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Effects of Chronic Alcohol and Nicotine Consumption on Aggressive behaviour of Adolescent Male Rats.

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Table of Contents

List of Figures and Tables **4**
Acknowledgements **5**
Abstract **6**
Introduction **7**
Prevalence Rates Among Adolescence 7
Alcohol 8
Nicotine 9
Concurrent Use 10
Significant Impacts During Adolescence 11
Alcohol 11
Nicotine 12
Concurrent Use 13
Aggression 14
Alcohol and Aggression 14
Theoretical Background 14
Individual Level 17
Psychopharmacology 18
Animal Studies 19
Alcohol-Induced Aggression during Adolescence 20
Nicotine and Aggression 23
Nicotine-Induced Aggression during Adolescence 24
Current Study 25
Method **26**
Subjects 26
Treatment 26
Behavioral Testing 27
Behavioral Categories 28
Data Analysis 29
Results **30**
Aggression Overtime 31
Aggression at Baseline 33
Aggression During Intoxication 35
Aggression at Withdrawal 37
Aggression Within each Treatment Condition 38
Discussion **42**

Overview of Results 42
Alcohol and Aggression 42
Nicotine and Aggression 43
Concurrent Use and Aggression 44
Summary of Hypothetical Predictions 47
Importance of Adolescent Research 47
Limitations and Advantages of Current Study 48
Future Recommendations 50
Conclusion 51
References **53**
Appendix A **58**

List of Figures and tables.

Figure 1. *Average aggressive behavior scores from each treatment condition at baseline (PND33), during treatment (PND43) and withdrawal (PND53). Higher scores indicate higher levels of aggressive behavior (bar graph).* **30**

Figure 2. *Average aggressive behavior scores from each treatment condition at baseline (PND33), during treatment (PND43) and withdrawal (PND53). Higher scores indicate higher levels of aggressive behavior (line graph).* **31**

Figure 3. *Overall mean aggression levels before treatment at PND33, during treatment at PND43 and withdrawal at PND53* **32**

Figure 4. *Mean aggression scores for each treatment condition at baseline (PND33). Higher scores represent more aggressiveness..* **33**

Figure 5. *Mean aggression scores for each treatment condition during treatment (PND43). Higher scores represent more aggressiveness.* **35**

Figure 6. *Mean aggression scores for each treatment condition during withdrawal (PND53). Higher scores represent more aggressiveness.* **38**

Table 1. *Numbers of subjects in each treatment condition from PND35-PND50.*..... **27**

Table 2. *Average Aggression Levels in Each Condition at PND33 (Baseline)*..... **34**

Table 3. *Correlation between aggression scores at baseline, during chronic treatment and withdrawal.* **34**

Table 4. *Average Aggression Levels in Each Condition During Intoxication at PND43.* **36**

Table 5. *Average Aggression Levels in Each Condition during withdrawal at PND53* **37**

Table 6. *Repeated within-subjects comparison of average aggression scores for each treatment condition between time 1, 2 and 3. Time 1 represents PND33 (baseline), time 2 PND43 (during treatment) and time 3 PND53 (withdrawal).* **39**

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ABSTRACT

Adolescent rats were chronically treated with alcohol, nicotine or the combination of both for 16 days. Aggression was examined before, during and following chronic treatment using the resident-intruder paradigm for anti-social behaviour. It was hypothesized that chronic alcohol consumption during adolescence will significantly heighten aggressive behaviour, whereas nicotine may have reverse effects. It was also predicted that chronic consumption of concurrent alcohol and nicotine will lead to the highest levels of aggressive behaviour. The results of the current study found no significant difference in the behaviours of the rats during baseline. However, when subjects were given alcohol alone, high doses of nicotine alone or the combination of alcohol and nicotine at either dose, aggression was significantly heightened compared to baseline. During withdrawal, a decline occurred for all groups, however alcohol alone had the strongest impact. In addition, the levels of aggression were significantly more frequent during chronic treatment when ethanol was consumed alone compared to the remaining conditions, including concurrent consumption. From the main findings of the current study it was concluded that, although chronic alcohol consumption (as predicted), increased aggressive behaviour among adolescent subjects, aggression was significantly less frequent when co-used with nicotine and when nicotine was consumed alone. Furthermore, chronic nicotine consumption at high compared to lower doses increased aggressive behaviour. These increases were not affected by the addition of alcohol. Further research is needed to determine reasons for the dose-dependent outcomes of chronically consuming nicotine alone or in combination with alcohol during adolescence.

INTRODUCTION

The combined use of nicotine and alcohol are very common. These drugs are the most commonly abused combination in the world and they continue to be a public health problem. The effects of co-use of alcohol and nicotine have not received enough attention especially when taken by adolescents. The current study aims to determine the effects of alcohol and nicotine exposure concurrently and independently during adolescence, with its consequential outcomes on aggressive behaviour before, during and after chronic exposure. Because there are only a few relevant scientific research investigations targeting aggressive behaviour in adolescents with respect to heavy smoking and/ or drinking, the present investigation was undertaken. Due to ethical and legal constraints, experimental research must involve animals for the investigation of many drug effects. Among the advantages of animal research are avoidance of self-report biases, memory inaccuracy, and the lack of causal explanations in cross-sectional research. Adolescence in humans is typically between 9 – 18 years of age and can be modified by cultural diversity and situational settings. Puberty represents physical changes associated with adolescent development of becoming sexually mature, simultaneously, it is also a period of social and psychological development from childhood to adulthood (Burnett, et al. 2011). The adolescent period in laboratory rats ranges from postnatal day (PND) 30 to PND60, which is reasonably equivalent to the teenage and early adult period in humans. In the current study, rats underwent experimentation from PND30 to PND53.

Prevalence

Prevalent rates of alcohol use, nicotine use, and concurrent consumption, are usually defined as the consumption frequency in single sessions and the frequency of these sessions over time (e.g. 20 cigarettes and/or 2 standard drinks daily). The concurrent and independent use of these drugs has become more and more noticeable among young people which highlights the importance for research. Initial nicotine and alcohol use is typically introduced early on in life and statistics suggest that consumption rates increases with age during adolescence and early adulthood.

Alcohol Consumption among Adolescence

For alcohol use, Johnston et al. (2009) showed that 37% of 8th grade students have attempted to consume alcohol and by 12th grade, the percentage rose to 72%, which is an increase of 35% within a 4-year span during adolescence. Past-month rates of getting drunk increased from 5% in 8th graders to 27% in 12th graders and having consumed 5 drinks in row in the past 2 weeks expanded from 8% to 25%. Results of the New Zealand health survey in 2015/16 showed that overall, 76% of adolescence (ages 15 – 24) had consumed alcohol in the past 12 months. To be precise, alcohol use rose from 57% in early adolescence (ages 15 – 17) to 84% in late adolescence (18 – 24 years). That is more than half the younger adolescent population consuming at least one or more beverages in the last year, and over 8 out of every 10 senior pupil. Furthermore, in 2017, the proportion of teenagers who reported consuming alcoholic beverages 30 days prior to being surveyed were 8%, 20% and 33% for 8th, 10th and 12th graders respectively (Monitoring the Future, 2018). From here, we can see that alcohol is a major influence within the young community, especially as teenagers become older. The real concern is heavy consumption per session and how often these vigorous sessions occur.

Excessive amounts of alcohol consumption among humans is well known in the literature as binge drinking or "binging". This is defined as having 4 or more standard drinks for females and 5 or more for males in a single session or within two hours (Stahre, et al. 2014). This style of drinking is most detrimental compared to other styles of drinking, which rose from 6.4% in 8th graders to 21.6% in 12th graders. Among American teenagers, binge drinking in the last two weeks increased from 3.7% in 8th grade to 16.6% in 12th grade (Monitoring the Future, 2018), which is almost 1 out of every 5 high school senior pupil. In 2013, binge drinking was higher for males (15.8 percent) compared to females (12.4 percent) aged 12 – 20 years. As alcohol use continuously increases with age, binging is also becoming more prevalent. Animal studies have compared the consumption rates in adolescents and adults. Adolescent rats have been shown to voluntarily consume significantly more ethanol compared to their adult counterparts, especially at high doses (Truxell et al., 2007). Bell and colleagues argue that when adolescent rats were exposed concurrently to multiple concentrations of alcohol (30%, 20% and 10%),

their intake increased by roughly 10g/kg per day (2003). In line with high prevalent rates, it is suggested that adolescents are most vulnerable to consuming large amounts of alcohol and being a first time user.

Nicotine Consumption among Adolescence

Chronic nicotine consumption has been well documented to have consequential outcomes in humans, and similar to alcohol, introduced more often during adolescence. Prevalence of smoking in New Zealand in 2015/16, from ages 15–17, showed that 6.1% are smokers (defined as having smoked more than 100 smokes in their lifetime), 7.7% females and 4.6% males. From ages 18-24, the percentage increased to 22.7% (28.8% males & 16.1% females), which is the highest of all age groups in New Zealand (Ministry of Health, 2016). For the population of daily smokers, 5.2% were 15-17 years old, 19.1% were from the ages 18-24, and 19.3% were 25-34. The trend then declines as ages increase after 34. Smokers were more prevalent in Maori and Pacific Islanders in New Zealand. A report by the National Institute on drug abuse showed that from 1995-2011, overall prevalence of cigarette uses in 8^{th}, 10^{th} and 12^{th} graders decreased annually. However, cigarette use increases dramatically from the 8^{th} to the 12^{th} grade (2016).

Levin and researchers showed that female adolescent rats consumed almost twice as much nicotine per kilogram of body weight than adult counterparts when nicotine self-administration began in adolescence (2003). Abreu-Villaca and colleagues found that mice exposed to high nicotine during adolescence (PND30-PND45) exhibited more novelty seeking behaviour, which is suggested alongside other behavioural traits to be associated with drug use and initiation of drug experimentation and/or transitions to drug addiction (2015). In 2010, over 60% of new smokers in America were under the age of 18. In line with alcohol abuse, one of the major statistical issues is the initializing of nicotine use and its positive correlation with age, where teenagers are also at the most risk. It makes sense that the probability of co-using nicotine and alcohol may be a huge risk teenagers face, since both smoking and drinking go hand in hand from early to late adolescence.

