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REVIEW

Methylenedioxymethamphetamine ('Ecstasy')-induced immunosuppression: a cause for concern?

Noreen T Boyle and Thomas J Connor

Neuroimmunology Research Group, Department of Physiology, School of Medicine, Trinity College Institute of Neuroscience, Trinity College, Dublin, Ireland

Correspondence
Dr Thomas Connor, Room 4.57, Lloyd Institute, Trinity College Institute of Neuroscience, University of Dublin, Trinity College, Dublin 2, Ireland.
E-mail: connort@tcd.ie

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Methylenedioxymethamphetamine (MDMA; 'Ecstasy') is a ring-substituted amphetamine and a popular drug of abuse. In addition to ability to induce euphoria, MDMA abuse is associated with a range of acute and long-term hazardous effects. This paper is focused on once such adverse effect: its ability to negatively impact on functioning of the immune system. Research demonstrates that MDMA has immunosuppressive properties, with both innate and adaptive arms of the immune system being affected. The ability of MDMA to suppress innate immunity is indicated by impaired neutrophil phagocytosis and reduced production of dendritic cell/macrophage-derived pro-inflammatory cytokines including tumour necrosis factor-alpha, interleukin (IL)-1β, IL-12 and IL-15. MDMA also suppresses innate IFN-γ production, and considering the role of IFN-γ in priming antigen-presenting cells, it is not surprising that MDMA reduces MHC class II expression on dendritic cells and macrophages, and inhibits co-stimulatory molecule expression. Paradoxically, studies demonstrate that MDMA elicits pro-inflammatory actions in the CNS by activating microglia, the resident innate immune cells in the brain. In terms of adaptive immunity, MDMA reduces circulating lymphocyte numbers, particularly $CD4^+$ T-cells; suppresses T-cell proliferation; and skews cytokine production in a Th_2 direction. For the most part, the immunosuppressive effects of MDMA cannot be attributed to a direct action of the drug on immune cells, but rather due to the release of endogenous immunomodulatory substances. In this regard, peripheral β-adrenoceptors and cholinergic receptors have been shown to mediate some immunosuppressive effects of MDMA. Finally, we discuss emerging evidence indicating that MDMA-induced immunosuppression can translate into significant health risks for abusers.

Abbreviations

5-HT_2 receptors, serotonin2 receptors; CD11b, cluster of differentiation 11b; CD40, cluster of differentiation 40; CD80, cluster of differentiation 80; CTL, cytotoxic T-lymphocyte; HIV, human immunodeficiency virus; HSV-2, herpes simplex virus-2; ICAM-1, intracellular adhesion molecule-1; IFN-γ, interferon-gamma; IgG, immunoglobulin G; IgG_1, immunoglobulin G1; IgG_{2a}, immunoglobulin G2a; IgM, immunoglobulin M; IL, interleukin; IL-1ra, interleukin-1receptor antagonist; IP-10/CXCL10, IFN-γ-inducible protein 10; KLH, keyhole limpet haemocyanin; LPS, lipopolysaccharide; MDA, methylenedioxyamphetamine; MDMA/Ecstasy, methylenedioxymethamphetamine; MHC class II, major histocompatibility complex class II; MLR, mixed lymphocyte reaction; NK cells, natural killer cells; pCPA, *p*-chlorophenylalanine; PHA, phytohaemagglutinin; SSRI, selective serotonin re-uptake inhibitor; STAT1, signal transducers and activators of transcription-1; TGF-β_1, transforming growth factor-beta1; Th_1, T-helper1; Th_2, T-helper2; TNF-α, tumour necrosis factor-alpha

Introduction

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 (Nunez-Iglesias *et al.*, 1996; Klein *et al.*, 1998;

Mellon and Bayer, 1998b; Pellegrino and Bayer, 1998a; Yu *et al.*, 2002; Friedman *et al.*, 2003; In *et al.*, 2005). Moreover, numerous pre-clinical reports indicate that a range of drugs of abuse result in diminished host resistance to infections (Nunez *et al.*, 1993; Baldwin *et al.*, 1998; Cabral and Dove Pettit, 1998; Donahoe and Vlahov, 1998; Freire-Garabal *et al.*, 1998; 1999; Gavrilin *et al.*, 2002; Cabral and Marciano-Cabral, 2004). Indeed, some investigators have implicated drug abuse as a co-factor in susceptibility to infection with HIV or other viruses (Nunez *et al.*, 1993; Baldwin *et al.*, 1998; Donahoe and Vlahov, 1998; Gavrilin *et al.*, 2002). Here, we review the evidence indicating that the widely abused recreational drug methylenedioxymethamphetamine (MDMA; 'Ecstasy') has suppressive effects on immune system functioning and can result in increased disease susceptibility.

Neurochemical and physiological actions of MDMA

MDMA is a ring-substituted amphetamine and has been a popular recreational drug of abuse for the last three decades, and was classified as a schedule I controlled drug by the US Drug Enforcement Administration in 1985, and is classified as a class A controlled drug under the UK misuse of drugs act (Hegadoren *et al.*, 1999; Green *et al.*, 2003). From a neurochemical standpoint, MDMA increases synaptic availability of serotonin and dopamine in a number of brain structures, via interaction with serotonin and dopamine transporters (Koch and Galloway, 1997; Kankaanpaa *et al.*, 1998). These neurochemical actions underlie the positive subjective effects of MDMA (Liechti and Vollenweider, 2001), which includes a relaxed euphoric state, emotional openness, increased empathy and a decrease in inhibitions. Following repeated administration, tolerance develops to the positive subjective effects of MDMA, thereby requiring users to consume larger quantities of the drug to achieve the same 'high' (Parrott, 2005; Baumann *et al.*, 2008; Jaehne *et al.*, 2008) (Figure 1).

In addition to the psychoactive properties that lead to its abuse potential, MDMA produces an array of physiological actions such as hyperthermia, acute sympathomimetic effects, such as increased heart rate and blood pressure, increased anxiety and increased activation of the hypothalamic–pituitary–adrenal axis resulting in increased circulating glucocorticoid concentrations (Green *et al.*, 2003). Experimental studies have concluded that many of these actions of MDMA occur secondary to central release of either serotonin or dopamine (Nash *et al.*, 1988; Liechti and Vollenweider, 2001; Mechan *et al.*, 2002).

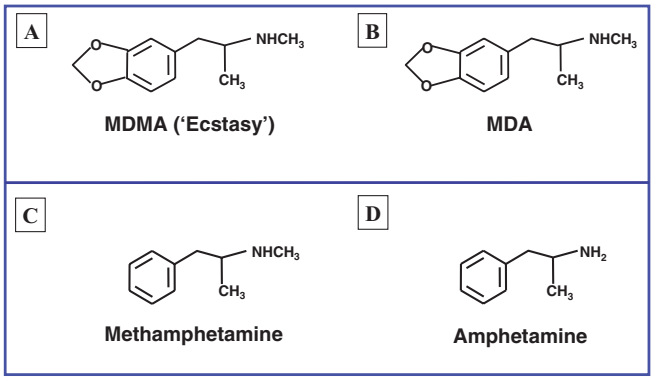


Figure 1

An illustration of the chemical structure of MDMA (A), its major metabolite MDA (B) and the parent amphetamines methamphetamine (C) and amphetamine (D).

Adverse effects induced by MDMA abuse

MDMA abuse is associated with serious adverse effects such as cardiac arrhythmias, hyperthermia, renal failure, hepatotoxicity, rhabdomyolysis, seizures and intracranial hemorrhage (Hegadoren *et al.*, 1999; Green *et al.*, 2003; Hall and Henry, 2006). In addition to these acute toxic effects, there is substantial evidence that MDMA can result in long-term neurotoxic effects on central serotonergic neurons (Stone *et al.*, 1987; Capela *et al.*, 2009), and that this may represent a predisposing factor to psychological disturbances/psychiatric disease (Montoya *et al.*, 2002; Durkin *et al.*, 2008). While the incidence and severity of acute or long-term adverse effects of MDMA are generally positively correlated with the extent of use, there is also evidence of idiosyncratic reactions to MDMA (Cole and Sumnall, 2003). One such factor that may influence the toxicity of MDMA is co-ingestion with other chemical substances. In this regard, recent evidence indicates that co-administration of caffeine with MDMA greatly exacerbates the acute and long-term toxicity of MDMA, ultimately resulting in death (McNamara *et al.*, 2006; 2007).

