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Acute 3,4-methylenedioxymethamphetamine (MDMA) administration produces a rapid and sustained suppression of immune function in the rat

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Abstract

(±)-3,4-methylenedioxymethamphetamine (MDMA;'Ecstasy') is a ring substituted phenylisopropylamine that is structurally related to both amphetamines and hallucinogens. The unique behavioural activating properties of MDMA have led to its widespread abuse. MDMA induces many neurochemical, behavioural and endocrine alterations which closely resemble those elicited by exposure to acute stress, suggesting that MDMA could be regarded as a 'chemical stressor'. In addition to the neurochemical, behavioural and endocrine effects of stressor exposure, it has been reported that stress produces alterations in immune function. However, to date the effects of MDMA on immune function have been restricted to in vitro investigations. In this study we report, for the first time, that acute in vivo administration 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 that persisted for at least 6 h following injection. These alterations in immune function were accompanied by a significant increase in plasma corticosterone concentrations 30 min post MDMA administration which had returned to baseline values within 6 h of drug administration. In addition, there was a significant depletion in cortical 5-HT concentrations both 30 min and 6 h after MDMA administration. The results of this study provide evidence that in addition to the well established toxic effects of MDMA on the central serotonergic system, a single administration of this widely abused drug induces a rapid and sustained suppression of immune function. © 1998

Keywords: MDMA; Ecstasy; Corticosterone; Immunosuppression; Lymphocyte proliferation; Leucocytes

1. Introduction

(±)-3,4-methylenedioxymethamphetamine (MDMA; 'Ecstasy') is a ring substituted phenylisopropylamine that is structurally related to both amphetamines and hallucinogens. The unique behavioural activating properties of MDMA have led to its widespread abuse in humans. In laboratory animals, acute MDMA administration causes an increase in the release of serotonin, increased locomotor activity, changes in body temperature and activation of both the hypothalamic pituitary adrenal (HPA) axis and sympathetic nervous system (SNS) (Stone et al., 1986; McNamara et al., 1995a,b; Gudelsky and Nash, 1996; Grob et al., 1996). Many of these

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MDMA-induced neurochemical, behavioural and endocrine alterations closely resemble those elicited by exposure to acute stress, suggesting that MDMA could be regarded as a 'chemical stressor'.

In addition to the neurochemical, behavioural and endocrine effects of stressor exposure, it has been reported that stress produces a suppression of immune function (Shavit et al., 1984; Connor et al., 1997) and alters immune cell distribution (Dhabhar et al., 1995; Millan et al., 1996; Connor et al., 1997). However, to date the effects of MDMA on immune function have been restricted to in vitro investigations (House et al., 1995); in these studies MDMA produced a suppression of IL-2 production at high doses, but an enhancement at lower doses, with intermediate doses failing to significantly alter this response. In addition, in vitro MDMA also produced a suppression of cytotoxic T-lymphocyte induction (House et al., 1995). B-lymphocyte function was not significantly altered by any of the MDMA concentrations used (House et al., 1995). With regard to the in vitro effects of MDMA on immune function, it is noteworthy that lymphocytes carry a high affinity serotonin transporter on their membrane (Faraj et al., 1994), which may facilitate the uptake of MDMA into these cells and permit MDMA to exert a direct effect on lymphocyte function.

Although in vitro data can be very valuable in assessing whether or not a substance such as MDMA has a direct effect on immune cells, such data also has its limitations for a number of reasons. Firstly, it is now well established that a dynamic equilibrium exists between the neuroendocrine and immune systems. Altered central nervous system (CNS) function can affect peripheral immunocompetence via the neuroendocrine and sympathetic nervous systems (Dantzer and Kelley, 1989; Black, 1994a). In addition, products of the immune system such as cytokines can modulate CNS function (Black, 1994b). MDMA produces profound effects on central serotonergic, and to a lesser extent noradrenergic, and dopaminergic neurotransmission (Commins et al., 1987; Piercey et al., 1990). One consequence of the effects of MDMA on central monoaminergic systems is the release of corticotropin-releasing factor (CRF) from the median eminence in the hypothalamus and consequently HPA-axis and SNS activation. It is well established that both increased HPA-axis and SNS activity can alter both leucocyte distribution and function (Bateman et al., 1989; Irwin, 1993; Ottaway and Husband, 1994; Dhabhar et al., 1995).

