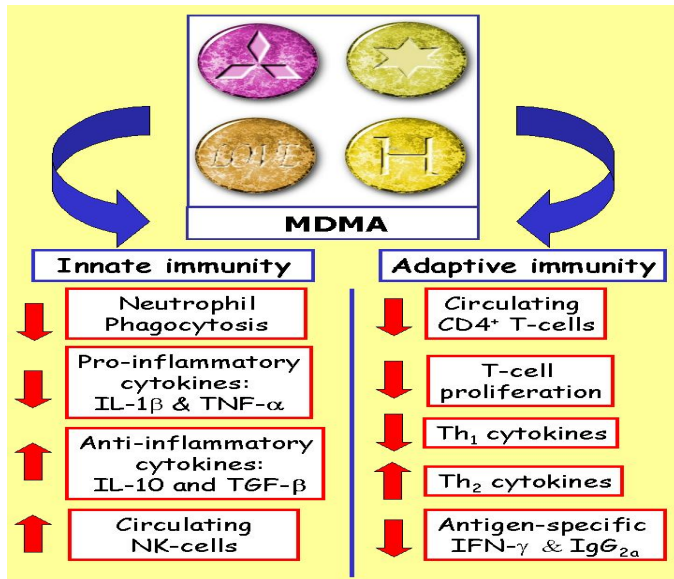
Figure 2: A diagrammatic summary of the effect of *in vivo* administration of MDMA on aspects of innate and adaptive immunity.

Table 1: A summary of the studies conducted concerning the impact of MDMA on the immune system

Parameter	Source	Stimulant	MDMA Conc./Dose	Effect	Ref.
Neutrophil phagocytosis	Rat blood	*Zymosan*	*In vivo:* 10 mg/kg (i.p.)/ In vitro (10 µg/ml)	**Decrease**	[28]
IL-1β production	Rat blood	*LPS*	*In vivo*: 10-20 mg/kg (i.p.)	**Decrease**	[30,31]
	Rat blood	*LPS*	*In vitro*: 1-10 µg/ml	**No change**	[31]
	Human blood	PHA	*In vivo*: 100 mg (p.o)	**Decrease**	[38]
TNF-α production	Rat blood	*LPS*	*In vivo*: 5-20 mg/kg (i.p.)	**Decrease**	[30,31]
	Rat blood	*LPS*	*In vitro*: 1-10 µg/ml	**No change**	[31]
	Mouse macrophages	*LPS*	*In vitro*: 0.0001–100µM	**No change**	[50]
IL-6 production	Mouse macrophages	*LPS*	*In vitro*: 0.0001–100µM	**No change**	[50]
	Human blood	PHA	*In vivo*: 100 mg (p.o)	**Decrease**	[38]
IL-10 production	Rat blood	*LPS*	*In vivo*: 1.25-10 mg/kg (i.p.)	**Increase**	[28]
	Rat blood	*LPS*	*In vitro*: 1-10 µg/ml	**No change**	[28]
	Rat blood	*Con A*	*In vivo*: 7.5 mg/kg (i.p.)	**Decrease**	[32]
	Human blood	*PHA*	*In vivo*: 100 mg (p.o)	**Increase**	[38]
TGF-β₁ production	Human blood	PHA	*In vivo*: 100 mg (p.o)	**Increase**	[38]
IFN-γ production	Rat blood	Con A	*In vivo*: 7.5 mg/kg (i.p.)	**Decrease**	[32]
	Human blood	PHA	*In vivo*: 100 mg (p.o)	**Decrease**	[38]
	Rat splenocytes	KLH	*In vivo*: 10 mg/kg (i.p.)	**Decrease**	[29]
IL-2 production	Rat blood	Con A	*In vivo*: 7.5 mg/kg (i.p.)	**Decrease**	[32]
	Human blood	PHA	*In vivo*: 100 mg (p.o)	**Decrease**	[38]
	Mouse splenocytes	Anti-CD3	*In vitro*: 0.0001µM	**Increase**	[50]
IL-4 production	Mouse splenocytes	Anti-CD3	*In vitro*: 0.0001–100µM	**Increase/Decrease**	[50]
	Human blood	PHA	*In vivo*: 100 mg (p.o)	**Increase**	[38]
NK cell numbers	Human blood	None	*In vivo*: 75-100mg (p.o)	**Increase**	[35,37-39]
NK cell activity	Mouse splenocytes	None/IL-2	*In vitro:* 0.0001-100µM	**Increase/Decrease**	[50]
Lymphocyte numbers	Rat blood	None	*In vivo*: 1.25-40 mg/kg	**Decrease: Lymphocytes**	[32,33]
	Human blood	None	*In vivo*: 75-125 mg	**Decrease: CD4⁺ T-cells**	[35,37-39]
T-cell proliferation	Rat blood	Con A/PHA	*In vivo*: 7.5-20 mg/kg	**Decrease**	[32-34]
	Human blood	PHA	*In vivo*: 75-125 mg	**Decrease**	[39]
CTL activity	Mouse splenocytes	None	*In vitro*: 100µM	**Decrease**	[50]
B-cell proliferation	Mouse splenocytes	Anti-IgM/IL-4	*In vitro:* 0.0001-100µM	**No change**	[50]
Antibody Production	Rat blood	KLH	*In vivo*: 5-10 mg/kg (i.p.)	**IgG₂ₐ: Decrease**	[29]

