Comparative neuropharmacology of the substituted amphetamines, p-methoxyamphetamine (PMA) & 3,4-methylenedioxymethamphetamine (MDMA)

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Abstract

Dramatic growth in substituted amphetamines ('Ecstasy') use since the 1980's has correlated with increased incidence of acute toxicity and residual neuropsychological deficits. This thesis aimed to characterise the acute neurochemical mechanisms and residual neurochemical alterations produced by p-methoxyamphetamine (PMA), which is usually sold as 'ecstasy' and is associated with greater acute toxicity than 3,4-methylenedioxymethamphetamine (MDMA). While both PMA and MDMA primarily modulate dopaminergic and serotonergic neurotransmission, little is known of the differences in the neurochemical effects of PMA within the central nervous system, *in vivo*. This thesis used *in vivo* chronoamperometry to elucidate the acute neurochemical alterations in monoaminergic pharmacology *in vivo* after local application of PMA or MDMA within discrete brain nuclei in anaesthetised rats. Measurement of evoked release of monoamines including serotonin (5-HT), and inhibition of neurotransmitter uptake via membrane transporters were assessed.

Initial studies compared pharmacodynamic responses of PMA and MDMA, showing PMA to have greater efficacy and potency for alteration of core body temperature in rats, a primary cause of acute toxicity, within minimal alteration in locomotion. Dose-response studies indicated local PMA application within striatum resulted in significantly greater 5-HT evoked release than MDMA, yet lesser dopaminergic release, as predicted by the pharmacodynamic data. Only PMA-evoked release could be partially blocked by pre-treatment with a 5-HT reuptake inhibitor (SERT). Differences in both the qualitative and quantitative nature of striatal evoked-release of 5HT and dopamine were noted for both drugs, which had not been previously seen. Both PMA and MDMA inhibited 5-HT clearance, but only MDMA inhibited dopamine clearance in striatum. Dose-response studies in the CA3 region of hippocampus indicated PMA was also more efficacious than MDMA in the inhibition of 5-HT clearance *in vivo*.

While the question of whether long term MDMA use induces selective neurodegeneration (reductions in serotonergic *in vitro* biomarkers) is still unclear, it was not known for PMA prior to this work. Repeated PMA administration was shown to result in reductions in cortical SERT (indicative of potential loss of 5-HT terminal axons), cortical 5-HT content was unaltered. A subsequent comprehensive study followed, comparing the residual effects of PMA or MDMA

administration on *in vitro* serotonergic biomarkers (markers of selective neurodegeneration) and SERT function *in vivo*. PMA administration resulted in reductions in hippocampal SERT binding and [^{3}H]-5HT synaptosomal uptake, correlating with *in vitro* biomarkers previously used. SERT function *in vivo* using chronoamperometric techniques was reduced, as would be predicted. However, hippocampal 5-HT content was again not reduced, indicating that selective neurodegeneration of 5-HT fibres may not in fact be occurring. MDMA administration reduced all measured *in vitro* serotonergic biomarkers, however SERT function *in vivo* was completely unaltered. These data indicate that reductions of *in vitro* biomarkers of 5-HT axonal degeneration do not necessarily predict the potential compensatory mechanisms that maintain SERT function *in vivo*. Compensatory mechanisms appear to exist *in vivo* to maintain clearance of extracellular 5-HT that may be disrupted or eliminated during tissue preparation for *in vitro* assays.

In summary, while PMA produced significantly greater alterations, compared to MDMA, in processes intrinsic to 5-HT neurotransmission in both striatum and hippocampus, the magnitude of these responses did not explain the significantly higher risk of acute toxicity seen clinically with PMA use. The second component of the thesis extended beyond prior work, investigating the potential neurodegenerative effects of PMA and MDMA through the assessment of changes in *key functional processes* in 5-HT neurotransmisson. It is hoped this will contribute to the subsequent characterisation of the mechanism(s) of functional compensation in 5-HT neurotransmission which may lead to more targeted treatments to modulate potential psychological/psychiatric deficits that occur in regular 'ecstasy' users.