Secondly, peripheral metabolism of MDMA may yield active metabolites which may have direct or indirect effects on the immune system. However, in vitro studies do not account for the effect of metabolites of MDMA on immune function.

To date, there is a lack of in vivo data on the immunological effects of MDMA. However, some data has been generated on the immunological effects of another serotonin releaser namely, D-fenfluramine. Clancy and Lorens (1996) reported that subchronic treatment with D-fenfluramine in rats caused an enhancement of cellular immune function when administered at low doses (0.6–1.8 mg/kg). In contrast, a suppression of immune function was evident after treatment with high doses (3.0–9.0 mg/kg) of D-fenfluramine. However, previous work from this laboratory demonstrated that although both MDMA and D-fenfluramine cause serotonin release after acute administration, D-fenfluramine lacks the behavioural activating properties of MDMA (McGarvey et al., 1995). In addition, previous research which examined the effect of MDMA's parent compound, D-amphetamine, on HPA-axis activity and immune function reported that systemic D-amphetamine administration produced a potent activation of the HPA-axis (McGarvey, 1995), provoked a suppression of natural killer cell activity, inhibited cytotoxic T-lymphocyte responses (Nunez-Iglesias et al., 1996) and increased lethality due to influenza virus infection in mice (Nunez et al., 1993).

As stressor exposure has been previously associated with alterations in circulating leucocyte counts and lymphocyte proliferation responses (Shavit et al., 1984; Dhabhar et al., 1995; Millan et al., 1996; Connor et al., 1997), the aim of the present study was to examine the effect of acute MDMA administration on both circulating leucocyte number and the functional responsiveness of lymphocytes to mitogenic stimulation with Concanavalin A (Con A). In addition, cortical serotonin (5-HT) and 5-hydroxyindole acetic acid (5-HIAA) concentrations and serum corticosterone concentrations were evaluated after MDMA administration. As MDMA-induced neurochemical, endocrine and immune changes may demonstrate differential times of onset, and differen-

tial response duration, in the present study these parameters were examined at two different time-points (30 min and 6 h) following acute MDMA administration. Previous research in this laboratory has shown that the dose of MDMA used in the present study (20 mg/kg; i.p.) produces optimal behavioural, neurochemical and endocrine alterations in rats (McNamara et al., 1995a,b), and was therefore used to assess the effects of MDMA on the immune system.

2. Methods

2.1. Subjects and procedures

Female Sprague–Dawley rats (Harlan Olac, Bicester) weighing approximately 220–250 g were housed five per cage and maintained on a 12 h:12 h light:dark cycle (lights on at 08.00 h) in a temperature controlled room (22–24°C). Food and water were available ad libitum at all times.

2.2. Drug administration

MDMA (Plaistow, Cork, Ireland) was dissolved in 0.89% NaCl to give a concentration of 20 mg/ml. 0.89% saline was administered alone as a vehicle to the control group. Both MDMA and vehicle were administered in an injection volume of 1 ml/kg using the intraperitoneal (i.p.) route, and consisted of three treatment groups:

Group 1: Control

Group 2: MDMA (20 mg/kg) sacrificed 30 min post administration

Group 3: MDMA (20 mg/kg) sacrificed 6 h post administration.

To eliminate any confounding effect of injection stress, all animals used in the study received two injections. Animals in Group 1 received a saline injection both 6 h and 30 min prior to sacrifice, animals in Group 2 received a saline injection 6 h prior to sacrifice and an MDMA injection 30 min prior to sacrifice and animals in Group 3 received an MDMA injection 6 h prior to sacrifice and a saline injection 30 min prior to sacrifice. Animals in the control group were sacrificed intermittently between the MDMA treated animals.