**In vivo effects of MDMA in animal models**

Innate immunity

Neutrophils are a subset of phagocytic cells that play a key role in the innate immune response. Neutrophil activation that occurs following phagocytosis is accompanied by an oxidative burst that produces reactive oxygen species, and destroys bacteria and fungi. Administration of MDMA (10 mg/kg) to rats has been shown to suppress the neutrophil oxidative burst in response to opsonised zymosan (a phagocytic stimulus), but not in response to chemical stimulation of protein kinase C using the phorbol ester PMA [27]. The fact that the PMA-induced oxidative burst was unaltered, suggests that the inhibitory effect of MDMA occurs upstream of protein kinase C activation, possibly by interfering with the phagocytic process. It is suggested that MDMA may down regulate the expression of molecules such as CR3 or FcR that are required to initiate phagocytosis of opsonized zymosan, however further studies are required in order to study the effect of MDMA on the expression of such cell surface molecules.

Cells of the innate immune system such as macrophages and dendritic cells produce pro-inflammatory cytokines such as IL-1β, TNF-α and IL-12 in response to stimulation with bacterial products such as LPS. These cytokines are of strategic importance in initiating and co-ordinating a large range of immune responses against invading pathogens [see 40-43]. MDMA administration to rats impairs the ability to respond to an *in vivo* immune challenge with bacterial lipopolysaccharide (LPS). Specifically, MDMA suppresses LPS-induced IL-1β and TNF-α production [28,30,31]. The suppression of TNF-α that occurs following MDMA

administration is the more profound and persists for longer than the suppression of IL-1β [31]. Whilst the rat does not produce IL-12 in response to LPS (Connor et al., unpublished data), we have recent data demonstrating that MDMA suppresses IL-12 production in the mouse following an in vivo LPS challenge (Boyle and Connor, Unpublished observations).

In addition, to producing pro-inflammatory cytokines, cells of the innate immune system also produce interleukin-10 (IL-10), an anti-inflammatory or immunosuppressive cytokine that inhibits several macrophage functions including pro-inflammatory cytokine production in response to lipopolysaccharide [44-47]. In a recent study it was demonstrated that MDMA provokes a dose-dependent increase in IL-10 following an in vivo LPS challenge in rats. This increase in IL-10 production occurs at doses of MDMA as low as 1.25 mg/kg, and closely parallels the suppression of TNF-α observed in response to MDMA [28]. However the ability of MDMA to suppress LPS-induced TNF-α and IL-1β is still evident following immunoneutralization of IL-10, indicating that the suppression of these pro-inflammatory cytokines occurs independent of increased IL-10 production [28]. In addition to suppressing the production pro-inflammatory cytokines, IL-10 also down-regulates expression of the antigen presenting molecule MHC class II, and the co-stimulatory molecule B7 expression on antigen presenting cells, thereby inhibiting antigen presentation producing anergy in the T-cell arm of the immune response [45,46]. Studies are ongoing in order to determine if the increase in LPS-induced IL-10 production observed following MDMA administration will have a significant impact on the antigen presenting or co-stimulatory capacity of macrophages or dendritic cells.
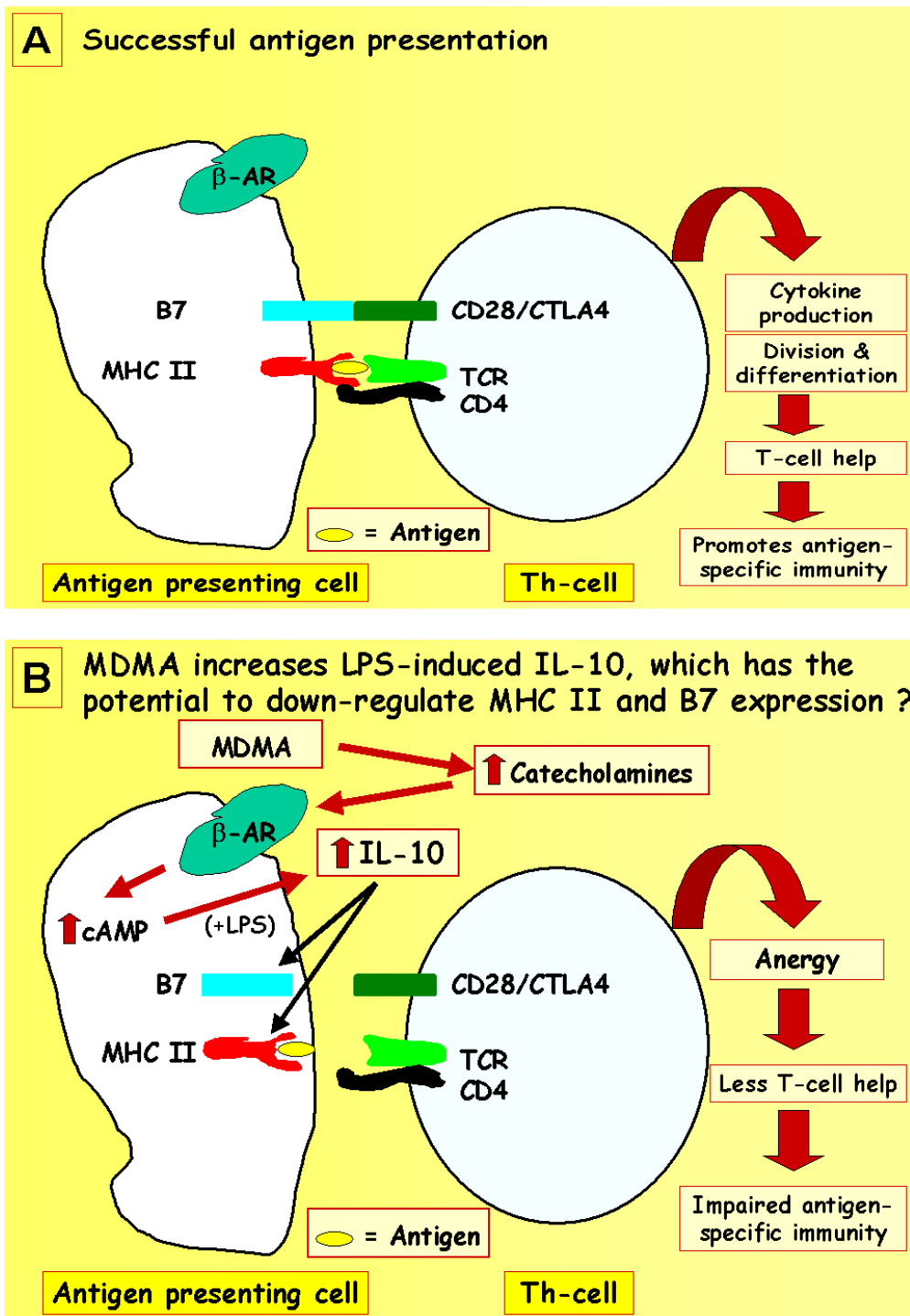
Figure 3: A schematic diagram depicting the potential of MDMA-induced increases in IL-10 production to interfere with antigen-presentation, and thereby produce anergy in the immune system