Research conducted over the last decade has demonstrated that the immune system is also a target of MDMA abuse. Specifically, studies indicate that MDMA suppresses aspects of innate and adaptive immunity in humans (Pacifici *et al.*, 1999; 2001a,b; 2002; 2004; 2007) and laboratory animals (Boyle and Connor, 2007; Connor *et al.*, 1998; 1999; 2000a,b; 2001a,b; de Paula *et al.*, 2009; Camarasa *et al.*, 2010). Moreover, emerging evidence indicates that MDMA-induced immunosuppression translates into significant health risks for abusers. The remainder of this paper focuses on the ability of MDMA to impact upon immune function, discusses the

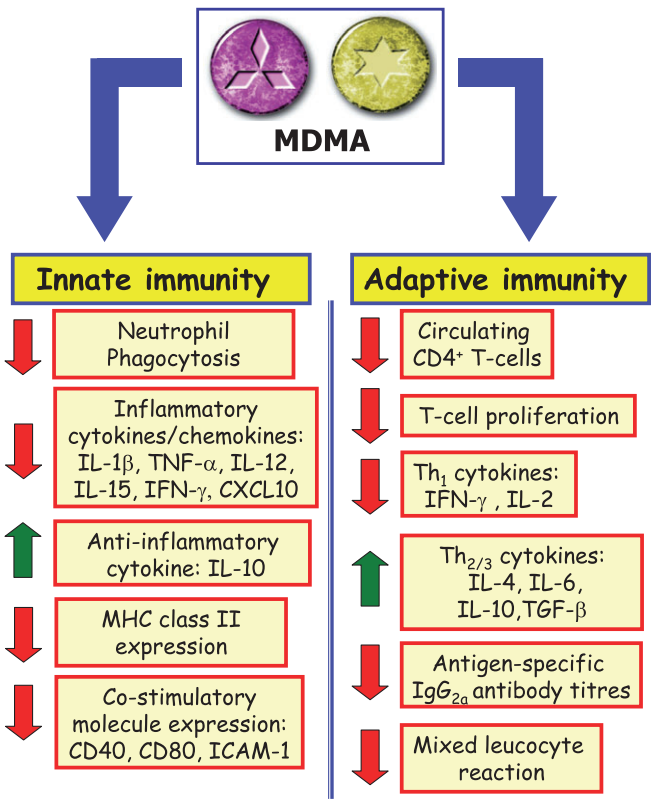


Figure 2

A diagrammatic summary of the effect of *in vivo* administration of MDMA on aspects of innate and adaptive immunity.

mechanisms and mediators underlying MDMA-induced immunosuppression and the ability of MDMA to result in reduced host resistance to disease. In addition, we discuss similarities between the effects of MDMA and the related drugs methamphetamine and D-amphetamine on immune functioning (Figure 2).

MDMA has immunosuppressive properties

MDMA-induced immunosuppression: evidence from studies conducted in laboratory animals

The first demonstration that MDMA had immunosuppressive properties following *in vivo* administration came from a pre-clinical study where MDMA (20 mg·kg^{-1}, i.p.) was shown to profoundly suppress lymphocyte proliferation in response to the T-cell mitogen concanavalin A, and this suppression of T-cell function was accompanied by a large reduction in circulating white blood cell numbers in rats which persisted for at least 6 h following drug administration (Connor *et al.*, 1998). In a subsequent study, it was observed that MDMA, its major metabolite MDA and also the related serotonin-releasing 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 (Connor *et al.*, 2000a).

While 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 (Connor *et al.*, 2001a). 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^{-1}) significantly suppressed KLH-specific IgG production (Connor *et al.*, 2001a). Therefore, while 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 switch to IgG_{2a}-secreting cells, while another pathway requires the Th_2-type cytokines interleukin (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.

Most subsequent animal studies examining immunosuppressive actions of MDMA focused on the impact of the drug on various aspects of innate immunity. Neutrophils are a subset of phagocytic cells that play a key role in the innate immune response, and are the first cells to be recruited to the site of infection (Quie and Mills, 1979; Quie, 1980). Neutrophil activation that occurs following phagocytosis is accompanied by an oxidative burst that produces reactive oxygen species, and destroys bac-

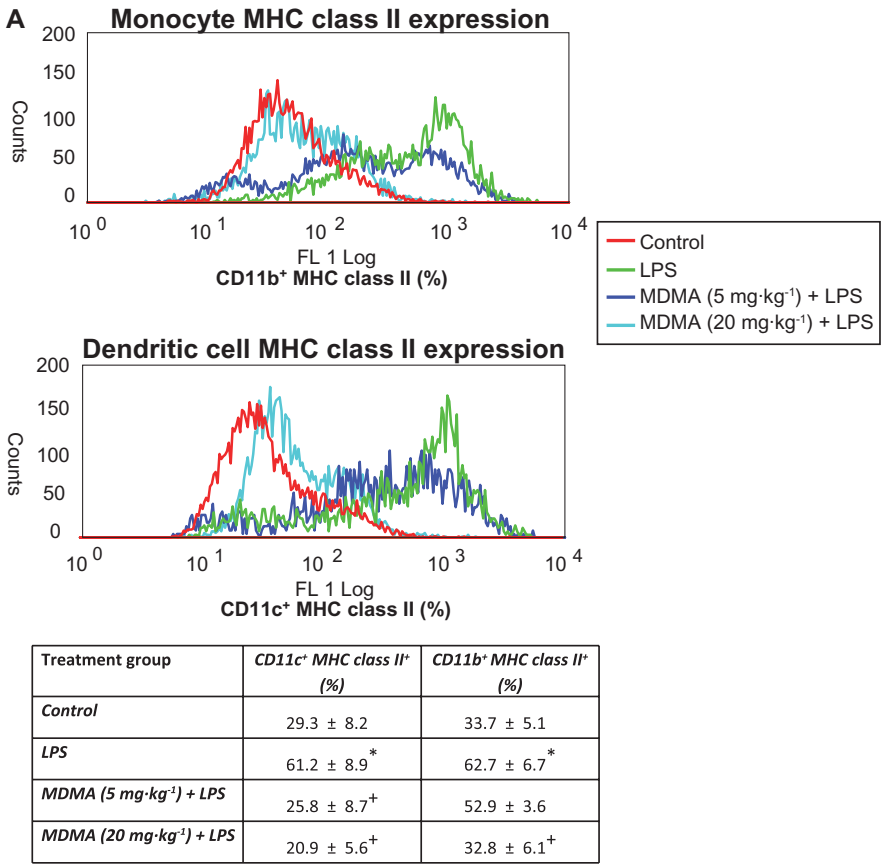
teria and fungi. Experimental studies show that MDMA suppresses neutrophil phagocytosis in both mice and rats (Connor *et al.*, 2004; de Paula *et al.*, 2009). For instance, in our study, we demonstrated that administration of MDMA (10 mg·kg^{-1}) to rats suppresses the neutrophil oxidative burst in response to opsonized zymosan (a phagocytic stimulus) (Connor *et al.*, 2004).

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 lipopolysaccharide (LPS). These cytokines are of strategic importance in initiating and co-ordinating a large range of immune responses against invading pathogens (Beutler, 1995; Trinchieri, 2003; Dinarello, 2009). MDMA administration to rats and mice impairs the ability to respond to an *in vivo* immune challenge with bacterial LPS. Specifically, MDMA suppresses LPS-induced IL-1β and TNF-α production in rats (Connor *et al.*, 2000b; 2001b; 2005; Camarasa *et al.*, 2010). The suppression of TNF-α that occurs following MDMA administration is the more profound and persists for longer than the suppression of IL-1β (Connor *et al.*, 2000b). The suppressive effect on TNF-α production is still evident following repeated treatment with MDMA, indicating that tolerance does not develop to its immunosuppressive effects (Connor *et al.*, 2005; Camarasa *et al.*, 2010). MDMA also suppresses production of the IFN-γ-inducing factors IL-12 and IL-15 in the mouse following an *in vivo* LPS challenge (Boyle and Connor, 2007). The suppressive effect of MDMA on IL-12 and IL-15 precedes and is correlated with a reduction in IFN-γ production that occurs in response to LPS (Boyle and Connor, 2007), and this was accompanied by impaired IFN-γ signalling indicated by reduced phosphorylation of the transcription factor STAT1, and reduced expression of the IFN-γ-inducible gene IP-10/CXCL10 (Boyle and Connor, 2007).

In addition to producing pro-inflammatory cytokines, cells of the innate immune system also produce IL-10, an anti-inflammatory or immunosuppressive cytokine that inhibits several macrophage functions including production of pro-inflammatory cytokines including TNF-α, IL-12 and IFN-γ (de Waal Malefyt *et al.*, 1991; Bogdan *et al.*, 1992; Ding *et al.*, 1993; Gerard *et al.*, 1993; Boyle and Connor, 2007). Our studies have demonstrated that MDMA induces a dose-dependent increase in IL-10 following an *in vivo* LPS challenge in both rats and mice (Connor *et al.*, 2005; Boyle and Connor, 2007), and that this effect persists following repeated treatment with MDMA (Connor *et al.*, 2005). In our recent study, we have demonstrated that IL-10 is a critical mediator of the ability of MDMA to suppress IL-12 and IFN-γ production in mice (Boyle and Connor, 2007). In addition to suppressing the production of 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 and producing anergy in the T-cell arm of the immune response (de Waal Malefyt *et al.*, 1991; Ding *et al.*, 1993). In this regard, recent data from our laboratory have demonstrated that in tandem with increasing IL-10 production, MDMA suppresses MHC class II expression and expression of the co-stimulatory molecules CD40, CD80 (B7.1) and ICAM-1 in mice (Figure 3). Moreover, when we examined the ability of splenocytes from MDMA-treated mice to act as stimulator cells in the mixed lymphocyte reaction (MLR), an assay that is commonly used to assess T-cell activation, we observed that administration of MDMA in combination with LPS suppressed the MLR (Boyle *et al.*, 2005). These data indicate that antigen-presenting cells from MDMA-treated mice are less effective in stimulating T-cell responses.

