



**INVESTIGATING THE INVOLVEMENT OF KAPPA
OPIOID RECEPTOR IN MEDIATING RELAPSE
RELATED TO MORPHINE/METHAMPHETAMINE
(POLY-DRUG) DEPENDENCE USING AN
IMMUNOHISTOCHEMISTRY TECHNIQUE**

BY

NUR SYAFINAZ BINTI WASLI

**A thesis submitted in fulfilment of the requirement for the
degree of Master in Pharmaceutical Sciences
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International Islamic University Malaysia**

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ABSTRACT

The upregulation of kappa opioid receptor (KOR) may result in dysphoria which could contribute to relapse towards various drugs of abuse. This research work is conducted to further investigate the involvement of KOR system in mediating relapse related to this poly-drug dependence at the brain level (striatum, amygdala, hippocampus, and prefrontal cortex). The reinstatement (relapse) model was initially developed for morphine (7.5 mg/kg), methamphetamine (1.0 mg/kg), and poly-drug (7.5 mg/kg and 1 mg/kg, respectively) using the conditioned place preference (CPP) paradigm. During reinstatement, a combination of 0.3 mg/kg buprenorphine and 1.0 mg/kg naltrexone (BUP/NTX) or saline was administered prior to the drug priming of morphine (2.5 mg/kg), methamphetamine (1.0 mg/kg), and poly-drug (2.5 mg/kg and 1 mg/kg, respectively). The change in KOR expression was quantitatively measured through the immunohistochemistry (IHC) technique by using the rabbit monoclonal antibody (EPR 18881) since it specifically binds at the KOR. Only the poly-drug group was investigated in order to evaluate the potential of this BUP/NTX treatment in IHC. The CPP results showed that the drug dependence models were successfully established in all groups, where the preference at the drug-paired compartment was significantly different ($p < 0.001$) compared to its baseline (23.45 ± 5.24 %, $n = 10$ vs. -8.55 ± 4.82 %, $n = 12$ [morphine]; 42.84 ± 6.83 %, $n = 12$ vs. -7.84 ± 4.31 %, $n = 14$ [methamphetamine]; and 34.91 ± 7.59 %, $n = 10$ vs. -11.16 ± 4.28 %, $n = 13$ [poly-drug]). During reinstatement, the BUP/NTX treatment successfully attenuated reinstatement to morphine (2.05 ± 11.04 %, $n = 11$ vs. -13.50 ± 5.18 %, $n = 13$, $p > 0.05$), but not for methamphetamine (35.03 ± 12.50 %, $n = 10$ vs. -6.75 ± 2.73 %, $n = 14$, $p < 0.05$). This treatment also successfully attenuated the reinstatement to poly-drug in the subgroup of mice that did not develop desensitisation behaviour (e.g., freezing behaviour), where the preference at the drug-paired compartment was not significantly different compared to its own baseline (19.14 ± 16.89 %, $n = 5$ vs. -16.14 ± 4.81 %, $n = 12$, $p > 0.05$). In IHC, only the striatum showed an increment in the KOR expression during reinstatement compared to post-conditioning in the saline group (33.390 ± 5.595 %, $n = 12$ vs. 16.730 ± 5.265 %, $n = 12$, $p < 0.01$). From the CPP results, it is suggested that the concomitant use of morphine and methamphetamine has triggered the opioid receptor system, which was not evidenced when methamphetamine alone was abused at low dose tested (1 mg/kg). Therefore, it is suggested that the KOR receptor system can be used as one of the targets to treat poly-drug dependence that involve opioid and methamphetamine.

