Mephedrone (4-甲基甲基卡西酮) 對大鼠制約地點偏好的影響

Effect of Mephedrone (4-methylnmethcathinone, 'meow') on Conditioned Place Preference in Rats

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係由本人指導撰述，同意提付審查。

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經本委員會審議，符合本校碩士資格標準。

論文口試委員會 委員

系主任
Constants

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中文摘要

背景 / 目的：

Mephedrone 之化學名為 4-methylmethcathinone(4-甲基甲基卡西酮)(4-MMC)，施用後有欣快、興奮等作用，會產生類似甲基安非他命與搖頭丸的效果，但因作用時間短，故施用者會不斷追加劑量。新興合成物質 mephedrone 的濫用已逐漸在全世界蔓延，目前 mephedrone 相關的藥物資料並不多，多半是來自濫用者的自我報告或是急診室的病歷紀錄，而不是實驗研究。這個實驗利用制約地點偏好(CPP)的模式來探討 4-甲基甲基卡西酮(mephedrone)對大鼠的影響。

方法：

本試驗利用公 SD 大鼠及制約性地點偏好 (Conditioned Place Preference)標準協定的動物模式，探討 mephedrone 的藥物成癮性與劑量反應測試。試驗分成給予生理食鹽水的控制組及四組給予不同 mephedrone 劑量的實驗組(5 mg/kg, 10 mg/kg, 20 mg/kg and 40 mg/kg)，每組各 3 隻。體重的測量分別是在試驗開始的第一天(習慣期)，及每次藥物給予前(條件化期)。偏好的變化是計算條件化前後，大鼠停留在給予藥物的那個空間的時間差(以秒計算)，並以重複量數單因子變異數分析統計其差異。

結果：

給予生理食鹽水的控制組及四組給予不同 mephedrone 劑量的實驗組的體
重增长並沒有顯著差異。於停留在給予藥物之空間的時間上，條件化前（第三天）五組皆無顯著差異（5 mg/Kg  P=1; .10 mg/Kg  P=0.674; 20 mg/Kg  P=0.876; 40 mg/Kg  P=0.975）。條件化後（第11天），給予低劑量 mephedrone 的兩組相較於給予生理食鹽水的控制組，並無顯著差異（5 mg/Kg  P=0.893; 10 mg/Kg  P=0.804），然而，在給予高劑量 mephedrone 的兩組，相較於給予生理食鹽水的控制組，卻有很顯著的差異（20 mg/Kg  P=0.012; 40 mg/Kg  P=0.001）。

結論:
結果呈現當老鼠急性、重複每日暴露於高劑量的 mephedrone，會出現酬償性反應。這個結果反映出 mephedrone 可能會有藥物依賴的成癮反應產生。至於確切的劑量、藥物給予次數、藥物需求間隔及戒斷反應，還有其分子生物訊號與藥物影響途徑，則是我們下一步要努力的目標。

關鍵詞：Mephedrone、4-Methylmethcathinone、4-甲基甲基卡西酮、條件化行為、制約性地點偏好
Abstract

**Background / Purpose :**

Mephedrone, also known as 4-methylmethcathinone (4-MMC), or 4-methylephedrone, is a synthetic stimulant drug of the amphetamine and cathinone classes. Mephedrone is hypothesized to possess abuse liability, abuse of the dangerous street drug has become commonplace in the Europe and United States, which has caused many deaths in Europe. Knowledge about the pharmacology of mephedrone has been obtained primarily from surveys of drug abusers and emergency room visits rather than experimental studies. The present study used the conditioned place preference (CPP) assays to investigate behavioral effects of mephedrone.

**Methods :**

An unbiased place conditioning protocol was used to examine the expression of the rewarding effects of mephedrone and related dose response. Male Sprague–Dawley rats were used in this experiment. Rats were divided into five groups, saline controlled group and four different doses of mephedrone treated groups (5 mg/kg, 10 mg/kg, 20 mg/kg, and 40 mg/kg), three rats in each group. The body weight of rats was taken on the beginning
of the habituation phase (day 1) and before the mephedrone injection during the conditioning phase. The change of preference was calculated as the difference (in seconds) between the time spent in the drug-paired compartment on the testing day and the time spent in this compartment in the pre-conditioning session. One-way, ANOVA, repeated measures were used to analyse the differences.

**Results :**

The mean body weight gain showed no significant difference among the saline controlled group and four mephedrone treated groups. The mean differences of the time spent in the drug-paired compartment of the five groups had no significant differences in day 3 (pre-conditioning) (5 mg/Kg $P=1$; 10 mg/Kg $P=0.674$; 20 mg/Kg $P=0.876$; 40 mg/Kg $P=0.975$). At day 11 (post-conditioning), there was no significant difference among low doses (5 mg/Kg $P=0.893$; 10 mg/Kg $P=0.804$), while with significant difference among high doses (20 mg/Kg $P=0.012$; 40 mg/Kg $P=0.001$) when they were compared with the saline controlled group.

**Conclusion :**

Our results indicated that previous repeated mephedrone exposure
enhanced subsequent CPP drug reward behavior.