2.3. Determination of brain biogenic amine concentrations

The rats were sacrificed by decapitation while under ether anaesthesia. After sacrifice, the brain was rapidly removed and the left frontal cortex was dissected on an ice cold plate (Popov et al., 1967). Concentrations of 5-HT and its metabolite 5-HIAA were measured by high performance liquid chromatography (HPLC) coupled with electrochemical detection (Seyfried et al., 1986). The frontal cortex was homogenised by sonication in 1.0 ml of mobile phase (pH 2.8) that was spiked with 20 ng/50 μl of N-methyl dopamine (Sigma Chemical, Poole, Dorset, U.K.) as an internal standard. The mobile phase contained 0.1 M citric acid (BDH Chemicals, Poole, Dorset, U.K.), 0.1 M sodium dihydrogen phosphate (BDH Chemicals, Poole, Dorset, U.K.), 1.4 mM octane-1-sulphonic acid (Sigma Chemical, Poole, Dorset, U.K.), 0.1 mM EDTA (BDH Chemicals, Poole, Dorset, U.K.) and 10% (v/v) methanol (Lab-Scan, Dublin, Ireland). The mobile phase was adjusted to pH 2.8 using 4 N NaOH (BDH Chemicals, Poole, Dorset, U.K.). Homogenates were centrifuged at 12,000 rpm in a Hettich Mikro/K refrigerated centrifuge for 15 min. A 20 μl sample of the supernatant was injected directly into a reverse phase column (LI Chrosorb RP-18, 25 cm $\times$ 4 mm internal diameter, particle size 5 μm) for separation of indoles and catecholamines (Flow rate 1 ml/min). An electrochemical detector (Shimadzu) was coupled to the HPLC system and was set at a potential of $+0.8$ V for the detection of monoamine neurotransmitters and metabolites. The neurotransmitters were quantified using a Merck-Hitachi D-2000 integrator. Neurotransmitter concentrations were expressed as ng of neurotransmitter per g fresh weight of brain tissue.

2.4. Serum corticosterone concentrations

Prior to sacrifice the animals were anaesthetised with diethyl ether (Lab Scan, Dublin, Ireland) and a blood sample was obtained by cardiac puncture into an anticoagulant free syringe. The blood was allowed to clot at room temperature. Serum corticosterone concentrations were measured using a modified method to that described previously (Glick et al., 1964). A corticosterone (Sigma Chemical, Poole,

Dorset, U.K.) stock solution (100 μg/dl) was prepared and diluted to produce a range of concentrations (10–80 μg/dl). Serum samples and corticosterone standards were then mixed in 600 μl dichloromethane (Lab Scan, Dublin, Ireland) for 15 s. 500 μl of the resulting dichloromethane extract phase was then transferred into a tube containing 400 μl of concentrated sulphuric acid (BDH Chemicals, Poole, Dorset, U.K.): absolute ethanol (Lab Scan, Dublin, Ireland) (65:35) and the tubes were mixed for 15 s using a vortex mixer. Samples were then placed in the dark for 45 min and a 300 μl aliquot of the lower phase was removed and the fluorescence measured at excitation 474 nm and emission 518 nm (Perkin Elmer LS-5 spectrophotofluorimeter). The results were expressed as μg corticosterone per dl of serum.

2.5. Total leucocyte counts

Prior to sacrifice the animals were anaesthetised with diethyl ether (Lab Scan, Dublin, Ireland) and a blood sample for the immune assays was obtained by cardiac puncture into a heparinized syringe. The total leucocyte counts were performed on heparinized whole blood samples using a haematology counter (Baker Diagnostics, Serono 9000).

2.6. Con A-induced lymphocyte proliferation

Heparinized blood was mixed with RPMI 1640 medium (Gibco Life Technologies, Scotland) (1:1 dilution) and layered onto 4 ml of Nycoprep (Nycomed AS, Olso, Norway) gradient. Following centrifugation (Beckman refrigerated benchtop centrifuge) at 600 *g* for 25 min at 20°C, the lymphocyte layer was removed and washed three times in RPMI 1640 medium. The cells were finally resuspended in complete RPMI 1640 medium (RPMI 1640 + 10% (v/v) heat inactivated fetal calf serum (Gibco Life Technologies, Scotland) + 2% (v/v) penicillin/streptomycin (Gibco Life Technologies, Scotland) and the number of lymphocytes was adjusted to 2×10^6/ml.