خلاصة البحث

التنظيم الرفعي للمستقبلات الأفيونية من نوع كابا (KOR) قد ينتج عنه حالة الديسفوريا أو الانزعاج والتي بإمكانها المساهمة في حالات الإبتكاس وتعاطي العديد من المخدرات. تم إجراء هذه الدراسة لمواصلة التحقيق في ارتباط KOR في التوسط في الانتكاسات المتعلقة بهذا الإدمان المتعدد المخدرات على مستوى الدماغ (المخطَّط، واللوزة، والحصين، وقشرة الفص الجبهي). تم تطوير نموذج الإرجاع أو الإبتكاس مسبقا لعقار المورفين (7.5 ملغم/كغم)، والميثامفيتامين (1 ملغم/كغم)، والعقارات المتعددة (7.5 ملغم/كغم و 1 ملغم/كغم، على التوالي) باستخدام نموذج المكان المكيف المفضل (CPP). أثناء عملية الإرجاع، تم إعطاء مزيج من 0.3 ملغم/كغم من البورينورفين و 1 ملغم/كغم من النالتريكسون (BUP/NTX) أو محلول ملحي قبل الشروع بإعطاء المورفين (2.5 ملغم/كغم)، والميثامفيتامين (1 ملغم/كغم)، والعقارات المتعددة (2.5 مغ/كغ و 1 مغ/كغ، على التوالي). تم قياس التغير في تعبير المستقبلات الأفيونية من نوع كابا كيميا من خلال التصوير الكيميائي الهيستولوجي المناعي باستخدام الأجسام المضادة الأحادية النسيلة للأرانب (EPR 18881) لارتباطها التحديدي على KOR. تم التحقيق فقط في مجموعة العقارات المتعددة من أجل تقييم إمكانية علاج (BUP/NTX) من خلال الطريقة الكيميائية الهيستولوجية المناعية. أظهرت النتائج أنه تم إنشاء نماذج الإدمان على المخدرات بنجاح في جميع المجموعات، حيث كان التفضيل في القسم المرتبط بالمخدرات مختلفا بشكل ملحوظ ($p < 0.001$) مقارنة مع مجموعة خط الأساس ($n = 10$ مقابل $55.8 \pm 4.82\%$ ، $n = 12$ [مورفين]؛ $42.84 \pm 6.83\%$ ، $n = 12$ مقابل $7.84 \pm 4.31\%$ ، $n = 14$ [ميثامفيتامين]؛ و $34.91 \pm 7.59\%$ ، $n = 10$ مقابل $11.16 \pm 4.28\%$ ، $n = 13$ [عقارات متعددة]). تم تخفيف الإرجاع للمورفين بنجاح بواسطة علاج الـ BUP/NTX ($n = 11$ مقابل $13.50 \pm 5.18\%$ ، $n = 13$ ، $p < 0.05$)، على خلاف الميثامفيتامين ($n = 10$ مقابل $12.50 \pm 35.03\%$ ، $n = 13$ ، $p > 0.05$). أدى هذا العلاج أيضا إلى تخفيف الإرجاع إلى العقارات المتعددة في المجموعة الفرعية من الفئران التي لم تطور سلوك نزع الحساسية (على سبيل المثال، سلوك التجمد)، حيث التفضيل في المقصورة المخدرات يقتصر لا تختلف اختلافا كبيرا بالمقارنة مع خط الأساس الخاص بها ($n = 5$ مقابل $16.14 \pm 4.81\%$ ، $n = 12$ ، $p > 0.05$). أشارت نتائج التحليل الكيميائي الهيستولوجي المناعي أن مخطط الدماغ وحده أظهر زيادة في تعبير KOR أثناء الإرجاع مقارنة مع حالة ما بعد التكييف في المجموعة المعالجة بالأملح ($n = 12$ مقابل $5.595 \pm 33.390\%$ ، $n = 12$ مقابل $5.265 \pm 16.730\%$ ، $n = 12$ ، $p < 0.01$). اقترح من نتائج الـ CPP أن الاستخدام الملازم للمورفين والميثامفيتامين أثار نظام مستقبلات الأفيونيات، والذي لم يكن واضحا عندما تعاطي الميثامفيتامين وحده في اختبار جرعة منخفضة (1 ملغ/كغ). ولذلك يقترح أنه بإمكان نظام مستقبلات KOR أن تستخدم كأحد الأهداف في علاج الإدمان على العقارات العديدة المشتملة على الأفيون والميثامفيتامين.

APPROVAL PAGE

I certify that I have supervised and read this study and that in my opinion, it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a thesis for the degree of Master in Pharmaceutical Sciences (Pharmacology).

.....
Irna Elina Ridzwan
Supervisor

.....
Marwan Saad Abdulrahman
Azzubaidi
Co-Supervisor

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a thesis for the degree of Master in Pharmaceutical Sciences (Pharmacology).

.....
Wan Mohd Azizi Wan Sulaiman
Internal Examiner

.....
Sharif Mahsufi Mansor
External Examiner

This thesis was submitted to the Department of Basic Medical Sciences and is accepted as a fulfilment of the requirement for the degree of Master in Pharmaceutical Sciences (Pharmacology).

.....
Muhamad Rusdi Ahmad Rusmili
Head, Department of Basic
Medical Sciences

This thesis was submitted to the Kulliyah of Pharmacy and is accepted as a fulfilment of the requirement for the degree of Master in Pharmaceutical Sciences (Pharmacology).

.....
Juliana Md. Jaffri
Dean, Kulliyah of Pharmacy

DECLARATION

I hereby declare that this thesis is the result of my own investigations, except where otherwise stated. I also declare that it has not been previously or concurrently submitted as a whole for any other degrees at IIUM or other institutions.

