THE USE OF BUPRENORPHINE/NALTREXONE COMBINATION TREATMENT IN ATTENUATING RELAPSE TO MORPHINE/METHAMPHETAMINE POLYDRUG-DEPENDENCE IN MICE

BY

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ABSTRACT

Currently, there is an increasing pattern of methamphetamine abuse among opioid users and yet there is no pharmacological treatment approved by Food and Drug Administration (FDA) to treat methamphetamine dependence. Since kappa-opioid receptor has been associated with relapse to many drugs of abuse, this receptor might have the potential to prevent relapse to both methamphetamine and opioid. Therefore, this study aims to investigate the involvement of the kappa-opioid receptor system in relapse related to morphine/methamphetamine (polydrug) addiction. Firstly, naltrexone was assessed in a tail-withdrawal test to ensure its ability in suppressing the mu-opioid receptor activity of buprenorphine in mice at 52 °C warm water bath. The tail-withdrawal test was also used to identify the minimum dose of methamphetamine that can produce analgesic effects in order to determine the dose responsible in activating the mu-opioid receptor. Both of these assays were crucial since this study aims to investigate the involvement of kappa-opioid receptor rather than the rest of opioid receptor (e.g. mu-opioid receptor) in preventing drug relapse. Next, conditioned place preference (CPP) test was used to investigate the ability of the treatment candidates (buprenorphine/naltrexone combination and norbinaltorphimine, nor-BNI) in drug relapse model in mice. Method validation and optimisation for the CPP test were conducted prior to the test to ensure that the conditioning and priming dose, confinement time as well as time interval applied in the test are optimum. Then, the CPP test was conducted to compare the ability of these two treatment regimes in attenuating reinstatement to morphine-, methamphetamine- and morphine/methamphetamine polydrug-dependence. Based on the result, 1.0 mg/kg naltrexone pretreatment successfully block the mu-opioid receptor agonist activity of 0.3 mg/kg buprenorphine by showing a decrease in tail-withdrawal latency in the tail-withdrawal test. This proved that the combination of buprenorphine/naltrexone treatment is able to mask the mu-opioid receptor agonism of buprenorphine and act as a functional kappa-opioid antagonist. Moreover, it was found that the minimum dose of methamphetamine that can produce analgesic effect in the tail-withdrawal test is at 5.0 mg/kg. Hence, 1.0 mg/kg methamphetamine used in the CPP test will have no effect on the mu-opioid receptor and any dependence induced at this dose is not related to the mu-opioid receptors activity. In the CPP test, the combination of 0.3 mg/kg buprenorphine and 1.0 mg/kg naltrexone pretreatment (ip) was able to attenuate morphine- (7.5 mg/kg, ip) and morphine/methamphetamine (7.5 mg/kg and 1.0 mg/kg, ip respectively) polydrug-reinstatement but not methamphetamine-reinstatement (1.0 mg/kg, ip). However, pretreatment with 10.0 mg/kg norbinaltorphimine (a selective kappa-opioid antagonist) only attenuated morphine-reinstatement but not methamphetamine- and morphine/methamphetamine polydrug-reinstatement. Therefore, it can be suggested that methamphetamine and morphine/methamphetamine polydrug-dependence may involve other important receptors and neurocircuit besides the kappa-opioid receptor. Further studies on immunohistochemistry and autoradiography may be crucial to rule out the involvement of kappa-opioid receptor in mediating relapse to morphine-, methamphetamine- or morphine/methamphetamine polydrug-dependence.