MDMA-induced immunosuppression: evidence from studies conducted in humans

Pacifici and co-workers have conducted a number of studies clearly demonstrating that MDMA has potent immunomodulatory properties following administration to human volunteers (Pacifici *et al.*, 1999; 2001a,b; 2002; 2004). In these studies, either placebo or MDMA (75–100 mg) was administered orally to recreational MDMA users in a controlled setting. The effect of ethanol consumption (0.8 mg·kg^{-1}) on immune function was also assessed, as was the effect of MDMA and ethanol co-administration. Studies were conducted in a double blind fashion, using a crossover (Latin square) design, where each participant received all of the treatments in separate experimental sessions, with a 1 week washout period between each session. The studies conducted by Pacifici and co-workers clearly demonstrate that MDMA suppresses the number of circulating CD4$^+$ T-cells, suppresses mitogen-stimulated T-cell proliferation and increases circulating numbers of natural killer (NK) cells (Pacifici *et al.*, 1999; 2001b). 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. Their studies also indicate that MDMA promotes a switch to a Th_2-type cytokine profile as indicated by reduced IFN-γ and IL-2 production, with a concomi-



Treatment group	*CD11c⁺ MHC class II⁺ (%)*	*CD11b⁺ MHC class II⁺ (%)*
Control	29.3 ± 8.2	33.7 ± 5.1
LPS	61.2 ± 8.9*	62.7 ± 6.7*
MDMA (5 mg·kg⁻¹) + LPS	25.8 ± 8.7+	52.9 ± 3.6
MDMA (20 mg·kg⁻¹) + LPS	20.9 ± 5.6+	32.8 ± 6.1+

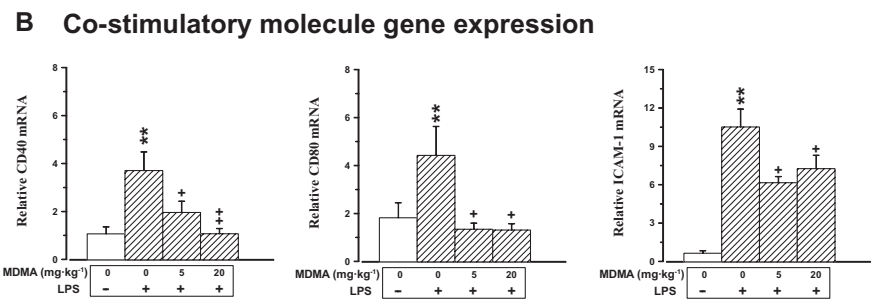


Figure 3

MDMA suppresses MHC class II expression and expression of the co-stimulatory molecules CD80, CD40 and ICAM-1. LPS (250 μg·kg^{-1}) was administered to mice immediately following MDMA administration, and the mice were sacrificed 8 h after injection for measurement of MHC class II expression on splenic monocytes and dendritic cells by flow cytometry (A) and splenic mRNA expression for the co-stimulatory molecules CD40, CD80 and ICAM-1 using real-time PCR (B). Data are expressed as mean ± SEM (n = 4–8). *P < 0.05, **P < 0.01 versus no LPS control group, ^{+}P < 0.05, ^{++}P < 0.01 versus LPS group (one-way ANOVA followed by a Newman–Keuls *post hoc* test).

tant increase in the Th_2 cytokines IL-4 and IL-6, and the T-regulatory cytokines IL-10 and TGF-β_1 (Pacifici *et al.*, 1999; 2001b). These immunosuppressive effects of MDMA were maximal 3–6 h following drug administration, and in some cases were evident 24 h later. In some instances, co-administration of alcohol further enhanced the immunosuppressive effects of MDMA. When two doses of MDMA (100 mg per dose) were administered 4 or 24 h apart, the immunosuppressive effects of MDMA were augmented following administration of the second dose. Furthermore, administration of two doses of MDMA 4 h apart produced longer-lasting immunosuppression that a single dose of MDMA when immune measures were assessed 24 h after treatment. In a second clinical trial, the second MDMA dose was administered 24 h 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 examined as late as 48 h after the second dose. Based on these findings, we conclude that repeated administration of MDMA with either a short or long time interval between doses increases both the magnitude and duration of MDMA-induced immunosuppression.

The clinical trials discussed so far were conducted in a controlled environment and have examined time-dependent effects of a single dose or two repeated doses of MDMA within a 24–28 h time-frame. The same research group has also studied baseline immunological parameters in recreational MDMA users at different time-points, compared with control subjects (Pacifici *et al.*, 2002; 2007). When baseline values were compared between MDMA users and controls, a significant reduction in NK cell numbers, T-helper cell numbers and lymphocyte proliferation could be attributed to consumption of MDMA (Pacifici *et al.*, 2007). These studies indicate that there is a sustained suppression in lymphocyte numbers and function, and NK cell numbers in chronic MDMA users; this reduction in NK cell numbers observed in chronic MDMA users is at variance with the increase in NK cell numbers observed following acute administration of MDMA to human volunteers (Pacifici *et al.*, 1999; 2001b).

Mechanisms by which MDMA can alter immune function

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

The most well-classified molecular targets for MDMA action are the transporter (uptake) sites for serotonin and dopamine. While 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 (Mossner and Lesch, 1998; Gordon and Barnes, 2003). In addition, recent evidence indicates that MDMA and related amphetamines bind to trace amine receptors (Bunzow *et al.*, 2001), and these receptors are expressed on immune cells (Nelson *et al.*, 2007). Consequently, MDMA can interact directly with molecular targets expressed on immune cells, and thereby has the potential to alter immune cell activity directly.

The first study to examine the immunomodulatory potential of MDMA was published 15 years ago by House and co-workers, and examined the impact of *in vitro* exposure to MDMA (0.0001–100 μM) on a number of immune parameters in splenocytes and peritoneal macrophages from B6C3F1 mice (House *et al.*, 1995). 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 100 μM MDMA and not altered by any of the five 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, and therefore cannot be regarded as a robust finding. 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 100 μM, but was unaltered at any of the other concentrations used. Finally, LPS-induced macrophage IL-6 or TNF production was not significantly altered by any concentration of MDMA; however, there was a slight but statistically non-significant suppression of TNF observed at 10 and 100 μM MDMA. In summary, the data generated by House *et al.* (1995) indicate 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, we 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 (Connor *et al.*, 2000b), and the inability of *in vitro* MDMA exposure to suppress LPS-induced TNF-α production in rat whole blood cultures was recently replicated by another group (Camarasa *et al.*, 2010). We also observed that *in vitro* exposure of LPS-stimulated mouse splenocytes to MDMA failed to mimic its ability to suppress IL-12 and IFN-γ production following an *in vivo* LPS challenge (Boyle and Connor, 2007). Similarly, we 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 (Connor *et al.*, 2005). In addition, the suppression of Con A-stimulated lymphocyte proliferation observed *ex vivo* in blood harvested from MDMA-treated rats cannot be mimicked by *in vitro* exposure to MDMA (Connor, unpubl. obs.). 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, suggesting that MDMA can elicit a direct effect on neutrophil phagocytosis (Connor *et al.*, 2004). In addition, a recent study reported that *in vitro* exposure of murine macrophages to MDMA for 24 h suppressed production of the pro-inflammatory cytokines IL-6, TNF-α and IL-12; the inflammatory chemokine RANTES; and the anti-inflammatory cytokine IL-10 induced by murine γ-herpes virus-68 (Nelson *et al.*, 2008). However, it is important to point out that in this study, the suppressive effect of MDMA was observed at 500 μM, a very high concentration of MDMA that is far in excess of what would be ever encountered by immune cells in the *in vivo* setting following ingestion of the drug.

Indirect mechanisms by which MDMA can impact upon the immune system

In addition to having a direct action on immune cells, MDMA has the potential to alter immune functioning via neuroimmune mechanisms. Specifically, it is well established that changes in CNS neurotransmitter function can alter immunity via changes in endocrine output and sympathetic nervous system activity (Dantzer and Kelley, 1989; Serafeim and Gordon, 2001). For instance, MDMA induces the release of the neurotransmitters serotonin and dopamine in the CNS (Koch and Galloway, 1997; Kankaanpaa *et al.*, 1998), and produces consequential downstream activation of peripheral immunomodulatory pathways such as the hypothalamic–pituitary–adrenal axis and the sympathetic nervous system (Nash *et al.*, 1988; Grob *et al.*, 1996). 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.