Co-use of Alcohol and Nicotine among Adolescence

The concurrent consumption of nicotine and alcohol varies according to age, gender and ethnic background. Data from the National institute of health in the US mention that men have higher rates of co-use than women, whereas the younger age-groups also have higher prevalence of concurrent use compared to adults (2007). Among ninth graders in Louisiana, 20% were co-users (Johnson, et al. 2009). It was mentioned that those who abuse or are dependent on alcohol are more likely to be heavy smokers (Falk. 2006). Furthermore, research comparing psychiatric and non-psychiatric adolescents showed that the use of alcohol and another illicit drug were significantly higher in psychiatric adolescents, compared to the general population (Mangerud, et al. 2014). Compared to teenagers from the general population, adolescents with alcohol and other drug (AOD) use disorder have higher rates of delinquent behaviour and reported higher rates of smoking and drinking (Myers & Kelly, 2006). More than a considerable number of alcohol users smoke cigarettes and vice versa, but psychiatric patients, hospitalized patients and adolescents smoke much more than non-alcohol users (Ceballos, 2006). Additionally, almost 20% or 1 out of every 5 students (of average age of 15 years) were shown to be co-users. This percentage is very high but not surprising (Jonson. 2009).

Anthony and Echeagaray-Wagner collected national data before 1997 and presented an epidemiologic analysis of alcohol and nicotine co-use and found a pattern (2000). These researchers showed that concurrent consumption of these two drugs peaked during later adolescence from low levels earlier on, but then starts to decline after the peak in early adulthood. In line with the rates of single alcohol and nicotine use, concurrent abuse is almost inevitable and will continue to be problematic in the young community.

Significant Impacts during Adolescence

Consequences of alcohol use in Adolescence

For an adolescent brain exposed to binging or heavy alcohol consumption there can be significant consequences. Firstly, the natural pharmacological and developmental properties of the adolescent brain are disrupted when alcohol is binged or consumed heavily. For example, Crews and colleagues argue that binge drinking during adolescence is more detrimental compared to binge drinking in adult rats. Binge drinking in juvenile adolescent rats resulted in significantly more damaged portions of the brain (e.g. associated frontal cortical olfactory regions, the anterior parts of the piriform, and perirhinal cortices) compared to adult rats exposed to binge drinking (Crews, et al. 2000). Adolescent binge drinking initiation reduced quantities of the frontal branches of the corpus callosum (e.g. forceps minor), and damaged and reduced axon myelin density located in the medial pre-frontal cortex (Vargas, et al. 2014). Adolescent mice chronically treated with alcohol showed significant reductions in physical growth (Zou, et al. 2009). Secondly, heavy alcohol use has memory and behavioural consequences. Adolescent rats that repeatedly consumed ethanol for five consecutive days performed worse on the Morris water maze memory test compared to saline treated rats. Even after cessation of treatment, adolescent rats still failed to catch up to the control rats (Sircar & Sircar, 2005). In support of this, Vargas et al. (2014) found that binge drinking during adolescence in male rats produced impaired performance on the T-maze working memory task in adulthood. Similar such rats performed poorly on spatial learning tasks (White & Swartzwelder, 2005). Zou et al (2009) showed that chronically treated mice produced accelerated acclimation to a novel environment and a novel companion mouse, which suggested social recognition and memory were impaired. There is no doubt that heavy acute and chronic alcohol consumption in both humans and animals negatively impacts adolescent working memory, spatial memory, physical development, brain maturity and cognitive development. Recovery after cessation of heavy use is also problematic.

Consequences of Nicotine use in Adolescence

Although research on adolescent nicotine abuse may not be as prevalent in the psychological literature as alcohol, the undeveloped brain is still at high risk for consequential outcomes of toxic substances such as nicotine. Animal studies have shown significant evidence suggesting nicotine-induced psychological and behavioural consequences. For example, mice treated with both high and low doses of nicotine showed greater anxiety-like behaviour in the elevated plus maze (Abreu-Villaca, et al. 2015; Iniquez, et al, 2009).

Interestingly, nicotine exposure during adolescence in rats heightened sensitivity to stress in the forced swim test and heightened a depressive-like state as shown by reduced sensitivity to natural reward such as sucrose (Iniquez, et al. 2009). These animal studies demonstrate the psychological impact of nicotine exposure at a young age. They are also consistent with human studies.

A cohort study examined the relationship between depression and anxiety symptoms with nicotine daily consumption and dependence in young adulthood. Interestingly, teenage smokers who experienced symptoms of depression and anxiety, significantly progressed to nicotine dependence (McKenzie, et al. 2010). In line with this, it is estimated that 44% of all cigarettes sold in the United States, are purchased by individuals with psychiatric disorders (National Institute on drug abuse. 2016). There seems to be either a mutual relationship between depression and anxiety with nicotine consumption in youth, or there is an unexplained effect of nicotine on emotion-specific brain regions in the brain. Further research is clearly needed.

Chronic nicotine consumption was shown to produce impaired cognitive performance during adolescence. Counotte et al. (2009) found that compared to adults, adolescent female rats chronically treated with nicotine displayed diminished attentional performance and increments in impulsive action. If this holds true, it may be of concern if nicotine is concurrently consumed with alcohol given the attentional deficits that alcohol also produces. In addition, other research suggested a significant initial learning impairment in adult rats with a history of nicotine consumption during adolescence (Barron. 2005) thereby indicating negative long-term effects of chronic nicotine use during adolescents. It is safe

to say that although research may be limited in enabling a causal explanation, similar to alcohol, nicotine use is still potentially hazardous for adolescent memory, learning and psychological development.

Consequences of co-use in Adolescence

The co-use of alcohol and tobacco use in adolescence is very common but remains very limited in the literature. There are recent studies suggesting a number of consequences. Compared to the impacts of either drug on its own, the concurrent use may lead to poorer outcomes including unsuccessful quitting attempts and a higher probability of developing a psychological disorder (Abreu-Villaca, 2017). The probability of developing co-use tendencies and high unsuccessful quitting attempts is highly likely considering it possesses a 'mutual relationship'. Not only do rates of single use increase with age, the two drugs may develop to co-exist naturally. For example, it has been shown that nicotine consumption during adolescence led to increases in alcohol intake for male adolescents but not for their female or adult counterparts (Larraga, et al. 2017). Other evidence suggests that nicotine use decreases blood alcohol levels in female rats (Parnell, et al. 2006), which may lead to increases in alcohol consumption. As mentioned earlier, it appears that mood disorders and co-use are strongly associated, but in which direction? One study showed that adolescents with mood disorders were more likely to be both smokers and alcohol users compared to healthy subjects (Saban, et al. 2010; Mangerud, et al. 2014). Mangerud and colleagues also mentioned that the abuse of these drugs may be a self-medicating or coping tool in alleviating ADHD symptoms and depressive symptoms experienced by individuals, thus increasing the need to consume more (2014). A longitudinal study by Mojtabai et al. (2013) showed a strong association between drinking, smoking and early onset of mood disorders in late adolescence/ early adulthood. There appears to be an unexplained association among co-use, single-use and psychological distress. In turn, the outcomes of mood disorders and stress may produce behavioural modifications such as increases in aggression, which is of most interest in the current study.

Aggression

Aggression is commonly defined as the "intent to create a noxious state for or harm the target or any action that is intended to harm (or threaten harm to) another individual" (Barthalow, 2018), whereas violence is depicted as "behaviours by individuals that intentionally threaten, attempt or inflict physical harm on others" (Asdigian, et al. 2002). It is generally understood that aggression and violence are highly correlated. Some argue that functional aggression is a strong predictor of violence in rats, and that the definition of violence can be understood as "an injurious form of offensive aggression that is out of control and out of context" (Koolhaas, et al. 2013, p. 1).

There are many forms of aggression in humans, for instance social/ indirect (e.g. starting rumours), verbal (e.g. face to face insults) and physical (dominant orientated and threatening behaviour) aggression (Landsford, 2017). However aggressive behaviour is specific to different species. For the purpose of the current study we will be evaluating offensive aggression in adolescent rats in relation to co-use and single use of alcohol and nicotine. Common aggression in laboratory rats (e.g. PVG/C species) include lateral threats, upright posture, chasing, pinning and clinching, which are threatening behaviours that predict physical attacks and biting vulnerable parts such as the throat, belly, and paws (Koolhaas, et al. 2013).

Alcohol and Aggression

Theoretical Background

There have been a number of different theoretical models that have been developed to explain the relationship between alcohol and aggression in humans. To really gain more insight into this relationship and models that specify its nature, it is best to mention some broader theories. Geen's theory developed in 1990 argues that the expression of aggression depends on the relationship between two common influences or factors. These are background factors and provocative environments. Background factors include variables such as physiology, temperament, exposure to violence, genetics and so forth. Provocative environments are situations that produce anger, increased arousal and stress.

For example, verbal attack, family conflicts, stressful environments, physical pain and so on. The interpretation of these situation by the individual (perceived as threatening or not) and the "background factors" imbedded in them, together influences on their reactions towards each situation, thus producing aggressive behaviour. Another broad theory suggests that it is not so much the direct effect of instigating factors (e.g. physically assaulted) that trigger aggression but more so the psychological stress that is endured by the individual which leads to experiencing 'negative affect' (Berkowitz, 1993). Negative affect is defined as an undesirable feeling experienced by the individual that can arise due to several factors including frustration, noise, insults, and attacks. This model suggests that experiencing negative affect triggers aggression-related cognitions and physiological reactions that are linked to fight and flight propensities (1993).