خلاصة البحث

هناك حاليا نمط متزايد من حالات تعاطي الميتامفيتامين بين مستخدمي الأفيونات، ولا يوجد حتى الآن علاج دوائي موافق علي من قبل إدارة الغذاء والدواء الأمريكية (FDA) لعلاج ادمان الميتامفيتامين .ارتبطت مستقبِلات كابا الأفيونية مع حالات الانتكاس في العديد من انواع تعاطي المخدرات، وقد يكون لدى هذا المستقبِل القدرة على منع حالة الانتكاس في إدمان الميثامفيتامين والأفيونات. لذلك هدفت هذه الدراسة إلى التعرف على دور نظام مستقبلات كابا الأفيونية في حالات الانتكاس المتعلقة بإدمان المورفين والميثامفيتامين (تناول عقاقير متعددة). أولا، تم تقييم النالتريكسون في اختبار سحب الذيل للتيقن من قدرتها في قمع نشاط مستقبلات ميو–أفيون من البوبرينورفين في الفئران عند 52 درجة مئوية. تم استخدام اختبار سحب الذيل أيضا لتحديد الحد الأدنى لجرعة المخدر التي يمكن أن ينتج عنها التأثيرات المسكنة من أجل تحديد الجرعة المسؤولة في تفعيل مستقبلات ميو–أفيون. هذه الاختبارات كانت مهمة جدا لأن هذه الدراسة هدفت إلى التحقيق في تورط مستقبلات كابا الأفيونية وليس غيرها من المستقبلات الأفيونية (مثل مستقبلات ميو–أفيون) في الوقاية من حالات الانتكاس. بعد ذلك، تم استخدام اختبار تفضيل المكان المهيأ (CPP) للتحقيق في قدرة المعالجين (مزيج البوبرينورفين والنالتريكسون والنوربينالتورفيمين، والغير BNI) في نموذج انتكاس المخدرات في الفئران. تم إجراء اختبارات التحقق وتحسين الأداء لاختبار الـ CPP قبل الاختبار الرسمي للتأكد من التكييف، والجرعات المناسبة، أوقات الحبس، وكذلك الفترات الزمنية التي طبقت في الاختبار هي الأمثل .ثم، وأجري اختبار الـ CPP لمقارنة قدرة المعالجين في معاملة اثنين في تخفيف العودة للمورفين، والميثامفيتامين ومزيج المورفين والميثامفيتامين .استنادا إلى النتائج، 1.0 ملغم /كغم من المعالجة المسبقة بالنالتريكسون كتلة بنجاح نشاط مستقبلات ناهض مو الأفيونية من 0.3 ملغم /كغم البوبرينورفين من خلال إظهار انخفاض في الكمون ذيل الانسحاب في اختبار الذيل الانسحاب .أثبت هذا أن الجمع بين العلاج البوبرينورفين / النالتريكسون قادر على حجب مستقبلات التحولي مو الأفيونية من البوبرينورفين ويكون بمثابة خصم كابا الأفيونية وظيفية .وعلاوة على ذلك، فقد وجد أن أقل جرعة من مخدر التي يمكن أن تنتج تأثير مسكن في اختبار الذيل الانسحاب هي عند 5.0 ملغم /كغم .وبالتالي، 1.0ملغم /كغم من الميثامفيتامين المستخدمة في الاختبار CPPلن يكون لها أي تأثير على مستقبلات مو الأفيونية وأي الاعتماد الناجم عند هذه الجرعة لا علاقة للنشاط مستقبلات كابا الأفيونية .في الاختبار حزب الشعب الكمبودي، فإن الجمع بين 0.3 ملغم /كغم البوبرينورفين و 1.0 ملغم /كغم من النالتريكسون المعالجة (الملكية الفكرية) قادرة على تخفيف المورفين (7.5 ملغ /كغ، والملكية الفكرية) والمورفين / الميثامفيتامين (7.5 ملغ /كغ و 1.0 ملغ / كغ، والملكية الفكرية على التوالي) تناول عقاقير متعددة، ولكن ليس إعادة الميتامفيتامين إعادة (1.0 ملغ /كغ، والملكية الفكرية .(ومع ذلك، فإن المعالجة مع 10.0 ملغ /كغ ولا–أخطار الحريق (انتقائي كابا الأفيونية خصم) الموهن فقط المورفين إعادة ولكن المورفين / الميثامفيتامين تناول عقاقير متعددة–إعادة .ولذلك، فإنه يمكن أن يكون اقترح ان الميثامفيتامين والمورفين / الميثامفيتامين تناول عقاقير متعددة الاعتماد قد تنطوي على مستقبلات أخرى مهمة الدوائر العصبية بالإضافة إلى مستقبلات كابا الأفيونية .قد يكون إجراء المزيد من الدراسات على المناعية وتصوير الإشعاع الذاتي حاسم لاستبعاد تورط مستقبلات كابا الأفيونية في التوسط الانتكاس إلى أو المورفين / الميثامفيتامين تناول عقاقير متعددة الاعتماد.