Lymphocyte proliferation was performed in triplicate as previously described (Mosmann, 1983; Luo et al., 1993). Briefly, 100 μl aliquots of the lymphocyte preparation were pipetted into wells of a 96 well microtiter plate. To each well was added either no mitogen for background transformation or concanavalin A (Con A) (Sigma Chemical, Poole, Dorset, U.K.) at a working concentration of 2.5 or 5.0 μg/ml. Cultures were incubated for 64 h at 37°C in a 5% CO_2 atmosphere prior to addition of 15 μl MTT (Promega, UK) per well, the cultures were then incubated for a further 8 h to allow the dye to be metabolized by actively proliferating cells. At the end of the culture period 100 μl of the solubilization reagent (Promega, UK) was added to each well of the 96 well microtitre plate and the plates were incubated overnight at 37°C in a 5% CO_2 atmosphere to facilitate complete solubilization of the MTT-formazan product. Microtitre plates were then read using an ELISA plate reader (Dynatech MR5000), at a wavelength of 550 nm, with a reference wavelength of 650 nm. The plates were shaken for 10 s prior to reading to ensure uniform colour distribution within the wells. Mean absorbance readings were calculated for each concentration of Con A and the background absorbance of unstimulated lymphocytes was subtracted from the mean absorbance of stimulated lymphocytes.

2.7. Statistical analysis of data

All data with the exception of the lymphocyte proliferation were analysed using a one-way analysis of variance. Lymphocyte proliferation data were analysed using a two-way analysis of variance. If any statistically significant change was found, post hoc comparisons were performed using Student Newman–Keuls multiple range test. Results were deemed significant when $P < 0.05$. Results are expressed as group mean ± standard error of the mean.

3. Results

3.1. Biogenic amine concentrations in the frontal cortex

There was a significant effect of MDMA administration on 5-HT ($F_{(2.27)} = 56.49$, $P < 0.001$) and 5-HIAA ($F_{(2,27)} = 7.09$, $P < 0.01$) concentrations in the frontal cortex. Post hoc analysis revealed a significant ($P < 0.05$) reduction in cortical 5-HT con-

Table 1
Effect of acute MDMA administration on 5-HT and 5-HIAA concentrations in the frontal cortex

Group	5-HT	5-HIAA
Control	448 ± 26	192 ± 8
MDMA (+30 min)	312 ± 31*	234 ± 54
MDMA (+6 h)	93 ± 6*	72 ± 1*

Data expressed as means ± SEM. Neurotransmitter concentrations are expressed as ng/g fresh weight of tissue. 5-MT: Serotonin; 5-HIAA: 5-hydroxyindoleacetic acid. * $P < 0.05$ vs. Control (Student Newman–Keuls multiple range test) ($n = 10$).

centrations both 30 min and 6 h post MDMA administration. In addition, cortical 5-HIAA concentrations were significantly ($P < 0.05$) reduced 6 h post MDMA administration (Table 1).

3.2. Serum corticosterone concentrations

There was a significant effect of MDMA administration on serum corticosterone concentrations ($F_{(2,27)} = 19.33$, $P < 0.05$). Post hoc analysis revealed a significant ($P < 0.05$) increase in serum corticosterone 30 min post MDMA administration. However, by 6 h post administration the concentrations were no longer significantly different from the control value (Fig. 1).

3.3. Total leucocyte counts

There was a significant effect of MDMA administration on total WBC counts ($F_{(2,27)} = 9.72$, $P <$ 0.001). Post hoc analysis revealed a significant ($P <$ 0.05) reduction in the total WBC count 30 min and 6 h after MDMA administration (Fig. 2).

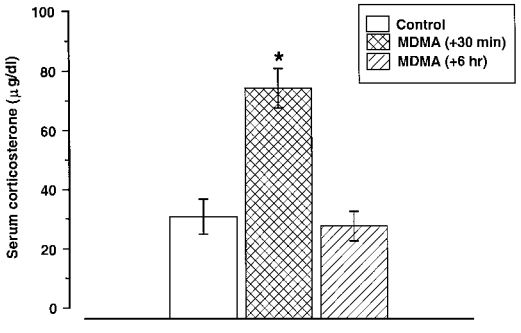

Fig. 1. Effect of acute MDMA administration on serum corticosterone concentrations. Data expressed as means ± SEM. * $P <$ 0.05 vs. Control (Student Newman–Keuls multiple range test) ($n = 10$).

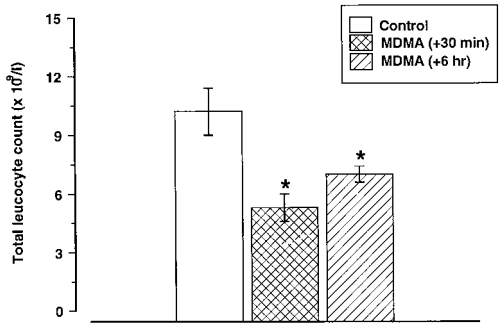

Fig. 2. Effect of acute MDMA administration on total leucocyte counts. Data expressed as means ± SEM. * $P < 0.05$ vs. Control (Student Newman–Keuls multiple range test) ($n = 10$).