White (1997) proposed three theoretical models to potentially explain the relationship between alcohol use and aggression during adolescence. The first is that alcohol use causes aggressive behaviour due predominately to the psychopharmacological properties of ethanol (e.g. attention deficits, disinhibition, poor judgement, etc.). The second explanation is that aggressive individuals are more likely to be driven into subcultures and social situation in which heavy alcohol consumption is encouraged, and the last model argues that there is a third factor (s) that links the two together (e.g. poor relationship with parents, temperament, etc.), for example heightened anxiety (Parrott, et al. 2012). Based on empirical evidence, the third suggestion is the best explanation, where the first two ideas may be incorporated into the third theory as a potential third factor. The first theory White mentions, proposes a direct effect of alcohol on aggression. If this was the case, everyone who consumes enough alcohol become aggressive, which is not entirely true. However the pharmacological impacts of acute alcohol intoxication is a significant factor that should always be considered. Giancola mentions the disinhibition model which also argues a similar position, in that the pharmacological properties of alcohol affects brain centres important for maintaining inhibitory control over behaviour (2002). Thus, allowing us to regulate our aggressive behaviour. Others have also developed cognitive theories to help explain these pharmacological disruptions to the brain. Pernanen (1976) talks about how alcohol "narrows the perceptual field" and that it diminishes the ability to recognize internal and external cues that provide crucial information about another person's intentions in risky situations. In this case, aggression may

arise when the individual misinterprets what another person is intending on doing or saying, underlined by alcohol-induced cognitive deficits.

Similar to the "narrows the perceptual field" idea, the attention allocation model suggests that this "narrowing" occurs around attention and that intoxicated peer's information processing is disrupted (Steele & Josephs, 1990). This leads to alcohol induced peers allocating their attention to the most obvious cues of a specific situation rather than multiple cues, where attention is restricted. For example, a patriot raising their tone in a bar may be perceived as threatening and portrayed by the intoxicated person as a form of verbal attack. However, other cues may also be present at the same time which may not be portrayed as offensive by a sober person, but becomes difficult to notice by intoxicated people (e.g. increasing tone due to loud music, non-offensive words used, friendly postures, etc.). This may trigger aggression by selectively adhering to the most salient external cue (MacDonald, et al. 2000) that may be offensive while naturally avoiding (due to intoxication) inhibitory or less threatening cues that are also present in a hostile inter-personal situation (Steele & Joseph, 1990). Both these attention-based theories stress that the main causes of aggression lie within not maintain sufficient attention to and normal brain processing of the outside world.

Phil et al. (1993) later suggested a 'biosocial model' of intoxication. This theory argues that alcohol consumption disrupts the performance of the prefrontal cortex (involved in higher cognitive processes and self-regulatory control) and its subcortical regions (e.g., the hippocampus) that are involved in "recognition of danger". In other words, alcohol has anxiolytic effects or reduces fear reactions that may lead to reduced inhibitory control of behavioural reactions, thus leading to aggressive responses.

Although these theories have pertinent points, Giancola (2000) developed a model that incorporates most cognitive theories mentioned earlier into a single and more unique construct, namely executive functioning. Giancola defines executive functioning as a "higher order" cognition involved in regulation and initiation of goal-directed behaviour. Cognitive aspects included in this "higher order" construct involve abstract reasoning, self- and social monitoring, ability to organize information, attentional control, information appraisal and so forth. The main ideas behind this model is firstly, that the relationship between alcohol and aggression is mediated by executive functioning and that acute

intoxication disrupts executive functioning thereby increasing the probability of aggression. The second is that the alcohol- aggression relationship is moderated by executive functioning, where individuals with low executive functioning are more likely to be less aggressive when intoxicated compared to persons with medium to high executive functioning.

Individual Level

As mentioned earlier, it is important to note that although it has been shown that alcohol consumption indirectly leads to increases in aggressive behaviour, not all consumers become aggressive when they are intoxicated. For this reason alone, we must consider and take into account factors at the individual level that may help describe alcohol-induced aggression. Giancola (2002) mentions a few individual variables to consider when evaluating alcohol related aggression including dispositional aggressivity, alcohol expectancies, drinking history, gender, hostile attribution biases, and biochemistry. Others may include anxiety, difficult temperament, personality disorders, level of executive function, and pharmacological properties (Heinz, et al. 2011; Giancola, et al. 2006; Parrot, et al. 2012).

For sex differences, White et al. (1993) found that young adult males reported engaging in more alcohol-related aggression including physical fights, forced sex, setting fires, vandalism and hurting someone, compared to females (1993). For females, a laboratory study found that when individuals consumed low doses of alcohol, verbal aggression (using an adjective checklist) was more frequent compared to males (Rohsenow & Bachorowski, 1986). These studies suggest that males and females may engage in different subsets of aggression that is related to alcohol consumption. Furthermore, evidence showed that when a low provoking situation was introduced in a laboratory setting, no sex differences were found (Bond & Lader, 1986). However, males were significantly more aggressive than females when exposed to high levels of provocation. A study showed that difficult temperament was positively associated with aggression for all subjects and increased intoxicated aggression for men only (Giancola, 2004). Giancola et al. (2006) then conducted a study with 21 to 30 year old male and female social drinkers and hypothesized that executive functioning would be a significant mediating factor for the relationship between difficult temperament and intoxicated aggression. They found that executive function successfully mediated the alcohol-induced aggression and difficult temperament relationship,

but for males only. Males from this study who scored higher on difficult temperament, were also higher on intoxicated aggression and this significant relationship was mediated by executive function. Parrott et al. (2012) found that during low provocation, intoxicated men who were higher on trait anxiety measures (State Trait Anxiety Inventory) displayed more aggression towards their opponent. It seems that specific situational context, and personality traits influences on sex differences in aggressive behaviour. Personality traits such as dispositional aggressivity, or the propensity to be aggressive across a variety of situations, is highly associated with marital violence (Leonard & Senchak, 1993). Similar to irritability, individuals with higher levels of dispositional aggressiveness compared to low or moderate levels, displayed heightened aggression during acute alcohol consumption (Giancola, 2002) and selected higher levels of shock even under low provocation situations (Bailey & Taylor, 1991). Another important assumption mentioned by many researchers is the "alcohol expectancy" theory which suggests that the alcohol-aggression relationship is better explained by the mere belief of an individual that alcohol leads to aggression, rather than by its pharmacological effects. Many have reviewed the influences of having such a belief and how it may trigger the alcohol-aggression relation (MacAndrew and Edgerton, 1969; Chermack & Giancola, 1997). There is still more research needed to on the relationship between alcohol expectancy and alcohol-induced aggression, as only a few have shown good support for this relationship (Chermack & Taylor, 1995).

Psychopharmacology

Biochemical properties of the brain is also an influential factor. In a study conducted in 1997. Berman and colleagues suggested that shortage of the brain neurotransmitter serotonin (5-HT) may be a significant factor in heightened aggression. These researchers argued that 5-HT is involved with behavioural inhibition. This may be a concern for individuals who consume heavy amounts of alcohol because it has been suggested that consumption of alcohol initially increases but later decreases 5-HT levels (Giancola, 2002). The role of 5-HT was further examined as a partially potential explanation for alcohol heightened aggression (Faccidomo, et al. 2008). Faccidomo and colleagues noted that the 5-HT receptor type b agonists CP-94,253 (which has been shown to reduce extracellular levels of 5-HT in striatum, hippocampus and prefrontal cortex) has anti-aggressive effects. Heinz et al. (2011) also

observed that alcohol-induced aggression in mice was accompanied by reductions in serotonergic receptor expressions, except for 5-HT3 receptors. The 5-HT deficiency hypothesis maintains that decreases in 5-HT should be linked with increased aggression. Therefore, Faccidomo, et al. (2008) investigated whether the 5-HT receptors type b in the orbitofrontal cortex, medial prefrontal cortex and dorsal raphe attenuated or weakened heightened aggression. Approximately 60% of mice showed more aggression after consuming 1 g/kg of alcohol, but aggressive and motor behaviours were both reduced significantly after the infusion of a 5-HT receptor b agonist (i.e. CP-94,253) in the dorsal raphe. However, after alcohol consumption, the same infusion into the medial prefrontal cortex, but not the orbitofrontal cortex, increased aggressive behaviour. This suggests that the type b 5-HT receptors in mPFC may selectively disinhibit aggressiveness in mice, but the use of a 5-HT1B agonist in mPFC increases aggression in subjects with a history of ethanol self-administration (Heinz, et al. 2011). Along with serotonin levels, other evidence indicates that higher testosterone levels in college students are linked to higher levels of physical aggression (Volavka, 1995). Despite the influences of other variables within the individual level, the biochemical balance in the brain and body is also influential in heightened aggression.

Animal Studies

In one laboratory study of the acute effects of alcohol on 'aggressive' vs 'non-aggressive' subjects, Miczek et al. (1992) treated rats with ethanol concentrations from 3% to 17% by diluting 100% ethanol with distilled water. After behavioural assessment using the resident intruder paradigm (to be described later), these authors identified individuals with reliable "aggression-heightened" effects and inspected moment-to-moment changes in the aggressive behaviour patterns of the subjects as a result of ethanol treatment. They showed that at low acute doses (0.1, 0.3, 1.0 g/kg) alcohol heightened attack behaviour in almost half of the total population, reliably suppressed attack behaviour (0.1 – 0.3, 1.0 g/kg) in 25% of rats, and had unreliable effects in the remaining 28% (Miczek, et al. 1992). Overall, regardless of alcohol dose and subgroup, if each subject engaged in aggressive behaviour, there was a significant sequence of aggression-related responses that occurred in bursts or rapidly in a short period of time. For example, when subjects engaged in aggressive behaviour, common patterns began with chasing the intruder, followed by a sideways threat, attack bite and ended with an aggressive posture. In heightened-

aggressive subjects (low dose), alcohol produced increases in frequency of attack, number of aggressive elements in burst and the time of these aggressive bursts without changing the rate of aggressive behaviour within a burst, number of aggressive burst during encounter, and latency to initiate aggressive behaviour (Miczek, et al. 1992). Furthermore, Tuominen etal. (1990) looked at both anxiety and aggressive traits in male rats selectively bred for alcohol preference. The second-generation rats were categorized as either alcohol-preferring or alcohol-avoiding rats. In the resident intruder paradigm, alcohol preferring and alcohol naïve subjects both displayed a shorter latency to the first aggressive act (e.g., duration of time before first bite, arched posture, etc.) and greater frequency of agonistic behaviour (duration of these behaviours did not differ) compared to alcohol-avoiding rats. Interestingly, defensive behaviour were also higher in alcohol-preferring rats than their alcohol-avoiding counterparts, which indicates an uneasy interpretation (Tuominen, et al. 1990). For example, both offensive and defensive behaviours were higher in alcohol preferring rats, compared to alcohol-avoiding rats, which does not provide a clear correlation between alcohol preference and aggression. Increases in defensive behaviour typically indicates decreases in aggressive tendencies or adhering towards a subordinate rather than dominant position. A strength of these studies was the targeting of specific responses and subject-orientated traits which provides a clearer understanding of the behaviours produced in intoxicated subjects. Along with different theories constituting cognitive, pharmacological, and contextual factors relating to alcohol-induced aggression, sex differences, biochemical properties and subject-orientated factors are all also important influences.