Adaptive immunity

Lymphocytes are the main subset of immune cell that mediate adaptive of specific immune responses. A number of studies indicate that MDMA suppresses lymphocyte function in rats. The first study to examine the effect of MDMA on immune function demonstrated that quite a high dose of MDMA (20 mg/kg, i.p.) produced a rapid (within 30 min) suppression of Con A-induced lymphocyte proliferation, and a profound reduction in the total leucocyte count in rats both of which persisted for at least 6 hours following injection [34]. In a subsequent study it was observed that MDMA, its major metabolite MDA, and also the related amphetamine derivative fenfluramine, suppressed circulating lymphocyte numbers, mitogen-stimulated T-cell proliferation and cytokine production, with MDA and fenfluramine being more potent than MDMA with respect to their immunosuppressive actions [32].

Whilst examining mitogen-stimulated lymphocyte responses gives a useful indication of lymphocyte function, it has the limitation that under normal physiological circumstances the immune system does not encounter mitogens, but rather encounters antigens. Consequently a study was conducted to assess the impact of MDMA administration on an antigen specific immune response to the soluble protein antigen keyhole limpet haemocyanin (KLH) in rats [29]. In this study, KLH-specific immunoglobulin production and KLH-specific cytokine production were assessed as indices of immunocompetence. MDMA did not alter the KLH-specific IgM response. In contrast, MDMA (5 and 10 mg/kg) significantly suppressed KLH-specific IgG production [29]. Therefore whilst MDMA failed to alter the initial generation of the antibody response, it profoundly inhibited antibody class switching from IgM to IgG. Two pathways for the genetic switch from IgM to IgG production were investigated. One pathway

requires the Th_1 type cytokine IFN-γ to stimulate the to IgG_{2a}-secreting cells, whilst another pathway requires the Th_2 type cytokines IL-4 and IL-6 to stimulate the switch to IgG_1-secreting cells. IgG_1 and IgG_{2a} levels were measured to determine if these two pathways were differentially affected. The results indicate that only IgG_{2a} levels were decreased following MDMA administration. Furthermore, this decrease in IgG_{2a} production was accompanied by decreased KLH-specific splenic IFN-γ production. Overall, these data indicate that MDMA alters the ability to switch from IgM to IgG_{2a} production, possibly by reducing production of the TH_1 cytokine IFN-γ. These data indicated that in addition to the ability of MDMA to suppress lymphocyte response to mitogenic stimuli, that it also suppresses the Th_1 arm of the immune response to an antigenic stimulus.

In vivo effect of MDMA in humans

Over the number of years Pacifici and co-workers have clearly demonstrated that MDMA is a potent immunomodulator in humans [35-39]. In these studies either placebo or MDMA (75-100 mg) were administered orally to recreational MDMA users in a controlled setting. The effect of ethanol consumption (0.8 mg/kg) on immune function was also assessed, as was the effect of MDMA and ethanol co-administration. These studies were conducted double blind, using a crossover (Latin Square) design. Each subject received all of the treatments in separate experimental sessions, with a 1-week washout period between each session. Blood samples were collected from the participants at a number of time intervals up to 24 hr following drug administration, or up to 48 hr after drug administration in the study that assessed the impact of repeated doses of MDMA on immune function.

