

**The Acute, Chronic, and Teratological Effects of
Methamphetamine on Aggressive Behaviour in Adolescent
Hooded Rats**

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By

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Abbreviations

CNS	Central Nervous System
DAT	Dopamine transporter
DSM-IV TR	The Diagnostic and Statistical Manual of Mental Disorders (4 th Edition, Text Revised)
ESR	Environmental Science & Research Limited
i.p.	Intraperitoneal injection
Mg/kg	Milligrams Per Kilogram (drug dosage)
METH	Methamphetamine
n	Number
PND	Post Natal Day
PET	Positron emission tomography
S	Saline
SEM	Standard Error of the Mean

Abstract

Methamphetamine is a widely abused psychostimulant often associated with aggressive, violent, and criminal behaviour. Research into the effects of adolescent methamphetamine use on aggressive behaviour is limited. This study aimed to establish whether methamphetamine would induce aggressive behaviour following an acute dosing regimen and a chronic dosing regimen. It also aimed to establish a teratological or delayed effect on adult behaviour. To investigate this 20 male and 20 female adolescent rats were equally divided into treatment and control conditions. The treatment condition received a single dose of methamphetamine (2mg/kg) on postnatal day (PND) 35 followed by twice daily doses of methamphetamine (2mg/kg) from PND 36-46. This was done via intraperitoneal injection. The control condition received comparable doses of saline. Animals were tested using the resident intruder test following the single dose, after the completion of the final dose, and again in early adulthood (PND 90). Results found an acute dosing regimen significantly reduced aggressive-like behaviour. A chronic dosing regimen increased aggressive-like behaviour however, this relationship was less clear. Finally, the results found increased aggressive behaviour in adult animals following methamphetamine use in adolescence. This provides preliminary evidence for a teratological effect and support for the neuronal imprinting theory.

1.0 Introduction

1.1 General Overview

Adolescence is a vulnerable period in which an individual makes the transition from childhood to adulthood. During this phase of development there is a dramatic increase in risk taking behaviour, including the initiation of substance use (Arnett, 1996). Substance use during adolescence can have damaging effects on both the brain and behaviour. These effects have been shown to outlast the substance use itself (Andersen & Navalta, 2004). This is called a teratological effect. There is currently insufficient research surrounding the behavioural effects of substance use when taken during adolescence. For this reason there is a need to research the effects of these drugs with a strong emphasis on those substances that are commonly abused.

Methamphetamine, an amphetamine derivative known as ice, meth, glass, shabu, or P, is a widely abused psychostimulant. Research released by the Organized and Financial Crime Agency (Organized and financial crime agency New Zealand [OFCANZ], 2010) identified Oceania as having one of the highest amphetamine-type substance user rates per capita in the world. In New Zealand young people, in conjunction with Maori and Pacific Islanders are alleged to be of greatest risk of encountering harm from methamphetamine use (New Zealand Police, 2009). In 2001 in a sample aged 13-45 years 11% of individuals reported using amphetamines at least once in the past 12 months (Wilkins, Sweetsur, & Casswell et al., 2006). Use during adolescence, aged 15-19 years, increased from 4.0% to 7.5% between 1998 and 2001 (Wilkins et al., 2006). Methamphetamine use is as common in females. In a sample of 350 American substance abusers over half, 56%, were female (Brecht, O'Brien, Von Mayrhauser, & Anglin et al., 2004).

Methamphetamine, synthesized from pseudoephedrine or ephedrine, is taken for its euphoric and wakefulness effects (Tyner & Fremouw, 2008). However chronic methamphetamine use can result in hostility, paranoia, hallucinations, and obsessive and aggressive behaviour (Hall & Hando 1994; Shearer, Sherman, Wodak, & Beek et al., 2002).

Currently there is minimal scientific literature on the short-term and longer-term outcomes of methamphetamine use during adolescence. Therefore, the primary aim of this research was to provide an assessment of the short-term and longer-term behavioural effects, namely aggression, that result from repeated adolescent exposure to methamphetamine.

1.2 Substance Use and Addiction

To understand the possible outcomes of repeated exposure to methamphetamine, the diagnostic criteria for substance abuse and substance dependence disorder are described below. Substance-related disorders can lead to significant personal, interpersonal, occupational, and social impairment. Substance abuse and substance dependence are two separate substance use disorders and these disorders are briefly described below. *The Diagnostic and Statistical Manual of Mental Disorders* (DSM-IV TR; APA, 2000) is the leading tool for diagnosing these disorders.

The DSM-IV TR defines substance abuse as a maladaptive pattern of substance use manifested by recurrent and significant adverse consequences related to the repeated use of a substance. For a diagnosis the user must display at least one of four criteria in a 12 month period. These include failure to fulfil major role obligations at work, school or home, repeated use in situations where it is physically hazardous, multiple legal problems, and

recurrent social and interpersonal problems. The symptoms must not meet criteria for substance dependence disorder.

Conversely the DSM-IV TR defines substance dependence as a cluster of cognitive, behavioural, and physiological symptoms indicating that the individual continues substance use despite significant substance-related problems. For a diagnosis the user must display at least one of seven criteria in a 12 month period. These include tolerance, withdrawal, taking larger amounts over a longer than intended period, persistent desire or unsuccessful effort to cut down or control use, and spending more time on activities necessary to obtain, use or recover from the substance. See the DSM-IV TR for a full description of all symptoms; APA, 2000. Before someone can have substance use disorder they must first undergo an experimentation phase with the drug. Some possible reasons for experimentation are covered below.

1.2.1 Initiation of Substance Use

The initiation of substance use and its experimentation is a diverse and complex occurrence. Various risk factors may include, but are not limited to, genetic, environmental, biological, and psychological factors. There is a large portion of literature reserved for the possible causes of substance use. For the purpose of this study only genetic and environmental factors will be considered.

Genetic and familial factors are biological characteristics that may impact on the initiation of substance use (Weinberg, Rahdert, Colliver, & Glantz, 1998). Numerous studies of monozygotic and dizygotic twins have shown drug use to be highly heritable for substances such as marijuana, amphetamines, heroin, and cocaine (Xian et al., 2000; Tsuang et al.,

1999). Behavioural genetic research proposes that 40% to 60% of the vulnerability towards developing drug dependence is heritable (Button et al., 2006; Han, McGue, & Iacono, 1999). Using twin studies Tsuang et al., (1999) investigated the likelihood that respondents who were exposed to amphetamines would proceed from exposure to initiation of use to regular use. This was done using conditional probabilities (Tsuang et al., 1999). The conditional probability of making the transition from exposure to initiation of use was 0.44. The conditional probability for the transition from initiation of use to regular use was 0.60 (Tsuang et al., 1999). These probabilities indicate that the process of moving from exposure to regular use is highly heritable for amphetamines.

Environmental risk factors that may influence the initiation of substance use include low socio-economic status, family mental health problems, parental and older sibling drug use, lack of parental warmth, physical and sexual abuse, poor school performance, and peer pressure (Berk, 2006; Prinstein, Boergers, & Spirito, 2001). Peer substances use is consistently found to be one of the highest predictors of substance use in adolescents (Hawkins, Catalano, & Miller, 1992). In a sample of youth aged 10-15 perceived peer alcohol and marijuana use predicted the onset of marijuana use (D'Amico & McCarthy, 2006).

Although research suggests many factors influence substance use it is commonly agreed that behaviours are the result of an interaction between numerous factors. Substance use has the potential to escalate into drug dependence, of which the mechanisms of action are covered below.

1.2.2 Mechanisms of Addiction

Drug addiction occurs when substance use shifts from being voluntary to being habitual and compulsive (Everitt & Robbins, 2005). For a drug to lead to dependency it must first reinforce people's behaviour (Cardinal & Everitt, 2004; Carlson, 2007). Initially it is positive reinforcement that mediates the effects of drugs. Positive reinforcement refers to the effect that certain stimuli have on the behaviours that precede them (Carlson, 2007). When behaviour, such as substance use, is regularly followed by an appetitive stimulus, such as pleasurable feelings or reduced stress, then that behaviour is reinforced and will occur more often.

The process by which positive reinforcement influences substance abuse is through instrumental conditioning (Cardinal & Everitt, 2004). This is the process by which behaviour is altered when there is a contingency between behaviour and a reinforcing outcome (Cardinal & Everitt, 2004). Drugs act as reinforcers as they increase the likelihood of responses that produce them (Everitt & Robbins, 2005). Drug abuse occurs initially because of its positive hedonic impact. Additionally, stimuli consistently present in the drug taking environment gain motivational power due to their association with the drug. These stimuli, such as drug paraphernalia, thereby elicit and support drug seeking and assist in relapse (Everitt & Wolf, 2002).

Various neuroanatomical substrates are responsible for the reinforcing effects of substance use. The neuronal pathways responsible for this are the same as those responsible for natural rewards such as food, drink, and sex (Cami & Farre, 2003). It is proposed that most substances of abuse stimulate the release of dopamine from neurons in the brain (Carlson, 2007). These dopaminergic neurons responsible for reinforcement are located in the

mesocortical and mesolimbic dopamine systems which originate in the ventral tegmental area (Cami & Farre, 2003). The mesolimbic system projects to limbic structures including the nucleus accumbens, amygdala and hippocampus. This system is implicated in reinforcement, memory and the euphoric and addictive properties of substances (Cami & Farre, 2003; Carlson, 2007). The mesocortical system projects from the ventral tegmental area to the prefrontal cortex, orbitofrontal cortex, and anterior cingulate. The mesocortical system is involved in the conscious experience of the effects of substance use, craving, and the compulsion to take substances (Cami & Farre, 2003).

The nucleus accumbens, located in the striatum, plays an important role in the reinforcing effects of stimuli (Cardinal & Everitt, 2004). During substance use the release of dopamine into the nucleus accumbens causes euphoria and reinforcement (Cami & Farre, 2003).

Research using positron emission tomography (PET) on human cocaine users showed a dramatic increase in dopamine transporters in the striatum (Volkow & Fowler, 2002).