Future studies that various factors such as dose, dosing frequency, and forced abstinence interval are needed to further assess the motor activating properties of mephedrone. Furthermore, we will examine phosphorylation levels of various relevant signaling molecules in the brain regions implicated in addictive behaviors after acute and repeated mephedrone administration.

**Keywords**: Mephedrone, 4-Methylmethcathinone, Conditioned Place Preference, CPP
Chapter 1. Introduction

Background

Food and Drug Administration, Department of Health, Executive Yuan, recently evaluated the abuse potential of mephedrone, an emerging synthetic abuse substance commonly known as “Meow”. "Meow" has caused many deaths in Europe. There was a 17-year-old girl died of suspected smoking "Meow" in Taiwan recently.

Mephedrone, also known as 4-methylmethcathinone (4-MMC), or 4-methylephedrone, is a synthetic stimulant and entactogen drug of the amphetamine and cathinone classes (Table 1). It is a synthetic substance based on the cathinone compounds found in the khat plant of eastern Africa. Figure 1 showed the comparison of the chemical structures among mephedrone, cathinone, amphetamine, and methamphetamine.

There are evidences of its availability in Europe since 2007, with seizures and detections of mephedrone reported in 28 European and neighbouring countries to date. The size and number of mephedrone seizures has increased year on year. Mephedrone is usually purchased by/sold to users in powder form in small sealed
plastic bags which are marked as ‘not for human consumption’ or ‘research chemical’. It is available under a number of brand names, including ‘plant feeder’, ‘plant food’ and ‘bath salts’. In Europe, it was more commonly sold as mephedrone or ‘plant food’. However it was more commonly sold as ‘bath salts’ in the US. Despite these brand names, mephedrone has no proven use as a plant food or as a bath/cosmetic product. Although the majority of mephedrone is sold in powder form, it is also available in tablets or encapsulated forms. Use of mephedrone is predominately by nasal insufflation, although some users report that this is associated with significant unwanted nasal effects. Therefore, some individuals may dissolve it in water/other drinks or swallow the powder wrapped in paper (known as ‘bombing’).

The intended effects of mephedrone have similar effect as methamphetamine and ecstasy, including increased alertness, euphoria, excitement, feeling of stimulation, and openness after use. There are numerous reports on different internet discussion forums describing the unwanted effects in relation to self-reported mephedrone use. Commonly reported unwanted effects include elevated body temperature, chest pain, convulsions, anxiety, sweating, hallucinations, paranoia, bruxism, and elevated heart rate and blood pressure. When these individual reports are combined, the overall pattern of described unwanted effects would be consistent with the acute sympathomimetic drug toxicity.
Because of the properties of euphoria and stimulation, a boom in the popularity of this chemical as a recreational drug across the UK, Republic of Ireland and mainland Europe had attracted substantial media and political interest during 2009. The possession and distribution of mephedrone are criminalized in many countries. It has become the fourth most popular street drug in the United Kingdom, behind marijuana, cocaine, and ecstasy in 2009\textsuperscript{10,13,15,16,17}.

In late 2009, UK newspapers began referring to the drug as Meow or Miaow (sometimes doubled as Meow Meow or Miaow Miaow). Slang names include M-Cat, Bubbles, Meow Meow, Meph and Drone\textsuperscript{18,19,20,21}.

**Importance**

Mephedrone is widely and legally available from suppliers on the Internet, where it has been openly sold in retail or bulk quantities, providing a higher potential for spread than other new substances previously encountered in Europe. Furthermore, the widespread media coverage on the substance and its potential health consequences may have led to increased awareness of the drug amongst young people in general, and established user groups in particular.
Before the introduction of the legislation, users generally obtained mephedrone via the internet. Now they buy it from street dealers, on average at double the price. We suspect that, in time, there are likely to be reductions in purity and increases in health harms\textsuperscript{22,23}.

There were increasing reports of mephedrone use, and that the majority of these were UK based\textsuperscript{4,24}. They typically were mephedrone specific websites, and would ship mephedrone to any country on request. After the control of mephedrone in the UK in April 2010, not only was there a significant decrease in the number of internet sites selling mephedrone, the majority of sites were no longer UK based. A significant proportion had changed to selling other "legal highs", which may in fact be mephedrone being sold covertly under different brand names\textsuperscript{13,14,25}. This puts the individual purchaser at risk of potential criminal conviction, since inadvertently they may be purchasing a substance that is controlled in the country where it is being supplied to\textsuperscript{23,26,27,28}.

There is still limited information available on the potential acute toxicity (harms) associated with the use of these emerging psychoactive substances. Gold standard evidence, such as animal studies or human clinical trials, is not available to users or healthcare professionals.

Users of novel psychoactive substances, such as mephedrone, often
post information on both the desired and unwanted effects seen following use on internet discussion forums and blogs. These user reports can often be very detailed, in some cases describing minute-by-minute physiological parameters and other desired/unwanted effects, along with information on the amount of substance used and the route(s) of use. Additionally, there is also the potential to undertake small sub-population level surveys to try and collate this information from a broader group of users. It is designed to capture data on trends in the use of recreational drugs and novel psychoactive substances; however, those conducting the survey often include additional questions relating to the acute harm associated with the use of drugs and emerging psychoactive substances. Both of these approaches are limited in that they are based on anecdotal self-reported use and harms, therefore, there is no analytical confirmation of the substance(s) used. In addition, the sub-population surveys often used to predetermine unwanted effects to facilitate data analysis, but, which may limit any additional responses.