Alcohol-Induced Aggression during Adolescence

As mentioned earlier, executive functioning has been shown to interact with aggressive levels. In boys and young adult males, low executive functioning was found to be associated with increased aggression involving fighting in normal pre-adolescent boys, as well as delinquent and physically aggressive behaviours in female adolescents (Giancola, 2002). Although aggression by definition, is not necessarily identified as violence, aggression is embodied in violence. Shephard et al (2006) concluded that drinking frequency and hitting others correlated significantly among the adolescent period.

Additionally, a New Zealand longitudinal study concluded that there was a strong association between adolescent drinking and violence, and that teenagers who abused alcohol were three times more likely to commit violence when compared to individuals who did not drink to excess (Fergusson et al., 1996). Furthermore, heavy episodic drinking in adolescent females resulted in a significant number of fights after consuming alcohol in a public location away from home (not specifically the usual drinking location) as opposed to private locations (Wells, et al. 2005). Adolescent males who drank more frequently and males who usually drink in public away from home were shown to be at more risk of alcohol-related aggression. Drinking frequency and volume was shown to be a significant confounding variable for heavy episodic drinking and fights during intoxication for human teenagers.

Interestingly, the association between alcohol and aggression were stronger in ages 12, compared to ages 15 and 18 (Sacco, et al, 2015). Similarly, Reyes et al (2011) found that higher levels of early alcohol use were linked to higher levels of dating aggression in early to middle adolescence but this association diminished in later adolescence. It may seem that alcohol-induced aggression is more prevalent in early to mid-adolescence. However in contrast to this finding, White et al. (1993) found that for male adolescents, as they age from 12 to 18 years old, alcohol use became a significant predictor of alcohol-related aggression. Early signs of aggressive behaviour for adolescent males was a better predictor for later intoxicated aggression compared with early alcohol use. Conversely, for female adolescents, 'early alcohol use' was a significant predictor of later alcohol-related aggression, which highlights an interesting sex difference within the adolescent population. Alcohol-related aggression may also be understood in terms of behavioural traits and age of onset in teenagers. A study by Huang, et al. (2001) showed that alcohol use and inter-personal aggression (i.e. assault with intentions to hurt, picked a fight, thrown rocks at people, etc.) were significantly associated with one another at ages 14, 15, 16 and 18 respectively (2001). They mention that inter-personal aggression at age 15 predicted alcohol use at 16 and that alcohol use at 16 predicted interpersonal aggression at 18. Huang, et al. (2001) also argue that the direction of the influences of aggression on alcohol use stayed positive as age increases, whereas when assessed in terms of the influences of alcohol use on aggression, associations were negative at early adolescence but positive later in late adolescence. These findings suggest that

aggressive tendencies at early ages seem to play influential roles in the development of alcohol related aggression in teenagers, and that these consequential outcomes can be modified by sex, age of onset and contextual-specific drinking locations. In line with the theory "alcohol expectancy", Brown, et al. (2010) studied adolescents aged 11 – 14 in a laboratory priming experiment and found that priming alcohol-related images significantly increased aggressive responding in early adolescence. This result Implies that the relationship between alcohol and aggression is learned early on (either through society, tv, culture, etc.), and that before the experience of alcohol related aggression, teenagers may already have developed a mental representation or a mere belief that a relationship between alcohol and aggression does in fact exist.

Male adolescent hamsters that voluntarily consumed 15% ethanol solution from PND25 to PND43 were much more aggressive (e.g. attacking behaviour) towards smaller intruders placed into their home cage after cessation of alcohol exposure (Ferris, et al. 1998). Specifically, subjects treated with ethanol as opposed to sucrose-yoked controls were twice as quick to bite intruders, and their frequency of biting was significantly higher. In contrast, Zou, et al. (2009) showed that chronic alcohol consumption from adolescence to adulthood reduced social play/ fight behaviour. However, this study used a paradigm of habituation/ dishabituation in which each experimental subject interacted with the same companion mouse four times before a fifth interaction with an unfamiliar mouse. By using a free-choice paradigm (choice between two drinking bottles), this study mimicked the natural settings of drinking among humans. It is clear that, for all age groups, alcohol abuse impacts negatively on brain development and cognitive ability (e.g. memory, learning, etc.), and can result in psychiatric symptoms, anxiety and aggression, anxiety), as well as leading to dependency and associated crime. However, in these respects, adolescents are the most vulnerable.

Nicotine and Aggression

The mechanisms underlying the relationship between acute nicotine consumption and increased aggression is still not clear. It seems as if the increases in aggression become greater when the drug is withdrawn, but reduced when acutely consumed. One study suggested that the anti-aggressive properties of nicotine may arise from action on the nicotinic acetylcholine receptor (nAChR) in the brain (Lewis, et al. 2015). NAChRs release neurotransmitters involved in aggressive behaviour (e.g. GABA, serotonin) all around the brain and are known to modulate higher-order cognitive and emotional processes such as anxiety, mood, attention and impulsivity, all of which may consequently impact on aggression.

It is possible that the link between nicotine dependency and aggression may be bidirectional. For example, baseline hostility and aggression can predict smoking several years later whilst reducing the likelihood of abstaining (Gruder, et al. 2013). This link is argued to exist on the basis that individuals smoke to cope with or regulate negative affect, thus reducing the tendencies to engage in anti-social behaviour. In a laboratory study, Jamner, et al. (1999) observed that among high hostile smokers, aggression traits were reduced to almost half when they were exposed to a nicotine patch, compared to a placebo patch. Furthermore, Driscoll and Baettig (1981) showed that nicotine consumption inhibited shock-induced fighting in "high frequency" fighting male rats. Low doses produced 'posturing' and higher doses resulted in increased 'no-reaction' or freezing. Waldbilling (1980) showed that intraperitoneal injections of nicotine led to suppressed 'mouse-killing' behaviour among rats, in a dose dependent manner. In terms of defensive aggression in mice, Johnson, et al. (2003) showed that nicotine had a dose-dependent impact that led to reductions in post-shock and inter-shock interval biting. These authors argued that the consumption of nicotine eases the negative impacts of provoking or stressful situations, which in return leads to reduced aggressive behaviour.

In consequence, nicotine consumption has shown to lead to aggressive responses during withdrawal. Saatcioglu and Erim (2009) conducted clinical interviews with Turkish male inpatients who were dependent smokers and alcoholics. Results found that compared to non-smoking inpatients, aggression was positively related to smoking in patients with an alcohol-dependence. A cross sectional study

conducted in a psychiatric hospital assessed withdrawal effects of nicotine and patient experiences of violence within a 6 month span. Although it has been proven that smoking is significantly higher for patients or individuals with mental illness, in a controlled setting like a psychiatric ward, effects of nicotine withdrawal was normally avoided by nurses. Inpatient nurses often reported supplying cigarettes to patients in fear of aggressive behaviour from or even being hit by by patients (Lawn & Pols, 2003). Parrot and Zeichner (2001) examined deprivation of nicotine in male participants and irritability traits on physical aggression. Aggression was measured using the Taylor Aggression Paradigm or TAP. As a result, individuals who were high on irritability and deprived of nicotine showed heightened physical aggression. It seems as if nicotine may have protective properties by reducing anti-social behaviour but leads to troublesome outcomes when withdrawn.

Nicotine-Induced Aggression during Adolescence

File and colleagues (2001) treated students with nicotine vapes and tested stress-induced mood changes and aggressive responses. Interestingly, nicotine reduced feelings of tiredness in females, but not for males. However, under stressful conditions, nicotine had calming effects in females but significantly increased aggression in males. Although it may seem as if nicotine under stressful situations may provide young females with stress relief, it may be a problem to the female population if they were to be introduced to cigarette smoking. If nicotine provides positive affects among females, it may become more difficult to maintain abstinence or quitting, which may then lead to developing dependence more rapidly. As for the young male population, nicotine consumption may be problematic in relation to the increases in alcohol-induced aggression. For example, most whom consume nicotine are more likely to start drinking, and given alcohol increases aggression, nicotine may indirectly heightened aggression via increased alcohol consumption. Aggression and delinquency among students were associated with increase smoking risk in both sexes. However, the link between increased smoking and depression was only significant for female adolescents (Whalen, et al. 2001). Cherek (1981) tested nicotine consumption of zero, low and high doses in older adolescents and young adults. He found that when subjects were given experimental cigarettes, the suppressing effects of nicotine on aggressive responses

were stronger in high than low doses. Cherek (1981) argued that the dose-dependent decline in aggressive responses may have been due to the suppression of deprivation effects or nicotine withdrawal, rather than to nicotine-induced reduction of aggression.