3.4. Con A-induced lymphocyte proliferation

The two-way ANOVA revealed a significant effect of MDMA administration ($F_{(2,54)} = 14.05$, $P <$ 0.001) on Con A-induced lymphocyte proliferation. However, no significant effect of Con A concentration was observed. Post hoc analysis showed that there was a significant ($P < 0.05$) reduction in Con A-induced lymphocyte proliferation both 30 min and 6 h after MDMA administration at both concentrations of Con A (Fig. 3).

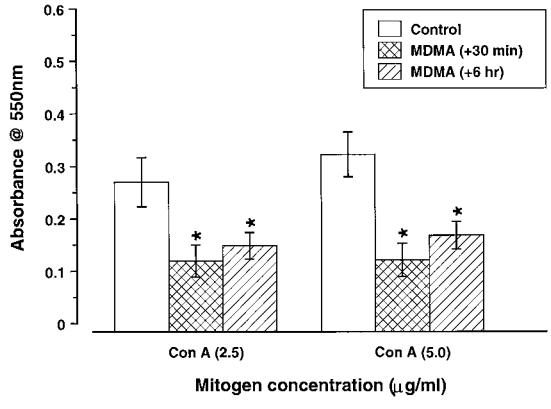

Fig. 3. Effect of acute MDMA administration on Con A-induced lymphocyte proliferation. Data expressed as means ± SEM. * $P <$ 0.05 vs. Control (Student Newman–Keuls multiple range test) ($n = 10$).

4. Discussion

These results demonstrate that acute administration of the substituted amphetamine MDMA produces a rapid and sustained suppression of mitogen stimulated lymphocyte proliferation and total leucocyte count in the female rat. In addition, 5-HT depletion in the frontal cortex was observed as a result of MDMA administration. The reduction in cortical 5-HT concentrations seen 30 min post MDMA administration was accompanied by a modest, but non-significant, increase in the 5-HIAA concentration. These alterations in cortical indoleamine concentrations reflect an increased release of 5-HT accompanied by increased synaptic metabolism of the amine. However, 6 h following MDMA administration there was a significant decrease in both 5-HT and 5-HIAA, which reflects a depletion of 5-HT stores and decreased 5-HT synthesis due to the inhibitory action of MDMA on tryptophan hydroxylase activity (Stone et al., 1987). These findings are consistent with previous reports which examined the effects of MDMA on 5-HT concentrations in rat brain (Stone et al., 1986; McGarvey et al., 1995; McNamara et al., 1995b).

Acute MDMA administration elicited a robust corticosterone response which was evident within 30 min and returned to baseline values within 6 h following administration. This result is consistent with previous findings (McGarvey et al., 1995; McNamara et al., 1995b). It is possible that this MDMA-induced increase in serum corticosterone concentrations could be responsible for the reduction in the total leucocyte count noted in this study. In support of this hypothesis it has been reported that adrenalectomy significantly attenuated stress-induced reductions in the total leucocyte count (Dhabhar et al., 1995). In addition, administration of exogenous corticosterone to non-adrenalectomized, non-stressed animals produced changes in leucocyte numbers that were similar in magnitude and duration to the changes induced by various stressors (Dhabhar et al., 1995; Connor et al., 1997). This suggests that it is the release of corticosterone from the adrenal cortex, and not adrenal catecholamines from the adrenal medulla, that mediate stressor-induced reductions in leucocyte numbers and possibly the MDMA-induced reduction in circulating leucocytes observed in the present study. It is well established that immune cell trafficking is central to the performance of the surveillance as well as effector functions of the immune system (Dhabhar et al., 1995). Therefore, such a reduction in the total blood leucocyte count in response to MDMA administration could potentially impair immune cell recruitment in vivo. From the results of the present study it is not clear how long it would take for the MDMA-induced reduction in circulating leucocyte numbers to return to control values, nor which subset(s) of leucocytes leave the peripheral blood in response to MDMA administration. However, it has been previously reported that corticosterone infusion causes lymphocytes to leave the peripheral blood without any significant alteration in neutrophil numbers (Dhabhar et al., 1995). Since MDMA administration causes a profound increase in circulating serum corticosterone, it is not unreasonable to suggest that the MDMA-induced reduction in leucocyte number seen in the present study may be due to a reduction in circulating lymphocytes.