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 Science (Pharmacology).

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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.

Maryam Saadah Binti Suhaimi

Signature……. Date ….....

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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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LIST OF ABBREVIATIONS

°C	Celsius
5-HT	Serotonin
APA	American Psychiatric Association
ATS	Amphetamine-Type Stimulants
Bup	Buprenorphine
cAMP	Cyclic Adenosine Monophosphate
CCTV	Closed-Circuit Television
CNS	Central Nervous System
CPP	Conditioned Place Preference
DA	Dopamine
DAT	Dopamine Transporter
DSM	Diagnostic and Statistical Manual of Mental Disorders
FDA	Food and Drug Administration
GABA	Gamma-Aminobutyric Acid
GluA1	AMPA-Type Glutamate Receptor
H_2O	Water
HIV	Human Immunodeficiency Virus
IACUC	Institutional Animal Care and Use Committee
ip	Intraperitoneal Injections
KOR	Kappa-Opioid Receptor
M3G	Morphine-3-Glucuronide
M6G	Morphine-6-Glucuronide
MAO-A	Monoamine Oxidase A
MDMA	3,4-Methylenedioxy-N-Methyl-Amphetamine (Ecstasy)

Meth	Methamphetamine
MMT	Methadone Maintenance Treatment
MOR	Mu-Opioid Receptor
MPE	Maximum Possible Effect
NA	Noradrenaline
NAcc	Nucleus Accumbens
NADA	National Anti-drugs Agency
NIDA	National Institute on Drug Abuse
NOP	Nociceptin Opioid Peptide
nor-BNI	Norbinaltorphimine
Ntx	Naltrexone
OF1	Oncins France 1
PFC	Prefrontal Cortex
PNS	Peripheral Nervous System
PUSPEN	Pusat Pemulihan Penagihan Narkotik
sc	Subcutaneous
SEM	Standard Error Of The Mean
STI	Sexually-Transmitted Infections
UNODC	United Nations Office on Drugs and Crime
VTA	Ventral Tegmental Area
WHO	World Health Organization

CHAPTER ONE

INTRODUCTION

1.1 RESEARCH BACKGROUND

Drug addiction is a chronic brain disorder which causes compulsive drug seeking and drug taking behaviour despite the harmful effects (National Institute on Drug Abuse (NIDA), 2014a). In the Diagnostic and Statistical Manual of Mental Disorders (DSM) developed by the American Psychiatric Association (APA), addiction has no specific diagnosis, but can be included in the category of substance use disorder. The symptoms of substance use disorder may include impaired control over individual actions, social impairment, risky use, tolerance and withdrawal (NIDA, 2014a). The most commonly abused substances are depicted in Table 1.1.

Table 1.1 The Most Commonly Abused Substances (NIDA, 2014a)

Drug classification	Examples
Opioids	Morphine and heroin
Psychostimulants	Cocaine, methamphetamine and amphetamine
Cannabis	Marijuana
Hallucinogen	Ecstasy and lysergic acid diethylamide (LSD)
Inhalants	Gasoline and spray paint

In 2013, it was estimated that 3.4 - 7.0 % (162 - 329 million) of the world's population aged 15 – 64 years old had used illicit drugs, with the highest abused drug reported for cannabis (2.7 - 4.9 %), followed by opioids (non-medical use of pharmaceutical opioids like oxycodone) (0.6 - 0.8 %), amphetamines (0.3 - 1.1 %), opiates (heroin and opium) (0.3 - 0.4 %), cocaine (0.3 - 0.4 %) and ecstasy (0.2 - 0.6 %)

(United Nations Office on Drugs and Crime (UNODC), 2015). In the same report, UNODC (2015) also revealed that between 2009 to 2013, cannabis, opiates and opioids were among the highest abused drugs, while amphetamines, ecstasy and cocaine abuse were reducing (Figure 1.1).