The absence of information and research findings has been a problem for all risk assessment exercises conducted by the Scientific Committee. Therefore, the risk assessment conclusions are inevitably
based on partial knowledge and, consequently, are tentative. The risk assessment on mephedrone was particularly difficult, due not only to limited data available on this substance, but also to the fact that there was very little similarity to other compounds which have been previously risk-assessed through the Council Decision mechanism.

Mephedrone’s specific effects are difficult to assess because it is primarily used in combination with substances like alcohol and other stimulants. Mephedrone is deemed to have similar physical effects as other stimulant drugs, in particular ecstasy (MDMA). However, its relatively short duration of action, leading to repeated dosing, is more analogous to cocaine. Evidence available suggests that it may be used as an alternative to illicit stimulants, that it has a high abuse liability and a potential to cause dependency\textsuperscript{30,31}. More in-depth studies would be required to explore in detail the dependence potential of this drug.

Mephedrone was exported from China to Europe, Australia and New Zealand, which has caused numerous deaths\textsuperscript{2,13,16}. It urgently needs to call for extensive attention, and prompts urgent action on the drug to be banned. We have to do something about it, and take the responsibility against it.
Aim

Currently, there are no national or international surveys collecting information on the acute toxicity associated with the use of mephedrone. Additionally, at this time, there is no ICD-10 specific code for acute mephedrone toxicity. Therefore, it is not possible to interrogate hospital admission databases to determine the frequency of hospital admissions\textsuperscript{11,3}.

Very little is known about the pharmacology or toxicity of mephedrone, as no studies have been published that establish such characteristics. To provide insight into the behavioral effects of mephedrone, the present study was designed to test the hypotheses that repeated mephedrone exposure elicits a conditioned place preference (CPP) in rats. Thus, the presented experiment aimed to explore the addiction and to estimate the dose response using the conditioned place preference (CPP) to monitor the mephedrone reward behavior.
Chapter 2. Review

Emergency Department presentation data

There are a number of case reports/series relating to individuals presenting to healthcare facilities with acute mephedrone toxicity\textsuperscript{12,32}. The first reported case of analytically confirmed acute toxicity was an individual who presented symptoms following oral ingestion and intramuscular injection of mephedrone powder. The main feature of these symptoms was sympathomimetic character\textsuperscript{14}. There was a case of a fatality in The Netherlands in an individual who was initially found with extreme agitation and aggression, and was described as “having injured himself severely by smashing windows in a rage of fury”\textsuperscript{33}. There was post mortem analytical confirmation of mephedrone use (femoral blood 5.1 mg/L post mortem), although “traces” of cocaine, MDMA and oxazepam were also detected. There was a report of an individual with Type 1 diabetes who presented with ketoacidosis following mephedrone use\textsuperscript{29}. There was also a report from the Republic of Ireland of an individual who developed myocarditis related to the unconfirmed use of mephedrone (there was analysis of the plant food reported to have been ingested, but no analysis of biological sample)\textsuperscript{32}. One report was of presumed “mephedrone-induced euvolaemic hypo-osmotic hyponatraemia with encephalopathy and raised intra-cranial pressure” in a 15 years old
Information on the unwanted effects in these individuals was extracted from the routine Emergency Department (ER) and Medical notes, rather than using a pro forma with pre-defined unwanted effects. The most common by observed unwanted effects on the presentation to the ER was agitation (38.9% of patients); other common effects were palpitations (25.0%), vomiting (13.9%), chest pain (12.5%), self-limiting pre-hospital seizures (6.9%) and headache (7.2%). In particular, there were no reports of skin discolouration or cool/cold peripheries in this series. Serum sodium concentrations were measured in 34 (47.2%) individuals, and were normal in 33 (97.1% of those measured). One patient who died following analytically confirmed mephedrone use had hyponatraemia with a sodium concentration of 125 mmol/L on the presentation to the ER. Subsequent review at the coroner's inquest and of the medical notes suggested that this was secondary to excess fluid intake and water intoxication.\textsuperscript{34,30}

**Animal models of mephedrone effects and toxicity**

There have been a number of recent publications describing the
use of animal models to investigate potential pharmacological mechanisms of activity and acute toxicity of mephedrone\textsuperscript{31,35,36}.