The first study conducted was a pilot study using four subjects. This study demonstrated that MDMA profoundly suppressed the number of circulating $CD4^+$ T-cells, suppressed mitogen stimulated T-cell proliferation an increased circulating numbers of NK-cells [39]. Although MDMA was shown to increase circulating NK-cell numbers the activity of these NK-cells was not assessed, consequently it is difficult to predict the effect of MDMA on overall NK-cell functionality. In a follow up study the ability of MDMA to reduce the number of circulating T-cells and increase circulating NK-cells was replicated and it was also reported that MDMA and promoted a switch to a Th_2 type cytokine profile and indicated by reduced IFN-γ and IL-2 production, with an concomitant increase in the Th_2 cytokines IL-4 and IL-6, and the T-regulatory cytokines IL-10 and TGF-β_1 [38]. These immunosuppressive effects of MDMA were maximal 3-6 hours following drug administration, and in some cases were evident 24 hours later. In some instances co-administration of alcohol further enhanced the immunosuppressive effects of MDMA.

In a more recent study Pacifici and colleagues assessed cell-mediated immune responses after administration of two repeated doses of MDMA (100 mg per dose) at 4-hour and 24-hour intervals [37]. As previously reported MDMA produced a time-dependent decrease in the number of $CD4^+$ T-helper cells, a decrease in the functional responsiveness of lymphocytes to mitogenic stimulation, and a simultaneous increase in natural killer cells. In the first clinical trial two MDMA doses were given 4 hours apart, and the immune changes produced by the first dose were enhanced following administration of the second dose. Furthermore administration of two doses of MDMA 4 hours apart produced longer lasting immunological deficits that a single dose of MDMA; in that 24 hours after treatment, significant residual

effects were observed for all the altered immune parameters after the administration of two MDMA doses, when compared to a single dose. In the second clinical trial, the second MDMA dose was administered 24 hours after the first dose, and produced immunological changes significantly greater than those induced by the initial drug administration, which seemed to show a delayed onset. In addition, significant residual effects were observed for all the immune parameters as late as 48 hours after the second dose. Based on these findings it can be concluded that repeated administration of MDMA with both a short and a long time interval between doses, increases both the magnitude and duration of MDMA-induced immunosuppression.

The clinical trails that were outlined above were conducted in a controlled environment. The same research group conduced a study in a non-controlled environment where they compared baseline immunological parameters in thirty recreational MDMA users that participated in their clinical trials, with a sample of twenty-four matched control subjects [see 36]. When baseline values were compared the only significant difference between MDMA users and controls was in NK cells numbers; with NK-cell numbers in MDMA users reduced to one-third of that seen in control subjects. In another study, leucocyte subsets from six habitual MDMA users from the thirty that participated in the clinical trials were followed up for a two-year period, and compared to eight matched control subjects [see 36]. At the beginning of this study, basal levels of leucocyte subsets (Total lymphocytes, Thelper cells, Cytotoxic T-cells and B cells) were equivalent between the six control subjects and eight MDMA abusers. In contrast, NK-cell numbers were significantly decreased in MDMA users, and remained decreased over the two-year duration of the study. One year after the initial observation, the total number of lymphocytes, CD4+ T-cells, CD19+ B-lymphocytes were all significantly reduced in MDMA

users compared to control subjects. Moreover, this suppression of leucocyte subpopulations in MDMA users became even more apparent two years following the initial observation. Based on these data the authors concluded that permanent alterations in immunological parameters are evident in MDMA abusers.

Whilst these non-controlled studies are interesting, the results obtained are somewhat difficult to interpret for two reasons. Firstly, it is not possible to ascertain if the suppression of immune parameters observed in the MDMA users is due to MDMA abuse per se. For instance, it is outlined in the methods of the study that the subjects enrolled in the clinical trails were all habitual users of cannabis with prior experience of cocaine or methamphetamine [36]. As it is well established that cannabis, cocaine and methamphetamine all have significant immunomodulatory properties in their own right [1,2,5,6], it is impossible to discount the fact that they may have contributed to the immunological changes observed in this experimental group. The second issue pertains to experimental design adopted in the 2 year follow up study. In this study the control subjects were not tested at 1 and 2 year intervals, thus comparisons were being made using historical data. For these reasons one must be cautious when interpreting these results.

Mechanisms by which MDMA can alter immune function

Direct effects of MDMA on immune cells: Evidence from in vitro studies

The molecular targets for MDMA's action are the transporter (uptake) sites for serotonin and dopamine. Whilst these transporter sites are located predominantly on pre-synaptic serotonergic and dopaminergic neurons respectively, there is now ample evidence the cells of the immune system also express transporter sites for both of these neurotransmitters [see

48,49]. Consequently MDMA can interact directly with immune cells, and thereby has the potential to alter their activity directly.