Once behaviour, such as substance dependence, has been established it must then be maintained. This occurs via negative reinforcement (Carlson, 2007). Negative reinforcement refers to when behaviour reduces an aversive stimulus. For example, substance use reduces stress or anxiety (Cami & Farre, 2003). In order to experience these negative effects of substances an individual must acquire tolerance to a substance and subsequently experience withdrawal effects.

Tolerance refers to a state of progressively decreasing responsiveness to a drug (Julien, 2001). When a drug is taken more frequently larger doses of that drug are required in order to achieve the effects such as euphoria, originally obtained by a smaller dose. There are two

main pharmacological mechanisms involved in the development of tolerance (Julien, 2001). These are metabolic tolerance and cellular-adaptive or pharmacodynamic tolerance. In metabolic tolerance the enzyme responsible for metabolizing the drug increases and therefore more of the drug is required to maintain the same amount in the body. In cellular-adaptive or pharmacodynamic tolerance receptors in the brain adapt to the presence of the drug. This occurs via a process called down regulation whereby neurons adapt by either reducing the number of receptors available to the drug or by reducing their sensitivity.

Once tolerance has been established the sudden removal of a drug will result in physical or psychological withdrawal effects (Carlson, 2007). Withdrawal effects for any drug are commonly the opposite of the effects of the drug itself. For example methamphetamine produces positive effects such as alertness, euphoria, and reduced appetite (Tyner & Fremouw, 2008). However when consumption is ceased an individual will experience fatigue, depression and increased appetite. These negative effects are only lessened by consuming the drug again. However in doing so the drug removes these negative effects and is therefore negatively reinforcing the drug taking behaviour. Withdrawal symptoms and tolerance share the same basic mechanism. That is, they are the body's attempt to compensate for the drug and remain at an optimal level. Both tolerance and withdrawal are essential for maintaining addiction (Cami & Farre, 2003).

Substance use and dependence are serious disorders which can lead to significant impairment. Substance use is reinforced via dopamine transporters in the mesocortical and mesolimbic systems. The nucleus accumbens is especially implicated in the rewarding effects of substances. Once substance use is acquired negative reinforcement, through tolerance and withdrawal, maintains it and drug use shifts to dependence.

1.3 Neurodevelopment: The Adolescent Point of Vulnerability

Adolescence is the transitional phase from childhood to adulthood. During this period certain developmental alterations are made in preparation for the transition from immaturity and dependence to maturity and independence (Spear, 2007). Commonly the onset of puberty coincides with the beginning of adolescence. However, it is important to note that the two are not synonymous (Spear, 2000). Puberty, or sexual maturation, is merely a specific developmental alteration that occurs as part of adolescence. The brain continues to develop after sexual maturation has been met (Spear, 2000). It is still unclear as to how long this adolescent brain development, known as the periadolescent period, continues. The onset of the periadolescent period in humans spans from roughly 12 to 18 years of age and may extend up to 25 years (Spear, 2000). The following sections will consider whether or not interference during this transitional period of brain development may have implications later in life.

During the adolescent period there is a need to gain the appropriate skills for independence. In order to acquire growth adolescents partake in sensation seeking and risk taking behaviours (Arnett, 1992; Arnett, 1996). Laviola, Adriani, Terranova, & Gerra (1999) suggest these traits are characterized by the continuing necessity to experiment with various novel and complex sensations. Such behaviours include aggression and other antisocial behaviours, school misconduct, and substance use. These types of behaviour are so common in adolescence that they are considered normal (Moffitt, 1993). Although these behaviours do have benefits they also have negative consequences. Specifically, research has shown that taking drugs during adolescence increases the risk of drug abuse and helps form long term abuse patterns in adulthood (Stansfield & Kirsstein, 2006).

Adolescence is a time of considerable neural restructuring and sculpting of the brain (Luciana, 2010; Spear, 2007). Brain regions responsible for basic functioning such as motor and sensory processes develop first (Casey, Tottenham, Listo, & Durston, 2005). This is followed by the development of areas associated with the control of thoughts and actions (Casey et al., 2005).

Neurodevelopment is evident in both cellular and anatomical structures (Carlson, 2007). Cellular development is marked by excessive synapse formation, receptors, and myelination. Neurons that are rarely stimulated lose their synapses in a process called synaptic pruning which occurs via apoptosis (Carlson, 2007). Around 40% of synapses are lost during this process in order to reach the adult level (Berk, 2006).

Anatomical structures that go through notable development include the prefrontal cortex, amygdala, nucleus accumbens, and hippocampus (Carlson, 2007). Each of these structures serves a different purpose in brain functioning. The prefrontal cortex is involved in formulating plans and strategies. Specifically the growth of the prefrontal cortex in adolescence allows for the development of abstract reasoning, affect and judgement making. However the prefrontal cortex is not fully developed until later in life and therefore neither is the ability to make mature judgements (Chambers, Taylor, & Potenza, 2003). The amygdala regulates emotional reactions. The nucleus accumbens is responsible for motivation, reinforcement, and addiction. The hippocampus is responsible for memory formation. (Andersen, 2003; Carlson, 2007; Spear, 2000). Research has also shown there to be sex differences in neurodevelopment. For example, females in mammalian species mature more rapidly than males and human females reach peak levels of grey matter earlier than males at 11.6 years versus 12.8 years (Andersen, 2003; Spear, 2000).

Exposure to drugs during the periadolescent phase alters the development of specific brain regions (Andersen & Navalta, 2004). As a result there is a negative impact on normal development. The below section will argue that substance use in this transitional phase can produce damaging consequences in adulthood.

1.3.1 Neuronal Imprinting Theory

Neuronal imprinting occurs when the effects of drug exposure outlast the drug itself (Andersen & Navalta, 2004). It is the notion that the long term effects of drug exposure in adolescence are delayed and express themselves in adulthood (Andersen, 2003). Specifically, drug exposure during adolescence alters the development of the particular brain regions where the drugs are active (Andersen & Navalta, 2004). This is proposed to be caused by the ‘normal developmental trajectory’ of the drug-affected brain circuit being altered in such a way that it differs from what would be predicted if the drug exposure had occurred in adulthood (Andersen & Navalta, 2004).

As mentioned previously, adolescence is a developmental period when the brain is undergoing major neurological changes. Drug exposure during this period will most likely have its greatest impact on brain regions that are undergoing active development. This is compared to those brain regions that have reached their adult status (Anderson & Navalta, 2004; Anderson 2003; Stansfield & Kierstein, 2003). The effect of chronic drug use in adolescence occurs by integrating drug-induced changes into permanent developmental adjustment.

Neuronal imprinting has been shown to occur with a range of drugs including marijuana, nicotine, alcohol, and stimulants (Andersen, 2003). One compelling example is that of exposure to methylphenidate in rats (Andersen, Arvanitogiannis, Pliakas, LeBlanc, & Carlezon, 2001). After repeated administration of methylphenidate throughout adolescence the rats were tested in adulthood on place conditioning, a procedure in which rats learn to associate drug effects with particular environments. Rats that were exposed to methylphenidate had a reduced responsiveness to cocaine's rewarding effects and an increased responsiveness to its aversive effects. Rats not exposed to the drug had the opposite response to cocaine's effects. A different sample of rats were treated with methylphenidate in adulthood and tested in later adulthood. These rats did not show an aversive response to cocaine. This research suggests that exposure to methylphenidate in adolescence, as compared to adulthood, has an enduring effect on brain and behaviour development.

Considering the research, it is likely that adolescent exposure to methamphetamine may produce behavioural changes in adulthood. These changes in adulthood would provide evidence for the neuronal imprinting theory.

1.4 Methamphetamine

Methamphetamine is a powerful psychostimulant belonging to the amphetamine class. This central nervous system stimulant is taken by a wide range of people for its psychoactive effects. Methamphetamine produces feelings of increased euphoria, focus, mental alertness, self esteem, confidence and reduced appetite (Anglin, Burke, Perrochet, Stamper, & Dawud-Noursi, 2000; Darke, Kaye, McKetin, & Dufrou, 2008). Typical after-effects include depression, fatigue, sleep disturbance, headaches and cravings (Anglin et al., 2000; Darke et

al., 2008). Methamphetamine is metabolized slower than other stimulants, such as cocaine, and produces these effects for up to 12 hours (Anglin et al., 2000).

Repeated and chronic methamphetamine use is associated with paranoia, hallucinations and delusions of persecution, obsessive and aggressive behaviours (Hall & Hando 1994; Shearer et al. 2002). In a New Zealand sample of 78 methamphetamine users the psychological problems commonly reported from frequent use were trouble sleeping (84%), short temper (58%), strange thoughts (56%), paranoia (55%), and anxiety (51%) (Wilkins, Girling, Sweetsur, & Butler, 2005).

The rate of methamphetamine use is becoming increasingly problematic. The estimated lifetime prevalence use in the United States of America for those aged 12 years and older is 4.9% (Substance Abuse and Mental Health Services Administration, Office of Applied Studies [OAS], 2005). However, little is known about the drug's long term effects and therefore more research is required to better understand the long term effects of adolescent exposure to methamphetamine.

Methamphetamine was first synthesised in the 1887 in Germany from ephedrine, a common decongestant (Tyner & Fremouw, 2008). Over many years methamphetamine has been used to treat narcolepsy, mild depression, postencephalitic parkinsonism, chronic alcoholism and obesity (Tyner & Fremouw, 2008). During World War II American, Japanese, and German soldiers also used methamphetamine to maintain alertness (Tyner & Fremouw, 2008).

During the 1980's methamphetamine became a commonly abused drug of choice and was used by a wide variation of people (Cartier, Farabee & Prendergast, 2006). Media reports soon emerged linking the drug to aggression, violence, and crime despite there being a lack

of literature to suggest this. Legal restrictions were implemented throughout the 1980's along with restricted access to products containing ephedrine.