Neurotransmitters as central drivers 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 rats (Connor *et al.*, 2000a; Connor and Kelly, 2002). For instance, fenfluramine suppressed circulating lymphocyte numbers, suppressed T-lymphocyte proliferation and cytokine production (Connor *et al.*, 2000b) and also suppressed production of the pro-inflammatory cytokines IL-1β and TNF-α in response to an *in vivo* LPS challenge (Connor and Kelly, 2002). Similarly, it has been demonstrated that selective serotonin re-uptake inhibitors suppress T-cell function, and this immunosuppressive effect is mediated via activation of central 5-HT_2 receptors (Pellegrino and Bayer, 2002). These findings all point 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 re-uptake inhibitor paroxetine was used to prevent MDMA from entering serotonergic neurons, thereby preventing MDMA-induced serotonin release (Connor *et al.*, 2001b). In the second study, rats were pretreated with the tryptophan hydroxylase inhibitor *p*-chlorophenylalanine (pCPA), an agent that reduces brain serotonin synthesis. The impact of both of these anti-serotonin strategies on MDMA-induced suppression of IL-1β and TNF-α in rats was evaluated. While paroxetine pretreatment completely blocked MDMA-induced serotonin depletion in both the frontal cortex and hypothalamus, it failed to alter the suppressive effects of MDMA on LPS-induced TNF-α secretion. It was also of interest that paroxetine treatment alone suppressed both LPS-induced IL-1β and TNF-α secretion by 27 and 50%, respectively, possibly by its ability to increase central serotonin concentrations (Connor *et al.*, 2001b). While the treatment regimen of pCPA used caused in excess of a 90% depletion of brain serotonin concentration, the suppressive effect of MDMA on LPS-induced IL-1β and TNF-α was 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^{-1}) on LPS-induced IL-1β and TNF-α production was not blocked by pretreatment with either paroxetine or pCPA (Dennedy *et al.*, 2000). However, in a later study using much lower doses of fenfluramine (1.25 and 2.5 mg·kg^{-1}), it was observed that the suppressive effect of fenfluramine on LPS-induced IL-1β production was blocked by pretreatment with pCPA, indicating that it was indeed a serotonin-dependent event (Connor *et al.*, 2003). 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 research is necessary to test this hypothesis.

In contrast to the animal studies outlined above that failed to elucidate a role for serotonin in MDMA-induced immunosuppression (Connor *et al.*, 2001b), a study conducted in humans demonstrated that pretreatment with the serotonin re-uptake inhibitor paroxetine could block some of the immunosuppressive effects of MDMA. Specifically, paroxetine pretreatment partially inhibited that ability of MDMA to suppress circulating CD4+ T_{helper} cell numbers, and to increase circulating NK cell numbers (Pacifici *et al.*, 2004). 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 phytohaemagglutinin (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. These results are consistent with an earlier finding that central 5-HT_2 receptors mediate the suppressive effect of the selective serotonin re-uptake inhibitor fluoxetine on T-cell function (Pellegrino and Bayer, 2002).

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 (Koch and Galloway, 1997; Kankaanpaa *et al.*, 1998). 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 and are potent dopamine releasers, elicit immunosuppressive effects in rodents (Freire-Garabal *et al.*, 1991; Pezzone *et al.*, 1992; Yu *et al.*, 2002). Future studies are required to evaluate the role of dopamine in MDMA-induced immunosuppression (Tables 1 and 2).

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 (Green *et al.*, 2003). It is of interest that previous studies demonstrated that depletion of serotonin concentrations with pCPA or blockade of serotonin release by pretreatment with SSRIs attenuates the locomotor stimulant effect of MDMA in rats (Callaway *et al.*, 1990). Therefore, while pretreatment with paroxetine or pCPA 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 (Connor *et al.*, 2001b). In addition, the fact that the non-psychostimulant amphetamine derivative fenfluramine elicits similar immunosuppressive effects to MDMA (Connor *et al.*, 2000a; Connor and Kelly, 2002) supports the view that the psychoactive and immunosuppressive properties of substituted amphetamines in rats are not necessarily linked.

A previous study conducted in humans reported that treatment with the SSRI citalopram blocked the positive mood, extraversion and self-confidence induced by MDMA (Liechti and Vollenweider, 2001). In addition, some of the immunosuppressive actions of MDMA in humans are blocked by serotonin transporter blockade with the related SSRI paroxetine (Pacifici *et al.*, 2004). However, despite these coincidental effects, the exact role that the euphoric effect of MDMA plays in its ability to suppress the immune system in humans is not clear.

Peripheral mediators of MDMA-induced immunosuppression

It is well established that MDMA activates both the hypothalamic–pituitary–adrenal axis and sympathetic nervous system (Nash *et al.*, 1988; Grob *et al.*, 1996; Connor *et al.*, 1999), and that the end products of these axes namely glucocorticoids and catecholamines have immunosuppressive properties (Bateman *et al.*, 1989; Elenkov *et al.*, 2000). 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 pretreatment 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 (Connor *et al.*, 2005; Boyle and Connor, 2007). Similarly, the

Table 1

Studies that have assessed the effect of MDMA on cytokine and chemokine production

Immune measure	Species	Stimulus	MDMA conc./dose	Effect	Reference
IL-1β	Rat blood	LPS	*In vivo*: 10–20 mg·kg^{-1} (i.p.)	Decrease	(Connor *et al.*, 2000b; 2001b)
	Rat blood	LPS	*In vitro*: 1–10 μg·mL^{-1}	No change	(Connor *et al.*, 2000b)
	Human blood	PHA	*In vivo*: 100 mg (p.o.)	Decrease	(Pacifici *et al.*, 2001b)
TNF-α	Rat blood	LPS	*In vivo*: 5–20 mg·kg^{-1} (i.p.)	Decrease	(Connor *et al.*, 2000b; 2001b)
	Rat blood	LPS	*In vitro*: 1–10 μg·mL^{-1}	No change	(Connor *et al.*, 2000b)
	Mouse macrophages	LPS	*In vitro*: 0.0001–100 μM	No change	(House *et al.*, 1995)
	Rat blood	LPS	*In vivo*: 0.1–20 mg·kg^{-1} (i.p.)	Decrease	(Camarasa *et al.*, 2010)
	Mouse blood	LPS	*In vivo*: 2.5–20 mg·kg^{-1} (i.p.)	Decrease	(Camarasa *et al.*, 2010)
	Rat blood	LPS	*In vitro*: 5–500 μM	No change	(Camarasa *et al.*, 2010)
	Mouse macrophages	γ–Herpes virus-68	*In vitro*: 500 μM	Decrease	(Nelson *et al.*, 2008)
IL-6	Mouse macrophages	LPS	*In vitro*: 0.0001–100 μM	No change	(House *et al.*, 1995)
	Mouse macrophages	γ–Herpes virus-68	*In vitro*: 100 μM–6 mM	Decrease	(Nelson *et al.*, 2008)
	Human blood	PHA	*In vivo*: 100 mg (p.o.)	Decrease	(Pacifici *et al.*, 2001b)
IL-10	Rat blood	LPS	*In vivo*: 1.25–10 mg·kg^{-1} (i.p.)	Increase	(Connor *et al.*, 2005)
	Rat blood	LPS	*In vitro*: 1–10 μg·mL^{-1}	No change	(Connor *et al.*, 2005)
	Rat blood	Con A	*In vivo*: 7.5 mg·kg^{-1} (i.p.)	Decrease	(Connor *et al.*, 2000a)
	Human blood	PHA	*In vivo*: 100 mg (p.o.)	Increase	(Pacifici *et al.*, 2001b)
	Mouse blood	LPS	*In vivo*: 5–20 mg·kg^{-1} (i.p.)	Increase	(Boyle and Connor, 2007)
	Mouse macrophages	γ-Herpes virus-68	*In vitro*: 500 μM	Decrease	(Nelson *et al.*, 2008)
IL-12	Mouse blood/spleen	LPS	*In vivo*: 5–20 mg·kg^{-1} (i.p.)	Decrease	(Boyle and Connor, 2007)
	Mouse splenocytes	LPS	*In vitro*: 1–50 μg·mL^{-1}	No change	(Boyle and Connor, 2007)
	Mouse macrophages	γ-Herpes virus-68	*In vitro*: 500 μM	Decrease	(Nelson *et al.*, 2008)
IL-15	Mouse spleen	LPS	*In vivo*: 5–20 mg·kg^{-1} (i.p.)	Decrease	(Boyle and Connor, 2007)
IL-18	Mouse spleen	LPS	*In vivo*: 5–20 mg·kg^{-1} (i.p.)	No change	(Boyle and Connor, 2007)
TGF-β_1	Human blood	PHA	*In vivo*: 100 mg (p.o.)	Increase	(Pacifici *et al.*, 2001b)
IFN-γ	Rat blood	Con A	*In vivo*: 7.5 mg·kg^{-1} (i.p.)	Decrease	(Connor *et al.*, 2000a)
	Human blood	PHA	*In vivo*: 100 mg (p.o.)	Decrease	(Pacifici *et al.*, 2001b)
	Rat splenocytes	KLH	*In vivo*: 10 mg·kg^{-1} (i.p.)	Decrease	(Connor *et al.*, 2001a)
	Mouse blood/spleen	LPS	*In vivo*: 5–20 mg·kg^{-1} (i.p.)	Decrease	(Boyle and Connor, 2007)
	Mouse splenocytes	LPS	*In vitro*: 1–50 μg·mL^{-1}	No change	(Boyle and Connor, 2007)
IL-2	Rat blood	Con A	*In vivo*: 7.5 mg·kg^{-1} (i.p.)	Decrease	(Connor *et al.*, 2000a)
	Human blood	PHA	*In vivo*: 100 mg (p.o.)	Decrease	(Pacifici *et al.*, 2001b)
	Mouse splenocytes	Anti-CD3	*In vitro*: 0.0001 μM	Increase	(House *et al.*, 1995)
IL-4	Mouse splenocytes	Anti-CD3	*In vitro*: 0.0001–100 μM	Increase/Decrease	(House *et al.*, 1995)
	Human blood	PHA	*In vivo*: 100 mg (p.o.)	Increase	(Pacifici *et al.*, 2001b)
IP-10 (CXCL10)	Mouse spleen	LPS	*In vivo*: 5–20 mg·kg^{-1} (i.p.)	Decrease	(Boyle and Connor, 2007)
RANTES (CCL5)	Mouse macrophages	γ-Herpes virus-68	*In vitro*: 500 μM	Decrease	(Nelson *et al.*, 2008)