In addition to the changes observed in leucocyte numbers in the present study, altered functional responsiveness of lymphocytes to a mitogenic stimulus was also apparent. There was a rapid (within 30 min) and sustained (for up to 6 h) suppression of Con A-induced lymphocyte proliferation as a result of acute MDMA administration. Con A is a T-lymphocyte mitogen (Woods and Greally, 1976) suggesting that the observed alterations in lymphocyte function were as a result of suppressed T-lymphocyte responsiveness. It is well established that glucocorticoids, including corticosterone, have immunosuppressive properties (Bateman et al., 1989), and many previous studies have implicated corticosterone to be a key mediator in stress-induced suppression of cell mediated immunity (CMI) (Shu et al., 1993; Song et al., 1994). However, others have demonstrated that stress-induced suppression of CMI occurs even in the absence of corticosterone secretion, suggesting that corticosterone secretion is not responsible for the impaired CMI after stress (Jaing et al., 1990; Jain et al., 1991). MDMA, through its action on the serotonergic system, causes the release of CRF from the nerve terminals in the median eminence. In addition to the role of CRF in HPA-axis activation, CRF also activates the sympathetic nervous system (SNS); it has been recently suggested that SNS

activation is an important contributory factor in stress-induced suppression of CMI (Irwin, 1993). However, from the results of the present study we cannot say whether the suppressive effect of MDMA on Con A-induced lymphocyte proliferation is mediated by increased SNS activity, or by elevated serum corticosterone concentrations. Further studies using treatment with adrenoceptor antagonists and glucocorticoid receptor antagonists/synthesis inhibitors prior to MDMA administration, should help to delineate the exact roles that increased SNS activity and increased HPA-axis activity play in MDMA-induced alterations in the lymphocyte function. The impaired lymphocyte proliferation response to Con A can not be attributed to a reduced number of circulating lymphocytes in the peripheral blood, as the lymphocytes were adjusted to a standard cell concentration (2×10^6 cells/ml) prior to mitogenic stimulation. However, the reduced responsiveness may be due to an alteration in lymphocyte subpopulations, such as a reduction in T-lymphocytes.

Apart from the hypothesis that reduced lymphocyte responsiveness may be due to a reduced number of T-lymphocytes, even in the absence of any significant alteration in T-lymphocyte numbers, there may be decreased functional responsiveness of lymphocytes to mitogenic stimulation. Such a decrease may occur in response to lymphocyte β-adrenoceptor activation by circulating catecholamines and a consequent increase in intracellular cyclic adenosine monophosphate (cAMP), as it is well established that increased intracellular cAMP concentrations produce a suppression of lymphocyte proliferation (Watson, 1975). It is noteworthy to mention that another stimulant drug of abuse, cocaine, produces a rapid and sustained suppression of Con A-induced lymphocyte proliferation in rats (Bayer et al., 1995). Cocaine administration in vivo, activates the HPA-axis and SNS and both of these mechanisms have been implicated to mediate the suppressive effects of this drug on the ex vivo lymphocyte proliferation response (Bayer et al., 1995).

In conclusion, acute MDMA administration produced a variety of time-dependent neurochemical, endocrine and immune alterations in the rat. The results of this study provide evidence that in addition to the well established toxic effects of MDMA on the central nervous system, a single administration of this widely abused drug induces a rapid and sustained suppression of immune function. These results add another dimension to the toxic effects of MDMA, implicating that abusers of this drug may have reduced immunocompetence and therefore display increased susceptibility to both infectious and neoplastic disease. In this regard it is of interest that there have been cases where abuse of MDMA closely preceded the development of meningococcal meningitis in humans (Prasad et al., 1994). However, to date there are no clinical studies available which examine ex vivo immunological parameters in MDMA abusers, and consequently it is not known if the doses of MDMA taken by recreational users have the ability to produce impairments in immune function like those observed in the present study.

References

Bateman, A., Singh, A., Kral, T., Solomon, S., 1989. The immune-hypothalmic–pituitary–adrenal–axis. Endo. Rev. 10, 92–112.