Current Study

There is a strong association between use of alcohol and tobacco and their effects on aggression. Matuszka (2017) showed that concurrent drinking and smoking among human adolescents were additively associated with elevated physical aggression compared to single substance users. Therefore, exposure to alcohol and nicotine (both together and alone) during adolescence became the subject of the present research using laboratory rats' animals as experimental subjects. To some extent this will help deal with the limitation of unreliable self-report measures in human research and will show changes in adolescent aggressive behaviour over time inside a controlled environment. The main idea was to examine the interaction effects of these commonly abused drugs. In line with previous evidence, we hypothesize that (1) alcohol intoxication leads to increased aggression, but (2) is reduced when it is consumed concurrently with nicotine, (3) Acute nicotine consumption leads to decreases in aggressive behaviour (especially at high doses) compared to pre and post treatment, but (4) heightened when consumed concurrently with alcohol, (5) Aggression during nicotine withdrawal is heightened, but (6) diminishes during alcohol withdrawal.

METHOD

Subjects

60 adolescent male rats (PVG/C) from the University of Canterbury Animal Facility were used as subjects. On reaching PND30 when the rats were early adolescents, they were weaned and caged in pairs, but physically isolated from each other. Cages were divided in half with wire mesh dividers. One rat was housed in each half of these cages with its own water bottle and food. Using this method of caging reduced the likelihood of future behaviour being influenced by repeated social interactions. In contrast, social isolation also appears to have negative implications. To minimize undesirable effects of social isolation, the dividers that separate the larger cage into two, enable subjects to see, smell and hear the other rat, but not physically interact with it.

Treatment

Alcohol and nicotine was administered via each rat's drinking water. Administering one bottle per rat enables a more accurate measurement of the quantity of ethanol and nicotine each rat consumes, which would be difficult if three or more subjects are present drinking from the same bottles. Nicotine was also administered through drinking bottles. From PND30, all rats were allowed five days for modification (PND30-PND34). This allowed all subjects to adjust to their new homes whilst developing a sense of territoriality.

Treatment with chronic alcohol and/ or nicotine began at PND35 and lasted for 16 days until subjects reached PND50. This will be termed the 'intoxication' period. When subjects reached the age of 35 days they were assigned to 6 separate treatment groups, including a control condition. During treatment, subjects were given access to either nicotine alone (at a high or low dose), ethanol alone, the combination of both, or a control condition (unadulterated drinking water) from PND35 to PND50.

1. **Ethanol**: 3g/kg or 2mL/100g (10% concentration) a day.
2. **Nicotine**: low dose (3mg/kg) and high dose (6mg/kg).

The table below shows the number of subjects within each treatment group. Each of the 6 conditions involved 10 subjects. Normal availability of food and water remained from PND30 to PND34, and when treatment began on PND35, subjects in five of the six groups were provided with drinking bottles containing a solution of ethanol, nicotine or the combination of both. Subjects in the control group had access to both food and unadulterated water throughout the whole experiment. Food was also available for all the treated subjects. Alcohol and nicotine drinking solutions were renewed every 48 hours between 1200hrs and 1500hrs starting from PND35. From PND51 onwards (the end of the intoxication period) until the completion of the study, solutions of treated subjects were replaced with unadulterated water. During the intoxication period, the rats' fluid consumption was recorded in order to subsequently determine the quantity of alcohol and/ or nicotine ingested.

Table 1. Numbers of subjects in each treatment condition from PND35-PND50.

Drinking Solution	*Water only*	*LOW Nicotine 3mg/kg*	*HIGH Nicotine 6mg/kg*
Water only	10 rats	10 rats	10 rats
Ethanol 3g/kg	10 rats	10 rats	10 rats

Behavioural testing

As mentioned earlier, subjects were tested for their aggressiveness using the resident-intruder paradigm before, during and 3 days after treatment (withdrawal) i.e., PND33, PND43 and PND53. All drinking bottles were removed 10 minutes before testing. Then the wire-mesh barrier was replaced with a barrier that restricted the resident's visual and olfactory access to the occupant of the other half of the cage. A session began with introduction of an unfamiliar 'intruder' rat into the experimental resident's living area. Each session ran for five minutes from when the "intruder" was introduced. The interactions

between the resident and intruder rat were recorded via a video camera mounted overhead (connected to a video recorder) for later viewing and scoring. The resident-intruder model tested each resident from 1800hr to 2200hrs each testing day and once testing had been completed, the barrier was replaced with the original wire mesh barrier.

Behavioural categories

Each rat's video record was played and viewed for 5 minutes. By means of a momentary time-sampling procedure, every 5 seconds (indicated by an auditory signal) the resident's aggression-related behaviour, with respect to the intruder, was noted. The categories of each residents behaviour is shown below.

For offensive behaviours:

a. **Bite attack** - resident bites intruder.
b. **Allogrooming** - resident aggressively grooms around the intruder's neck and shoulder area, involving teeth.
c. **Pinning** - resident places intruder in a supine or submissive position and releases contact with the ground by at least two paws.
d. **Boxing & kicking** - resident boxes with front paws or kicks intruder with hind paws with or without contact.
e. **Social exploration & pursuit** - resident explores and chases intruder around the cage.
f. **Offensive sideways posture or lateral threat** - resident is positioned sideways over the intruder which is in a submissive posture or sideways where the intruder is trapped and its front is facing the resident's side.
g. **Mutual upright posture and rearing** - both rats stand on hind legs and face each other.

For defensive behaviours:

a. **Submission latency** - latency to the first submissive response.
b. **Submissive posture** - freeze, defensive upright posture, non-social exploration, rearing, flight & move away.

While in the current study the focus was on offensive aggression, the residents' social exploration (i.e. resident sniffs the intruder's anogenital area & body/ tail, follows intruder around) and resting (i.e. inactive, or non-social behaviours) were also noted. Every 5 seconds, the behaviour or posture of the resident at that given moment was recorded as a score of 1. For example, if the resident was inactive at 5, 10 and 15 seconds before performing a lateral threat towards the intruder at 20 seconds, a 3 will be recorded for "non-social" and a 1 for "lateral threat" after 20 seconds has passed. The next behaviour will be recorded for 25, 30 seconds and so on until the 5 minutes is over. At the completion of each testing session, the frequency of every response/ posture was counted. The total of all postures and responses under the category "aggression" became the overall aggression score for each resident rat.

Data Analysis

All aggression scores were subjected to a 3 (treatment type; water, ethanol and nicotine) by 6 (treatment group) analysis of variance (ANOVA). To test if the treatment groups had significantly different outcome scores compared to one another at each age, a one-way ANOVA was applied. This will enable comparisons of the average aggression levels of each treatment group at PND33, PND43 and PND53 independently. To determine the effects of treatment type on behavioural outcomes across all three ages (time), a repeated measures ANOVA was used with treatment type as the between-groups effect and day of behavioural testing as the within-groups effect. This enabled assessment of the changes of aggression over time relative to treatment type. When appropriate, specific groups for significant main effects and interactions were compared with LSD Fisher post-hoc tests.

RESULTS

From records of the average daily consumption of drinking fluids and body weights of the rats, it was estimated that the approximate ranges of doses consumed each day of alcohol and nicotine, alone and in combination, were: alcohol = 2.7-3.1 g/kg, low dose nicotine = 2.5-2.8 mg/kg, high dose nicotine = 5.1-5.9 mg/kg. For the first analysis, aggression levels with increasing age was assessed for all subjects. This involved a 3 by 6 repeated measures analysis of variance with two independent factors (time with three levels and treatment with six levels). Figure 1 outlines the average aggression levels of the subjects in each treatment group at different ages. Figure 2 displays this comparison as a line graph.

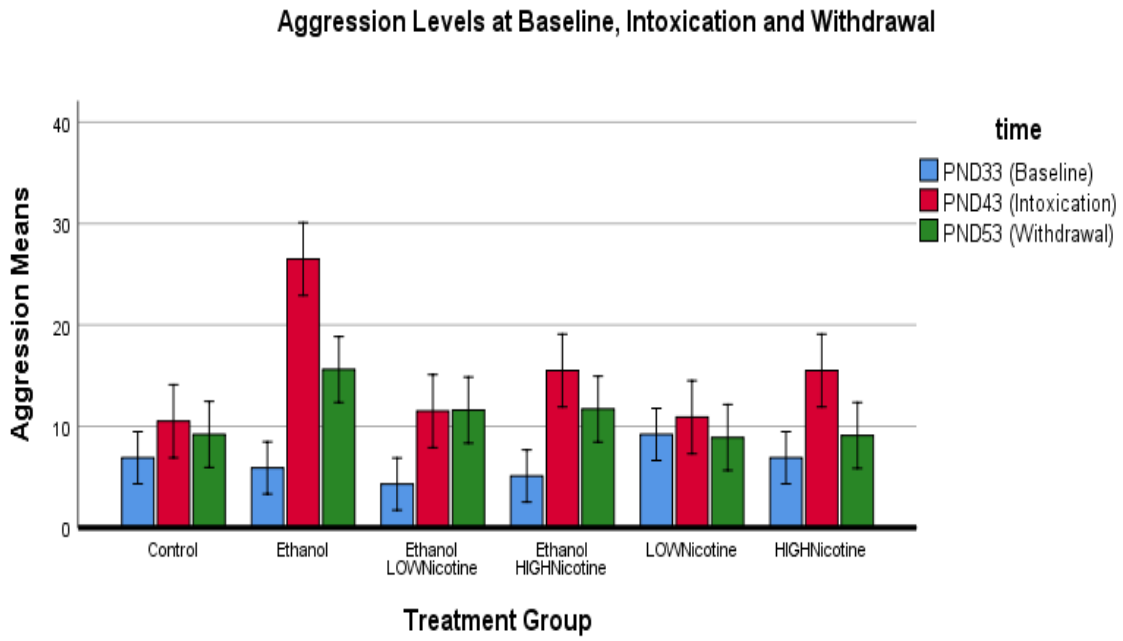

Figure 1. Average aggressive behaviour scores from each treatment condition at baseline (PND33), during treatment (PND43) and withdrawal (PND53). Higher scores indicate higher levels of aggressive behaviour