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“In the name of Allah, the Most Compassionate, the Most Merciful. Praise be to Allah, Lord of the universe, and peace and prayers be upon His final Prophet and Messenger”

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TABLE OF CONTENTS

Abstract	ii
Abstract in Arabic	iii
Approval page	iv
Declaration	vii
Copyright Page.....	viii
Acknowledgements	viii
Table of Contents	ix
List of Tables	xiii
List of Figures	xiv
List of Equations	xviii
List of Abbreviations	xix
CHAPTER ONE: INTRODUCTION	1
1.1 Research Background	1
1.2 Research Problem	5
1.3 Significance of Study.....	5
1.4 Objective(s) of Research.....	6
1.4.1 General Objective.....	6
1.4.2 Specific Objectives.....	6
1.5 Research Hypothesis.....	6
CHAPTER TWO: LITERATURE REVIEW	7
2.1 Overview of Drug Addiction	7
2.1.1 Stages of Drug Addiction.....	7
2.2 Brain Region in Drug Addiction	11
2.2.1 Striatum.....	12
2.2.1.1 Brief anatomy of striatum.....	12
2.2.1.2 The role of striatum in addiction	13
2.2.2 Amygdala	15
2.2.2.1 Brief anatomy of amygdala	15
2.2.2.2 Role of amygdala in addiction.....	16
2.2.3 Prefrontal Cortex.....	17
2.2.3.1 Brief anatomy of prefrontal cortex	17
2.2.3.2 The role of prefrontal cortex in addiction.....	18
2.2.4 Hippocampus.....	21
2.2.4.1 Brief anatomy of hippocampus	21
2.2.4.2 The role of hippocampus in addiction	22
2.3 Neurocircuitry of Drug Addiction	24
2.3.1 Opioid and its Neurocircuitry Addiction.....	27
2.3.1.1 Opioids	27
2.3.1.2 Neurocircuitry of opioids addiction.....	28
2.3.2 Psychostimulant and its Neurocircuitry of Addiction.....	30
2.3.2.1 Psychostimulant.....	30
2.3.2.2 Neurocircuitry of psychostimulant addiction	31
2.3.3 Overlapping of Poly-drug Neurocircuits.....	35

2.4 Treatment in Drug Addiction.....	35
2.4.1 Methadone.....	36
2.4.2 Buprenorphine.....	37
2.4.3 Combination of Buprenorphine and Naltrexone.....	38
2.5 Kappa Opioid Receptor (KOR) System in Drug Addiction.....	39
2.5.1 KOR Activation in Mediating Addiction.....	39
2.5.2 KOR as a Target for Reinstatement (Relapse) Prevention.....	41
2.5.3 Kappa Antagonist Treatment for Morphine/Methamphetamine (Poly-drug) Addiction.....	43
2.6 Animal Model in Drug Addiction Study.....	44
2.6.1 Conditioned Place Preference (CPP) Test.....	44
2.7 Quantifying the Involvement of the Kappa Opioid Receptor (KOR) in Addiction Cycle.....	48
2.7.1 Immunohistochemistry (IHC) Technique.....	49
CHAPTER THREE: METHODOLOGY.....	55
3.1 Materials.....	55
3.1.1 Subjects.....	55
3.1.2 Drugs and Chemicals.....	55
3.2 Conditioned Place Preference (CPP) Test.....	56
3.2.1 Apparatus.....	56
3.2.2 Procedures.....	57
3.2.3 Data Analysis.....	60
3.3 Histopathology.....	62
3.3.1 Equipments.....	62
3.3.2 Chemicals.....	62
3.3.3 Reagents for Immunohistochemistry.....	62
3.3.4 Poly-L-lysine Glass Slide Preparation.....	63
3.3.5 Tissue Processing.....	63
3.3.6 Immunohistochemistry (IHC).....	66
3.3.6.1 Deparaffinization.....	66
3.3.6.2 Pretreatment.....	67
3.3.6.2.1 Preparation of antigen retrieval solution, (pH 9.0, concentration 10×).....	68
3.3.6.3 Staining procedure.....	68
3.3.6.3.1 Preparation of primary antibody.....	69
3.3.6.4 Counterstaining.....	69
3.3.6.5 Dehydration process.....	70
3.3.7 Optimisation of Primary Antibody Concentration.....	70
3.3.8 Image Acquisition.....	71
3.3.9 Interpretation of the IHC Slide.....	71
3.3.10 Counting Method Validation.....	72
3.3.11 Statistical Analysis.....	73
CHAPTER FOUR: RESULTS AND FINDINGS.....	74
4.1 Conditioned Place Preference (CPP) Test.....	74
4.1.1 Establishment of a Drug Dependence Model.....	74
4.1.1.1 Morphine-dependent group.....	74
4.1.1.2 Methamphetamine-dependent group.....	75