The principle mechanisms of action for methamphetamine are very similar to those for cocaine. Amphetamines, including methamphetamine, stimulate the release of synthesized catecholamines in the CNS (Homer et al., 2008). Amphetamines cause neuronal storage vesicles to release dopamine, norepinephrine, and serotonin neurotransmitters into the synapse (Homer et al., 2008). Amphetamines then inhibit the uptake of these neurotransmitters by membrane transporters as well as inhibiting monoamine oxidase (Homer et al., 2008). Although the serotonin transporter is involved dopamine and norepinephrine transporters are the most influenced. *In vitro* studies of chronic methamphetamine users found reduced levels of dopamine, tyrosine hydroxylase and dopamine transporter (DAT) in the nucleus accumbens, caudate nucleus, and putamen (Wilson et al., 1996). *In vivo* studies have found detoxified methamphetamine users to have reduced DAT, and Dopamine D2 receptor binding in the caudate, putamen, accumbens and prefrontal cortex (Volkow et al., 2001; Volkow & Fowler, 2001). Another *in vivo* study using PET scans found abstinent methamphetamine users had reduced serotonin levels in the CNS (Sekine et al., 2006).

A growing number of studies now show that methamphetamine is highly neurotoxic (Homer et al., 2008). Various anatomical toxicities have been reported in the limbic system, such as reduced hippocampus size, white matter replacing grey matter, corpus callosal changes, and reduced levels of presynaptic dopamine and serotonin transporters (See Barr et al., 2006, for a review). These anatomical changes correlate with behavioural changes that persist long after drug cessation. For example damage to the dopamine and serotonin systems would have wide ranging neural and behavioural effects as these neurotransmitters play a vital role

in physiological and behavioural systems including regulating mood, anxiety and behaviour, such as aggression. Dopamine, norepinephrine, and serotonin also constitute a reward system in the brain that influences abuse potential. The neurotoxicity findings of methamphetamine suggest it may produce a neuronal imprinting effect.

Methamphetamine is acknowledged to be among one of the most addictive substances known and has high levels of abuse potential (Rothman & Baumann, 2003). Methamphetamine has been shown to induce conditional place preference and to elicit self-administration of the drug. Taylor and Horger (1999) studied the reinforcing properties of amphetamines in male rats previously trained to self-administer cocaine and found amphetamine acted as a reliable reinforcer. There was a five-fold increase in the self-administration of methamphetamine, via cannula placements in the nucleus accumbens, in cocaine pre-treated versus control rats. Mendrek, Blaha, and Phillips (1997) studied whether pre exposure to methamphetamine would enhance the motivation to self administer methamphetamine. Using a fixed-ratio schedule methamphetamine pre-treated rats self administered methamphetamine more so than controls.

Research on the short-term and longer-term outcomes of repeated adolescent exposure to methamphetamine is very limited. Research by Shintomi (1975) measured the short-term effects of methamphetamine in seven adult male rats. The study showed that hyperactivity and fighting were increased when rats were administered a higher dose of 5 mg/kg versus a lower dose of 2.5 mg/kg. These behaviours were apparent 20 minutes after a subcutaneous injection and diminished after 60 minutes. A more recent study by Sokolov, Schindler, and Cadet (2004) compared the short-term and longer-term effects of exposure to methamphetamine using adult male mice. Their research showed that mice treated

chronically with increasing twice daily injections over 8 weeks (up to 6 mg/kg each) showed significantly more fighting behaviour (83%) than those treated with one acute intraperitoneal injection (6 mg/kg) of methamphetamine (25%). Sokolov et al., (2004) employed the resident intruder paradigm to measure aggression.

Although both studies mentioned above demonstrate aggressive behaviour after exposure to methamphetamine this was done using small numbers of adult male rats and mice. However, a local study by Johnson (2010) has provided some preliminary results using adolescent male and female rats. In his study, adolescent rats of both sexes were administered 1 or 2 mg/kg of methamphetamine and subsequently tested for aggression. The research found that an acute dosing regimen did not significantly increase aggression when compared to controls. This study highlighted the short-term effects of adolescent exposure to methamphetamine. It is then possible to extend this study and measure the long-term outcomes of adolescent exposure to methamphetamine.

Methamphetamine is a commonly abused psychostimulant being consumed by New Zealand adolescents. Methamphetamine has a high abuse potential and is regarded as being one of the most addictive substances. However there remains a lack of research regarding both the short-term and longer-term outcomes of methamphetamine use in this demographic.

1.5 Aggression

Methamphetamine has been closely linked to aggressive behaviour (Baskin-Sommers & Sommers, 2006; Boles & Miotto, 2003; Cartier et al., 2006; Herring, Schaefer, Gudelsky, Vorhees, & Williams, 2008; Homer et al., 2008; McCormick & Smith, 1995; McEllistrem, 2003; Sheridan et al., 2004; Sokolov et al., 2004; Sommers & Baskin, 2006; Zweben et al.,

2004). Methamphetamine users are described as being high risk offenders due to their frequent and erratic violent behaviour (Tyner et al., 2008). In a New Zealand sample of 78 frequent users 23% reported experiencing violent behaviour from their methamphetamine use (Wilkins et al., 2005). In another sample of 137 regular methamphetamine users in New Zealand, 42% had committed a violent crime in their lifetimes (Wilkins, Griffiths, & Sweetsur, 2009). A study by Sommers and Baskin (2006) found that 27% of methamphetamine users had engaged in violent behaviour while under the influence of methamphetamine. They found 51% of these incidents occurred within domestic relationships and 29% were drug related. Incidents also included random acts of violence and gang-related violence.

Although methamphetamine is commonly associated with aggression and violence, this relationship still remains poorly understood. McKetin and colleagues (2006) proposed that this relationship is credible for three reasons. Firstly, experimental evidence suggests that chronic use may increase aggressive behaviours. Secondly, an acute dose may enhance an aggressive response if someone is provoked. Finally, methamphetamine use increases the likelihood of psychosis which itself is associated with aggressive behaviours.

Research commonly agrees that there are two primary modes of aggressive and violent behaviour. These are affective and predatory aggression (McEllistrem, 2003). This study will focus on the former. Affective defence is an aggressive response based upon the presence of fear and or threat that may be real or perceived. The goal is to reduce or eliminate the threat object from the environment. This form of aggression occurs in humans and animals.

Research suggests aggression is biological in nature with neurotransmitters including GABAergic, serotonergic, noradrenergic and dopaminergic systems being strongly implicated (McEllistrem, 2003). Recent research has suggested increased aggression in humans occurs via serotonin depletion. In a recent study by Sekine et al. (2006) chronic methamphetamine users were found to have both higher levels of aggression than non-drug using controls and decreased levels of serotonin in areas of the brain that are involved in the regulation of aggression, including the orbitofrontal cortex, anterior cingulate cortex, and temporal cortex. Sekine and colleagues (2006) also found that the level of serotonin depletion in these areas of the brain correlated with the magnitude of aggression among methamphetamine users. There is also support for the role of cognitive, behavioural, and environmental factors (Weinshenker & Siegal, 2002). Boles and Miotto (2003) report the pharmacological effects of methamphetamine, such as agitation, paranoia and psychosis, and the systemic violence factors are responsible for aggression.

The resident intruder test measures territorial aggression. That is, aggression that occurs when an intruder enters into an area that an animal has determined to be its own domain (Weinshenker & Siegal, 2002). The test was formulated specifically to engender agonistic behaviour in laboratory rats (Miczek, 1979). Aggressive behaviours seen in the test are similar to those seen in humans, including bite attacks, threats, defence, submission, and flight. The resident intruder test has been effectively used to measure aggressive behaviours in rats, mice, humans and hamsters (Ferris et al., 1997; Johnson, 2010; Malkesman, Maayan, Weizman, & Weller, 2006; Miczek, 1978; Miczek, & O'Donnell, 1978; Ogawa, Lubahn, Korach, & Pfaff, 1997; Sokolov & Cadet, 2006).

1.7 Present Study

The present study investigated the short-term and long-term behavioural effects of adolescent exposure to methamphetamine. On post natal day (PND) 35 male and female rats were treated with a single dose of methamphetamine (2 mg/kg) or saline. From PND 36-46 the rats were treated with twice daily doses of methamphetamine (2 mg/kg) or saline. Subjects were tested after the initial dose, after the completion of the final dose and again in early adulthood (PND 90) in a resident intruder test. Sex differences in behaviour were recorded because of sex differences in brain maturation (Andersen, 2003). As male and female brains develop differently, exposure to methamphetamine is likely to affect each sex differently in the long-term.

2.0 The Aims and Hypotheses of this Study

The aim of this research was to provide an original contribution to the scientific literature regarding the short-term and long-term outcomes of adolescent exposure to methamphetamine. To date there has been a lack of literature regarding methamphetamine use in adolescence. This is an important cohort to examine not only because of the immediate consequences of abusing methamphetamine but also because of the potential long-term consequences.

Aggression was specifically studied as this behaviour is strongly associated with methamphetamine use, both in the literature and the media.

It was expected that aggressiveness would increase with a longer exposure to methamphetamine use in adolescence. That is, an acute dosing regimen of methamphetamine would have little effect on aggressiveness. This would support previous research suggesting that a single dose of methamphetamine does not increase aggressive behaviour. It was expected that a chronic dosing regimen of methamphetamine would increase aggressiveness. This finding would be consistent with previous research and society's belief that methamphetamine produces aggression and crime. It was also expected that methamphetamine use during adolescence would increase aggressiveness in adulthood. The demonstration of a teratological effect would provide further support for the neuronal imprinting theory and the neurotoxicity of methamphetamine.

3.0 Method

3.1 Subjects

Subjects were 40 male and 40 female PVG/C hooded rats from the Animal Facility, Department of Psychology, University of Canterbury, New Zealand. On post natal day (PND) 30, the pups were weaned and caged in 475 x 280 x 230 mm plastic cages in groups of two to four of the same sex. The temperature and humidity were controlled (rh 22°C ± 2°C and rh 48% ± 10%, respectively) on a 12 hour light/dark cycle (lights on at 0800). All animals had free access to water and food (commercial rat pellets) for the duration of the study. All subjects and procedures were approved by the University of Canterbury Animal Ethics Committee (see Appendix A).

On PND 30, the 80 subjects were randomly assigned to an intruder group, a control group and a treatment group. The intruder group consisted of 20 males and 20 females and remained housed in groups of two to four for the duration of the experiment. These rats were used only for testing purposes and received no treatment. The control and treatment groups consisted of 10 males and 10 females each. They were separated into individual cages (of the same measurement) on PND 30 until treatment and testing had been completed on PND 46. On PND 46 they were re-caged in groups of two to four until PND 83 when they were separated again into individual cages.