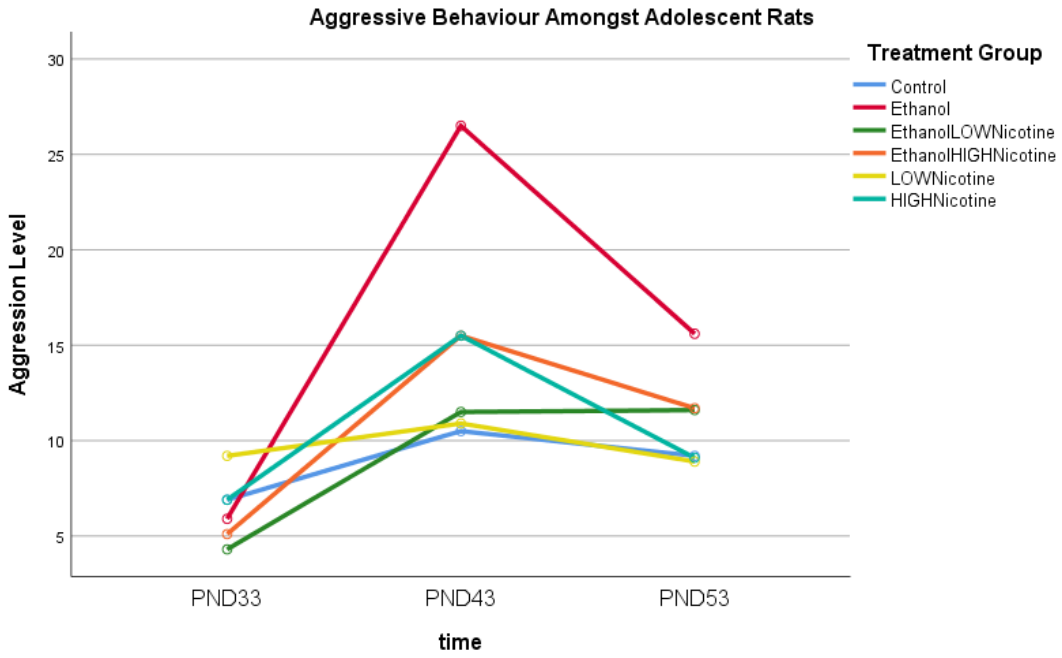

Figure 2. Average aggressive behaviour scores from each treatment condition at baseline (PND33), during treatment (PND43) and withdrawal (PND53). Higher scores indicate higher levels of aggressive behaviour.

For the repeated measures factorial ANOVA, within-subject's contrasts indicated that there was an overall significant main effect of time on aggressive behaviour ($p < 0.05$, *df* (2) $F = 56.69$). There was a difference in aggression between PND33 & PND43 (F (1, 54) = 102.08, $p < 0.00$), as well as between PND43 & PND53 (F (1, 54) = 24.81, $p < 0.00$). Partial eta squared indicated that there was a large effect size. For the independent time factor, Lavene's test of equality of error of variances and Mauchly's test of sphericity was not statistically significant. Therefore, it can be assumed that the homogeneity of variances and the assumption of sphericity had been violated. This suggests that there was considerable variance in the data, specifically within each treatment condition. Average aggression levels for all subjects at each age equalled to 6.38 in PND33, 15.07 in PND43 and 11.02 in PND53, with standard deviations of 4.19, 7.77 and 5.45. Pairwise comparisons showed that average aggression levels in PND33 were significantly lower than aggression levels at PND43 ($p < 0.05$, SE = 0.86,) and PND53 ($p < 0.05$, SE = 0.77). A significant difference was also found between PND43 and 53 ($p < 0.05$, SE =

0.81). These results indicate that over time, regardless of the treatment type, aggressive behaviour of adolescent rats peaked during treatment before slightly declining as their age increased.

A repeated measures analysis of variance also showed an overall significant interaction effect of time and treatment (F (5, 54) = 6.27, p < 0.00). This interaction between the two independent variables was significant for both inter-time periods, namely between PND33 & PND43 and between PND43 & PND53. The exact pattern will be discussed later in detail. Figure 3 displays average aggression scores of all subjects at separate ages. From this graph it can be seen that aggression was highest during treatment and lowest at PND33.

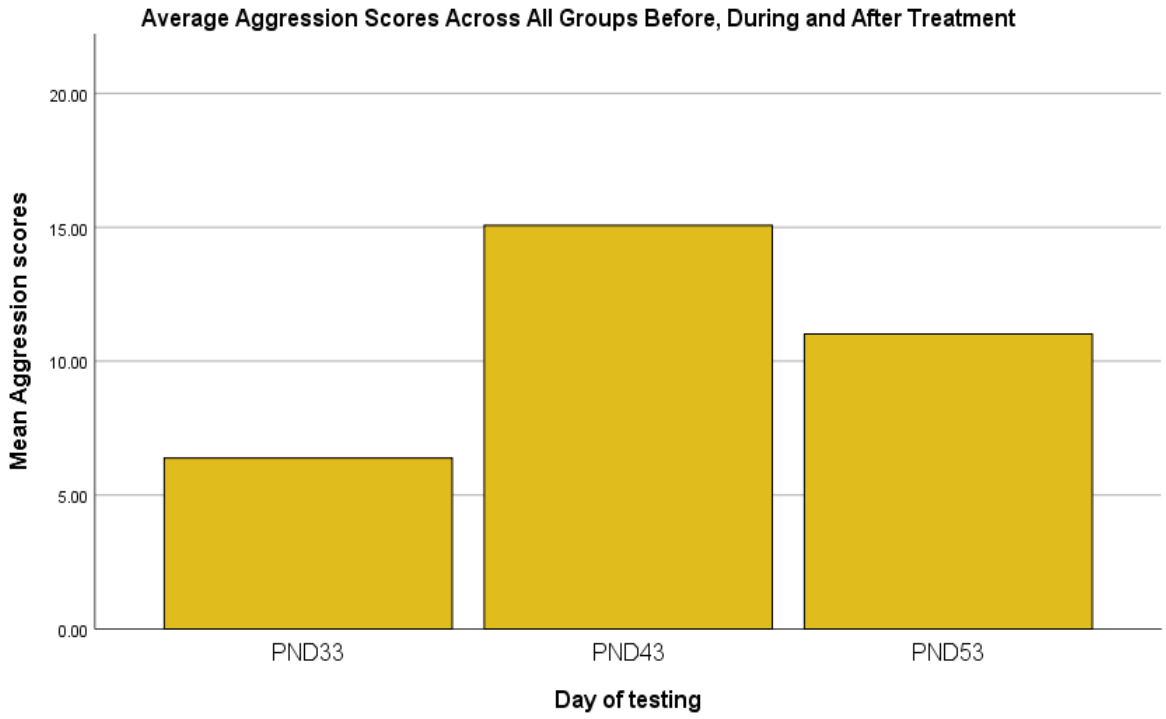

Figure 3. Overall mean aggression levels for all subjects before treatment at PND33, during treatment at PND43 and withdrawal at PND53.

Aggression at Baseline

To narrow down the analysis based on treatment type, the mean aggression scores of each treatment group at each of the three ages was compared. A one way between-groups analysis of variance was performed for each postnatal day separately. Firstly, analyses showed no overall main effect of treatment on aggression during baseline in PND33 ($F(5, 54) = 1.79$, $p > 0.05$), which suggest all subjects had relatively equal aggression levels before being assigned to a treatment condition. However, based on the LSD post hoc test for each mean comparison in PND33, there were a couple of significant difference between groups. Adolescent rats that were soon randomly assigned to the low nicotine dose condition had significantly higher aggression scores when compared to subjects soon assigned to Ethanol + low nicotine ($p = 0.009$, m difference = 4.9, SE = 1.81) and ethanol + high nicotine ($p = 0.028$, m difference = 4.1, SE = 1.81) before treatment in PND33. Figure 4 suggests that two days before these subjects were given a low dose of nicotine (PND33), aggressive behaviour was more frequent than subjects from the remaining groups, but only two comparisons reached significance. However, there was no overall significant difference between all groups.

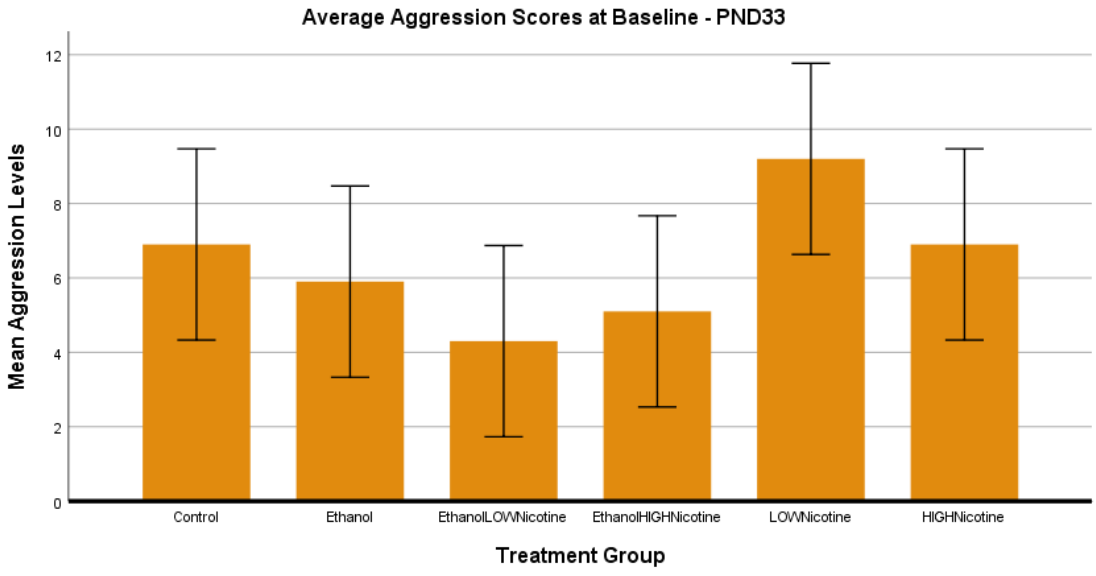

Figure 4. Mean aggression scores for each treatment condition at baseline (PND33). Higher scores represent more aggressiveness.