The effect of mephedrone on the uptake of serotonin (5-HT) and dopamine has been investigated using isolated synaptosomes from rats\textsuperscript{35}. Overall, these studies demonstrated that mephedrone inhibited the uptake of both serotonin and dopamine and that mephedrone has both affinity in serotonin and dopamine membrane transporters and receptors (5-HT2 and D2 receptors). This uptake study suggests that mephedrone has a similar effective profile to that of other amphetamine-like compounds. In a rat model, using microdialysis at the nucleus accumbens, mephedrone administration resulted in an increase in both extracellular concentrations of serotonin and dopamine, and the effect was greater for serotonin\textsuperscript{37}. Although repeated administration of mephedrone resulted in hyperthermia similar to repeated MDMA administration, there were no long-term changes in striatal or cortical amine concentrations that were seen with repeated MDMA administration. A further microdialysis study in the rat nucleus accumbens, demonstrated that mephedrone and amphetamine had similar effects on increasing dopamine concentrations (496% increase and 412% increase, respectively) whereas MDMA had a moderate effect (235% increase). Mephedrone and MDMA had similar effects on serotonin concentrations (941% and 911%, respectively) compared to
amphetamine (165% increase) \(^{31}\).

Rats given mephedrone in a human like binge use pattern, developed stimulant like hyperthermia and locomotor stimulation \(^{38}\), and resulted in an increase in locomotor activity and a reduction in social preference \(^{39}\). Subsequent histological analysis of the rat brain, to determine the pattern of brain activation, demonstrated that this was comparable to the combined pattern seen in methamphetamine and MDMA use. It was concluded that mephedrone had a similar effect profile as a MDMA/methamphetamine hydrid \(^{39}\).

**Chapter 3. Methods**

**Animals**

Male Sprague–Dawley rats (150–300 g; Lasco) were initially housed in groups of four per cage, but were separated on the day prior to pretreatment. They were housed in a temperature-controlled (21°C) and humidity-controlled (79%) colony room under a 12-h light/dark cycle (lights on at 07:00 hours) with food and water available *ad libitum*. All experimental protocols were consistent with the Office of Laboratory Animal Welfare regulations and were approved by the Animal Ethics Committee of China Medical
University.

**Drug Administration**

Mephedrone (Toronto Research Chemicals Inc.) was dissolved in sterile saline and administered subcutaneously (s.c.). All vehicle injections consisted of sterile saline. Animals were separated into five groups by different doses of mephedrone administration, 5 mg/kg, 10 mg/kg, 20 mg/kg and 40 mg/kg. The control group received an equivalent volume of saline.

**Conditioned Place Preference (CPP)**

Conditioned place preference (environmental place conditioning) is a commonly used technique to evaluate preferences for environmental stimuli that have been associated with a positive or negative reward. In general, this procedure involved several trials where the animal was presented with the positive stimulus (e.g., food or effects of a drug of abuse) paired with placement in a distinct environment containing various cues (e.g., tactile, visual, and/or olfactory). When later tested in the normal state, approaches and the amount of time spent in the compartments previously associated with the positive stimulus served as an indicator of preference and a measure of reward learning.

Each place conditioning apparatus consisted of an open field
enclosed in separate light- and sound-attenuating chambers and contained three distinct sensory environments. The floor of the open field consisted of interchangeable halves made of one of two textures. The combination of floor textures was selected on the basis of previous studies\textsuperscript{40} showing that rats spent an average of about 50% time on each floor type during preference tests. Thus, the apparatus was "unbiased". Specifically, the floors were either a "grid" made from stainless-steel rods or a "hole" floor made from perforated stainless-steel. Prior to each session the open field and floors were cleaned using a 70\% EtOH solution. Rats were conditioned using a modified standard place conditioning procedure \textsuperscript{41}.

There are numerous advantages of the conditioned place preference and aversion protocol. It is methodologically simple and only requires two to three weeks to perform all steps of the procedure \textsuperscript{42}. It allows both rewarding and aversive effects to be tested and it provides unique information about the motivational effects of unconditioned stimuli \textsuperscript{43}. In drug testing the conditioned reward or aversive effects can be tested in a drug free state where the animals will not be impaired due to drug use \textsuperscript{42}. The testing is also sensitive to the effects of low drug doses \textsuperscript{42}. Conditioned place preference is well suited to measure the temporal profile of drugs as well as the
aversive effects of withdrawal.

**Procedures**

The flow chart of the procedures of the study are presented on Figure 2.

Place conditioning consisted of the following four phases. (Figure 3)

1. Habituation phase (2 days): on two 30-minute sessions (1 session/day), rats were able to freely explore the entire open field apparatus using a non-discriminative flat plastic floor.

2. Pre-conditioning bias test (1 day): to determine whether a pre-existing bias existed, a single 10-minute test session was conducted during which rats were allowed to freely explore the open field with half of the arena containing the "grid" floor and half of the arena containing the "hole" floor. The amount of time spent in each compartment was recorded to assess the unconditioned preference and then rats were returned to their home cages. Any rat showing a strong unconditioned preference (7 minutes) was excluded.

3. Conditioning (7 days): the conditioning sessions were conducted twice each day with a 6-hour intervening interval. During conditioning sessions, rats received an s.c. injection and confined for 30 minutes to the assigned
compartment. After 6 hours, rats were administered the second conditioning treatment and confined to the other compartment for 30 minutes. Rats were divided into four treatment groups. The mephedrone paired group received mephedrone (5 mg/kg, 10 mg/kg, 20 mg/kg, and 40 mg/kg) in one side of the CPP chamber (mephedrone paired) and saline in the other side (nondrug-paired) of the chamber. The treatment compartments were assigned according to a counterbalanced design, such that one half of the rats in each experimental group were conditioned with mephedrone to the spontaneously preferred side and the other half to the spontaneously nonpreferred side. The order of administration of mephedrone and saline was counterbalanced. The saline control group received saline in both sides of the CPP chamber.