The first study to examine the immunomodulatory potential of MDMA was published almost 10 years ago by House and co-workers, and examined the impact of *in vitro* exposure to MDMA (0.0001–100μM) on a number of immune parameters in splenocytes and peritoneal macrophages from B6C3F1 mice [50]. In this study T-cell function was assessed by anti-CD3-induced IL-2 and IL-4 production, B-cell function was assessed by measuring proliferation, natural immunity was assessed by measuring NK-cell cytotoxicity, T-cell effector function was evaluated as a function of cytotoxic T-lymphocyte (CTL) activity, and macrophage function was assessed by measuring production of the pro-inflammatory cytokines IL-6 and TNF. *In vitro* exposure to MDMA had no effect on B-cell proliferation. In terms of T-cell function, production of the Th_1 cytokine IL-2 was enhanced by 0.0001 μM MDMA, suppressed by 100μM MDMA, and not altered by any of the 5 intermediate concentrations. Production of the Th_2 cytokine IL-4 was not altered by exposure to any concentration of MDMA examined. Basal and IL-2 augmented NK cell cytotoxicity were enhanced at concentrations of MDMA between 0.0001 and 0.1 μM, however this effect was evident only at one of the three effector:target cell ratios employed. Conversely, IL-2 stimulated NK-cell activity was significantly suppressed by MDMA (10 μM), but again this effect was evident only at one of the three effector:target cell ratios employed in the assay. CTL induction was significantly suppressed at a concentration of 100μM, but was unaltered at any of the other concentrations used. Finally, LPS-induced macrophage IL-6 or TNF production were not significantly altered by any concentration of MDMA, however there was a slight but statistically non-significant

suppression of TNF observed at 10 and 100μM MDMA. In summary, the data generated by House and co-workers (1995) [50] indicates that *in vitro* exposure to MDMA has variable, and for the most part modest, effects on the immune system depending on the dose employed, and the specific immune parameter under investigation.

In a subsequent study Connor et al., (2000) [31] observed that *in vitro* exposure of LPS-stimulated diluted rat blood to MDMA failed to mimic its ability to suppress the pro-inflammatory cytokines IL-1β and TNF-α following an *in* vivo LPS challenge; thereby supporting *in vitro* findings of House et al., (1995) [50]. It was also reported that the ability of MDMA to increase LPS-induced IL-10 production *in vivo*, was not mimicked by *in vitro* exposure of LPS-stimulated diluted whole blood cultures to the drug [28]. In addition, the suppressive effect of MDMA on Con A-stimulated lymphocyte proliferation that is observed *in vivo* cannot be mimicked by *in vitro* exposure to MDMA (Connor, Unpublished observations). These data suggest that the potent immunosuppressive actions of MDMA observed following *in vivo* administration are not due to a direct action of the drug on immune cells, and are likely to be due to the release of endogenous immunomodulatory substances that occurs in response to MDMA. In contrast to these findings, both *in vivo* and *in vitro* exposure to MDMA elicit similar suppressive effects on the zymosan-induced oxidative burst in rat neutrophils, indicating that MDMA can elicit a direct effect on neutrophil phagocytosis [27].

Indirect mechanisms by which MDMA can impact upon the immune system

In addition to having a direct effect on immune cells, it is possible that a drug such as MDMA that alters neurotransmission within the central nervous system (CNS) could alter

immunocompetence via a CNS-mediated mechanism. Specifically, it is well established that changes in CNS neurotransmitter function can alter immunity via changes in endocrine output and sympathetic nervous system activity [51,52]. MDMA stimulates the release of the neurotransmitters serotonin and dopamine in the central nervous system [17,18], and produces consequential downstream activation of peripheral immunomodulatory pathways such as the hypothalamic pituitary adrenal axis and the sympathetic nervous system [23,53]. Therefore when addressing the underlying physiological mechanisms that mediate the immunosuppressive effects of MDMA, it is necessary to consider central neurotransmitters that drive downstream responses, and also peripheral neurotransmitters and hormones that are the ultimate mediators impacting on immune cells.

Central mediators of MDMA-induced immunosuppression

As the predominant neurochemical action of MDMA is to release serotonin within the CNS, it is logical to assume that central serotonin release may mediate the actions of MDMA on the immune system. In this regard it was demonstrated that the related amphetamine compound fenfluramine that is a selective releaser of serotonin produced qualitatively similar suppressive effects to MDMA on a number of immunological measures in a rats [32,54]. For instance fenfluramine suppressed circulating lymphocyte numbers, suppressed T-lymphocyte proliferation and cytokine production [32], and also suppressed production of the pro-inflammatory cytokines IL-1β and TNF-α in response to an in vivo LPS challenge [54]. It was also of interest that fenfluramine was more potent than MDMA at inducing serotonergic changes, and also at provoking immunosuppressive effects [32]. All of this data pointed towards serotonin release as a mediator of the immunosuppressive effects of MDMA in rats.

In order to evaluate the role of serotonin in MDMA-induced immunosuppression, two pharmacological strategies that inhibit MDMA-induced serotonin release were employed. Firstly, the selective serotonin reuptake inhibitor paroxetine was used to prevent MDMA from entering serotonergic neurons, thereby preventing MDMA-induced serotonin release [30]. In the second study rats were pre-treated with the tryptophan hydroxylase inhibitor p-chlorophenylalanine (pCPA); an agent that reduces brain serotonin concentrations by approximately 90%. The impact of both of these anti-serotonin strategies on MDMA-induced suppression of IL-1β and TNF-α in rats was evaluated. Whilst paroxetine pre-treatment completely blocked MDMA-induced serotonin depletion in both the frontal cortex and hypothalamus, it failed to alter the suppressive effects of MDMA on lipopolysaccharide-induced TNF-α secretion. It was also of interest that paroxetine treatment alone suppressed both lipopolysaccharide-induced IL-1β and TNF-α secretion by 27% and 50%, respectively, possibly by its ability to increase central serotonin concentrations [30]. The treatment regimen of pCPA used caused in excess of a 90% depletion of brain serotonin concentrations. However, the suppressive effects of MDMA on LPS-induced IL-1β and TNF-α were equivalent in both saline and pCPA-treated groups. In all these data indicated that the immunosuppressive effects of MDMA occur by a mechanism(s) independent of serotonin release. In a similar manner it was observed that the suppressive effect of fenfluramine (10 mg/kg) on LPS-induced IL-1β and TNF-α production were not blocked by pre-treatment with either paroxetine or pCPA [55]. However in a later study using much lower doses of fenfluramine (1.25 and 2.5 mg/kg), it was observed that the suppressive effect of fenfluramine on LPS-induced IL-1β production was blocked by pre-treatment with pCPA, indicating that it was indeed a serotonin-dependent event [56]. In a similar fashion it is also possible that serotonin may contribute to the