Declaration

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

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Daws L. C., Irvine R. J., **Callaghan P. D.**, Toop N. P., White J. M. and Bochner F. (2000) Differential behavioural and neurochemical effects of para-methoxyamphetamine and 3,4-methylenedioxymethamphetamine in the rat. Prog Neuropsychopharmacol Biol Psychiatry 24, 955-977.

Callaghan P. D., Irvine R. J. and Daws L. C. (2005) Differences in the *in vivo* dynamics of neurotransmitter release and serotonin uptake after acute para-methoxyamphetamine and 3,4-methylenedioxymethamphetamine revealed by chronoamperometry. Neurochem Int 47, 350-361.

Callaghan P. D., Farrand K., Salem A., Hughes P., Daws L. C. and Irvine R. J. (2006) Repeated administration of the substituted amphetamine p-methoxyamphetamine produces reductions in cortical 5-HT transporter binding but not 5-HT content, unlike 3,4-methylenedioxymethamphetamine. Eur J Pharmacol 546, 74-81.

Callaghan P. D., Owens W. A., Javors M. A., Sanchez T. A., Jones D. J., Irvine R. J. and Daws L. C. (2007) *In vivo* analysis of serotonin clearance in rat hippocampus reveals that repeated administration of p-methoxyamphetamine (PMA), but not 3,4-methylenedioxymethamphetamine (MDMA), leads to long-lasting deficits in serotonin transporter function. J Neurochem 100, 617-627.

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Paul Damian Callaghan, 25/8/08

Statement of Authorship and Contribution

Publication 1:

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Author Contribution:

Mr Callaghan had a major input in the experimental design, conducted all *in vivo* electrochemical experimental procedures, statistical analysis and graphical presentation of the data collected, and was involved in preparation of the manuscript for submission.

Miss Toop was involved in the experimental design, conducted telemetric studies and contributed to the interpretation of the data collected, and preparation of the manuscript.

Professor Bochner was involved in the experimental design, and contributed to the interpretation of the data collected and preparation of the manuscript.

Professor White was involved in the experimental design, and contributed to the interpretation of the data collected and preparation of the manuscript.

Associate Professor Irvine was involved in the experimental design, conducted telemetric studies and contributed to the interpretation of the data collected and preparation of the manuscript.

Associate Professor Daws was involved in the experimental design, contributed to the interpretation of the data collected and preparation of the manuscript

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Associate Professor Daws was involved in the experimental design, and contributed to the interpretation of the data collected and preparation of the manuscript

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Author Contribution:
Mr. Callaghan had a major input in the experimental design, conducted all HPLC analysis for experiment 2, conducted all statistical analysis and graphical presentation of the data collected, and prepared the manuscript for submission.
Dr. Farrand was involved in the experimental design, conducted experimental procedures for experiment 1, and interpretation of the data collected.
Mr. Hughes was involved in the experimental design and conducted animal procedures for experiment 2.
Dr. Salem was involved in the experimental design, contributed to the experimental procedures, interpretation of the data collected and preparation of the manuscript.
Associate Professor Irvine was involved in the experimental design, contributed to the experimental procedures, interpretation of the data collected and preparation of the manuscript.
Associate Professor Daws was involved in the experimental design, and contributed to the interpretation of the data collected and preparation of the manuscript.

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Publication 4:

Callaghan P. D., Owens W. A., Javors M. A., Sanchez T. A., Jones D. J., Irvine R. J. and Daws L. C. (2007) *In vivo* analysis of serotonin clearance in rat hippocampus reveals that repeated administration of p-methoxyamphetamine (PMA), but not 3,4-methylenedioxymethamphetamine (MDMA), leads to long-lasting deficits in serotonin transporter function. J Neurochem 100, 617-627.
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Author Contribution:
Mr Callaghan had a major input in the experimental design, conducted all animal procedures, *in vivo* electrochemical procedures, autoradiography procedures, preparation of samples for HPLC analysis, conducted all statistical analysis and graphical presentation of the data collected, and prepared the manuscript for submission.
Mr. Owens was involved in the autoradiographic procedures, and interpretation of the data collected.
Ms. Sanchez conducted *in vitro* hippocampal 5-HT and noradrenaline uptake procedures, and interpretation of the data collected.
Professor Jones was involved in the experimental design of *in vitro* hippocampal 5-HT and noradrenaline uptake procedures, contributed to the experimental procedures and interpretation of the data collected.
Professor Javors was involved in conducting HPLC analysis of extracted 5-HT samples, and interpretation of the data collected.
Associate Professor Irvine was involved in the experimental design, and contributed to the interpretation of the data collected and preparation of the manuscript.
Associate Professor Daws was involved in the experimental design, and contributed to the interpretation of the data collected and preparation of the manuscript.