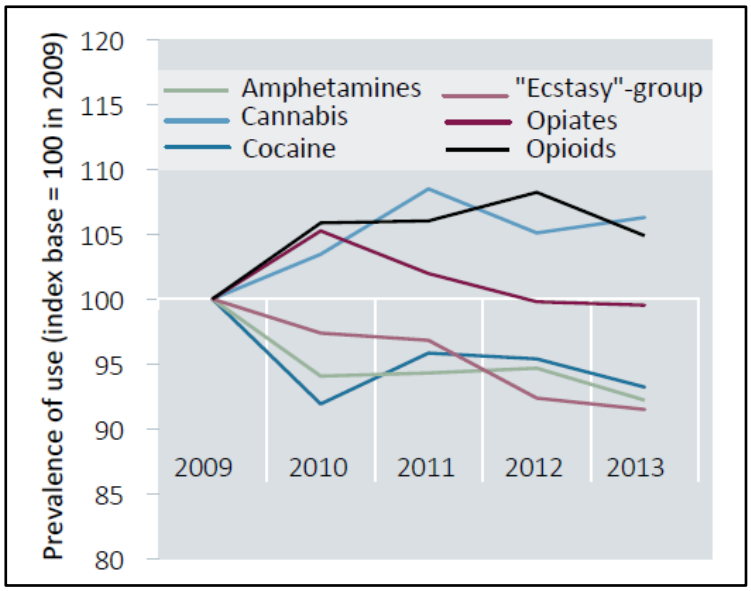

Figure 1.1 World Drug Report 2015 for the Year of 2009 - 2013 (UNODC, 2015)

In Malaysia, opioids (heroin and morphine) and methamphetamine are ranked as the most abused drugs from 2010 to 2014 (Figure 1.2) (National Anti-drugs Agency (NADA), 2014). In 2014, NADA reported that 64.8 % (14,496) of total drug addicts were addicted to opioids, followed by methamphetamine (18.4 %), cannabis (8.6 %) and ATS (amphetamine-type stimulants which include ecstasy and amphetamine) (7.9 %). About 37.5 % (8,295) from the total of 22,355 reported cases were relapse related with the highest relapse cases being due to opioids and methamphetamine addiction (78.9 % and 11.2 % respectively) (NADA, 2014).

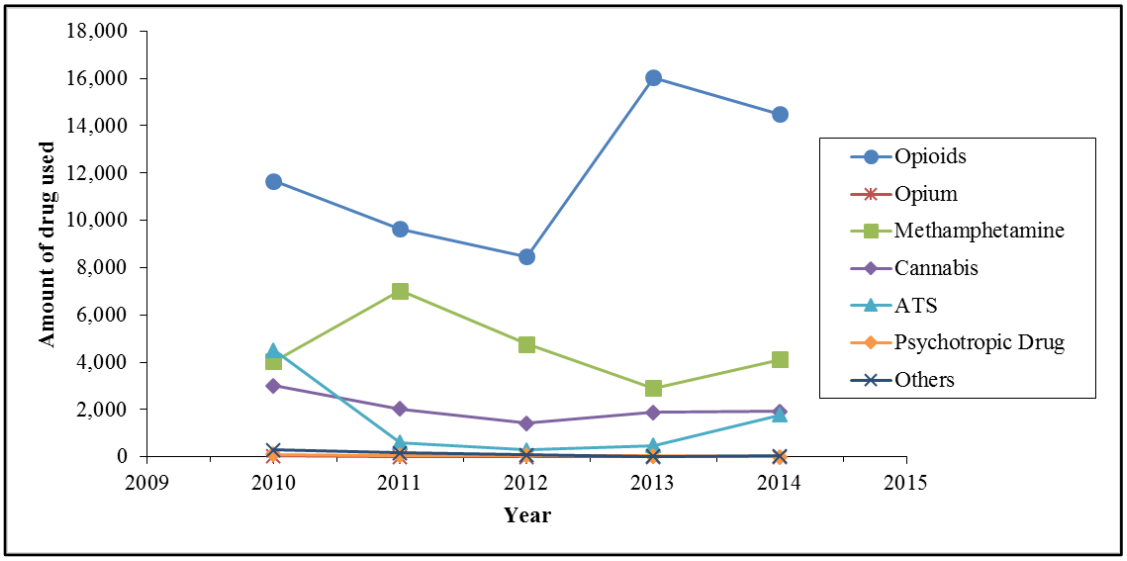

Figure 1.2 Drug Abuse Statistics, for the Year of 2010 – 2014 (NADA, 2014)