Table 2

Studies that have assessed the effect of MDMA immune cell numbers and functions

Immune measure	Species	Stimulus	MDMA conc./dose	Effect	Reference
Lymphocyte numbers	Rat blood	None	*In vivo*: 1.25–40 mg·kg^{-1}	Decrease: Lymphocytes	(Connor *et al.*, 1999; 2000a)
	Human blood	None	*In vivo*: 75–125 mg	Decrease: CD4^{+} T-cells	(Pacifici *et al.*, 1999; 2001a,b; 2004)
NK cell numbers	Human blood	None	*In vivo*: 75–100 mg (p.o.)	Increase (acute) Decrease (long term)	(Pacifici *et al.*, 1999; 2001a,b; 2004; 2007)
T-cell proliferation	Rat blood	Con A/PHA	*In vivo*: 7.5–20 mg·kg^{-1}	Decrease	(Connor *et al.*, 1998; 1999; 2000a)
	Human blood	PHA	*In vivo*: 75–125 mg	Decrease	(Pacifici *et al.*, 1999)
NK cell activity	Mouse splenocytes	None/IL-2	*In vitro*: 0.0001–1 μM	Increase	(House *et al.*, 1995)
Neutrophil phagocytosis	Rat blood	Zymosan	*In vivo*: 10 mg·kg^{-1} (i.p.) *In vitro* (10 μg·mL^{-1})	Decrease	(Connor *et al.*, 2004; de Paula *et al.*, 2009)
	Mouse blood	PMA/*Staphylococcus aureus*	*In vivo*: 10 mg·kg^{-1} (i.p.)	Decrease	(de Paula *et al.*, 2009)
	Mouse blood	PMA/*S. aureus*	*In vitro*: 66–6670 ng·mL^{-1}	No change	(de Paula *et al.*, 2009)
CTL activity	Mouse splenocytes	None	*In vitro*: 100 μM	Decrease	(House *et al.*, 1995)
B-cell proliferation	Mouse splenocytes	Anti-IgM/IL-4	*In vitro*: 0.0001–100 μM	No change	(House *et al.*, 1995)
Antibody production	Rat blood	KLH	*In vivo*: 5–10 mg·kg^{-1} (i.p.)	IgG_{2a}: decrease	(Connor *et al.*, 2001a)
MHC class II expression	Mouse monocytes and dendritic cells	LPS	*In vivo*: 5–20 mg·kg^{-1} (i.p)	Decrease	Figure 3, this article
CD40 expression	Mouse spleen	LPS	*In vivo*: 5–20 mg·kg^{-1} (i.p)	Decrease	Figure 3, this article
ICAM-1 expression	Mouse spleen	LPS	*In vivo*: 5–20 mg·kg^{-1} (i.p)	Decrease	Figure 3, this article
CD80 expression	Mouse spleen	LPS	*In vivo*: 5–20 mg·kg^{-1} (i.p)	Decrease	Figure 3, this article

suppressive effect of MDMA on production of the inflammatory cytokine IFN-γ is mediated by β-adrenoceptors; in contrast, suppression of other pro-inflammatory cytokines including TNF-α and IL-1β occurred independent of β-adrenoceptor activation (Connor *et al.*, 2005). Consistent with this observation, a recent study reported a key role for nicotinic acetylcholine receptors in mediating the suppressive effect of MDMA on TNF-α production *in vivo* in both rats and mice (Camarasa *et al.*, 2010). This is an interesting finding and supports an emerging literature that cholinergic signalling via the vagus nerve can suppress aspects of the innate immune system (Wang *et al.*, 2003).

While MDMA is a potent stimulator of peripheral glucocorticoid release (Nash *et al.*, 1988), our studies to date using adrenalectomy and the glucocorticoid receptor antagonist RU38486 do not support a role for glucocortiocids in mediating the immunosuppressive effects of MDMA in rats (Connor *et al.*, 2005) or mice (Boyle and Connor, unpubl. obs.).

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

Based on the studies outlined above, it is clear that MDMA suppresses aspects of innate and adaptive immunity, and that there is evidence of sustained suppression of immune function in chronic MDMA users. Thus, the possibility exists that 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, TNF-α and IFN-γ can have a significant impact on host resistance to infectious disease. Specifically, TNF-α knockout mice have reduced host resistance to *Listeria monocytogenes* infection (Pasparakis *et al.*, 1996), and antagonism of IL-1 receptors with IL-1ra interferes with host resistance to infection with *Mycobacterium avium* (Denis and Ghadirian, 1994). Moreover, the clinical literature clearly demonstrates that treatment with anti-TNF-α agents including infliximab and etanercept results in an increased susceptibility to infectious diseases (Keane, 2005; Strangfeld *et al.*, 2009; Furst, 2010). The critical role played by IFN-γ in antimicrobial defence is demonstrated by the increased susceptibilities of IFN-γ and IFN-γ receptor knockout mice to a variety of infectious organisms, particularly to intracellular organisms such as *Listeria* and *Mycobacteria* (Shtrichman and Samuel, 2001). Of course, IFN-γ also plays a key role in antiviral immunity via its ability to promote IgG_{2a} production, the dominant antibody isotype responsible for complement-mediated lysis reactions. In addition to its role in host resistance to infection, innate IFN-γ also plays a significant role in anti-tumour immunity (Kim *et al.*, 2000; Tannenbaum and Hamilton, 2000; Ikeda *et al.*, 2002). Thus, it is possible that MDMA, by suppressing production of inflammatory cytokines, could reduce host resistance to bacterial and viral infections, and also negatively impact upon cancer progression. The ability of MDMA to suppress neutrophil function is a cause for concern, considering their key role in the early stages of host defence (Quie and Mills, 1979; Quie, 1980). Also, the long-term depletion in NK cell numbers reported following MDMA abuse in humans is of concern, considering the important role that NK cells play in cell-mediated immunity, and more particularly in tumour surveillance (Albertsson *et al.*, 2003; Farag *et al.*, 2003). In addition, NK cells are a critical component of the innate immune response to infection, largely due to their ability to elaborate large quantities of IFN-γ prior to the development of an effective adaptive immune response (Farrar and Schreiber, 1993). Similarly, in the context of the adaptive immune response, the suppressive action of MDMA on T-cell function is a cause for concern.

To date, there is only one study that has examined the impact of MDMA on host resistance to infection. In this study, Pennock and co-workers demonstrate that treatment of female mice with MDMA (10 $mg \cdot kg^{-1}$) for 5 days increases susceptibility to herpes simplex virus-2 (HSV-2) infection following vaginal inoculation. Increased susceptibility to infection in MDMA-treated mice was characterized by an earlier onset of disease, and the fact that a lower virus inoculum concentration was required to establish infection in 50% of mice. This increase in disease susceptibility was accompanied by higher viral titer in the genital tract of MDMA-treated mice (Pennock *et al.*, 2009). These are important data and suggest that MDMA abuse can increase susceptibility to sexually transmitted diseases such as HSV. These findings of Pennock *et al.* are consistent with previous studies indicating that administration of D-amphetamine, the parent compound of MDMA, reduced host resistance to infection by influenza A virus and the bacteria *L. monocytogenes* (Freire-Garabal *et al.*, 1991; Nunez *et al.*, 1993).