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Auxiliary publications not for assessment for this thesis

The following publications would be presented as auxiliary publications, indicating my current research contribution to the field of the pharmacology of stimulants and drug abuse. These publications will not be presented for assessment for the degree of Doctor of Philosophy.

Kaminskas L.M., Irvine R.J., Callaghan P.D., White J.M. and Kirkbride P. 2002. The contribution of the metabolite p-hydroxyamphetamine to the central actions of p-methoxyamphetamine. Psychopharmacology (Berl) 160, 155-160. Impact factor: 3.625 (2006)

Daws L.C., Callaghan P.D., Moron J.A., Kahlig K.M., Shippenberg T.S., Javitch J.A. and Galli A. 2002. Cocaine increases dopamine uptake and cell surface expression of dopamine transporters. Biochem Biophys Res Commun 290, 1545-1550. Impact factor: 2.855 (2006)

Irvine, R.J., Keane, M., Felgate. P., McCann, U.D., Callaghan, P.D., White, J.M. 2005. Plasma Drug Concentrations and Physiological Measures in 'Dance Party' Participants. Neuropsychopharmacology. 31(2), 424-30. Impact factor: 5.889 (2006)

Abbreviations, prefixes and symbols

PMA	p-methoxyamphetamine
MDMA	3,4-methylenedioxymethamphetamine
CNS	Central nervous system
POHA	p-hydroxyamphetamine
5-HT	5-hydroxytryptamine
5-HIAA	5-hydroxy-indolacetic acid
MAO	Monoamine oxidase
SERT	5-HT transport protein
DAT	Dopamine transport protein
IC_{50}	Concentration of 50% inhibitory response
EC_{50}	Concentration of 50% response
E_{max}	Maximal response
MTA	Myoclonic twitch activity
PKC	Protein kinase C
NET	Noradrenaline transporter protein
KO	Knockout
GABA	gamma-hydroxybutyric acid
LD_{50}	Dose resulting in 50% lethality
CYP	Cytochrome P450 isozyme
MDA	Methylenedioxyamphetamine
HHMA	Dihydroxymethamphetamine
HHA	Dihydroxyamphetamine
OHMDMA	6-hydroxymethylenedioxymethamphetamine
THMA	2,4,5-trihydroxymethamphetamine
COMT	catechol-O-methyl transferase

MMAI	5-methoxy-6-methyl-2-aminoindan
BID	Twice daily
GFAP	Glial acidic fibrillary protein
PBR	Peripheral benzodiazepine receptor
kDa	Kilodalton
NO	Nitric oxide
nNOS	neural nitric oxide synthase
L-DOPA	L-3,4-dihydroxyphenylalanine
m-CPP	m-chlorophenylpiperazine
CSF	Cerebrospinal fluid
PET	Positron emission tomography
SPECT	Single photon emission computed tomography
OCT	Organic cation transporter
mRNA	Messenger ribonucleic acid

And the world's concerns with its rights and wrongs
Shall seem but small things
Poet or painter, a singer of songs,
Thine art is all things.

For the wine of life is a woman's love
To keep beside thee
But the love of Art is a thing above
A star to guide thee.

As the years go by with thy love of Art
All undiminished
Thou shalt end thy days with a quiet heart
Thy work is finished.

Passage from *'Ambition and Art'*, by A.B. 'Banjo' Patterson