Based on the World Health Organization (WHO) Guidelines (2009), there are two main options for the treatment of opioid addiction. It was either detoxification followed by a long-term maintenance on replacement therapy, or rapid detoxification followed by relapse prevention treatments. As for opioid dependence treatment, there are three main types of medication available which are opioid agonists (oral methadone liquid or sublingual buprenorphine tablets), opioid antagonists (naloxone or naltrexone) and alpha-2 adrenergic agonist (clonidine) (Mclellan, Lewis, Brien, & Kleber, 2000; WHO, 2009). The opioid agonists and antagonists act directly on the mu-opioid receptor (the same receptor that caused opioid dependence), whereas the alpha-2 adrenergic agonist acts by decreasing noradrenaline (NA) activity in the central nervous system (which is increased during withdrawal) (Mclellan et al., 2000; Kirchmayer et al., 2002; Nutt & Lingford-Hughes, 2008). Since most drugs of abuse (such as cocaine, amphetamine, methamphetamine and cannabis) have no approved pharmacological intervention, another possible non-pharmacological treatment is psychosocial intervention (cognitive and behavioural approaches and contingency management

techniques), which has low effectiveness and lacks scientific support (El-Guebaly, Carra, & Galanter, 2015).

1.2 RESEARCH PROBLEM

High relapse incidence (around 55-80 %) were reported among opioid addicts after completed methadone maintenance treatment (MMT) (Tkacz, Severt, Cacciola, & Ruetsch, 2011), making it crucial to revise the current treatment for opioid dependence. Since kappa-opioid receptor is said to be related to relapse of many drugs of abuse (Butelman, Yuferov, & Kreek, 2012), study on the potential treatment to overcome relapse of drug taking should be focused on this receptor. In addition, many drug addicts are addicted to multiple drugs of abuse from different pharmacological classifications, termed as polydrug addiction (Minozzi et al., 2011). There is an increasing pattern of psychostimulants abuse among patients who underwent methadone maintenance treatment (Shaffer & LaSalvia, 1992; Shariatirad, Maarefvand, & Ekhtiari, 2013). Moreover, it was reported that addicts usually take methamphetamine together with morphine to mask the side effects of the drugs (Ito, Mori, Namiki, Suzuki, & Sawaguchi, 2007; Preliminary interview at PUSPEN Gambang, 2013). Thus, not only relapse is a big issue in drug addiction, but polydrug addiction should also be considered as well. Till now, there is no pharmacological treatment approved by Food and Drug Administration (FDA) to treat methamphetamine and polydrug dependence (Pereira & Gough, 2011). Since buprenorphine/naltrexone combination pretreatment showed an ability to attenuate drug-primed reinstatement in cocaine- and morphine-dependent rats (Cordery et al., 2014), it is possible that this drug combination may also possess the ability to attenuate morphine/methamphetamine polydrug dependence as cocaine and methamphetamine are classed in the same pharmacological classification of drug which

is psychostimulant, although their neurocircuit might be slightly different. Therefore, this study aims to find the possible drug candidate to treat polydrug addiction related to morphine/methamphetamine and to prevent relapse upon successful detoxification.

1.3 SIGNIFICANCE OF STUDY

The findings of this study will contribute greatly to the benefits of society considering that morphine and methamphetamine are the highest abused drugs for the past 5 years, while polydrug-abuse is becoming popular recently. Treatment that can attenuate relapse of drug taking after successful cessation would be beneficial to ensure effectiveness of the treatment choices. This study aid in developing new treatment option for drug addiction that can enhance the effectiveness of the current treatment and may reduce relapse incidence. It will reveal if opioid receptors can be manipulated to overcome this issue. The researchers can also use the findings as a guide to synthesise a new compound that selectively target the receptors that responsible to cause drug relapse. This study can also be a guide in assessing other possible treatment options related to addiction and relapse. It may provide a clear framework for research in drug addiction field. Therefore, the effectiveness of pharmacological intervention for drug addiction and relapse can be improved.