On PND 35 subjects were administered one dose of saline or methamphetamine. From PND 36 to 46 subjects were administered two daily doses of saline or methamphetamine. All doses were administered at 0900 and 1500 hours. The age of drug exposure was selected to represent the periadolescent stage of development in rats (Spear, 2000). The subjects were

tested behaviourally for aggression on PND 35, 20 minutes after treatment, on PND 46, 20 minutes after the final treatment, and on PND 90. Testing at PND 35 enabled examination of the effects of an acute dosing regimen in early adolescence. Testing at PND 46 enabled examination of the effects of a chronic dosing regimen in late adolescence. Testing at PND 90 enabled adult assessment of behavioural effects of methamphetamine exposure in adolescence, after a long period without drug. These three stages will be referred to as acute effects, chronic effects, and teratological effect in the results section of this report.

3.2 Drugs and Rationale for Doses

Methamphetamine was donated in a pure form crystal by Environmental Science & Research Limited (ESR, Wellington, New Zealand). The crystals were crushed and dissolved in sterile 0.9% saline to produce a dose of 2 mg/kg. On PND 35, animals in the control and treatment group received an intraperitoneal (i.p.) injection of either saline or methamphetamine, in a volume of 1 mg/kg. From PND 36 to 46 the same animals received two daily i.p. injections of either saline or methamphetamine. Hence on day 1 the treatment animals received 2 mg/kg of methamphetamine and every day there after (for a further 10 days) they received a total of 4 mg/kg daily. This resulted in a cumulative dose of 42 mg/kg. Control animals received the same amount of saline. Treatment at day 1 represented an acute dosing regimen. Treatment for the total 11 days represented a chronic dosing regimen (see Table 1). Intraperitoneal administration was selected for ease of drug delivery. The animals' body weights were recorded prior to each injection and injection volumes were adjusted accordingly.

The acute dose of 2 mg/kg was based on a recent study completed at Canterbury University assessing aggression in rats treated with a single dose of methamphetamine (Johnson, 2010). A dose of 4 mg/kg per day (given in two 2 mg/kg doses) was selected for the chronic dosing regimen in this study as it represented a low to medium dose of methamphetamine. In earlier research, doses of methamphetamine shown to be behaviourally effective have ranged from as low as 2 mg/kg per day to as high as 30 mg/kg per day (Brennan et al., 2007, Sokolov, 2004; Vorhees, 2005). For example, a medium dose of 5 mg/kg per day was shown to produce increased levels of fighting behaviour (Shintomi, 1975). Due to high neurotoxicity a multiple-day dosing procedure of two or four doses of methamphetamine per day, is generally used for all chronic dosing regimens (Vorhees, 2005).

3.3 Apparatus and Behavioural Measures

This experiment made use of an empirically-supported test of aggressive-like behaviour, the resident intruder test. In the resident intruder test, a more aggressive animal will take less time to initially bite and display more bite attacks, allogrooming, anogenital contact, boxing, pursuit, and pinning directed at another animal. A more aggressive animal will also display more offensive sideways posture and mutual upright posture.

The experimental room was controlled at 22°C \pm 2°C, 48% \pm 10% humidity and 44 lx dim lighting. All testing took place between 0900 and 1800 hours during the light phase of the animals' light/dark cycle. Previous research had indicated that changing bedding shortly or a few days before testing almost completely eliminated any significant findings as the animals become less territorial. Therefore, tests were completed on day 6 or 7 after changing bedding (Sokolov et al., 2003)

To control for habituation effects the test was completed three times, each with a significant period of time separating them. Testing following the chronic dosing regimen occurred 10 days after testing following the acute dosing regimen. Testing in early adulthood on PND 90 occurred 44 days after testing following the chronic dosing regimen. In addition each animal treated with methamphetamine or saline was never tested against the same intruder animal more than once.

3.31 Resident Intruder Test

The resident intruder test reliably measures agonistic behaviour without the use of aversive manipulations such as shock, food deprivation and brain surgery (Miczek, 1979). A more aggressive animal will display more agonistic behaviour towards the intruder animal. The resident intruder test was conducted in the residents' home cage where it had been singly housed for 5 to 6 days prior. The plastic cages measured 475 x 280 x 230 mm and were illuminated by dim (44 lx) fluorescent lighting, 1,390 mm above the cage. The lids of the home cages were removed during the test. The intruder animal was introduced into the home cage, 20 minutes following injection, for 15 minutes (900 seconds). Timing began as soon as the intruder animal touched the bottom of the home cage.

Latency before the first bite attack was recorded and was assumed to be 900 seconds where no bite attack occurred. Other behaviours recorded included the frequency of bite attacks, allogrooming, anogenital contact, pinning, pursuit, lunging, escapes, offensive sideways posture, mutual upright posture, and boxing and kicking (measured as one). Boxing and kicking were measured as one behaviour as they were deemed to be very similar. Boxing involved the use of the animal's front legs and kicking involved the use of the animals back legs. In a previous study only boxing had been measured (Malkesman et al., 2006) however

kicking occurred just as frequently. The categories of aggressive behaviour are described below;

- Latency to first bite attack – time elapsed before the first bite attack.
- Bite attack – resident rat bites intruder rat.
- Allogrooming – resident rat aggressively grooms (involving teeth) around the intruder's shoulder and neck area.
- Anogenital contact – resident rat sniffs the anogenital region of the intruder.
- Pinning – resident rat places intruder rat in a supine position and releases contact with the ground with at least two paws.
- Boxing and kicking – resident rat boxes with front paws or kicks with hind paws (behaviour is still counted if no contact is made).
- Pursuit – resident rat chases intruder rat around the cage.
- Offensive sideways posture – resident rat rears on hind legs over the intruder rat who is in a submissive posture or lying on side with eyes closed.
- Mutual upright posture – both rats stand on hind legs and face one another.

If intervals between the occurrences of two aggressive acts exceeded 3 seconds the two behavioural acts were scored as two separate aggressive acts (Ogawa et al., 1997). A 3 second timer, regularly tested for accuracy, was used throughout the resident intruder test.

If one of the animals were deemed to be overly distressed or injured they were separated for a few seconds, and then the test was continued. This was done by removing the intruder rat from the test cage and placing it back into the home cage.

Each treatment animal completed the test three times. This was after the acute dosing regimen, after the chronic dosing regimen, and again at PND 90. In the former cases testing occurred 20 minutes after the appropriate treatment had been given (Shintomi, 1975). To control for habituation effects the treatment animals were not exposed to the same intruder more than once.

4.0 Statistical Analyses

All raw data were analysed by separate 2(treatment) x 2(sex) analyses of variance (ANOVA's) using the Statview statistical programme. The ANOVA's were performed on each measure at each of the three testing periods. Methamphetamine treatment effects are presented in graphical form as means \pm standard error of the means (SEM's) (See Figures 1-9). Due to sex differences in brain development it seemed likely that each sex might express aggressive behaviour differently over the course of the study. Sex differences were examined using averages of both trials, and the results are presented in tabular form. There were no significant treatment x sex interactions.

5.0 Results

5.1 Effects of Methamphetamine Treatment

5.1.1 Acute Effects

Figures 1, 2, and 3 below show the level of each aggressive behavioural measure after the acute dosing regimen of methamphetamine or saline in early adolescence. As shown in Figure 1A below, the methamphetamine group took significantly longer to bite than controls after an acute testing regime $F(1,36) = 37.29, p < .0001$. This indicates the control group showed significantly more aggressive behaviour than the methamphetamine group. Figure 1B and 1C shows the control group performed significantly more bite attacks and allogrooming than the methamphetamine group $F(1,36) = 43.48, p < .0001$, and $F(1,36) = 73.25, p < .0001$ respectively. These results indicate significantly more aggressive behaviour in the control group.

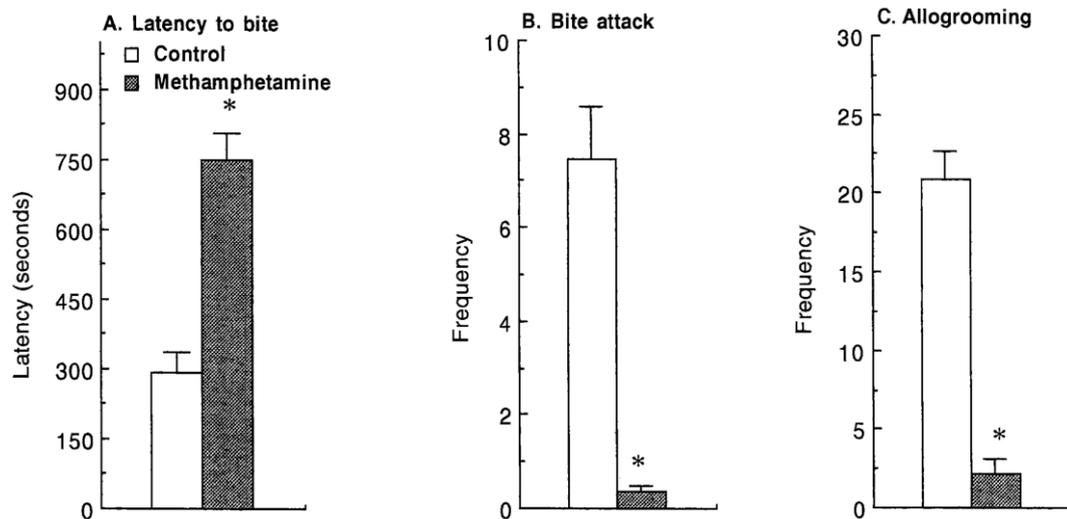


Figure 1. A. Latency to bite (in seconds), B. Frequency of bite attacks, and C. Frequency of allogrooming for control (n=20) and methamphetamine (n=20) groups as measured in early adolescence. The error bars show standard errors of the means. *significantly different from control group (p<.05).