In addition to these pre-clinical findings, studies have used structured questionnaires to examine the incidence of infections in MDMA abusers. One study reported a significantly higher rate of mild infections (common cold, acute pharyngitis and sinusitis and uncomplicated urinary tract infections) in regular MDMA/*Cannabis* users compared

with regular *Cannabis* users alone or control subjects (Pacifici *et al.*, 2007), thus indicating that regular MDMA use may play a causal role in the incidence of infections. Another report presented the results of a survey of 282 Ecstasy users that participated in a WWW study (Parrott *et al.*, 2002). 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 (Parrott *et al.*, 2002).

It is also possible that both the environment in which MDMA is consumed and/or the psychoactive effects that MDMA induces could synergize 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 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 behaviours (unprotected anal intercourse) in a population of gay/bisexual men sampled from three New York dance clubs (Klitzman *et al.*, 2000). 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.

Some clinical case reports also support a role for MDMA-induced immunosuppression resulting in increased susceptibility to infectious disease. For instance, Zwick and colleagues reported a case of herpes zoster ophthalmicus (shingles of the eye) in a 24-year-old, and otherwise healthy, man, which developed immediately after an Ecstasy binge where he consumed MDMA three times daily for 4 days (Zwick *et al.*, 2005). As this condition is extremely rare in individuals under the age of 50 years, and as reactivation of *Varicella zoster* (the virus that causes this condition) is a potential complication of immunosuppression, the authors suggest that MDMA-induced immunosuppression could be a causal factor in the development of herpes zoster ophthalmicus in this individual (Zwick *et al.*, 2005). Another report highlighted cases where MDMA abuse in humans closely preceded the development of meningococcal meningitis (Prasad *et al.*, 1994).

MDMA drives an inflammatory response in the CNS: a role in the neurodegenerative actions of MDMA

Recent studies demonstrate that MDMA induces activation of microglia; the resident innate immune cells of the CNS that are of the same lineage as peripheral monocytes. Microglial activation was characterized by increased expression of the microglial activation marker CD11b, increased production of the microglial-derived pro-inflammatory cytokine IL-1β and activation of the inflammatory signalling molecule NFκB (Orio *et al.*, 2004; 2009). Microglial activation has be reported to be a pharmacologically specific marker for neurotoxic amphetamines (Thomas *et al.*, 2004), and co-administration of caffeine with MDMA, which is known to exacerbate its neurotoxic actions (McNamara *et al.*, 2006), also augments the degree of microglial activation induced by MDMA (Khairnar *et al.*, 2010). Significantly, treatment with an inhibitor of microglial activation, minocycline, prevents MDMA-induced serotonergic neurotoxicity in frontal cortex, striatum and hippocampus, and prevents dopaminergic neurotoxicity in the striatum (Zhang *et al.*, 2006; Orio *et al.*, 2009). These data indicate that a microglial inflammatory response contributes to the neurotoxic actions of MDMA in these brain regions. Thus, while MDMA elicits immunosuppressive/anti-inflammatory effects in the peripheral immune system in response to various immune cell stimulants, it is clear that in the brain MDMA elicits an inflammatory response in microglia that contributes to its neurotoxic actions.

Conclusions

Research conducted over the last decade clearly demonstrates that MDMA elicits immunosuppressive effects in humans and laboratory animals, a property that it shares with other drugs of abuse (Nunez-Iglesias *et al.*, 1996; Klein *et al.*, 1998; Mellon and Bayer, 1998a; Pellegrino and Bayer, 1998b; Yu *et al.*, 2002; Friedman *et al.*, 2003). 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 recent studies have implicated catecholaminergic β-adrenoceptors and nicotinic acetylcholine receptors in mediating some of the suppressive effects of MDMA on immune functioning (Connor *et al.*, 2005; Boyle and Connor, 2007; Camarasa *et al.*, 2010). In addition, there is emerging pre-clinical and clinical evi-

dence to suggest that the immunosuppressive actions of MDMA lead to reduced host resistance to infections. In addition to the peripheral immunosuppressive actions of MDMA, recent evidence indicates that MDMA induces an inflammatory reaction in the brain by activating microglia and that microglial activation contributes to the neurotoxic actions of this drug.

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Conflict of interest

The authors have no conflict of interest to declare.

References

Albertsson PA, Basse PH, Hokland M, Goldfarb RH, Nagelkerke JF, Nannmark U *et al.* (2003). NK cells and the tumour microenvironment: implications for NK-cell function and anti-tumour activity. Trends Immunol 24: 603–609.

Baldwin GC, Roth MD, Tashkin DP (1998). Acute and chronic effects of cocaine on the immune system and the possible link to AIDS. J Neuroimmunol 83: 133–138.

Bateman A, Singh A, Kral T, Solomon S (1989). The immune-hypothalamic–pituitary–adrenal axis. Endocr Rev 10: 92–112.

Baumann MH, Clark RD, Franken FH, Rutter JJ, Rothman RB (2008). Tolerance to 3,4-methylenedioxymethamphetamine in rats exposed to single high-dose binges. Neuroscience 152: 773–784.

Beutler B (1995). TNF, immunity and inflammatory disease: lessons of the past decade. J Investig Med 43: 227–235.

Bogdan C, Paik J, Vodovotz Y, Nathan C (1992). Contrasting mechanisms for suppression of macrophage cytokine release by transforming growth factor-beta and interleukin-10. J Biol Chem 267: 23301–23308.

Boyle NT, Connor TJ (2007). MDMA ('Ecstasy') suppresses the innate IFN-gamma response *in vivo*: a critical role for the anti-inflammatory cytokine IL-10. Eur J Pharmacol 572: 228–238.

Boyle NT, Todryk SM, Connor TJ (2005). Methylenedioxymethamphetamine (MDMA; 'Ecstasy') suppresses interleukin (IL)-12 and increases IL-10 production *in vivo*, and results in reduced stimulator capacity in the mixed lymphocyte reaction. Ir J Med Sci 174: 1–40.

Bunzow JR, Sonders MS, Arttamangkul S, Harrison LM, Zhang G, Quigley DI *et al.* (2001). Amphetamine, 3,4-methylenedioxymethamphetamine, lysergic acid diethylamide, and metabolites of the catecholamine neurotransmitters are agonists of a rat trace amine receptor. Mol Pharmacol 60: 1181–1188.

Cabral GA, Dove Pettit DA (1998). Drugs and immunity: cannabinoids and their role in decreased resistance to infectious disease. J Neuroimmunol 83: 116–123.

Cabral GA, Marciano-Cabral F (2004). Cannabinoid-mediated exacerbation of brain infection by opportunistic amebae. J Neuroimmunol 147: 127–130.

Callaway CW, Wing LL, Geyer MA (1990). Serotonin release contributes to the locomotor stimulant effects of 3,4-methylenedioxymethamphetamine in rats. J Pharmacol Exp Ther 254: 456–464.

Camarasa J, Ros C, Pubill D, Escubedo E (2010). Tumour necrosis factor alpha suppression by MDMA is mediated by peripheral heteromeric nicotinic receptors. Immunopharmacol Immunotoxicol 244: 344–353.

Capela JP, Carmo H, Remiao F, Bastos ML, Meisel A, Carvalho F (2009). Molecular and cellular mechanisms of ecstasy-induced neurotoxicity: an overview. Mol Neurobiol 39: 210–271.

Cole JC, Sumnall HR (2003). Altered states: the clinical effects of Ecstasy. Pharmacol Ther 98: 35–58.

Connor TJ, Kelly JP (2002). Fenfluramine-induced immunosuppression: an *in vivo* analysis. Eur J Pharmacol 455: 175–185.

Connor TJ, McNamara MG, Finn D, Currid A, O'Malley M, Redmond AM *et al.* (1998). Acute 3,4-methylenedioxymethamphetamine(MDMA) administration produces a rapid and sustained suppression of immune function in the rat. Immunopharmacology 38: 253–260.

Connor TJ, McNamara MG, Kelly JP, Leonard BE (1999). 3,4-Methylenedioxymethamphetamine (MDMA; Ecstasy) administration produces dose-dependent neurochemical, endocrine and immune changes in the rat. Hum Psychopharmacol 14: 95–104.

Connor TJ, Kelly JP, Leonard BE (2000a). An assessment of the acute effects of the serotonin releasers methylenedioxymethamphetamine, methylenedioxyamphetamine and fenfluramine on immunity in rats. Immunopharmacology 46: 223–235.

Connor TJ, Kelly JP, McGee M, Leonard BE (2000b). Methylenedioxymethamphetamine (MDMA; Ecstasy) suppresses IL-1beta and TNF-alpha secretion following an *in vivo* lipopolysaccharide challenge. Life Sci 67: 1601–1612.

Connor TJ, Connelly DB, Kelly JP (2001a). Methylenedioxymethamphetamine (MDMA; 'Ecstasy') suppresses antigen specific IgG2a and IFN-gamma production. Immunol Lett 78: 67–73.