immunosuppressive effect of low doses of MDMA, and that this may be overridden by dopaminergic influences when higher doses are employed. However, further studies are necessary in order to substantiate this hypothesis. In addition is possible that some aspects of MDMA-induced immunosuppression may be serotonin dependent, whilst others may occur by a serotonin independent mechanism.

In contrast to the studies outlined above that failed to elucidate a role for serotonin in MDMA-induced immunosuppression in rats [30], a recent study in humans demonstrated that pre-treatment with the serotonin reuptake inhibitor paroxetine could block some of the immunosuppressive effects of MDMA. Specifically, paroxetine pre-treatment partially inhibited that ability of MDMA to suppress circulating CD4+ T_{helper} cell numbers, and to increase circulating NK-cell numbers [35]. In addition, paroxetine totally abolished the suppressive effect of MDMA on lymphocyte proliferation and IL-2 production induced by the T-cell mitogens Con A and PHA, and blocked the ability of MDMA to enhance PHA-stimulated production of the anti-inflammatory cytokines IL-10 and TGF-β. In all, these data support a role for serotonin release in mediating the suppressive effect of MDMA on human T-cell function.

In addition to the potent serotonin-releasing properties of MDMA, it is also well established that MDMA releases dopamine within the CNS, although with less potency [17,18]. Thus, it is possible that dopamine release may play a role in the immunosuppressive effects of MDMA. In this regard, it was previously demonstrated that both d-amphetamine and methamphetamine; two psychostimulants that are structurally related to MDMA (see Figure 1) and are potent

dopamine releasers, elicit immunosuppressive effects in rodents [6,57,58]. Future studies will evaluate the role of dopamine in MDMA-induced immunosuppression.
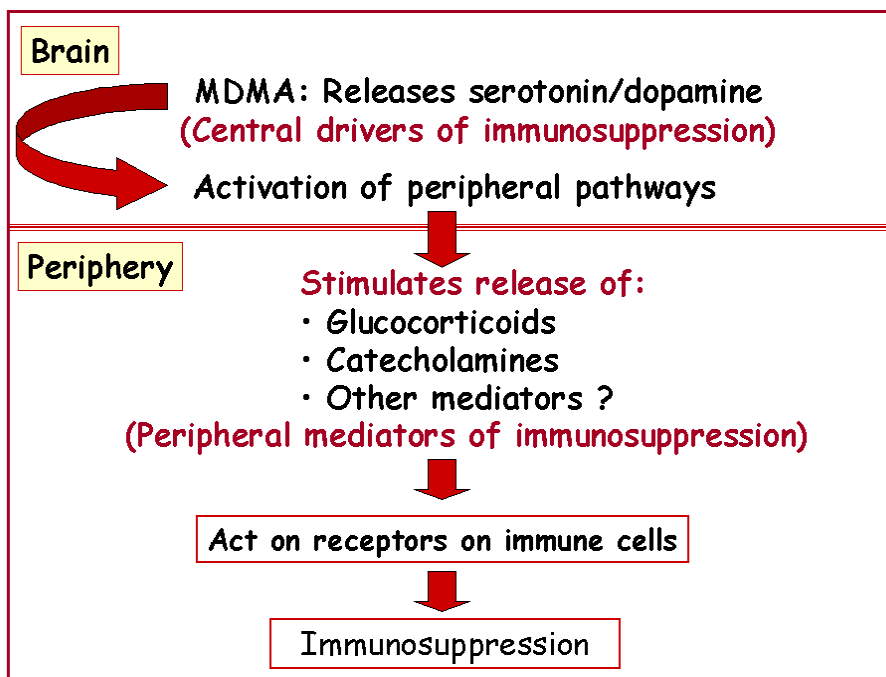
Does behavioural stimulation play a role in the immunosuppressive effect of MDMA ?

MDMA provokes a variety of euphoric effects in humans and behavioural hyperactivity in laboratory animals [see 15]. It is of interest that previous studies demonstrated that depletion of serotonin concentrations with *p*-chlorophenylalanine or blockade of serotonin release by pre-treatment with SSRI's attenuate the locomotor stimulant effect of MDMA in rats [19]. Therefore whilst pretreatment with paroxetine or *p*-chlorophenylalanine attenuates the behavioural effects of MDMA, the immunosuppressive effects (at least on pro-inflammatory cytokine production) still persist, indicating a dissociation between the behavioural and immunosuppressive effects of MDMA [30]. In addition, the fact that the non-psychostimulant amphetamine derivative fenfluramine elicits similar immunosuppressive effects to MDMA [32,54,56], supports the view that the psychoactive and immunosuppressive properties of substituted amphetamines in rats are not necessarily linked.