Below Figure 2A and 2B shows the control group made significantly more anogenital contact than the methamphetamine group $F(1,36) = 26.53, p < .0001$ and performed significantly more pinning $F(1,36) = 38.76, p < .0001$ after the acute administration. Figure 2C shows the control group displayed only slightly more boxing and kicking than the methamphetamine group. This difference was not significant $F(1,36) = 1.8, p < .188$.

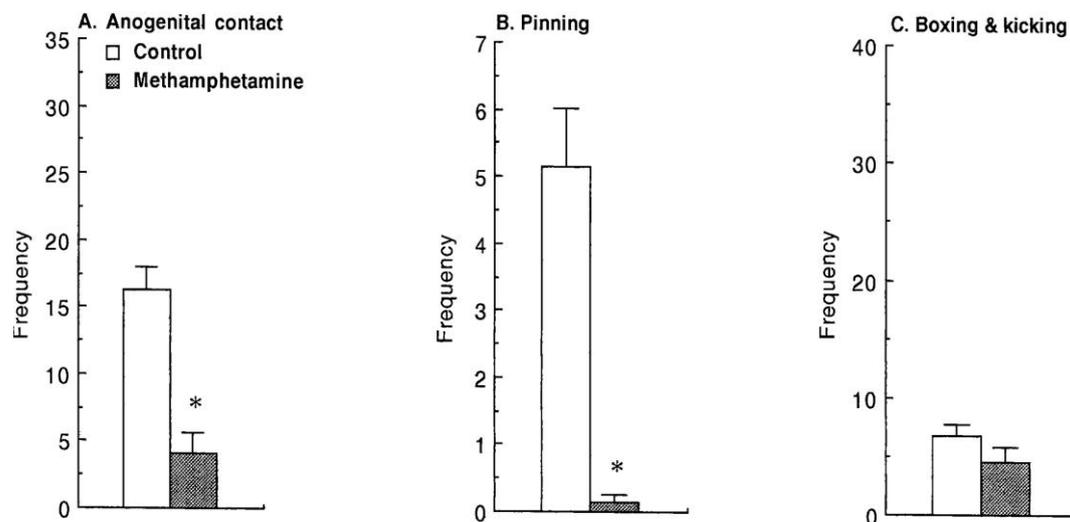


Figure 2. A. Frequency of anogenital contact, B. Frequency of pinning, and C. Frequency of boxing and kicking for control (n=20) and methamphetamine (n=20) groups as measured in early adolescence. The error bars show standard errors of the means. *significantly different from control group ($p < .05$).

As shown in Figure 3A the control group engaged in significantly more pursuit than the methamphetamine group $F(1,36) = 38.66, p < .0001$. Figure 3B shows the control group displayed more offensive sideways posture than the methamphetamine group, but this difference was not significant $F(1,36) = 1.59, p < .215$. Figure 3C shows the control group displayed significantly more mutual upright posture than the methamphetamine group $F(1,36) = 21.16, p < .0001$.

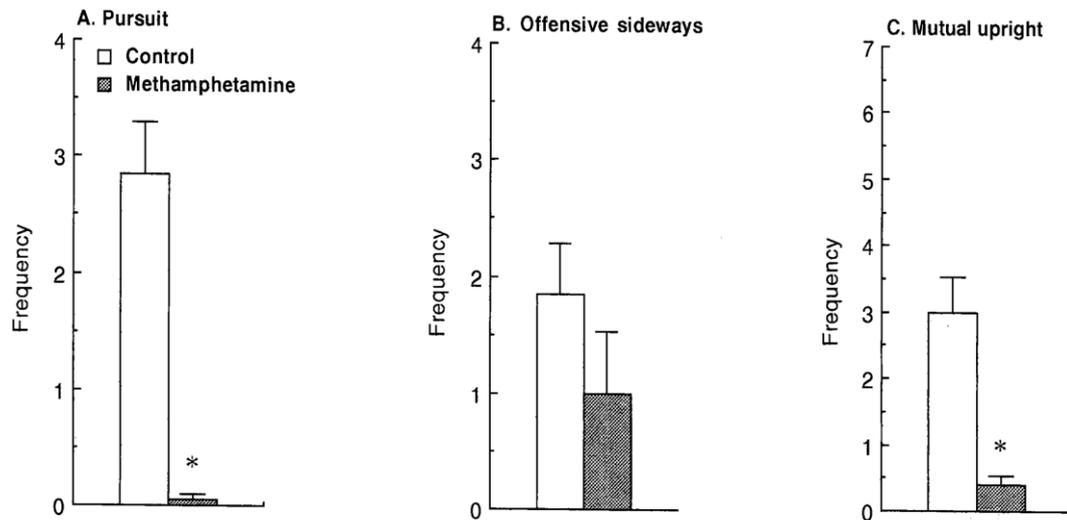


Figure 3. A. Frequency of pursuit, B. Frequency of offensive sideways posture, and C. Frequency of mutual upright posture for control (n=20) and methamphetamine (n=20) groups as measured in early adolescence. The error bars show standard errors of the means. *significantly different from control group ($p<.05$).

Overall the results suggest that, following an acute dosing regimen, the control group displayed significantly more aggressive behaviour than the methamphetamine group. The difference between the two groups was significant for all measures of behaviour except for boxing and kicking, and offensive sideways posture.

5.1.2 Chronic Effects

Figure 4, 5, and 6 below show the level of each aggressive behaviour following the chronic dosing regimen when tested in late adolescence. Figure 4A shows the methamphetamine group took longer to initially bite than the control group indicating reduced aggression. However this difference was not significant $F(1,36) = 3.13, p<.085$. Figure 4B and 4C show the control group displayed significantly more aggressive behaviour than the methamphetamine group performing significantly more bite attacks $F(1,36) = 5.24, p<.028$ and allogrooming $F(1,36) = 19.68, p<.0001$.

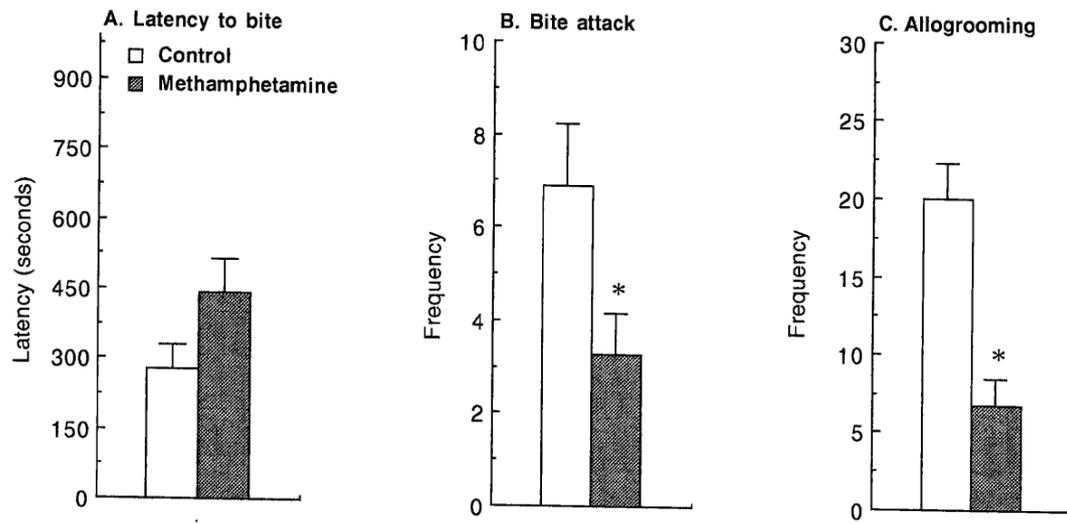


Figure 4. A. Latency to bite (in seconds), B. Frequency of bite attacks, and C. Frequency of allogrooming for control (n=20) and methamphetamine (n=20) groups as measured in late adolescence. The error bars show standard errors of the means. *significantly different from control group (p<.05).

Figure 5A and 5B show the control group performed significantly more anogenital contact $F(1,36) = 4.6, p < .0388$, and pinning $F(1,36) = 4.47, p < .0415$ after a chronic testing regime. Figure 5C demonstrates the methamphetamine group performed significantly more boxing and kicking than the control group when tested in late adolescence $F(1,36) = 12.51, p < .001$.

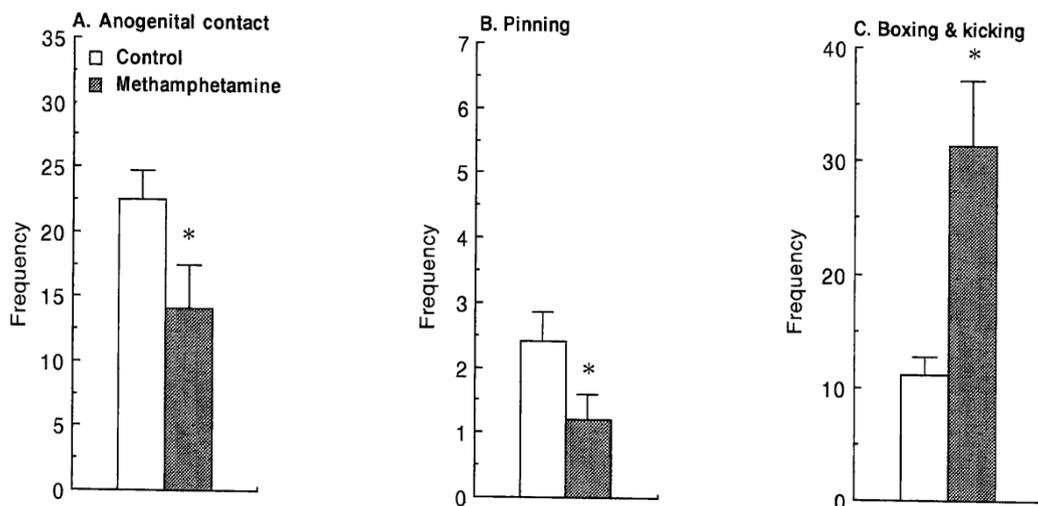


Figure 5. A. Frequency of anogenital contact, B. Frequency of pinning, and C. Frequency of boxing and kicking for control (n=20) and methamphetamine (n=20) groups as measured in late adolescence. The error bars show standard errors of the means. *significantly different from control group ($p < .05$).