1.4 OBJECTIVES OF STUDY

1.4.1 General Objective:

To study the involvement of kappa-opioid receptor system in relapse related to morphine/methamphetamine polydrug addiction.

1.4.2 Specific Objectives:

a) To evaluate the ability of naltrexone to block any mu-opioid receptor agonism of buprenorphine.

b) To evaluate the ability of buprenorphine/naltrexone combination treatment in attenuating reinstatement to morphine-, methamphetamine- and morphine/methamphetamine polydrug-dependent mice.

c) To evaluate the independent role of selective kappa-antagonist in attenuating reinstatement to morphine-, methamphetamine- and morphine/methamphetamine polydrug-dependent mice.

1.5 RESEARCH HYPOTHESIS

Antagonism of the kappa-opioid receptor may attenuate reinstatement following morphine-, methamphetamine- and morphine/methamphetamine polydrug withdrawal.

CHAPTER TWO

LITERATURE REVIEW

2.1 OVERVIEW OF DRUG ADDICTION

Drug addiction can be defined as a chronic and relapsing brain disease in which compulsive drug-seeking and drug-use persists despite the serious negative consequences (NIDA, 2014b). According to Le Moal and Koob (2007), the term addiction is previously represented by both physical dependence (discontinuation of prolong drug usage resulting in an intense physical disturbance) and psychological dependence (drug produces rewarding effect and compulsive behaviour that urges regular drug administration, to feel pleasure or avoid discomfort). Currently, it is well accepted that dependence itself means; "A physiological state that can occur with regular drug use and results in withdrawal symptoms when drug use is abruptly discontinued" (NIDA, 2014b). Therefore, addiction and dependence are actually interrelated to one another, and these terms are often interchangeable.

The rate of relapse in drug addiction was similar to other chronic illnesses, such as asthma, hypertension and type I diabetes (NIDA, 2014b), making it important to find the effective treatment for addiction and relapse. Generally, drug addiction affects individual health, mentally (depression and anxiety) and physically (withdrawal symptoms like irritability, insomnia and hot flashes). Furthermore, drug addicts is known to be the major agent for transmitting the infectious diseases such as Human Immunodeficiency Virus (HIV), sexually-transmitted infections (STI), hepatitis, and tuberculosis. Some of these diseases are easily spread through needle sharing which can pose a threat to public health. Other than that, illegal drug use also has a high impact on

socioeconomic status of the country through health care, productivity loss, crime, imprisonment and drug enforcement (Bauer, Soares, & Nielsen, 2015).

2.1.1 Stages of Drug Addiction

Drug addiction developed through a complex interaction of neurobiological components, genetic variables, environmental factors and social aspects (Le Moal & Koob, 2007; Koob & Volkow, 2010; Volkow, Wang, Fowler & Tomasi, 2012). All these factors are also involved in the establishment of drug addiction through the three stages of addiction which occurs in a continuous cycle (Figure 2.1). Drug use begins with either social drug-taking or acute reinforcement, then advances to the escalating or compulsive use, dependence, withdrawal, and usually relapses to the drug-taking; and the cycle continues (Le Moal & Koob, 2007). However, addicts occasionally can stay on recovery stage for a longer time with the help of proper medication and treatment.

The first stage in the addiction cycle (Figure 2.1) is the 'binge/intoxication' stage where drug taking activity gives a positive reinforcement to the addicts, usually named as rewarding effect (e.g. enhance performance and reduce pain). The second stage refers to the 'withdrawal/negative effect' stage where the previous motivating effect (positive reinforcement) of the drug has been shifted towards avoiding the negative effects of the drug withdrawal. The third stage, termed as the 'preoccupation/anticipation' stage is concerned about the drug craving and compulsive drug seeking behaviour (Koob, 2006a).

In order to investigate the causes of repetitive cycle of addiction, different experimental studies can be useful at different stages of addiction. Table 2.1 summarises the appropriate animal laboratory models of the different stages of the drug addiction cycle based on a review by Koob (2009). Current study used conditioned place