(4) Post-conditioning bias test (1 day): rats were placed in the central gray chamber and allowed to freely roam the entire apparatus. Time spent in each test compartment was recorded separately for each rat for 10 minutes. The change in preference was calculated as the difference (in seconds) between the time spent in the drug-paired compartment on the testing day and the time spent in this compartment in the preconditioning session.

Statistics

One-way ANOVA, repeated measures was used to analyze the
differences of the mean body weight among the day 1, day 4 and day 10, and of the mean time spent in the drug-paired compartment between the pre-condition (day 3) and post condition (day 11). These statistical analyses were performed using the PC version of IBM SPSS Statistics 19. A two-sided \( P \)-value of less than 0.05 was considered significant.

**Chapter 4. Results**

Figure 4 shows the structure map of whole study and the results in summary. The means and the standard deviation of body weights of the saline controlled group and the four mephedrone treated groups, 5 mg/Kg, 10 mg/Kg, 20 mg/Kg, and 40 mg/Kg, among the three different measuring days are presented on Table 2. The body weight of rats was taken on the beginning of the habituation phase (day 1), and before the mephedrone injection during the conditioning phase. Two rats showed the strong unconditioned preferences in the pre-conditioning bias test were excluded. The mean body weight of these two rats was also presented on the last raw of the Table 2, for comparison. There was no significant difference among the saline controlled group, the other four different doses of mephedrone groups, 5 mg/Kg, 10 mg/Kg, 20 mg/Kg, and 40 mg/Kg, and the two excluded rats in the body weight.
Comparing the mean body weight of day 10 and day 1 (Table 3, Figure 5), while we compared the day 4 with day 1, there were significant difference among five groups. There were significant difference in saline controlled groups, the dose of 5mg/Kg, and 20mg/Kg, (saline controlled group $P=0.029$; 5mg/Kg $P=0.004$; 20mg/Kg $P=0.039$).

The mean body weight of all the mephedrone treated groups was not significantly different from that of the saline controlled group (Table 2). Also, the mean of the body weight of the two excluded rats was compared on the last raw of Table 2. As other mephedrone treated groups, there was no significant difference, while comparing the mean difference of the body weight of the excluded rats with the saline controlled group.

The curve chart of Figure 6 shows the trends of the body weights of the saline controlled group and the other four different dose treated groups, 5 mg/Kg, 10 mg/Kg, 20 mg/Kg, and 40 mg/Kg, among the three different measuring days. The mean body weight of the two excluded rats was also presented on Figure 6, for presenting that there is no significant difference among the saline controlled group, the four different doses of mephedrone groups and the two excluded rats in the body weight.
The change of preference was calculated as the difference (in seconds) between the time spent in the drug-paired compartment on the post–conditioning session (day 11) and the pre-conditioning session (day 3). Table 4 show the difference between the post–conditioning session (day 11) and the pre-conditioning session (day 3) of the time spent in the drug-paired compartment of the saline controlled group and the groups of low mephedrone doses, 5 mg/Kg, 10 mg/Kg. And there were significant differences in the groups of the dose of 20 mg/Kg ($P=0.009$), and the dose of 40 mg/Kg ($P=0.007$). Two rats showed the strong unconditioned preferences in the pre-conditioning bias test were excluded (454 seconds and 172 seconds, respectively). To show the reason why these rats had been excluded, the mean of the time stayed in the drug-paired compartment of these two rats was presented in the last row of table 4.

Table 5 shows the mean differences of the time spent in the drug-paired compartment of the saline controlled group and the four mephedrone treated groups in day 3 (pre-conditioning) and day 11 (post-conditioning). There was no significant differences in day 3 ($P=0.975$). In day 11 (post-conditioning), there was no significant difference among treatments with low doses (5 mg/Kg $P=0.893$; 10 mg/Kg $P=0.804$), while there was a significant difference in high doses (20 mg/Kg $P=0.012$; 40 mg/Kg $P=0.001$) treated groups.
The curve chart of Figure 7 presents the significant difference of time spent in the drug-paired compartment in the high dose groups compared the day 11 (post-conditioning) and the day 3 (pre-conditioning). There were significant differences in the group of dose 20 mg/Kg ($P=0.009$), and in the group of dose 40 mg/Kg ($P=0.007$). The time spent in low doses of the mephedrone treated groups was not significantly different between the day 11 (post-conditioning) and day 3 (pre-conditioning). (saline controlled $P=0.396$; 5 mg/Kg $P=0.720$; 10 mg/Kg $P=0.625$).

The bar chart of Figure 7 compares the time spent in the drug-paired compartment among the groups of the saline controlled and four different mephedrone doses, 5 mg/Kg, 10 mg/Kg, 20 mg/Kg, and 40 mg/Kg, in the pre-conditioning (day 3) (black bar) and post-conditioning (day 11) (white bar). There was no significant difference in the saline controlled group ($P=0.396$), the dose of 5 mg/Kg ($P=0.720$), and the dose of 10 mg/Kg ($P=0.625$). And there were significant differences in the groups of the dose of 20 mg/Kg ($P=0.009$), and the dose of 40 mg/Kg ($P=0.007$).