Table 2. Average Aggression Levels in Each Condition at PND33 (Baseline)

Treatment Condition	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
				Lower Bound	Upper Bound		
CONTROL	6.9	3.3	1	4.6	9.3	3	12
ETHANOL	5.9	5.4	1.7	2.1	9.7	1	16
ETHANOL + LOW NICOTINE	4.3	4	1.3	1.4	7.2	0	13
ETHANOL + HIGH NICOTINE	5.1	3.9	1.2	2.3	7.9	0	11
LOW NICOTINE	**9.2**	3.5	1.1	6.7	11.7	3	14
HIGH NICOTINE	6.9	3.9	1.2	4.1	9.7	0	14

Note. Each subject in each condition (n = 10) was tested once on their aggressive behaviour using the resident-intruder paradigm from 1800hr to 2200hr.

A correlation matrix was calculated using IMB SPSS 25 (shown in table 3) to assess if there was a relationship between aggression scores across the three age groups. Results showed that aggression in PND33 and PND43 were not significantly associated (r = -0.00). However, there was a significant moderate association between aggression in PND43 and PND53 (r = 0.47), suggesting statistically that there is a connection between aggression scores during treatment and withdrawal.

Table 3. Correlation between aggression scores at baseline, during chronic treatment and withdrawal.

		PND33 (**Baseline**)	PND43 (**Intoxication**)	PND53 (**Withdrawal**)
PND33 (**Baseline**)	-Pearson Correlation:	1	-.004	.047
	-Sig. (2-tailed):		.973	.720
	-N	60	60	60
PND43 (**Intoxication**)	-Pearson Correlation:	-.004	1	**.472****
	-Sig. (2-tailed):	.973		.000
	-N:	60	60	60
PND53 (**Withdrawal**)	-Pearson Correlation:	.047	**.472****	1
	-Sig. (2-tailed):	.720	.000	
	-N:	60	60	60

**. Correlation is significant at the 0.01 level (2-tailed).

Aggression during Intoxication

For mean aggression levels during treatment in PND43, results produced a significant between groups main effect (F (5, 54) = 11.3, p = 0.00). Which suggests that there was an impact of treatment type on aggressive behaviour in PND43. Scores of adolescent subjects whom were treated with ethanol differed significantly from the rest. Ethanol or alcohol mean scores were significantly higher than mean scores from low nicotine (p = 0.00, m difference = 15.6), high nicotine (p = 0.00, m difference = 2.54), ethanol + low nicotine (p = 0.00, m difference = 15), ethanol + high nicotine (p = 0.00, m difference = 11) and water only (p = 0.00, m difference = 16). Furthermore, adolescent subjects that were administered water only throughout the experiment had significantly lower aggression levels in PND43 than subjects administered with high doses of nicotine (p = 0.05, m difference = 5) and ethanol concurrently with high doses of nicotine (p = 0.05, m difference = 5). To evaluate overall aggression levels in adolescent rats nine days after first administration of alcohol and/ or nicotine (PND43), adolescent rats given alcohol alone, high doses of nicotine alone and the two simultaneously, showed more frequent aggressive behaviours and postures when compared to the remaining groups.

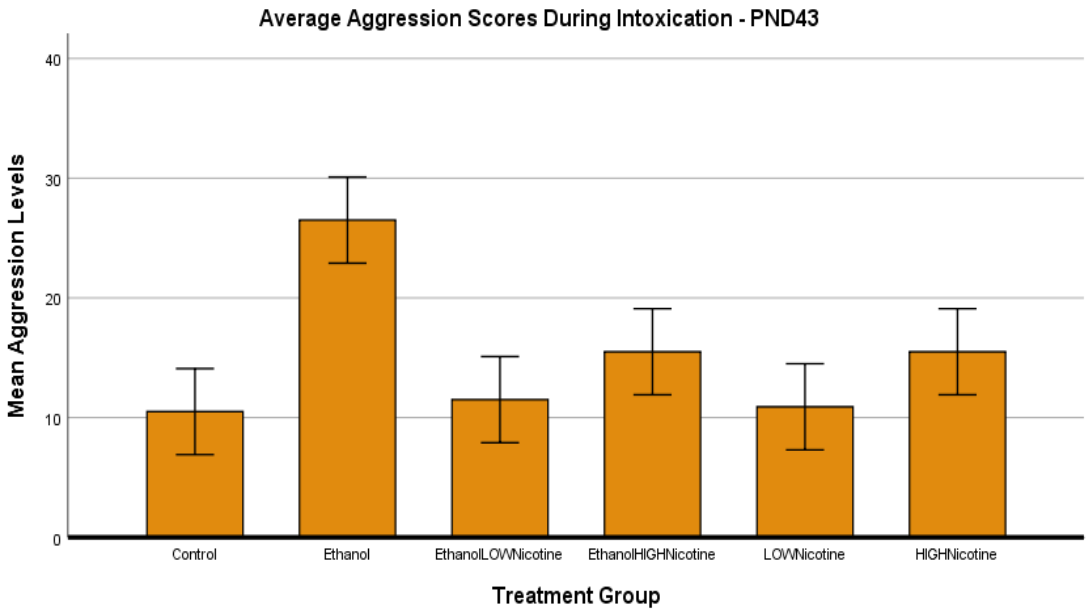

Figure 5. Mean aggression scores for each treatment condition during treatment (PND43). Higher scores represent more aggressiveness.

Based on the results, alcohol intoxication led to heightened aggression. A contrast comparison was used to examine if the addition of nicotine at both doses was a significant influence on heightened alcohol-related aggression and the results were significant ($p < 0.05$). When alcohol was consumed concurrently with low doses of nicotine ($m = 11.5$) and high nicotine ($m = 15.5$), results showed a significant difference ($F (1, 54) = 34.98$, $p < 0.05$). Concurrent treatment displayed lower levels of aggression compared to alcohol treatment. This procedure was then repeated for the contribution of alcohol on nicotine-related aggression during PND43 and results were not significant ($F (1, 54) = 0.02$, $p < 0.87$). This suggests that alcohol-induced aggression in PND43 was significantly influenced by the addition of nicotine at either dose. Interestingly, the impacts of nicotine on aggression had no significant effect when alcohol was present. In other words, the inclusion of alcohol does not modify the levels of aggression in both high and low doses of nicotine. Main observations should target low nicotine dose with and without alcohol, and high nicotine dose with and without alcohol. Figure 5 above and table 4 below displays the results.

Table 4. **Average Aggression Levels in Each Condition During Intoxication at PND43**

					95% Confidence Interval for Mean			
	N	Mean	Std. Deviation	Std. Error	Lower Bound	Upper Bound	Minimum	Max
CONTROL	10	10.50	5.503	1.740	6.56	14.44	3	20
ETHANOL	10	26.50	8.423	2.664	20.47	32.53	19	44
ETHANOL + LOW NICOTINE	10	11.50	5.911	1.869	7.27	15.73	3	19
ETHANOL + HIGH NICOTINE	10	15.50	5.061	1.600	11.88	19.12	7	24
LOW NICOTINE	10	10.90	4.533	1.433	7.66	14.14	5	17
HIGH NICOTINE	10	15.50	3.308	1.046	13.13	17.87	10	20

Note. Each subject in each condition (n = 10) was tested once on their aggressive behaviour using the resident-intruder paradigm from 1800hr to 2200hr.

Aggression at Withdrawal

The final between groups comparison of means was carried out for aggression levels during withdrawal or after treatment, PND53. A significant between groups main effect was found ($p = 0.04$, $F(5, 54) = 2.53$). Post hoc comparisons indicate that during withdrawal, adolescent rats that were chronically exposed to alcohol during the fifteen day treatment prior to, had significantly higher levels of aggressive behaviour compared to subjects that were exposed to high doses of nicotine ($p = 0.006$, m difference = 6.5), nicotine at low doses ($p = 0.005$, m difference = 6.7), and control ($p = 0.007$, m difference = 6.4). Aggression in alcohol withdrawal did not significantly differ to aggression levels in concurrent alcohol-nicotine subjects during withdrawal. Excluding the alcohol group, the remaining conditions did not reach statistical significance when compared with one another. Based on the ANOVA contrasts comparing withdrawal effects of nicotine-related aggression with or without the addition of alcohol and vice-versa.

. **Table 5. Average Aggression Levels in Each Condition during withdrawal at PND53**

				95% Confidence Interval for Mean			
	N	Mean	Std. Deviation	Std. Error	Lower Bound	Upper Bound	Minimum	Maximum
CONTROL	10	9.20	4.872	1.541	5.72	12.68	3	18
ETHANOL	10	15.60	4.766	1.507	12.19	19.01	11	24
ETHANOL + LOW NICOTINE	10	11.60	7.427	2.349	6.29	16.91	3	25
ETHANOL + HIGH NICOTINE	10	11.70	3.713	1.174	9.04	14.36	4	19
LOW NICOTINE	10	8.90	5.363	1.696	5.06	12.74	2	18
HIGH NICOTINE	10	9.10	3.725	1.178	6.44	11.76	3	15

Note. Each subject in each condition (n = 10) was tested once on their aggressive behaviour using the resident-intruder paradigm from 1800hr to 2200hr.