Bayer, B.M., Mulroney, S.E., Hernandez, M.C., Ding, X.Z., 1995. Acute infusions of cocaine result in time- and dose-dependent effects on lymphocyte responses and corticosterone secretion in rats. Immunopharmacology 29, 19–28.

Black, P.H., 1994a. Central nervous system–immune system interactions: Psychoneuroendocrinology of stress and its immune consequences. Antimicrob. Agents Chemother. 38, 1–6.

Black, P.H., 1994b. Immune system–central nervous system interactions: Effect and immunomodulatory consequences of immune system mediators on the brain. Antimicrob. Agents Chemother. 38, 7–12.

Clancy, J.J., Lorens, S., 1996. Subchronic and chronic exposure to D-fenfluramine dose-dependently enhances splenic immune functions in young and old male Fischer-344 rats. Behav. Brain Res. 73, 355–358.

Commins, D.L., Vosmer, G., Virus, R.M., Woolverton, W.L., Schuster, C.R., Seiden, L.S., 1987. Biochemical and histological evidence that methylenedioxymethamphetamine (MDMA) is toxic to neurons in rat brain. J. Pharmacol. Exp. Ther. 241, 338–345.

Connor, T.J., Kelly, J.P., Leonard, B.E., 1997. Forced swim test-induced neurochemical, endocrine and immune changes in the rat. Pharmacol. Biochem. Behav., in press.

Dantzer, R., Kelley, K.W., 1989. Stress and immunity: An integrated view of relationships between the brain and the immune system. Life Sci. 44, 1995–2008.

Dhabhar, F.S., Millar, A.H., McEwen, B.S., Spencer, R.L., 1995. Effects of stress on immune cell distribution, dynamics and hormonal mechanisms. J. Immunol. 154, 5511–5527.

Faraj, B.A., Olkowski, Z.L., Jackson, R.T., 1994. Expression of a high-affinity serotonin transporter in human lymphocytes. Int. J. Immunopharmacol. 16, 561–567.

Glick, D., Von Redlich, D., Levine, S., 1964. Fluorometric determination of corticosterone and cortisol in 0.02–0.05 millilitres of plasma or submilligram samples of adrenal tissue. Endocrinology 74, 653–655.

Grob, S.C., Russell, E.P., Chang, L., Ernst, T., 1996. Psychobiologic effects of 3,4-methylenedioxymethamphetamine in humans: Methodological considerations and preliminary observations. Behav. Brain Res. 73, 103–107.

Gudelsky, G.A., Nash, J.F., 1996. Carrier-mediated release of serotonin by 3,4-methylenedioxymethamphetamine: Implications for serotonin–dopamine interactions. J. Neurochem. 66, 243–249.

House, R.V., Thomas, P.T., Bhargava, H.N., 1995. Selective modulation of immune function resulting from in vitro exposure to methylenedioxymethamphetamine (Ecstasy). Toxicology 96, 59–69.

Irwin, M., 1993. Stress-induced immune suppression. Role of the autonomic nervous system. Ann. NY Acad. Sci. 692, 203–218.

Jain, R., Zwickler, D., Hollander, C.S., Brand, H., Saperstein, A., Hutchinson, A., Brown, C., Audhya, T., 1991. Corticotropin-releasing factor modulates the immune response to stress in the rat. Endocrinology 128, 1329–1336.

Jaing, C.G., Morrow-Tesch, J.L., Belier, D.I., Levy, E.M., Black, P.H., 1990. Immunosuppression in mice induced by cold water stress. Brain Behav. Immun. 4, 278–291.

Luo, Y.M., Cheng, X.J., Yuan, W.X., 1993. Effects of ginseng root saponins and ginsenoside Rb1 on immunity in cold water swim stress mice and rats. Acta Pharmacol. Sin. 14, 401–404.

McGarvey, C.M., 1995. The effects of pharmacological modulation of the serotonergic system on some neuroendocrine, neurochemical and behavioural parameters in the rat. Ph.D. Thesis, Department of Pharmacology, Univ. Coll. Galway, Natl. Univ. Ireland.

McGarvey, C., Kelly, J., O'Donnell, J.M., Leonard, B.E., 1995. Neuroendocrine and behavioural effects of the serotonin releasers 3,4-methylenedioxymetamphetamine and fenfluramine in rats. J. Serotonin Res. 4, 257–265.