The diagram of Figure 8 shows the time spent by the rats in the drug-paired compartment. The time spent on day 3 (pre-conditioning) had no significant difference among the saline controlled group and
other four mephedrone, 5 mg/Kg \((P=1)\), 10 mg/Kg \((P=0.674)\), 20 mg/Kg \((P=0.876)\), and 40 mg/Kg \((P=0.975)\) treated groups. On the day 11 (post-conditioning), comparing the saline controlled group with the mephedrone group of dose 5 mg/Kg \((P=0.893)\), and the dose 10 mg/Kg \((P=0.804)\), there were no significant difference. However, there were significant differences with dose 20 mg/Kg \((P=0.012)\), and dose 40 mg/Kg \((P=0.001)\) when compared with the other groups tested.

Chapter 5. Discussion

In this presented study, the dose response of mephedrone on body weight changes and conditioned place preference responses were evaluated.

The body weight gains had no significant difference among the saline controlled group and the mephedrone treated groups. A single dose repeated and acute administration of mephedrone in high doses (20 mg/Kg/day and 40mg/Kg/day, once daily for 7 days) resulted in the development of behavioral sensitization and significantly increased CPP scores. It is also found that, mephedrone-induced rewarding effects were demonstrable in both high doses of 20 mg/Kg and 40 mg/Kg. And this indicated that rats under the repeated mephedrone administration in
high-dose may be addictive to mephedrone.

In 2011, the first case died of suspected smoking mephedrone had been reported in Taiwan, there were few articles published about mephedrone then. We mentioned about the needs of call for extensive attention on the drug. Being a pilot study of the emerging synthetic abuse substance, the dose response of mephedrone on the body weight changes and CPP response were only evaluated. Extensive investigation on the addictive liability of mephedrone should be conducted.

Another common model for assessing the rewarding properties of drugs is the self-administration paradigm. As the name suggests, this paradigm consists of recording the number of times an animal produces a response, for example a lever press, that results in an infusion of drug, which is usually given intravenously. The self-administration paradigm is an important tool for screening drugs for abuse potential and to elucidate the rewarding effects of drugs. Although the conditioned place preference and self-administration paradigms both measure the rewarding properties of drugs, there are important differences between these two models. An important contrast between these two paradigms is the difference in methodological procedures. Unlike the CPP paradigm, the
self-administration paradigm requires surgical implantation of a catheter, usually for intravenous drug administration, and an extensive operant training history. Moreover, in CPP, the subjective effects of the drug are present prior to the task, whereas in the self-administration paradigm, a subject is learning a task where responses produce near-immediate effects from drug administration. The latter appears to be most similar of these two models to drug use in humans.

Initial reports suggested that mephedrone was a "non-addictive" alternative to methamphetamine, cocaine, and other stimulants, but there is increasing evidence indicating that it causes compulsive use patterns that are similar to or stronger than those of other recreational euphoric stimulants.

Given the pharmacology of mephedrone, it is unlikely that long-term use would be associated with a physical dependency and withdrawal syndrome. However, similar to other classical and novel sympathomimetic recreational drugs, there is a potential that mephedrone could be associated with the development of psychological dependence. Mephedrone availability and use have only been significantly noted during 2009 and 2010. Therefore, given its short history on the recreational drug scene, it is not possible to determine the true prevalence of dependency in chronic long-term users. Given that mephedrone is not likely to be
associated with a physical dependence syndrome, there is unlikely to be any specific management in terms of a withdrawal syndrome. Users with psychological dependence may require medical treatment for their symptoms on discontinuation. But, a routine withdrawal program would not be recommended as for other drugs such as gamma-hydroxybutyrate (GHB), gamma-butyrolactone (GBL), opioids, and ethanol. In those individuals with problematic use of mephedrone, there is likely to be a need for ongoing psychological support after discontinuation of use to prevent relapse.

Chapter 6. Conclusion

The current experiments provided the first evidence that mephedrone produces CPP in rats. The preference shift detected following mephedrone conditioning suggests that the drug exhibits rewarding properties that are consistent with a risk of abuse liability. Although more extensive experiments are required to compare hedonic effects of mephedrone and established drugs of abuse, the current results do provide insight into the reward profile of mephedrone. For example, mephedrone produced CPP following the
conditioning trials, and CPP can also be detected following a similar number of trials with cocaine, amphetamine, and methamphetamine \(^{51,52,53}\). One difference between mephedrone and established psychostimulants may be the threshold dose required to elicit CPP. Mephedrone only produced a significant preference shift in rats following conditioning with the higher doses of 20 and 40mg/Kg. A preference shift in rats was observed following conditioning with lower doses of 5 and 10 mg/kg, but the effects did not reach statistical significance. In contrast, CPP can be elicited by much lower doses of cocaine, amphetamine, methamphetamine, and MDMA \(^{54}\). For instance, MDMA can produce CPP in rats at a dose range of about 6–10 mg/kg, with the preference shift beginning to wane at about 20 mg/kg \(^{55,56,57}\). Methamphetamine produces biphasic effects, with low doses producing reward and high doses producing aversion \(^{58}\).