4.1.1.3 Morphine/methamphetamine (poly-drug)-dependent group	75
4.1.2 Establishment of A Drug Reinstatement (Relapse) Model	77
4.1.2.1 Morphine-dependent group (control)	77
4.1.2.2 Morphine-dependent group (combination of buprenorphine/naltrexone treatment).....	78
4.1.2.3 Methamphetamine-dependent group (control)	80
4.1.2.4 Methamphetamine-dependent group (combination of buprenorphine/naltrexone treatment).....	81
4.1.2.5 Morphine/methamphetamine (poly-drug)-dependent group (control)	82
4.1.2.6 Morphine/methamphetamine (poly-drug)-dependent group (combination of buprenorphine/naltrexone treatment).....	84
4.2 Immunohistochemistry (IHC).....	87
4.2.1 Optimisation of Primary Antibody Concentration in Hippocampus	87
4.2.2 Quantifying the Kappa Opioid Receptor Expression in Morphine/methamphetamine (poly-drug)-dependent Group	88
4.2.2.1 Prefrontal cortex	88
4.2.2.1.1 Microscopic view of prefrontal cortex.....	89
4.2.2.2 Striatum	91
4.2.2.2.1 Microscopic view of striatum	92
4.2.2.3 Hippocampus	94
4.2.2.3.1 Microscopic view of hippocampus	95
4.2.2.4 Amygdala	97
4.2.2.4.1 Microscopic view of amygdala.....	98
4.2.3 Counting Method Validation in IHC Group	101

CHAPTER FIVE: DISCUSSION.....103

5.1 Conditioned Place Preference as a Tool to Investigate Drug Reinstatement (Relapse) and its Potential Treatment.....	103
5.2 Buprenorphine / Naltrexone as a Potential Treatment for a Single Drug and Poly-drug Dependence	104
5.2.1 Buprenorphine/Naltrexone Treatment in Single Drug Dependence.....	104
5.2.1.1 Buprenorphine/naltrexone treatment in morphine dependence	104
5.2.1.2 Buprenorphine/naltrexone treatment in methamphetamine dependence	106
5.2.1.3 Buprenorphine/naltrexone treatment in morphine/methamphetamine (poly-drug) dependence	107
5.3 The Changes in the Kappa Opioid Receptor Expression in Morphine/Methamphetamine (Poly-drug) Dependence.....	108
5.3.1 The Kappa Opioid Receptor Expression in Striatum.....	108
5.3.2 The Kappa Opioid Receptor Expression in Amygdala and Hippocampus	109
5.3.3 The Kappa Opioid Receptor Expression in Prefrontal Cortex.....	112

CHAPTER SIX: CONCLUSION	113
6.1 Conclusion	113
6.2 Limitation of Studies	113
6.3 Future Studies	114
REFERENCES.....	115
APPENDIX I: ANIMAL ETHICS APPROVAL	131
APPENDIX II: PRESENTATION, PUBLICATION AND CERTIFICATE ..	132

LIST OF TABLES

<u>Table No.</u>		<u>Page No.</u>
3.1	Preparation of Stock Solution of Drugs for CPP Test	56
3.2	Mice Grouping for CPP Test	60
3.3	Concentration of Primary Antibody Dilution and Incubation Time Tested	70
4.1	The Number of Mice Used in the Experiments during Baseline and Post-conditioning	77
4.2	The Number of Mice Used in the CPP Experiments, Excluded at Each Stage, and Time Taken to Reach Extinction	86
4.3	The Number of Sample (Brain Tissue) Used in the IHC Experiment at Each Stage	100
4.4	The Total Number of Positive and Negative Cells, Percentage of Positive Cells at Different CPP Stages in Hippocampus Region by Comparing the Investigator and the Double-blinded Person Findings	102

LIST OF FIGURES

<u>Figure No.</u>		<u>Page No.</u>
1.1	World Drug Report 2016 from Year 1998 to 2014 (UNODC, 2016)	2
1.2	Statistic of Drug Usage from Year 2010 to 2016 (NADA, 2016)	2
2.1	Stages of Drug Addiction Comprising of Binge, Withdrawal, and Preoccupation Stage (Koob & Moal, 2008)	8
2.2	Action of Various Addictive Drugs at Ventral Tegmental Area and Nucleus Accumbens (Nestler, 2005)	9
2.3	Four Major Lobes of the Brain (WHO, 2004)	11
2.4	Stages in Drug Addiction and the Major Brain Regions Involved. (Adapted from Herman & Roberto, 2015)	12
2.5	Subregions of Basal Ganglia	13
2.6	The Location of Extended Amygdala in the Brain	15
2.7	Location of Prefrontal Cortex in the Brain	17
2.8	Subregions of Prefrontal Cortex	18
2.9	Projection from Medial Prefrontal Cortex to Other Brain Regions Related to Drug Seeking Behaviour and Fear Conditioning (Peters et al., 2009)	20
2.10	Location of Hippocampus in the Brain	21
2.11	The Components of Hippocampus	21
2.12	Limbic System that Controls the Reward Circuit (Taylor, Lewis, & Olive, 2013)	24
2.13	Reward Pathway which Involved Dopamine Release from Ventral Tegmental Area (VTA) (NIDA, 2016c)	25
2.14	Mesocorticolimbic Pathway which Consists of the Mesolimbic and Mesocortical Limbic Pathways (Arias-Carrión et al., 2010)	26
2.15	Mechanism of Dopamine Release in Opioid Addiction	28
2.16	G-protein Coupled Receptor (GPCR) Family	30