Connor TJ, Dennedy MC, Harkin A, Kelly JP (2001b). Methylenedioxymethamphetamine-induced suppression of interleukin-1beta and tumour necrosis factor-alpha is not mediated by serotonin. Eur J Pharmacol 418: 147–152.

Connor TJ, O'Mahony S, Kelly JP, Harkin A (2003). Augmentation of serotonergic function suppresses pro-inflammatory cytokine production in response to an *in vivo* immune challenge. Ir J Med Sci 172 (Suppl. 2): 23.

Connor TJ, O'Shaughnessy D, Kelly JP (2004). Methylenedioxymethamphetamine 'MDMA; Ecstasy' suppresses neutrophil phagocytosis. Fundam Clin Pharmacol 18 (Suppl. 1): 90.

Connor TJ, Harkin A, Kelly JP (2005). Methylenedioxymethamphetamine suppresses production of the proinflammatory cytokine tumor necrosis factor-alpha independent of a beta-adrenoceptor-mediated increase in interleukin-10. J Pharmacol Exp Ther 312: 134–143.

Dantzer R, Kelley KW (1989). Stress and immunity: an integrated view of relationships between the brain and the immune system. Life Sci 44: 1995–2008.

Denis M, Ghadirian E (1994). Interleukin-1 is involved in mouse resistance to *Mycobacterium avium*. Infect Immun 62: 457–461.

Dennedy MC, Connor TJ, Harkin A, Kelly JP, O'Donnell JM (2000). Does serotonin mediate the immunosuppressive effects of methylenedioxymethamphetamine and fenfluramine in rats? Ir J Med Sci 169: 360.

Dinarello CA (2009). Immunological and inflammatory functions of the interleukin-1 family. Annu Rev Immunol 27: 519–550.

Ding L, Linsley PS, Huang LY, Germain RN, Shevach EM (1993). IL-10 inhibits macrophage costimulatory activity by selectively inhibiting the up-regulation of B7 expression. J Immunol 151: 1224–1234.

Donahoe RM, Vlahov D (1998). Opiates as potential cofactors in progression of HIV-1 infections to AIDS. J Neuroimmunol 83: 77–87.

Durkin S, Prendergast A, Harkin A (2008). Reduced efficacy of fluoxetine following MDMA ('Ecstasy')-induced serotonin loss in rats. Prog Neuropsychopharmacol Biol Psychiatry 32: 1894–1901.

Elenkov IJ, Wilder RL, Chrousos GP, Vizi ES (2000). The sympathetic nerve – an integrative interface between two supersystems: the brain and the immune system. Pharmacol Rev 52: 595–638.

Farag SS, VanDeusen JB, Fehniger TA, Caligiuri MA (2003). Biology and clinical impact of human natural killer cells. Int J Hematol 78: 7–17.

Farrar MA, Schreiber RD (1993). The molecular cell biology of interferon-gamma and its receptor. Annu Rev Immunol 11: 571–611.

Freire-Garabal M, Balboa JL, Nunez MJ, Castano MT, Llovo JB, Fernandez-Rial JC *et al*. (1991). Effects of amphetamine on T-cell immune response in mice. Life Sci 49: PL107–PL112.

Freire-Garabal M, Nunez-Iglesias MJ, Rey-Mendez M, Pereiro-Raposo MD, Riveiro P, Fernandez-Rial JC *et al*. (1998). Effects of amphetamine on the development of Moloney sarcoma virus-induced tumors in mice. Oncol Rep 5: 381–383.

Freire-Garabal M, Nunez MJ, Balboa J, Rodriguez-Cobo A, Lopez-Paz JM, Rey-Mendez M *et al*. (1999). Effects of amphetamine on development of oral candidiasis in rats. Clin Diagn Lab Immunol 6: 530–533.

Friedman H, Newton C, Klein TW (2003). Microbial infections, immunomodulation, and drugs of abuse. Clin Microbiol Rev 16: 209–219.

Furst DE (2010). The risk of infections with biologic therapies for rheumatoid arthritis. Semin Arthritis Rheum 39: 327–346.

Gavrilin MA, Mathes LE, Podell M (2002). Methamphetamine enhances cell-associated feline immunodeficiency virus replication in astrocytes. J Neurovirol 8: 240–249.

Gerard C, Bruyns C, Marchant A, Abramowicz D, Vandenabeele P, Delvaux A *et al*. (1993). Interleukin 10 reduces the release of tumor necrosis factor and prevents lethality in experimental endotoxemia. J Exp Med 177: 547–550.

Gordon J, Barnes NM (2003). Lymphocytes transport serotonin and dopamine: agony or ecstasy? Trends Immunol 24: 438–443.

Green AR, Mechan AO, Elliott JM, O'Shea E, Colado MI (2003). The pharmacology and clinical pharmacology of 3,4-methylenedioxymethamphetamine (MDMA, 'Ecstasy'). Pharmacol Rev 55: 463–508.

Grob CS, Poland RE, Chang L, Ernst T (1996). Psychobiologic effects of 3,4-methylenedioxymethamphetamine in humans: methodological considerations and preliminary observations. Behav Brain Res 73: 103–107.

Hall AP, Henry JA (2006). Acute toxic effects of 'Ecstasy' (MDMA) and related compounds: overview of pathophysiology and clinical management. Br J Anaesth 96: 678–685.

Hegadoren KM, Baker GB, Bourin M (1999). 3,4-Methylenedioxy analogues of amphetamine: defining the risks to humans. Neurosci Biobehav Rev 23: 539–553.

House RV, Thomas PT, Bhargava HN (1995). Selective modulation of immune function resulting from *in vitro* exposure to methylenedioxymethamphetamine (Ecstasy). Toxicology 96: 59–69.

Ikeda H, Old LJ, Schreiber RD (2002). The roles of IFN gamma in protection against tumor development and cancer immunoediting. Cytokine Growth Factor Rev 13: 95–109.

In SW, Son EW, Rhee DK, Pyo S (2005). Methamphetamine administration produces immunomodulation in mice. J Toxicol Environ Health A 68: 2133–2145.

Jaehne EJ, Salem A, Irvine RJ (2008). The effect of long-term repeated exposure to 3,4-methylenedioxymethamphetamine on cardiovascular and thermoregulatory changes. Psychopharmacology (Berl) 201: 161–170.

Kankaanpaa A, Meririnne E, Lillsunde P, Seppala T (1998). The acute effects of amphetamine derivatives on extracellular serotonin and dopamine levels in rat nucleus accumbens. Pharmacol Biochem Behav 59: 1003–1009.

Keane J (2005). TNF-blocking agents and tuberculosis: new drugs illuminate an old topic. Rheumatology (Oxford) 44: 714–720.

Khairnar A, Plumitallo A, Frau L, Schintu N, Morelli M (2010). Caffeine enhances astroglia and microglia reactivity induced by 3,4-methylenedioxymethamphetamine ('Ecstasy') in mouse brain. Neurotox Res 17: 435–439.

Kim S, Iizuka K, Aguila HL, Weissman IL, Yokoyama WM (2000). *In vivo* natural killer cell activities revealed by natural killer cell-deficient mice. Proc Natl Acad Sci USA 97: 2731–2736.

Klein TW, Friedman H, Specter S (1998). Marijuana, immunity and infection. J Neuroimmunol 83: 102–115.

Klitzman RL, Pope HG, Jr, Hudson JI (2000). MDMA ('Ecstasy') abuse and high-risk sexual behaviors among 169 gay and bisexual men. Am J Psychiatry 157: 1162–1164.

Koch S, Galloway MP (1997). MDMA induced dopamine release *in vivo*: role of endogenous serotonin. J Neural Transm 104: 135–146.

Liechti ME, Vollenweider FX (2001). Which neuroreceptors mediate the subjective effects of MDMA in humans? A summary of mechanistic studies. Hum Psychopharmacol 16: 589–598.

McNamara R, Kerans A, O'Neill B, Harkin A (2006). Caffeine promotes hyperthermia and serotonergic loss following co-administration of the substituted amphetamines, MDMA ('Ecstasy') and MDA ('Love'). Neuropharmacology 50: 69–80.

McNamara R, Maginn M, Harkin A (2007). Caffeine induces a profound and persistent tachycardia in response to MDMA ('Ecstasy') administration. Eur J Pharmacol 555: 194–198.

Mechan AO, Esteban B, O'Shea E, Elliott JM, Colado MI, Green AR (2002). The pharmacology of the acute hyperthermic response that follows administration of 3,4-methylenedioxymethamphetamine (MDMA, 'Ecstasy') to rats. Br J Pharmacol 135: 170–180.

Mellon RD, Bayer BM (1998a). Evidence for central opioid receptors in the immunomodulatory effects of morphine: review of potential mechanism(s) of action. J Neuroimmunol 83: 19–28.

Mellon RD, Bayer BM (1998b). Role of central opioid receptor subtypes in morphine-induced alterations in peripheral lymphocyte activity. Brain Res 789: 56–67.