The Risk Assessment reveals limited scientific evidence and points out that further studies are needed on the overall health and social risks of mephedrone. However, because of its stimulant properties, its ability to produce dependence in users, its potential attractiveness, the risk to health, the lack of medical benefits, and the need to apply precaution, mephedrone should be controlled.

Future studies that various factors such as dose, dosing frequency, and forced abstinence intervals are needed to further assess the motor
activating properties of mephedrone. In the presented experiments all the rats had been sacrificed immediately after behavioral testing and their brains were rapidly extracted and different brain regions were dissected out and frozen at -80°. Further, we will also examine the phosphorylation levels of various key signaling molecules in the brain regions implicated in addictive behaviors after acute and repeated mephedrone administration in the future.


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34. Wood DM, Dargan PI. Novel Psychoactive Substances: How to
Understand the Acute Toxicity Associated With the Use of These Substances. Therapeutic drug monitoring 2012.


51. Soderman AR, Unterwald EM. Cocaine-induced mu opioid receptor occupancy within the striatum is mediated by dopamine D2 receptors. Brain research 2009;1296:63-71.


Appendix

Table 1. The review of the mephedrone

<table>
<thead>
<tr>
<th><strong>Mephedrone</strong></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Chemical Name</td>
<td>4-methylmethcathinone</td>
</tr>
<tr>
<td>Synonyms / Colloquial Terms</td>
<td>Miaow, Drone, 4-MMC, MMCat, MD3, Roxy, Mefedron (Norway), Krabba (Sweden), Meow Meow/Miaow Miaow (UK esp. Brighton and Hove), Bubbles (UK esp. Scotland), Meph, Rush, Plant feeder, White Magic, Challenge (=mephedrone+ketamine)</td>
</tr>
<tr>
<td>Type</td>
<td>Chemical</td>
</tr>
<tr>
<td>Origin</td>
<td>Mephedrone is a phenethylamine research chemical (RC) with a relatively short history of human consumption related to cathinone (the active ingredient of the khat plant). Forum chatter about mephedrone seems to have begun in or after 2007.</td>
</tr>
<tr>
<td>Active Constituents</td>
<td>2-Methylamino-1-(4-methylphenyl)propan-1-one</td>
</tr>
<tr>
<td>Status</td>
<td>Novel</td>
</tr>
<tr>
<td>IUPAC Name</td>
<td>2-Methylamino-1-(4-methylphenyl)propan-1-one</td>
</tr>
<tr>
<td>Molecular Formula</td>
<td>C_{11}H_{15}NO</td>
</tr>
<tr>
<td>Molecular Weight</td>
<td>177.242 g/mol</td>
</tr>
<tr>
<td>Routes</td>
<td>Oral, insufflation, IV, rectal, smoking</td>
</tr>
</tbody>
</table>
Table 2. The comparison of the mean body weights of the saline controlled group and other four different doses of mephedrone treated groups in the three weight taking days.

<table>
<thead>
<tr>
<th></th>
<th>Control Group</th>
<th>5mg/Kg</th>
<th>10mg/Kg</th>
<th>20mg/Kg</th>
<th>40mg/Kg</th>
<th>Excluded Rats</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mean</td>
<td>SD</td>
<td>p-value</td>
<td>mean</td>
<td>SD</td>
<td>p-value</td>
</tr>
<tr>
<td>Day 1</td>
<td>184.33</td>
<td>12.34</td>
<td>1.000</td>
<td>190.33</td>
<td>7.77</td>
<td>0.973</td>
</tr>
<tr>
<td>Day 4</td>
<td>207.67</td>
<td>3.79</td>
<td>0.982</td>
<td>206.00</td>
<td>15.13</td>
<td>1.000</td>
</tr>
<tr>
<td>Day 10</td>
<td>289.33</td>
<td>3.79</td>
<td>0.861</td>
<td>276.00</td>
<td>13.00</td>
<td>0.636</td>
</tr>
</tbody>
</table>
Table 3. The comparison of the body weight gain in the different groups.

<table>
<thead>
<tr>
<th></th>
<th>Day 1</th>
<th></th>
<th>Day 4</th>
<th></th>
<th>Day 10</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>Control Group</td>
<td>184.33</td>
<td>12.34</td>
<td>207.67</td>
<td>3.79</td>
<td>289.33</td>
<td>3.79</td>
</tr>
<tr>
<td>5mg/Kg</td>
<td>182.33</td>
<td>3.79</td>
<td>202.00</td>
<td>5.00</td>
<td>279.67</td>
<td>4.04</td>
</tr>
<tr>
<td>10mg/Kg</td>
<td>190.33</td>
<td>7.77</td>
<td>206.00</td>
<td>15.13</td>
<td>276.00</td>
<td>13.00</td>
</tr>
<tr>
<td>20mg/Kg</td>
<td>194.33</td>
<td>5.69</td>
<td>211.00</td>
<td>2.65</td>
<td>287.00</td>
<td>8.19</td>
</tr>
<tr>
<td>40mg/Kg</td>
<td>186.00</td>
<td>5.20</td>
<td>199.67</td>
<td>5.77</td>
<td>277.00</td>
<td>0.12</td>
</tr>
</tbody>
</table>

* p<0.05 as the mean body weight on day 4 compared to that on day 1.