2.17	Chemical Structures of the Monoamine Neurotransmitters, Amphetamine Type Stimulants and Cocaine (Richards et al., 2014)	33
2.18	Mechanism of Dopamine Release into the Synaptic Cleft in Methamphetamine Addiction (Kish, 2008)	34
2.19	Mechanism of Mood Modulation by the Opioid Receptor System (Carroll & Carlezon, 2013)	42
3.1	A Three-Compartments Conditioned Place Preference (CPP) Box (upper view)	57
3.2	Schematic Timeline in CPP Procedure	58
3.3	Glass Slides Were Soaked with 10 % of Poly-L-lysine Solution for 20 Minutes	63
3.4	The Brain Were Fixed in 10 % Formalin as Preservative Reagent	64
3.5	(a) Embedding Machine for the Preparation of Tissue Block (b) Tissue Block	65
3.6	Microtome Machine for the Tissue Cutting Procedure	66
3.7	Commercial Microwave for Antigen Retrieval Procedure	67
4.1	The CPP Test for Morphine-dependent Group. Data were presented as mean \pm SEM ($n = 10$) and analysed using paired-samples t -test. *** indicates an extremely significant difference from baseline ($p < 0.001$)	74
4.2	The CPP Test for Methamphetamine-dependent Group. Data were presented as mean \pm SEM ($n = 11$) and analysed using paired-samples t -test. *** indicates an extremely significant difference from baseline ($p < 0.001$)	75
4.3	The CPP Test for Morphine/methamphetamine (poly-drug)-dependent Group. Data were presented as mean \pm SEM ($n = 10$) and analysed using paired-samples t -test. *** indicates an extremely significant difference from baseline ($p < 0.001$)	76
4.4	The CPP Test for Morphine-dependent Control Group (reinstatement model). Data were presented as mean \pm SEM ($n = 14$) and analysed using paired-samples t -test. *** indicates an extremely significant difference from baseline ($p < 0.001$)	78
4.5	The CPP Test for Morphine-dependent Treated Group (reinstatement model). Data were presented as mean \pm SEM ($n = 14$) and analysed using paired-samples t -test. *** indicates an extremely significant difference from baseline ($p < 0.001$)	79

- 4.6 The CPP Test for Methamphetamine-dependent Control Group (reinstatement model). Data were presented as mean \pm SEM ($n = 14$) and analysed using paired-samples t -test. * indicates a significant difference from baseline ($p < 0.05$) and *** indicates an extremely significant difference from baseline ($p < 0.001$) 81
- 4.7 The CPP Test for Methamphetamine-dependent Treated Group (reinstatement model). Data were presented as mean \pm SEM ($n = 14$) and analysed using paired-samples t -test. * indicates a significant difference from baseline ($p < 0.05$) and *** indicates an extremely significant difference from baseline ($p < 0.001$) 82
- 4.8 The CPP Test for Morphine/methamphetamine-dependent (poly-drug) Control Group. Data were presented as mean \pm SEM ($n = 14$) and analysed using paired-samples t -test. ** indicates a very significant difference from baseline ($p < 0.01$). *** indicates an extremely significant difference from baseline ($p < 0.001$) 83
- 4.9 The CPP Test for Morphine/methamphetamine (poly-drug)-dependent Buprenorphine/naltrexone Treated Group (reinstatement model). Data were presented as mean \pm SEM ($n = 14$) and analysed using paired-samples t -test. * indicates a significant difference from baseline ($p < 0.05$), ** indicates a very significant difference from baseline ($p < 0.01$) and *** indicates an extremely significant difference from baseline ($p < 0.001$) 85
- 4.10 Primary Antibody Used at Different Concentrations. Microscopic view under 40 \times magnification at different concentration ratio of primary antibody with 30 minutes of incubation time; (a) concentration ratio at 1:100 (b) concentration ratio at 1:300 (c) concentration ratio at 1:500 (d) concentration ratio at 1:1000 87
- 4.11 The Expression of the Kappa Opioid Receptor in Prefrontal Cortex at Different Stages of CPP. Data were presented as mean \pm SEM and analysed using unpaired-samples t -test ** indicates a very significant difference from post-conditioning ($p < 0.01$) and *** indicates an extremely significant difference ($p < 0.001$) 89
- 4.12 Prefrontal Cortex Region Viewed Under Different Magnifications at Different CPP Stages. (a) 4 \times magnification (b) 10 \times magnification and (c) Post-conditioning (40 \times magnification) (d) Reinstatement (saline) (40 \times magnification) (e) Reinstatement (BUP/NTX) (not freezing) (40 \times magnification) (f) Reinstatement (BUP/NTX) (freezing) (40 \times magnification) 90
- 4.13 The Expression of the Kappa Opioid Receptor in Striatum at Different Stages of CPP. Data were presented as mean \pm SEM and analysed using unpaired-samples t -test. * indicates a significant difference ($p < 0.05$) from post-conditioning ** indicates a very significant