A previous study conduced in humans reported that treatment with the SSRI citalopram blocked the positive mood, extraversion and self-confidence induced by MDMA [21]. In addition, some of the immunosuppressive actions of MDMA in humans are blocked by serotonin transporter blockade with the related SSRI paroxetine [35]. However, despite these co-incidental effects, the exact role that the euphoric effect of MDMA plays in its ability to induce immunosuppression in humans is not clear.

Does hyperthermia play a role in the immunosuppressive effect of MDMA ?

Since MDMA is known to induce hyperthermia it is pertinent to address the possibility that the ability of MDMA to alter body temperature could contribute to its immunomodulatory effects. However, based on evidence from the published literature it appears unlikely that MDMA-induced hyperthermia plays a significant role in its immunomodulatory effects. For instance, the ability of MDMA to promote an immunosuppressive cytokine phenotype (reduced TNF-α and increased IL-10), and a suppression of lymphocyte proliferation are observed at doses as low as 1.25 mg/kg [28,33]; a dose that does not alter body temperature [59]. In addition, whilst both MDMA and the related amphetamine fenfluramine provoke similar immunosuppressive responses in rats, they provoke opposite thermal responses with fenfluramine eliciting a hypothermic response [60], and MDMA eliciting a hyperthermic response [23].

Brain

MDMA: Releases serotonin/dopamine
(Central drivers of immunosuppression)
Activation of peripheral pathways

Periphery

Stimulates release of:
· Glucocorticoids
· Catecholamines
· Other mediators ?
(Peripheral mediators of immunosuppression)

Act on receptors on immune cells

Immunosuppression

Figure 4: A schematic diagram outlining potential central and peripheral mediators of MDMA-induced immunosuppression

Peripheral mediators of MDMA-induced immunosuppression: A role for catecholamines or glucocorticoids?

It is well established that MDMA activates both the hypothalamic pituitary adrenal axis and sympathetic nervous system [23,33,53], and that the end products of these axes namely glucocorticoids and catecholamines have immunosuppressive properties [61,62]. Therefore it was plausible to suggest that MDMA could elicit its immunosuppressive actions by increasing the release of these endogenous negative immunoregulators. Consistent with this hypothesis, it was demonstrated that the increase in IL-10 induced by MDMA could be blocked by pre-treatment with the β-adrenoceptor antagonists propranolol and nadolol, indicating that the MDMA-induced enhancement of IL-10 production was mediated by β-adrenoceptor activation, presumably in response to MDMA-induced catecholamine release [28]. In contrast, the suppression of pro-inflammatory cytokines TNF-α and IL-1β induced by MDMA occurred independent of β-adrenoceptor activation [28]. However the possibility still exists that catecholamines may mediate the suppression of pro-inflammatory cytokines induced by MDMA by acting on other adrenoceptor subtypes such as the α_1-adrenoceptor.

With respect to the role of glucocorticoids in MDMA-induced immunosuppression, it was observed that pre-treatment of rats with the glucocorticoid receptor antagonist mifepristone failed to block the suppressive effect of MDMA on LPS-induced IL-1β and TNF-α production (Connor, unpublished data). Moreover in a recent study it was observed that adrenalectomy failed to block the ability of MDMA to suppress TNF-α, and increase IL-10 production [28]. These data clearly demonstrate that glucocorticoids do not mediate the ability of MDMA to promote an immunosuppressive cytokine phenotype in rats. However further studies are

required to determine if glucocorticoids play a role in the suppressive effect of MDMA on other immunological parameters such as mitogen stimulated T-cell responses, or antigen-specific T-cell responses and antibody production.

The fact that MDMA maintained the ability to increase LPS-induced IL-10 production in adrenalectomized rats indicates that this response is not mediated by adrenal catecholamines, suggesting that noradrenaline may modulate LPS-induced IL-10 production by direct innervation of immune organs such as the spleen. Studies are currently underway to investigate this possibility. In contrast to the animal studies, to date there has been a paucity of research with respect to peripheral mediators of MDMA-induced immunosuppression in humans. Whilst it has been demonstrated that MDMA activates the hypothalamic pituitary adrenal axis and sympathetic nervous system in humans [39,53], the effect of glucocoticoid receptor antagonists/synthesis inhibitors or adrenoceptor antagonists have not been examined in human studies.

A role for peripheral serotonin or dopamine in the immunosuppressive actions of MDMA ?

Serotonin

Immune cells have ample opportunity to be exposed to serotonin that is released from platelets in the periphery [see 48]. In addition to platelet-derived serotonin, it was previously suggested that sympathetic nerve terminals in lymphoid tissues take up serotonin, which can be released upon later stimulation of these nerves [49]. Moreover, it is well established that serotonin receptors are present on a variety of immune cells [see 48,49], and that serotonin has immunomodulatory properties, with some studies indicating that it enhances, and others

indicating that it suppresses, aspects of immune function [63,64,65]. However, despite the immunomodulatory potential of serotonin, there is no evidence to suggest that MDMA releases serotonin from peripheral stores. In fact the few studies that have examined the impact of MDMA on platelet or plasma serotonin concentrations have not observed any change following MDMA administration [66,67].