** p<0.01 as the mean body weight on day 4 compared to that on day 1.

*** p<0.001 as the mean body weight on day 10 compared to that on day 1.
Table 4. The time spent in the drug-paired compartment.

<table>
<thead>
<tr>
<th>Group</th>
<th>Day3 n</th>
<th>Mean</th>
<th>SD</th>
<th>Day11 n</th>
<th>Mean</th>
<th>SD</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control Group</td>
<td>3</td>
<td>303.67</td>
<td>7.02</td>
<td>3</td>
<td>285.33</td>
<td>32.72</td>
<td>0.396</td>
</tr>
<tr>
<td>5 mg/Kg</td>
<td>3</td>
<td>307.00</td>
<td>8.19</td>
<td>3</td>
<td>319.00</td>
<td>53.33</td>
<td>0.72</td>
</tr>
<tr>
<td>10 mg/Kg</td>
<td>3</td>
<td>345.00</td>
<td>39.40</td>
<td>3</td>
<td>326.67</td>
<td>45.24</td>
<td>0.625</td>
</tr>
<tr>
<td>20 mg/Kg</td>
<td>3</td>
<td>332.67</td>
<td>22.37</td>
<td>3</td>
<td>440.00</td>
<td>32.23</td>
<td>0.009**</td>
</tr>
<tr>
<td>40 mg/Kg</td>
<td>3</td>
<td>321.67</td>
<td>56.62</td>
<td>3</td>
<td>509.33</td>
<td>31.50</td>
<td>0.007**</td>
</tr>
<tr>
<td>Excluded</td>
<td>2</td>
<td>313.00</td>
<td>199.40</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td></td>
</tr>
</tbody>
</table>

** p<0.01 as day 11 compared to day 3.
Table 5. The comparison of the mean time spent of the saline controlled group with the other four doses of mephedrone in day 3 and day 11.

<table>
<thead>
<tr>
<th>Control Group n=3</th>
<th>5mg/Kg n=3</th>
<th>10mg/Kg n=3</th>
<th>20mg/Kg n=3</th>
<th>40mg/Kg n=3</th>
</tr>
</thead>
<tbody>
<tr>
<td>mean</td>
<td>SD</td>
<td>mean</td>
<td>SD</td>
<td>mean</td>
</tr>
<tr>
<td>Day 3</td>
<td>303.67</td>
<td>7.02</td>
<td>307.00</td>
<td>8.19</td>
</tr>
<tr>
<td>Day 11</td>
<td>285.33</td>
<td>32.72</td>
<td>319.00</td>
<td>53.33</td>
</tr>
</tbody>
</table>

*p<0.05 as the mephedrone treated group of dose 20 mg/Kg compared to saline controlled group.

**p<0.01 as the mephedrone treated group of dose 40 mg/Kg compared to saline controlled group.
Figure 1. The comparison of the chemical structures among mephedrone, cathinone, amphetamine, and methamphetamine.
Figure 2. The flow chart of the study procedures
Figure 3. The CPP procedures in this study
1. The mean body weight was no significant difference among the groups.
2. The time spent in the drug paired compartment had a significant difference in high-dose compared to the saline controlled group.

Figure 4. The structure map of the study.
Figure 5. The comparison of the mean body weights gain in the different groups.

Data are expressed as mean ± SD (n=3, per group). *** p<0.001 as the mean body weight on day 10 compared to that on day 1. ** p<0.01 as the mean body weight on day 4 compared to that on day 1. * p<0.05 as the mean body weight on day 4 compared to that on day 1.
Figure 6. The comparison of the mean body weights of the different groups in the three weight taking days.

Comparing the mean body weight of the saline controlled group and other four different doses of the mephedrone treated groups in the three different measuring days. For presenting the unbiased condition in the body weight, the body weights of the saline controlled group and the excluded two rats were also compared. Data are expressed as mean ± SD (n=3, per group).
Figure 7. The mean time spent in the drug-paired compartment between the day 3 and day 11.

The time spent in the drug-paired compartment among the groups of the saline controlled and four different mephedrone doses, in the pre-conditioning (day 3) and post-conditioning (day 11). Data are expressed as mean ± SD (n=3, per group) ***p<0.01 as the mean time spent in the drug-paired compartment of day 11 compared to day 3 in the five groups.
Figure 8. The mean time spent in the drug-paired compartment among the five groups.

The mean time spent in the drug-paired compartment among the saline controlled group and other different doses of mephedrone treated groups. The data of Day 3 and day 11 are expressed as mean ± SD (n=3, per group).

*p<0.05 and **p<0.01 as the high doses of mephedrone treated groups (20mg/Kg, and 40mg/Kg) compared to the saline controlled group in the day 3 and day 11.