Below, Figures 6A and 6B show the control group performed more aggressive behaviour than the methamphetamine group but this was not significant for pursuit $F(1,36) = 3.11, p < .0864$ and offensive sideways posture $F(1,36) = 1, p < .324$. Figure 6C shows the methamphetamine group performed more aggressive behaviour than controls, but, again this was not significant $F(1,36) = 2.19, p < .147$.

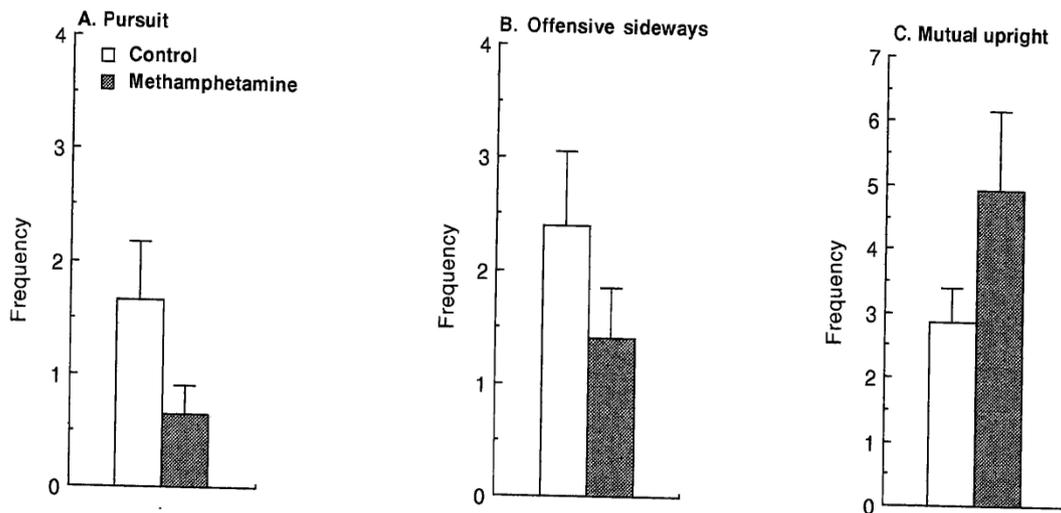


Figure 6. A. Frequency of pursuit, B. Frequency of offensive sideways posture, and C. Frequency of mutual upright posture for control (n=20) and methamphetamine (n=20) groups as measured in late adolescence. The error bars show standard errors of the means. *significantly different from control group ($p<.05$).

The above results show a trend towards the control group displaying more aggressive behaviour than the methamphetamine group. However, these findings are not consistent across all or most behaviours assessed and many differences did not reach statistical significance. Overall, the results suggest that, with the exception of boxing & kicking, and mutual upright posture chronic treatment with methamphetamine appeared to decrease (rather than increase) aggressive behaviour. However this decrease was less clear than the decrease seen following the acute dosing regimen.

5.1.3 Teratological Effects

Figures 7, 8, and 9 below demonstrate the level of aggressive behaviour when the groups were assessed in early adulthood after receiving both acute and chronic dosing regimen in adolescence. Figure 7A below shows the methamphetamine group took significantly less time to initially bite than the control group $F(1,36) = 4.2, p<.0478$. This indicates increased aggression in the methamphetamine group. In figure 7B the methamphetamine group

showed a significantly greater number of bite attacks than the controls $F(1,36) = 4.96$, $p < .0324$. Figure 7C shows the methamphetamine group performed significantly more allogrooming than controls $F(1,36) = 20.87$, $p < .0001$.

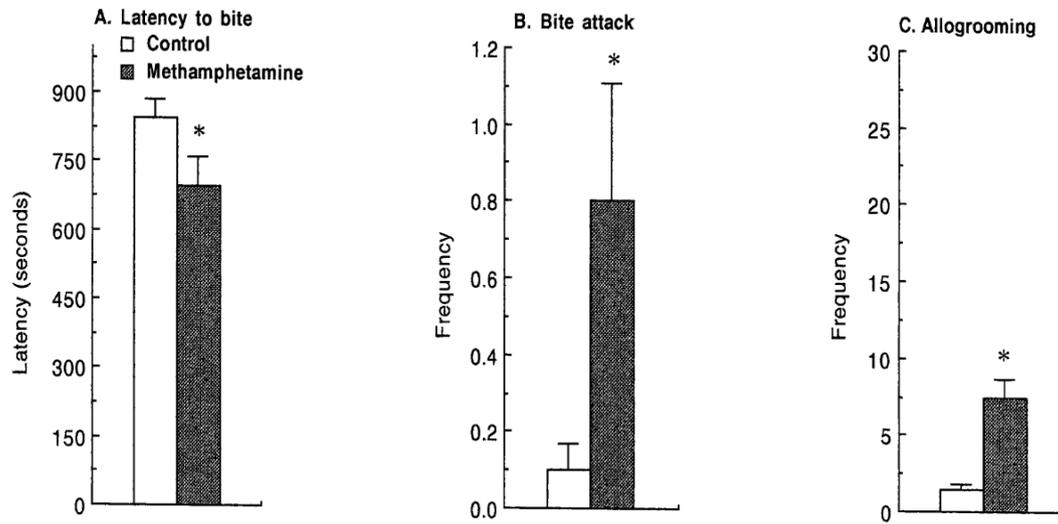


Figure 7. A. Latency to bite (in seconds), B. Frequency of bite attacks, and C. Frequency of allogrooming for control (n=20) and methamphetamine (n=20) groups as measured in early adulthood. The error bars show standard errors of the means. *significantly different from control group ($p < .05$).

As seen below, Figures 8A and 8B show the methamphetamine group displayed more aggressive behaviour than the control group, and performed significantly more anogenital contact $F(1,36) = 17.73$, $p < .0002$, and pinning $F(1,36) = 41.5$, $p < .0001$. Figure 8C shows there was a marginal difference between the groups for boxing and kicking $F(1,36) = 3.86$, $p < .0573$.

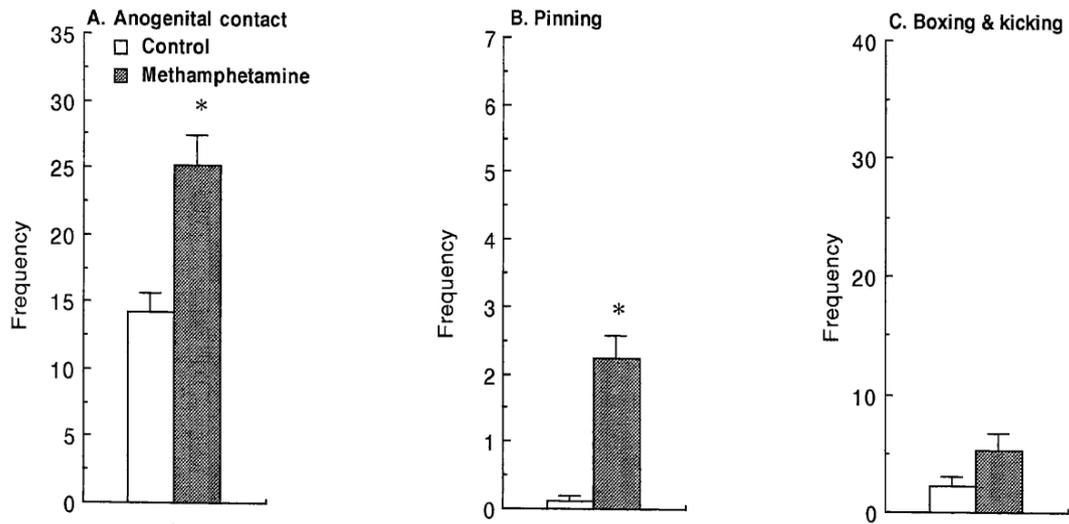


Figure 8. A. Frequency of anogenital contact, B. Frequency of pinning, and C. Frequency of boxing and kicking for control (n=20) and methamphetamine (n=20) groups as measured in early adulthood. The error bars show standard errors of the means. *significantly different from control group ($p < .05$).

Figures 9A and 9C show the methamphetamine group engaged in significantly more pursuit $F(1,36) = 4.5, p < .0408$ and mutual upright posture $F(1,36) = 32.5, p < .0001$, than the controls. Figure 9B shows the methamphetamine group displayed more offensive sideways posture than controls, but this was not significant $F(1,36) = 1, p < .324$.

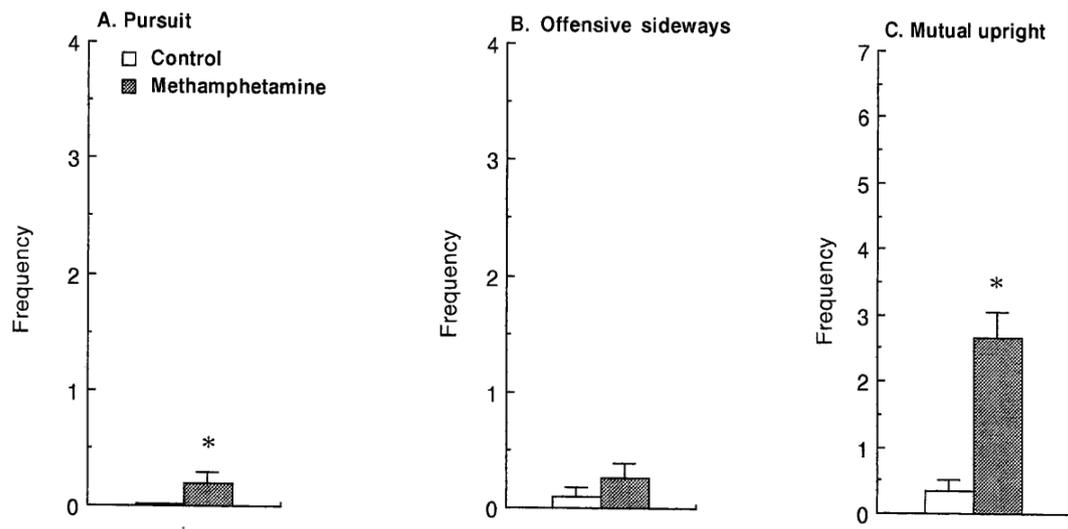


Figure 9. A. Frequency of pursuit, B. Frequency of offensive sideways posture, and C. Frequency of mutual upright posture for control (n=20) and methamphetamine (n=20) groups as measured in early adulthood. The error bars show standard errors of the means. *significantly different from control group ($p<.05$).