	difference ($p < 0.01$), and *** indicates an extremely significant difference ($p < 0.001$)	92
4.14	Striatum Viewed Under Different Magnifications at Different CPP Stages. (a) 4× magnification (b) 10× magnification and (c) Post-conditioning (40× magnification) (d) Reinstatement (saline) (40× magnification) (e) Reinstatement (BUP/NTX) (not freezing) (40× magnification) (f) Reinstatement (BUP/NTX) (freezing) (40× magnification)	93
4.15	The Expression of the Kappa Opioid Receptor in Hippocampus at Different Stages of CPP. Data were presented as mean \pm SEM and analysed using unpaired-samples t -test. *** indicates an extremely significant difference from post-conditioning ($p < 0.001$)	95
4.16	Hippocampus Viewed Under Different Magnifications at Different CPP Stages. (a) 4× magnification (b) 10× magnification and (c) Post-conditioning (40× magnification) (d) Reinstatement (saline) (40× magnification) (e) Reinstatement (BUP/NTX) (not freezing) (40× magnification) (f) Reinstatement (BUP/NTX) (freezing) (40× magnification)	96
4.17	The Expression of the Kappa Opioid Receptor in Amygdala at Different Stages of CPP. Data were presented as mean \pm SEM and analysed using unpaired-samples t -test. *** indicates an extremely significant difference from post-conditioning ($p < 0.001$)	98
4.18	Amygdala Viewed Under Different Magnifications at Different CPP Stages. (a) 4× magnification (b) 10× magnification and (c) Post-conditioning (40× magnification) (d) Reinstatement (saline) (40× magnification) (e) Reinstatement (BUP/NTX) (not freezing) (40× magnification) (f) Reinstatement (BUP/NTX) (freezing) (40× magnification)	99
4.19	The Percentage of Positive Cells Counted by the Investigator and Double-blinded Person. Data were presented as mean \pm SEM and analysed using unpaired-samples t -test whenever appropriate	101

LIST OF EQUATIONS

<u>Equations No.</u>		<u>Page No.</u>
3.1	Correction Factor = Duration of test \div Total time in A + B	61
3.2	% Preference = (Time in A \div Duration of Test) \times Correction Factor \times 100 %	61
3.3	% Preference = (Time in B \div Duration of Test) \times Correction Factor \times 100 %	61
3.4	Volume of AR (mL) = Final volume (mL) \div Concentration of AR (10 X)	68
3.5	Volume of diluted AR (mL) = Volume of AR (mL) + Distilled water (mL)	68
3.6	Volume of Ab (μ L) = Final volume (μ L) \div Dilution factor	69
3.7	Volume of diluted Ab (μ L) = Volume of Ab (μ L) + Ab diluent (μ L)	69
3.8	% Positive cells = (Positive cells \div Total cells) \times 100 %	73

LIST OF ABBREVIATIONS

5-HT	Serotonin
AMG	Amygdala
AR	Antigen Retrieval
ATS	Amphetamine-Type Stimulants
BA	Basal
BNST	Bed Nucleus of the Stria Terminalis
BUP	Buprenorphine
CA	<i>cornu ammonis</i>
CaMKII α	Calcium/calmodulin-dependent Protein Kinase II α Isoform
cAMP	cyclic Adenosine Monophosphate
CE	Central
CeA	Central Nucleus of the Amygdala
CNS	Central Nervous System
CPP	Conditioned Place Preference
CREB	cAMP Response Element-Binding
CRF	Corticotropin-Releasing Factor
DA	Dopamine
DAB	3,3'-Diaminobenzidine
DAT	Dopamine Transporter
DOR	Delta Opioid Receptor
DPX	Distyrene Plasticizer Xylene
DS	Dorsal Striatum
FDA	Food and Drug Administration
GABA	γ -Aminobutyric Acid
GDP	Guanosine Diphosphate
GPCR	G-Protein Coupled Receptor
GTP	Guanosine Triphosphate
HIV	Human Immunodeficiency Virus
HPC	Hippocampus
IHC	Immunohistochemistry

IL	Infralimbic
Ip	Intraperitoneal Injection
ITC	Intercalated
K ⁺	Potassium Ion
KOR	Kappa Opioid Receptor
LA	Lateral
LTD	Lateral Dorsal Tegmentum
MDMA	3,4-Methylenedioxy-N-Methyl-Amphetamine (Ecstasy)
MMT	Methadone Maintenance Treatment
MOR	Mu Opioid Receptor
mPFC	medial Prefrontal Cortex
NAc	Nucleus Accumbens
NAc-Sh	Transition zone in the medial shell subregion of the Nucleus Accumbens
NADA	National Anti-Drug Agency
NE	Norepinephrine
NOP	Nociceptin Opioid Peptide
nor-BNI	Norbinaltorphimine
NTX	Naltrexone
oPFC	orbital Prefrontal Cortex
PCR	Polymerase Chain Reaction
PFC	Prefrontal Cortex
PL	Prelimbic
PPT	Peduncular Pontine Tegmentum
rCMgIC	regional Cerebral Metabolic Rate for Glucose
<i>SEM</i>	Standard Error of the Mean
UNODC	United Nations Office on Drug Crime
VMAT-2	Vesicular Monoamine Transporter-2
VTA	Ventral Tegmental Area