Dopamine

Catecholamines, including dopamine, are present in lymphocytes, macrophages and neutrophils; the first two of which have been shown capable of synthesising dopamine [see 48]. In addition, noradrenergic sympathetic fibres in the spleen can take up circulating dopamine particularly during stress, which may then be released on sympathetic activation, and act upon immune cells in the vicinity of release [see 48]. Thus immune cells can come in contact with dopamine in the periphery. From a functional perspective a number of studies have demonstrated the presence of dopamine receptors on various immune cells, and indicate that dopamine has immunosuppressive properties [68-71]. However despite these findings the impact of MDMA on dopamine release in the periphery has not been studied, and therefore although dopamine has immunosuppressive properties it is difficult to say if it plays a role in MDMA-induced immunosuppression.

Thus further studies are required in order to determine if peripheral serotonin or dopamine play a role in MDMA-induced immunosuppression.

MDMA abuse – A stressor on the immune system

As many of the physiological changes elicited by MDMA closely resemble those induced by psychological or physical stress, it is suggested that exposure to MDMA could be regarded as a "chemical stressor" on the immune system. Exposure to stress is known to provoke similar immunological changes in both in laboratory animals and in man to those induced by MDMA. For instance, acute stress promotes an immunosuppressive cytokine phenotype in response to LPS [72,73]. Stress also suppresses lymphocyte proliferation and promotes a Th2 type cytokine response [74-79], and increases circulating NK-cell numbers [79-81].

Does MDMA-induced immunosuppression translate into a significant health risk for abusers?

Based on the studies outlined above, it is clear that MDMA suppresses both innate and adaptive immunity in animals and humans. Therefore it is possible the immunosuppressive effects of MDMA could lead to an abnormal immune response at times of infection or illness. For instance, pre-clinical studies have demonstrated that a deficiency in pro-inflammatory cytokines such as IL-1 or TNF-α can have a significant impact on host resistance to infectious disease. Specifically, TNF-α knockout mice have reduced host resistance to Listeria monocytogenes infection [82], and antagonism of IL-1 receptors with IL-1ra interferes with host resistance to infection with Mycobacterium avium [83]. In addition, considering the important role of Th$_1$-driven IgG$_{2a}$ antibody production in antiviral immunity [see 29], it is possible that MDMA abuse could interfere with reduce host resistance to viral infections. Also the long-term depletion in NK-cell numbers reported following MDMA abuse in humans gives

rise to concern considering the important role that NK-cells play in cell-mediated immunity, and more particularly in tumour surveillance [84,85]. Having all this said, it is difficult to predict the impact of MDMA-induced immunosuppression on disease susceptibility, as we don't know what degree of functional reserve is present within the immune system, and also as there are no data available regarding the impact of MDMA administration on host resistance in infection models. However, it was previously demonstrated that administration of d-amphetamine the parent compound of MDMA reduced host resistance to infection by influenza A virus and the bacteria *Listeria monocytogenes* [11,57]. Nonetheless, studies examining susceptibility to bacterial/viral infections are required in order to determine as to whether impairments in innate and adaptive immunity induced by MDMA have a major impact on infectivity and disease progression.

There are very few studies in the clinical literature that have examined the incidence of infections in MDMA abusers. However one recent report presented the results of a survey of 282 Ecstasy users that participated in a WWW study [86]. The sample was comprised of 109 novice users (1-9 occasions), 136 moderate users (10-99 occasions), and 36 heavy users (+100 occasions). In this study yes/no responses were recorded to a series of questions covering problems experienced when drug-free which were attributed by the respondents to their Ecstasy use. In this survey, infections were cited as one of the problems that were significantly associated with the extent of Ecstasy use [86]. In addition, there have been cases where MDMA abuse in humans closely preceded the development of meningococcal meningitis [87].

It is also possible that both the environment in which MDMA is consumed, and/or the psychoactive effects that MDMA induces, could synergise with the immunosuppressive effects

of the drug, to result in increased susceptibility to infectious disease. For instance MDMA use has traditionally been associated with the rave dance club scene; a crowded and environment where teenagers congregate. Such an environment is optimal for transmitting airborne infection between individuals. In addition, the results of a recent study indicated that MDMA abuse was strongly and significantly associated with high-risk sexual behaviors (unprotected anal intercourse) in a population of gay/bisexual men sampled from three New York dance clubs [88]. Thus when one combines such environmental factors with the immunosuppressive effect of MDMA, it is reasonable to suggest that MDMA users may have a higher risk of developing infectious disease in comparison to drug-free subjects.

Conclusions

Research conducted over the last five years has clearly demonstrated that administration of MDMA to both animals and humans has profound immunosuppressive effects; a property that it shares with other drugs of abuse [1-6]. For the most part the immunosuppressive effects of MDMA are not due to a direct action of the drug on immune cells, but rather due to the release of endogenous immunomodulatory substances, and it is suggested that exposure to MDMA could be regarded as a "chemical stressor" on the immune system. Further studies are required in order to elucidate the central and peripheral mediators of MDMA-induced immunosuppression. In addition, assessment of the effect of MDMA in pre-clinical host resistance models, and further clinical research is required before any definitive statement can be made on the propensity of MDMA-induced immunosuppression to translate into increased disease susceptibility.

Acknowledgements

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