Overall the above the results suggest that the methamphetamine group displayed significantly more aggressive behaviour than the control group when assessed in early adulthood. These findings suggest support for a teratological effect.

5.2 Sex Effects

Table 2 below shows sex effects for all measures of aggressive behaviour. There were only two behaviours that were found to have a significant sex effect. Females performed significantly more boxing and kicking $F(1,36)=4.17, p<.05$, and offensive sideways posture $F(1,36)=5.44, p<.05$. For the most part, there was very little difference between the rate of male and female aggressive behaviour.

Table 2. Means (standard error of the mean) and results of F tests for latency to bite and frequency of bite attacks, allogrooming, anogenital contact, boxing, pinning, pursuit, offensive sideways posture and mutual upright posture for male (n=20) and female (n=20) rats at the three different testing ages (acute, chronic and teratological). * indicates a significant sex effect.

Acute Treatment	Male (n=20)	Female (n=20)	F (1,36)	P
Latency to Bite	530.20 (67.94)	507.80 (78.94)	0.05	n.s.
Bite Attacks	2.90 (0.74)	4.90 (1.40)	1.59	n.s.
Allogrooming	11.25 (2.77)	11.75 (2.48)	0.02	n.s.
Anogenital Contact	8.00 (1.90)	11.85 (2.34)	1.63	n.s.
Boxing & Kicking	5.05 (1.12)	6.10 (1.31)	0.38	n.s.
Pinning	1.75 (0.59)	3.55 (0.99)	2.44	n.s.
Pursuit	1.25 (0.42)	1.65 (0.48)	0.40	n.s.
Offensive Sideways Posture	0.90 (0.26)	1.50 (0.46)	1.31	n.s.
Mutual Upright Posture	1.85 (0.53)	1.55 (0.44)	0.19	n.s.
Chronic Treatment	Male (n=20)	Female (n=20)	F (1,36)	P
Latency to Bite	362.45 (67.16)	354.60 (68.17)	0.01	n.s.
Bite Attacks	4.00 (0.84)	6.15 (1.47)	1.61	n.s.
Allogrooming	13.75 (2.50)	13.00 (2.63)	0.04	n.s.
Anogenital Contact	14.90 (2.68)	21.70 (3.08)	2.77	n.s.
Boxing & Kicking	16.65 (3.79)	25.85 (5.34)	1.98	n.s.
Pinning	1.30 (0.36)	2.30 (0.49)	2.70	n.s.
Pursuit	0.75 (0.25)	1.55 (0.53)	1.86	n.s.
Offensive Sideways Posture	1.15 (0.34)	2.65 (0.69)	3.76	n.s.
Mutual Upright Posture	3.85 (0.93)	3.90 (1.03)	0.00	n.s.
Teratological Effects	Male (n=20)	Female (n=20)	F (1,36)	P
Latency to Bite	841.15 (36.12)	696.90 (65.50)	3.72	n.s.
Bite Attacks	0.20 (0.12)	0.70 (0.31)	2.29	n.s.
Allogrooming	4.20 (0.94)	4.65 (1.32)	0.08	n.s.
Anogenital Contact	17.85 (2.18)	23.55 (2.46)	3.00	n.s.
Boxing & Kicking*	2.20 (0.55)	5.40 (1.47)	4.17	<.05
Pinning	1.10 (0.30)	1.25 (0.38)	0.10	n.s.
Pursuit	0.10 (0.07)	0.10 (0.07)	0.00	n.s.
Offensive Sideways Posture*	0.00 (0)	0.35 (0.15)	5.44	<.05
Mutual Upright Posture	1.15 (0.30)	1.85 (0.46)	1.62	n.s.

6.0 Discussion of Results

The present study exposed adolescent rats to either saline or methamphetamine. Both groups received treatment throughout the adolescent stage of development (PND 35 – PND 46). On PND 35 the methamphetamine group received one initial 2 mg/kg i.p injection of methamphetamine. For a further 10 days (PND 36-46) the methamphetamine group received 2 mg/kg i.p. injections twice daily. The control group received comparable doses of saline on the same days. The rats were subsequently tested in one empirically validated measure of aggression after the acute dosing regime in early adolescence, after the completion of the chronic dosing regime in late adolescence, and again in early adulthood when possible behavioural teratological effects of the drug were assessed.

6.1 Summary of Results

The results revealed a variation of differences in the drug treated animals and saline controls. This means that exposure to methamphetamine influenced the aggressive-like behaviour of the animals in some, but not all measures of the resident intruder test. The results are summarized below.

Acute methamphetamine exposure in early adolescence significantly reduced or had no effect on aggressive-like behaviour when compared to controls. That is, an acute dosing regimen of methamphetamine, when compared to saline, significantly reduced aggressive-like behaviour for measures of latency to bite, bite attacks, allogrooming, anogenital contact, pinning, pursuit, offensive sideways posture, and mutual upright posture. The methamphetamine-treated and control animals preformed a similar amount of boxing and kicking.

Chronic exposure to methamphetamine during adolescence significantly decreased several measures of aggressive-like behaviour, but not all. Animals administered a chronic dosing regimen of methamphetamine performed significantly more boxing and kicking. They also performed marginally more bite attacks and mutual upright posture than controls. However, controls performed significantly more allogrooming, anogenital contact, and pinning. Controls also performed more pursuit and offensive sideways posture, however these were not significant. There was no significant effect for latency to bite, however the methamphetamine group did take longer, indicating less aggression than controls.

These findings suggest that an acute dosing regimen may not produce aggressive-like behaviours. A chronic dosing regimen may influence aggressive-like behaviours to some degree, however this relationship is unclear. Nevertheless, the results do suggest some support for the main hypothesis, namely that aggressiveness, in adolescence, would increase with a longer exposure to methamphetamine.

The second aim of this research was to establish whether exposure to methamphetamine would have long term behavioural teratological effects. Adult animals that were exposed to methamphetamine during adolescence displayed a degree of aggressive-like behaviours. The methamphetamine-treated group, compared to controls, engaged in significantly more allogrooming, anogenital contact, pinning, pursuit and mutual upright posture. The methamphetamine group also took significantly less time to initially bite, compared to controls. The frequency of bite attacks, boxing and kicking, and offensive sideways posture were similar for both the treated and control animals. The findings from these measures support the possibility that methamphetamine use during adolescence can increase aggressiveness in adulthood.

6.1.1 Sex Differences

Overall, this research found little evidence of differences in aggressive behaviour between males and females. When tested in early adulthood females performed significantly more boxing and kicking and offensive sideways posture than males. One possible reason for this finding is that females are considered to be more active than males (Archer, 1975). Therefore increased aggressive behaviour may actually reflect increased activity. While some differences were noted, for the most part, males and females performed similar rates of aggressive behaviour.

6.2 Methodological Limitations

To determine the degree to which the findings from this study can be generalized the limitations and strengths need to be considered. One limitation of this study was the dose levels of methamphetamine used. The dose used in the acute phase (2 mg/kg) was consistent with other research using methamphetamine (Herbert & Hughes, 2009; Johnson, 2010; McFadden & Matuszewich, 2007). However, the dose used in the chronic phase (4 mg/kg) was less than what had been used in other research (Davidson, Lee & Ellinwood, 2004; Sovolov et al., 2004; Vorhees et al., 2004). This may partially explain why increased aggression was not consistently found following the chronic dosing regimen.

Another limitation of this study may have been the intraperitoneal route of administration used for methamphetamine and saline. In humans, methamphetamine is commonly inhaled via smoke form therefore producing differences in rapidity and onset of drug action (Maxwell, 2005). However, as both groups received i.p. exposure to saline or methamphetamine any alterations in behaviour should not be attributed to route of administration but to the effect of methamphetamine on behaviour.

A further limitation was that the same group of animals were used for both experimental groups, and therefore the teratological effects are the cumulative result of an acute and chronic dosing regimen combined. As a result the adulthood outcome of an acute dosing regimen was not investigated.

As with all animal studies, the issue arises of how well these results apply to humans. This research was intended to be a preliminary study investigating the effects of methamphetamine use in adolescence. Therefore to directly apply these results to humans further study is needed.

6.3 Methodological Strengths

The use of animals, and in particular rats, has several key advantages. Rats develop much more quickly than humans do. Rats reach periadolescence at PND 35 and adulthood at PND 90 (Anderson, 2003). This aging process made it possible to study the effects of methamphetamine across a large age span in a short period of time. Animal models also allow for more control of possible effects of confounding variables. In this study, the rats were housed in a contained environment in an animal facility. This allowed for the elimination of extraneous factors that may have influenced the relationship between methamphetamine use and aggression. These factors include, but are not limited to, drug concentration, time of drug administration, peer group, and parental practices (Weinberg et al., 1998). Controlling for these confounding variables increases the likelihood that any behavioural effects are primarily due to methamphetamine exposure.

A further strength of this study was the purity of the methamphetamine used.

Methamphetamine is often produced by “cooks” in clandestine laboratories using over the

counter medication (ephedrine) and general household chemicals. What is bought on the street as the end product is no longer pure methamphetamine and is instead mixed with a host of other ingredients and substances. Therefore street methamphetamine varies substantially. This study used 90-100% pure crystal methamphetamine, donated from Environmental Science & Research Limited (ESR, Wellington, New Zealand). Using pure methamphetamine ensures that the results of this study were due to methamphetamine and were not confounded by other ingredients or substances. The use of pure methamphetamine also controlled for poly drug use.

A further strength of this study is the use of an empirically supported measure of aggressive behaviour. The resident intruder test has been effectively used to measure aggressive behaviour in a range of animals (Ferris et al., 1997; Johnson, 2010; Malkesman et al., 2006; Miczek, 1978; Miczek, & O'Donnell, 1978; Ogawa et al., 1997; Sokolov & Cadet, 2006).