CHAPTER ONE

INTRODUCTION

1.1 RESEARCH BACKGROUND

Drug addiction is a chronic and relapsing brain disorder that can cause an uncontrolled compulsion to drug seeking behaviour despite of its negative consequences such as negative emotional state (e.g., dysphoria and anxiety) and withdrawal syndrome (Koob & Volkow, 2010; Trigo, Martin-García, Berrendero, Robledo, & Maldonado, 2010). The commonly abused drugs include alcohol, heroin, methamphetamine, cannabis (marijuana), ketamine, tobacco (nicotine), and inhalants. All these drugs can to lead harmful risks such as addiction, drugged driving, and infectious diseases (NIDA, 2016b).

Based on the latest worldwide statistics from the United Nations Office on Crime (UNODC), the amphetamine-type stimulants (ATS) is the highest drug seized by the authority since 2009. It also reported that methamphetamine was the main drug from ATS class that being seized, with the South and East Asia, as well as North America being the leading countries (UNODC, 2016). This was followed by opioids, cocaine, and cannabis (Figure 1.1) (UNODC, 2016).

In Malaysia, the National Anti-Drug Agency (NADA) in 2016 reported that opioids and methamphetamine are ranked first and second for the mostly abused drugs from the year of 2010 to 2016, with the recent usage percentage of 53.47 % and 31.82 % in 2016, respectively. It was followed by other amphetamine-type stimulants (10.69 %) and cannabis (3.89 %) as shown in Figure 1.2 (NADA, 2016). However, NADA has

separately classified methamphetamine from ATS, unlike UNODC, for the drug classification.

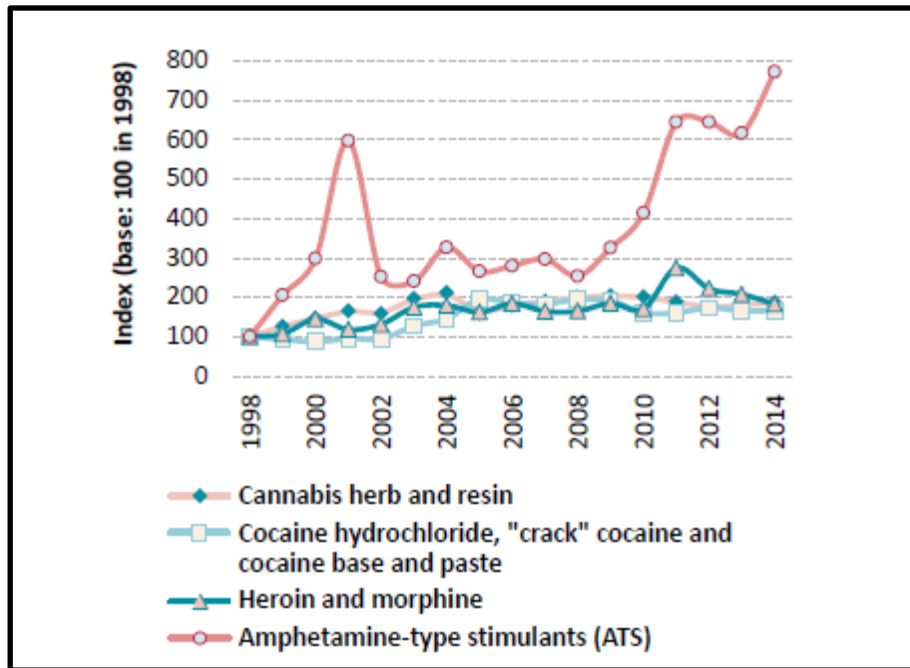


Figure 1.1 World Drug Report 2016 from Year 1998 to 2014 (UNODC, 2016)