Montoya AG, Sorrentino R, Lukas SE, Price BH (2002). Long-term neuropsychiatric consequences of 'Ecstasy' (MDMA): a review. Harv Rev Psychiatry 10: 212–220.

Mossner R, Lesch KP (1998). Role of serotonin in the immune system and in neuroimmune interactions. Brain Behav Immun 12: 249–271.

Nash JF Jr, Meltzer HY, Gudelsky GA (1988). Elevation of serum prolactin and corticosterone concentrations in the rat after the administration of 3,4-methylenedioxymethamphetamine. J Pharmacol Exp Ther 245: 873–879.

Nelson DA, Tolbert MD, Singh SJ, Bost KL (2007). Expression of neuronal trace amine-associated receptor (Taar) mRNAs in leukocytes. J Neuroimmunol 192: 21–30.

Nelson DA, Nirmaier JL, Singh SJ, Tolbert MD, Bost KL (2008). Ecstasy (3,4-methylenedioxymethamphetamine) limits murine gammaherpesvirus-68 induced monokine expression. Brain Behav Immun 22: 912–922.

Nunez MJ, Fernandez-Rial JC, Couceiro J, Suarez JA, Gomez-Fernandez DE, Rey-Mendez M *et al*. (1993). Effects of amphetamine on influenza virus infection in mice. Life Sci 52: PL73–PL78.

Nunez-Iglesias MJ, Castro-Bolano C, Losada C, Pereiro-Raposo MD, Riveiro P, Sanchez-Sebio P *et al*. (1996). Effects of amphetamine on cell mediated immune response in mice. Life Sci 58: PL29–PL33.

Orio L, O'Shea E, Sanchez V, Pradillo JM, Escobedo I, Camarero J *et al*. (2004). 3,4-Methylenedioxymethamphetamine increases interleukin-1beta levels and activates microglia in rat brain: studies on the relationship with acute hyperthermia and 5-HT depletion. J Neurochem 89: 1445–1453.

Orio L, Llopis N, Torres E, Izco M, O'Shea E, Colado MI (2009). A study on the mechanisms by which minocycline protects against MDMA ('Ecstasy')-induced neurotoxicity of 5-HT cortical neurons. Neurotox Res (in press).

Pacifici R, Zuccaro P, Farre M, Pichini S, Di Carlo S, Roset PN *et al*. (1999). Immunomodulating properties of MDMA alone and in combination with alcohol: a pilot study. Life Sci 65: PL309–PL316.

Pacifici R, Zuccaro P, Farre M, Pichini S, Di Carlo S, Roset PN *et al*. (2001a). Effects of repeated doses of MDMA ('Ecstasy') on cell-mediated immune response in humans. Life Sci 69: 2931–2941.

Pacifici R, Zuccaro P, Hernandez Lopez C, Pichini S, Di Carlo S, Farre M *et al*. (2001b). Acute effects of 3,4-methylenedioxymethamphetamine alone and in combination with ethanol on the immune system in humans. J Pharmacol Exp Ther 296: 207–215.

Pacifici R, Zuccaro P, Farre M, Pichini S, Di Carlo S, Roset PN *et al.* (2002). Cell-mediated immune response in MDMA users after repeated dose administration: studies in controlled versus noncontrolled settings. Ann N Y Acad Sci 965: 421–433.

Pacifici R, Pichini S, Zuccaro P, Farre M, Segura M, Ortuno J *et al.* (2004). Paroxetine inhibits acute effects of 3,4-methylenedioxymethamphetamine on the immune system in humans. J Pharmacol Exp Ther 309: 285–292.

Pacifici R, Zuccaro P, Farre M, Poudevida S, Abanades S, Pichini S *et al.* (2007). Combined immunomodulating properties of 3,4-methylenedioxymethamphetamine (MDMA) and *Cannabis* in humans. Addiction 102: 931–936.

Parrott AC (2005). Chronic tolerance to recreational MDMA (3,4-methylenedioxymethamphetamine) or Ecstasy. J Psychopharmacol 19: 71–83.

Parrott AC, Buchanan T, Scholey AB, Heffernan T, Ling J, Rodgers J (2002). Ecstasy/MDMA attributed problems reported by novice, moderate and heavy recreational users. Hum Psychopharmacol 17: 309–312.

Pasparakis M, Alexopoulou L, Episkopou V, Kollias G (1996). Immune and inflammatory responses in TNF alpha-deficient mice: a critical requirement for TNF alpha in the formation of primary B cell follicles, follicular dendritic cell networks and germinal centers, and in the maturation of the humoral immune response. J Exp Med 184: 1397–1411.

de Paula VF, Ribeiro A, Pinheiro ML, Sakai M, Lacava MC, Lapachinske SF *et al.* (2009). Methylenedioxymethamphetamine (Ecstasy) decreases neutrophil activity and alters leukocyte distribution in bone marrow, spleen and blood. Neuroimmunomodulation 16: 191–200.

Pellegrino T, Bayer BM (1998a). *In vivo* effects of cocaine on immune cell function. J Neuroimmunol 83: 139–147.

Pellegrino TC, Bayer BM (1998b). Modulation of immune cell function following fluoxetine administration in rats. Pharmacol Biochem Behav 59: 151–157.

Pellegrino TC, Bayer BM (2002). Role of central 5-HT(2) receptors in fluoxetine-induced decreases in T lymphocyte activity. Brain Behav Immun 16: 87–103.

Pennock JW, Stegall R, Bubar MJ, Milligan G, Cunningham KA, Bourne N (2009). 3,4-Methylenedioxymethamphetamine increases susceptibility to genital herpes simplex virus infection in mice. J Infect Dis 200: 1247–1250.

Pezzone MA, Rush KA, Kusnecov AW, Wood PG, Rabin BS (1992). Corticosterone-independent alteration of lymphocyte mitogenic function by amphetamine. Brain Behav Immun 6: 293–299.

Prasad N, Cargill R, Wheeldon NM, Long CC, McDonald TM (1994). 'Ecstasy' and meningococcal meningitis. Infect Dis Clin Pract 3: 122–123.

Quie PG (1980). The phagocytic system in host defense. Scand J Infect Dis Suppl Suppl. 24: 30–32.

Quie PG, Mills EL (1979). Bactericidal and metabolic function of polymorphonuclear leukocytes. Pediatrics 64: 719–721.

Serafeim A, Gordon J (2001). The immune system gets nervous. Curr Opin Pharmacol 1: 398–403.

Shtrichman R, Samuel CE (2001). The role of gamma interferon in antimicrobial immunity. Curr Opin Microbiol 4: 251–259.

Stone DM, Merchant KM, Hanson GR, Gibb JW (1987). Immediate and long-term effects of 3,4-methylenedioxymethamphetamine on serotonin pathways in brain of rat. Neuropharmacology 26: 1677–1683.

Strangfeld A, Listing J, Herzer P, Liebhaber A, Rockwitz K, Richter C *et al.* (2009). Risk of herpes zoster in patients with rheumatoid arthritis treated with anti-TNF-alpha agents. JAMA 301: 737–744.

Tannenbaum CS, Hamilton TA (2000). Immune-inflammatory mechanisms in IFNgamma-mediated anti-tumor activity. Semin Cancer Biol 10: 113–123.

Thomas DM, Dowgiert J, Geddes TJ, Francescutti-Verbeem D, Liu X, Kuhn DM (2004). Microglial activation is a pharmacologically specific marker for the neurotoxic amphetamines. Neurosci Lett 367: 349–354.

Trinchieri G (2003). Interleukin-12 and the regulation of innate resistance and adaptive immunity. Nat Rev Immunol 3: 133–146.

de Waal Malefyt R, Haanen J, Spits H, Roncarolo MG, Velde A, Figdor C *et al.* (1991). Interleukin 10 (IL-10) and viral IL-10 strongly reduce antigen-specific human T cell proliferation by diminishing the antigen-presenting capacity of monocytes via downregulation of class II major histocompatibility complex expression. J Exp Med 174: 915–924.

Wang H, Yu M, Ochani M, Amella CA, Tanovic M, Susarla S *et al.* (2003). Nicotinic acetylcholine receptor alpha7 subunit is an essential regulator of inflammation. Nature 421: 384–388.

Yu Q, Zhang D, Walston M, Zhang J, Liu Y, Watson RR (2002). Chronic methamphetamine exposure alters immune function in normal and retrovirus-infected mice. Int Immunopharmacol 2: 951–962.

Zhang L, Shirayama Y, Shimizu E, Iyo M, Hashimoto K (2006). Protective effects of minocycline on 3,4-methylenedioxymethamphetamine-induced neurotoxicity in serotonergic and dopaminergic neurons of mouse brain. Eur J Pharmacol 544: 1–9.

Zwick OM, Fischer DH, Flanagan JC (2005). 'Ecstasy' induced immunosuppression and herpes zoster ophthalmicus. Br J Ophthalmol 89: 923–924.