6.4 Relationship to Previous Findings

One of the findings from this research was that an acute dose of methamphetamine did not increase aggressive behaviour. This is consistent with previous research. Johnson (2010) exposed adolescent male rats to a single 1 mg/kg or 2 mg/kg of methamphetamine. The animals were aged between PND 41-50 which is similar to adolescence in humans. The animals were observed in the resident intruder test using a slightly different design to the one used in this study. Of the eight behavioural measures used to assess aggressive behaviour, six indicated less aggressive behaviour in the methamphetamine group, and two indicated mixed findings. Overall, the results showed that an acute dose of methamphetamine did not increase aggressive behaviour in male adolescent rats. On the contrary, it may have reduced aggression.

Another finding from this study was that a chronic dosing regimen of methamphetamine did not clearly increase aggressive behaviour. This is inconsistent with previous research. Sokolov, Schindler and Cadet (2004) exposed adult male mice to either an acute dosing regimen or chronic dosing regimen and then assessed aggressive behaviour using the resident intruder test. In the acute regime mice were treated with a single 6 mg/kg injection of methamphetamine. The chronic dosing regimen consisted of increasing twice daily injections over 8 weeks (up to 6 mg/kg each) of methamphetamine. The study found no increase in aggressive behaviour following the acute administration, which is consistent with the findings from this study. In contrast, chronic administration significantly increased the number of animals that initiated bite attacks and shortened the latency before the first attack. These results indicate that chronic use of methamphetamine increases aggressive behaviour.

One possible explanation for this inconsistency is the pattern of dosing and the dose of methamphetamine. Sokolov and colleagues (2004) gradually increased the dose of methamphetamine in the chronic dosing regimen, mimicking more closely the increased dose of a chronic human user. They also administered up to 6 mg/kg compared to the 4 mg/kg used in this study. It is possible that a slightly higher dose of methamphetamine is responsible for the increase in aggressive behaviour. This may partially explain why, in the current research, there was a non significant trend towards increased aggression in the methamphetamine group. Another explanation for the inconsistency was that their study used mice rather than rats. It may be possible that the effect found in their study does not apply to rats.

Previous research indicates differences in the processing of drugs at different ages (Laviola et al., 1999). Therefore, another explanation may be that the rats in the chronic administration group developed tolerance to the methamphetamine and thereby, repeated drug injections may have resulted in a reduction in the magnitude of the drug's effects (Laviola et al., 1999). The higher dose or gradually increasing dose used in previous research may have removed this effect.

Another finding from this study was that chronic methamphetamine use in adolescence increased aggressive behaviour in adulthood. This teratological effect is consistent with research suggesting methamphetamine is highly neurotoxic, in areas including the limbic system, dopamine system, and serotonin system (Barr et al., 2006). Toxicities in these areas damage important physiological and behavioural systems that are responsible for regulating mood, anxiety, and behaviour including aggression.

7.0 Overall Discussion

The findings from this research met some, but not all expectations. An acute dosing regimen of methamphetamine in adolescence did not increase aggressive behaviour, thereby supporting previous research. Overall, a chronic dosing regimen of methamphetamine during the adolescent period did not clearly increase aggressive behaviour. This finding conflicts with previous research demonstrating increased aggression following a chronic dosing regimen. Finally, methamphetamine use throughout adolescence increased aggressive behaviour in adulthood, following cessation of the drug. This finding provides preliminary evidence for a teratological effect of methamphetamine. The following section will discuss the theoretical significance, possible explanations for, and implications of these results. Finally, future directions for continuing research will be discussed.

7.1 Neuronal Imprinting

One aim of this study was to demonstrate neuronal imprinting of methamphetamine. That is, it was expected that methamphetamine exposure throughout adolescence would produce detrimental effects well into adulthood, following a sustained period of abstinence. In support of the expectations, this study demonstrated methamphetamine exposure in adolescence significantly increased aggressiveness in adulthood. This provides evidence for the theory.

This finding suggests methamphetamine altered the development of particular brain regions where the drug was active. As neurological analysis of the animals was not completed, it is unclear which brain regions were involved. Methamphetamine is thought to increase levels of dopamine, norepinephrine, and serotonin (Homer et al., 2008). It is suggested that

increased levels of these neurotransmitters in adolescence results in differing amount being produced in adulthood. Research around the neurotoxicity of methamphetamine has suggested exposure influences the limbic system, including the hippocampus, grey matter, and corpus callosum (Barr et al., 2006). It is proposed that adolescent exposure produces alterations in these brain regions and these persist into adulthood. These changes to neurotransmitter systems and brain regions may combine to produce lasting differences in adulthood aggressive behaviour (Sekine et al., 2006).

As mentioned previously, a reduction in serotonin is believed to influence aggressive behaviour (Sekine et al., 2006). The results from this research suggest that increases in the serotonin system following methamphetamine exposure in adolescence may alter serotonin levels in adulthood thereby influencing aggression.

7.2 Implications of Research and Application of Results

The results from this research have substantial implications for society given the impact methamphetamine exposure may have on adolescents. These results indicate that increasing the understanding of how methamphetamine affects behaviour may correct misguided assumptions about its use and have a positive influence on how adolescents choose to use and abuse drugs. By dispelling the myth that a one-off dose of methamphetamine will produce profound and long lasting changes in behaviour society will gain a much clearer understanding of how the drug influences behaviour, especially when it is repeatedly used.

Adolescence is a time marked by experimentation, sensation seeking, and risk taking behaviour. As a result adolescents are often exposed to and use various substances. Substance use is particularly problematic during this time because it increases the risk of drug

use in adulthood and the risk of developing co morbid psychological disorders such as anxiety and mood disorders (Laviola et al., 1999; Stansfield & Kirstein, 2006). It is also a period of ongoing neurodevelopment where the brain is undergoing a number of changes (Spear, 2007).

Perhaps the strongest implication of this research is the teratological finding which suggests that while methamphetamine use during adolescence may not produce detrimental effects at the time of use, it will likely produce adult changes in aggressive behaviour. This finding has major implications for New Zealand society given the financial and psychological cost that aggressive behaviour, crime, and mental health disorders pose. This research supports the need for increased education and treatment programmes targeting adolescents.

8.0 Future Research

To establish the relationship between methamphetamine and aggression the limitations of this study should be addressed. Future studies should administer differing doses of methamphetamine in the chronic dosing regimen to establish whether aggression occurs in a dose-dependent manner. The use of a gradually increasing dosing regimen should also be used to mimic the pattern of human methamphetamine users more closely. Future studies should also investigate different routes of administration and the possible effects this may have on the results.

Possible initiatives that could be employed to improve future research include observing treated rats further into their development to establish how permanent the teratological effects are. Neurological analysis of the treated animals would provide further information for the teratological and neurotoxic effects of methamphetamine exposure. This could be done post mortem via examination of the brain. Future research could investigate whether an acute dose of methamphetamine has any teratological effects by administering acute and chronic doses to different groups.

In future it would be useful to video record testing in the resident intruder test. This would allow a re-examination of responses as to ensure recording was correct. These recordings could also be examined by a third party to ensure inter-rater reliability. Future research should measure boxing and kicking as two separate aggressive behaviours. This follows the current literature on rat behaviour and is consistent with the observations from this research. When compared to all other aggressive behaviours measured in this study the effect that

methamphetamine exposure had on boxing and kicking behaviour was less clear. The separation of these two behaviours in future research may assist in clarifying this.

The current lack of literature surrounding the effects of adolescent drug use should be addressed. Particular focus on this age group is essential as adolescence is a developmental period where drug experimentation is common and the developing brain is undergoing a number of changes. This research has provided preliminary evidence for a delayed effect of methamphetamine in adulthood but more research needs to be conducted to understand this relationship more clearly. It is important that research around drug exposure across the adolescent period of development continues.

9.0 Conclusions

The results from this research conclude that an acute dose of methamphetamine during adolescence does not increase aggressive behaviour. A chronic dosing regimen of methamphetamine throughout adolescence may increase the likelihood of some aggressive behaviour but overall it does not appear to increase aggression. This research also concluded that methamphetamine use throughout adolescence, and following cessation, increases aggressive behaviour in adulthood.

Methamphetamine has been strongly linked to aggressive behaviour and is often associated with violent crime. This research provides further information that can assist in understanding this relationship. This research also highlights the risks associated with methamphetamine use in adolescence, and provides support for increased education and/or treatment services targeting this age group. Overall, it appears that a one-off use of methamphetamine or ongoing use of a relatively small dose may not increase aggressive behaviour. However, more notably, it influences behaviour in adulthood following a long period of no drug use. This finding provides support the neuronal imprinting theory.

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Appendix A

AEC Ref: 2010/07R

17 May 2010

Courtney Lowther
Department of Psychology
UNIVERSITY OF CANTERBURY

Dear Courtney

I am pleased to inform you that the Animal Ethics Committee (AEC) has approved your application entitled: "The effects of acute and chronic Methamphetamine exposure on adolescent rats."

Approval has been granted:

- (a) for the use of 80 rats
- (b) for your research project to be undertaken from 18 May 2010 to 31 October 2010. If you require an extension of this period please contact the AEC Secretary.

As part of AEC's new Code of Ethical Conduct all applicants receiving approval to work on animals are required to provide a final report at the completion of their project. The purpose is to provide the AEC with a record of your use of animals and what was achieved by your research project. We are very much interested in your findings and to learn what you have achieved. Following the completion date indicated above you are asked to provide this report using the new Final Report form which is available at the AEC web site (<https://intranet.canterbury.ac.nz/research/ethics.shtml>).

On an annual basis the University is legally required to provide to MAF statistical data on all animal manipulations undertaken in a calendar year. To assist us in collating this information you are also required to complete and return to the AEC Secretary the attached MAF Animal Manipulation Statistical form 30 days after the completion of this project, or once every three years, whichever ever comes first. If no animals have been manipulated in your project please provide a "Nil" return. Please also find enclosed a copy of the Animal Welfare (Records and Statistics) Regulations 1999 for your information, together with a list of Animal Type Codes and brief guideline notes for your assistance.

Yours sincerely

Associate Professor Jim Briskie
Chair
Animal Ethics Committee