McNamara, M.G., Kelly, J.P., Leonard, B.E., 1995a. Thermoregulatory and locomotor activity responses of 3,4-methylenedioxymethamphetamine (MDMA) in male and female rats. J. Serotonin Res. 4, 275–282.

McNamara, M.G., Kelly, J.P., Leonard, B.E., 1995b. The effect of acute MDMA administration on body temperature, serum corticosterone and neurotransmitter concentrations in male and female rats. Human Psychopharmacol. 10, 373–383.

Millan, S., Gonzalez-Quijano, M.I., Giordano, M., Soto, L., Martin, A.I., Lopez-Calderon, A., 1996. Short and long restraint differentially affect humoral and cellular immune functions. Life Sci. 59, 1431–1442.

Mosmann, T., 1983. Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. J. Immunol. Meth. 65, 55–63.

Nunez-Iglesias, M.J., Castro-Bolano, C., Losada, C., Perciro-Raposo, M.D., Riveiro, F., Sanchez-Sebio, P., Mayan-Santos, J.M., Rey-Mendez, M., Freire-Garabal, M., 1996. Effects of amphetamine on cell-mediated immune response in mice. Life Sci. 58, PL29–33.

Nunez, M.J., Fernandez-Rial, J.C., Coucciro, J., Suarez, J.A., Gomez-Fernandez, D.E., Rey-Mendez, M., Freire-Garabal, M., 1993. Effects of amphetamine on influenza virus infection in mice. Life Sci. 52, PL73–78.

Ottaway, C.A., Husband, A.J., 1994. The influence of neuroendocrine pathways on lymphocyte migration. Immunol. Today 15, 511–517.

Piercey, M.F., Lum, J.T., Palmer, J.R., 1990. Effects of MDMA ('ecstasy') on firing rates of serotonergic, dopaminergic and noradrenergic neurons in the rat. Brain Res. 526, 203–206.

Popov, N., Pohle, W., Rosler, V., Matthies, H., 1967. Regionale verteilung von gamma-aminobuttersaure, glutaminsaure, asparaginsaure, dopamin, noradrenalin and serotonin in rattenhirn. Acta Med. Biol. Germ. 18, 695–702.

Prasad, N., Cargill, R., Wheeldon, N.M., Long, C.C., McDonald, T.M., 1994. 'Ecstasy' and meningococcal meningitis. Infect. Dis. Clin. Pract. 3, 122–123.

Seyfried, C.A., Adam, G., Greve, T., 1986. An automated direct-injection HPLC method for the electrochemical/fluorometric quantitation of monoamines and related compounds optimized for the screening of large numbers of animals. Biomed. Chromatogr. 1, 78–88.

Shavit, Y., Lewis, J.W., Terman, G.W., Gale, R.P., Liebeskind, J.C., 1984. Opioid peptides mediated the suppressive effects of stress on natural killer cell cytotoxicity. Science 223, 188–190.

Shu, J., Stevenson, J.R., Zhou, X., 1993. Modulation of cellular immune responses by cold water swim stress in the rat. Dev. Comp. Immunol. 17, 357–371.

Song, C., Kelly, J.P., Leonard, B.E., 1994. The effect of stressful behavioral exposure on endocrine and immune parameters in the rat. Stress Med. 10, 239–245.

Stone, D.M., Stahl, D.C., Hanson, G.R., Gibb, J.W., 1986. The effects of 3,4-methylenedioxymethamphetamine and 3,4-methylenedioxyamphetamine on monoaminergic systems in rat brain. Eur. J. Pharmacol. 128, 41–48.

Stone, D.M., Merchant, K.M., Hanson, G.R., Gibb, J.W., 1987. Immediate and long-term effects of 3,4-methylenedioxymethamphetamine on serotonin pathways in the brain of rat. Neuropharmacology 26, 1677–1683.

Watson, J., 1975. The influence of intracellular levels of cyclic nucleotides on cell proliferation and the induction of antibody synthesis. J. Exp. Med. 141, 97–111.

Woods, R.J., Greally, G.F., 1976. A microculture technique in the evaluation of lymphocyte transformation by mitogens and specific antigens in humans. Ir. J. Med. Sci. 145, 369–377.