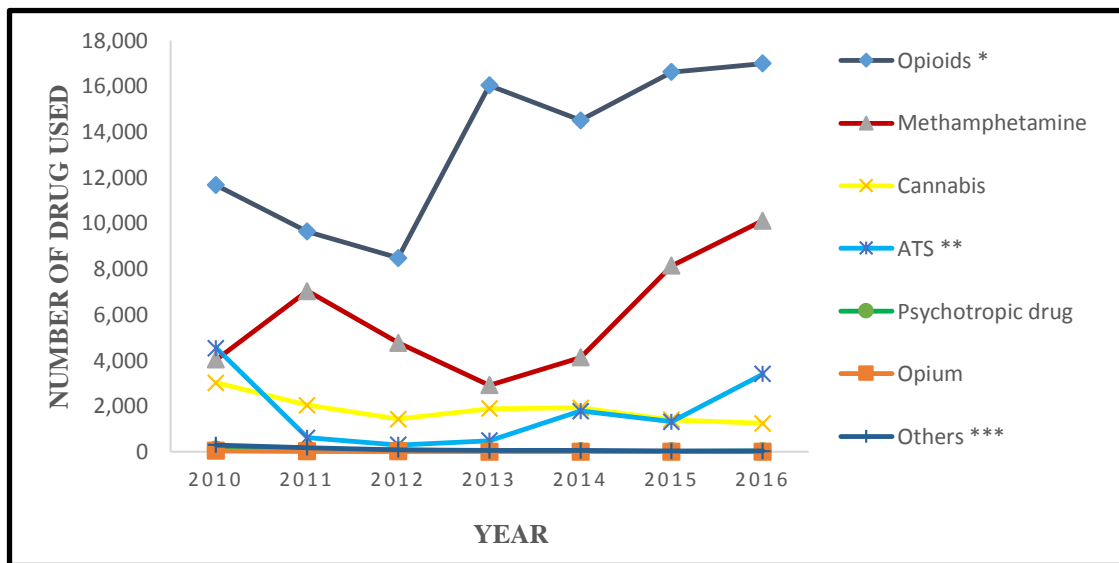


Figure 1.2 Statistic of Drug Usage from Year 2010 to 2016 (NADA, 2016)

Therefore, drug addiction remains as a worldwide concern, especially the ATS addiction. The UNODC reported that the prevalence of methamphetamine usage was high in Asia and there was a high demand for methamphetamine addiction treatment (UNODC, 2015). Still, there is no available treatment offered for methamphetamine addiction till today.

In contrast, few treatments have been approved by the FDA for opioid dependence case, mainly methadone and buprenorphine. Due to its cost, methadone is commonly used as the first line treatment for opioids dependence to substitute the illicit opioids (Steketee & Kalivas, 2011; Ward, Bell, Mattick, & Hall, 1996). Although methadone is proven to be safe and effective to treat opioid dependence, this drug still has its own limitation. One of the limitation is high incidence of relapse was found (around 55-80 %) following cessation of substitution therapy with the methadone (full mu-opioid agonist), where it is thought to be due to the kappa overdrive syndrome (Rothman et al., 2000; Tkacz et al., 2011).

The latest finding showed that there is an increasing pattern of methamphetamine addiction among the methadone patients after enrolling into MMT program (Shariatirad, Maarefvand, & Ekhtiari, 2013). These patients started to take methamphetamine while on methadone maintenance therapy in order to feel good, as self-medication for depression, and also to get high by shifting between different classes of drugs (Shaffer & LaSalvia, 1992; Shariatirad et al., 2013). This created another problem where the addicts started abusing more than one class of drugs which leads to poly-drug dependence (Trujillo, Smith, & Guaderrama, 2011).

The most commonly abused drugs by the poly-drug addicts are morphine and methamphetamine (Liu, Lin-Shiau, Chang, & Lan, 2015). Most of the addicts took these

drugs combination due to the greater effect known as “speedball”, as compared to a single drug (Trujillo et al., 2011).

To date, there is no FDA-approved treatment for methamphetamine dependence. Hence, this poly-drug dependence has becoming a serious health problem that needs attention, since its abuse is increasing and there is no available treatment to treat this poly-drug dependence (Pereira et al., 2011).

Buprenorphine is one of the FDA-approved treatments for opioids dependence (Cruciani & Knotkova, 2013). However, the use of buprenorphine is believed to be less than optimal because of its expensive cost. Buprenorphine is a partial mu opioid receptor agonist (MOR), while antagonist at the kappa (KOR) and delta (DOR) opioid receptors (Gerra et al., 2004). It is also a partial agonist at the nociceptin opioid receptor (NOP) (Lutfy & Cowan, 2008). The MOR activity of buprenorphine is the main key in treating opioid dependence, similar to methadone. Meanwhile, the KOR antagonist (the receptor of interest in this study) is strongly believed to counteract with the negative mood state (e.g., dysphoria) that experienced by the addicts due to drug withdrawal (Cruciani & Knotkova, 2013). Few studies had suggested that buprenorphine might be effective in reducing morphine, cocaine, and alcohol dependence (Lutfy et al., 2003; Montoya et al., 2009).

Back to the receptor of interest (the KOR), previous studies showed that there was a link between the drug relapse and KOR activity, including opioids and psychostimulants (Butelman, Yuferov, & Kreek, 2012). The activation of KOR that results in stress and dysphoria was believed contributes to drug relapse (Butelman et al., 2012). Due to the successful of buprenorphine/naltrexone treatment among the cocaine-dependent rats (Cordery et al., 2014), it is believed this treatment might be beneficial to prevent relapse related to methamphetamine dependence as well. Recent study