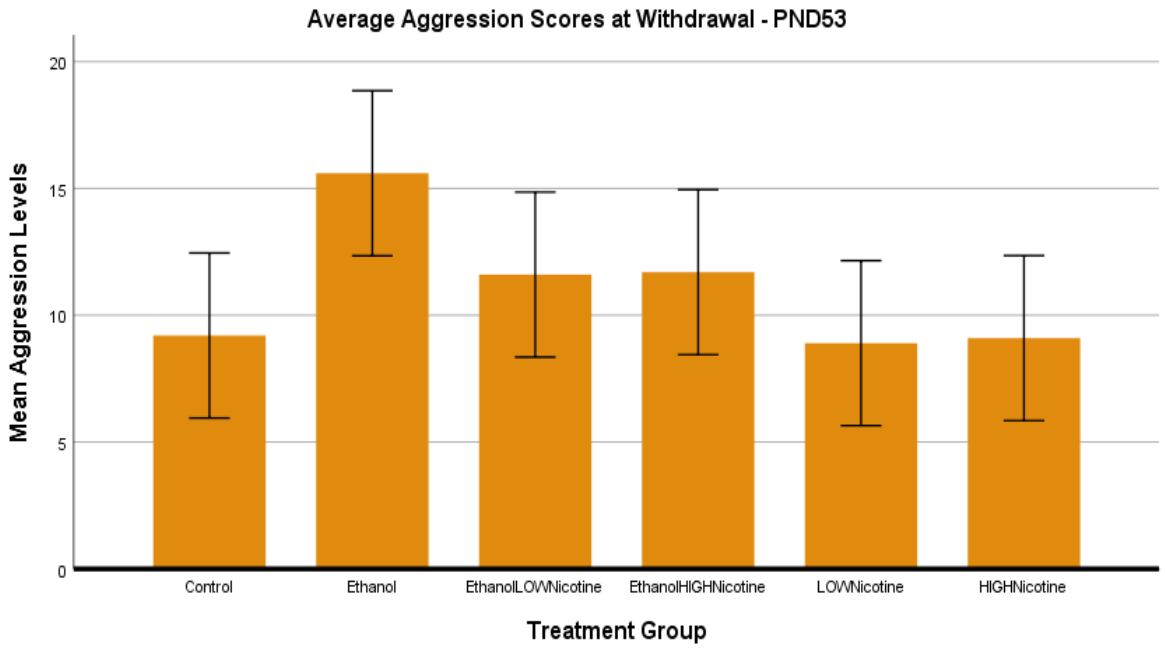

Figure 6. Mean aggression scores for each treatment condition during withdrawal (PND53). Higher scores represent more aggressiveness

Aggression in late adolescent rats treated with nicotine at both low (m = 8.9) and high doses (m = 9.1) were not significantly different to aggression levels in subjects given concurrent nicotine-alcohol (m = 11.6 & 11.7). Figure 6 and table 5 displays the results.

Aggression within Each Treatment Condition

To assess this pattern of whether subjects in each treatment condition performed aggressively or not overtime, a post hoc pairwise comparison was examined. Table 6 shows these comparisons. Time 1 refers to PND33, time 2 to PND43 (during treatment) and time 3 PND53 (withdrawal). Average aggression levels increased significantly and dramatically when subjects were given alcohol treatment on PND43 compared to baseline or PND33 ($p < 0.05$, SE = 2.11) with a mean difference of 20.6. Aggression levels for alcohol subjects later declined significantly after treatment on PND53 ($p < 0.05$, SE = 1.99), with a mean difference of 10.9, but remain significantly higher than mean aggressive scores in PND33 (before treatment).

TABLE 6

Repeated within-subjects comparison of average aggression scores for each treatment condition between time 1, 2 and 3. Time 1 represents PND33 (baseline), time 2 PND43 (during treatment) and time 3 PND53 (withdrawal).

Treatment Group	(I) Time	(J) Time	Mean Difference (I-J)	Standard. Error	Significance [b]	95% Confidence Interval for Difference [b] Lower Bound	Upper Bound
Control	1	2	-3.60	2.11	.09	-7.82	.62
		3	-2.30	1.90	.23	-6.10	1.50
	2	1	3.60	2.11	.09	-.62	7.82
		3	1.30	1.99	.52	-2.69	5.29
	3	1	2.30	1.90	.23	-1.50	6.10
		2	-1.30	1.99	.52	-5.29	2.69
Ethanol	1	2	-20.60*	2.11	**.000**	-24.82	-16.38
		3	-9.70*	1.90	**.000**	-13.50	-5.90
	2	1	20.60*	2.11	**.000**	16.38	24.82
		3	10.90*	1.99	**.000**	6.91	14.89
	3	1	9.70*	1.90	**.000**	5.90	13.50
		2	-10.90*	1.99	**.000**	-14.89	-6.91
Ethanol + LOW dose Nicotine	1	2	-7.20*	2.11	**.000**	-11.42	-2.98
		3	-7.30*	1.90	**.000**	-11.10	-3.50
	2	1	7.20*	2.11	**.001**	2.98	11.42
		3	-.10	1.99	.96	-4.09	3.89
	3	1	7.30*	1.90	**.000**	3.50	11.10
		2	.10	1.99	.96	-3.89	4.09

Treatment Group	(I) Time	(J) Time	Mean Difference (I-J)	Standard. Error	Significance [b]	95% Confidence Interval for Difference [b] Lower Bound	Upper Bound
Ethanol + HIGH dose Nicotine	1	2	-10.40*	2.11	**.000**	-14.62	-6.18
		3	-6.60*	1.90	**.001**	-10.40	-2.80
	2	1	10.40*	2.11	**.000**	6.18	14.62
		3	3.80	1.99	.06	-.19	7.79
	3	1	6.60*	1.90	**.001**	2.80	10.40
		2	-3.80	1.99	.06	-7.79	.19
LOW dose Nicotine	1	2	-1.70	2.11	.42	-5.92	2.52
		3	.30	1.90	.88	-3.50	4.10
	2	1	1.70	2.11	.42	-2.52	5.92
		3	2.00	1.99	.32	-1.99	5.99
	3	1	-.30	1.90	.88	-4.10	3.50
		2	-2.00	1.99	.32	-5.99	1.99
HIGH dose Nicotine	1	2	-8.60*	2.11	**.000**	-12.82	-4.38
		3	-2.20	1.90	.25	-5.99	1.60
	2	1	8.60*	2.11	**.000**	4.38	12.82
		3	6.40*	1.99	**.002**	2.41	10.39
	3	1	2.20	1.90	.25	-1.60	5.99
		2	-6.40*	1.99	**.002**	-10.39	-2.41

Note: Based on estimated marginal means *. The mean difference is significant at the .05 level. Significant scores are in bold and displayed to 3 d.p. whereas all numbers are within 2 d.p.

When subjects were treated with both alcohol and low dose of nicotine simultaneously (group 2), aggression significantly increased from PND33 to PND43 (during treatment) with a mean score difference of 7.2 ($p < 0.05$, SE = 2.11). Compared to aggression scores during intoxication in PND43, aggression levels takes a slight drop during withdrawal but was not significant. Mean aggression scores from PND53 were however, significantly higher than baseline PND33 ($p < 0.05$, SE = 1.9) with a mean

difference of 7.3. For the group that received the combination of alcohol and high doses of nicotine, aggression also increased significantly on PND43 compared to baseline ($p < 0.05$, SE = 2.11) with a mean difference of 10.4. These levels later decreased on PND53 during withdrawal but were not significantly lower compared to PND43. Aggression during withdrawal for concurrent alcohol and high nicotine were significantly higher than average scores before treatment on PND33 ($p < 0.05$, SE = 1.89, m difference = 6.6). Interestingly, during withdrawal PND53, concurrent alcohol-high nicotine and alcohol-low nicotine were not significantly different, but had very similar scores.

For subjects given high doses of nicotine only (group 5), aggressive behaviour increased significantly on PND43 compared to baseline ($p < 0.05$, SE = 2.11, m difference = 8.6) and was also significantly higher than aggression scores during withdrawal ($p = 0.002$, SE = 1.99) with a mean difference of 6.4. Mean scores during withdrawal and pre-treatment for group 5 (high dose nicotine) was not significantly different from one another. Similar to the controls, there was no significant difference in aggression scores of subjects that received low doses of nicotine over the three ages. Table 2 displays this in more detail.

DISCUSSION

In summary, the current study found that aggression levels varied depending on whether alcohol, nicotine or the combination of both was consumed overtime. On average, as their age increased the adolescent rats' aggressive behaviour became more obvious and peaked during the 'intoxication' period in mid-adolescence before declining in levels relative to treatment type during withdrawal in late adolescence. Although all conditions showed this trend across the three ages, there were distinctions to consider. The heightened mean aggression levels that occurred for all subjects during the 'intoxication' period' (PND43) was shown to be predominantly affected by the treatment type. For example, compared to baseline, aggression increased during chronic consumption for all groups including control, but was significant in 4 out of the 6 conditions. That is, aggression levels were significantly higher during treatment for subjects treated with alcohol, high doses of nicotine alone and consumption of both drugs relative to baseline and withdrawal. These results from these four groups may have strengthened the significant main effects of time and the high levels of aggression in PND43 as opposed to baseline and withdrawal. The decline in mean aggression scores during withdrawal from chronic treatment was only marginal for low doses of nicotine alone or in combination with alcohol and control. In line with increasing prevalent rates of alcohol and nicotine use, it can be concluded that intoxication from chronic consumption of alcohol and nicotine in combination or alone (PND43), should be addressed in further research.

Alcohol

Based on the results of our current investigation and in line with past evidence (Giancola, 2002; Miczek, et al. 1992; Shephard, et al. 2006; Giancola, 2004; Reyes, et al. 2011; Huang, et al. 2001), acute alcohol consumption alone played a major part in overall aggressive behaviour. Subjects that were intoxicated from chronic alcohol consumption, behaved more aggressively towards intruders, specifically displaying high frequencies of lateral threats, biting, boxing-kicking and upright postures compared to residents who were intoxicated by nicotine alone, in combination with alcohol and those given water. Not surprisingly, dramatic increases in aggressive behaviour during alcohol intoxication takes a toll

when adolescence reached withdrawal. Interestingly, aggression after treatment of alcohol in PND53 was significantly reduced, but remained at a high level compared to the alternative conditions. This suggests that the negative impacts of experiencing chronic alcohol consumption on adolescent behaviour remains troublesome during withdrawal (3 days after chronic consumption) compared to the withdrawal effects of nicotine use and water alone. Although the withdrawal effects of alcohol did not intensify aggressive behaviour after treatment, the level of aggression at PND53 was still significantly higher than baseline scores, and still considerably higher than the overall mean aggression scores of the remaining groups across all three ages. The aggression levels of subjects that experienced alcohol intoxication during withdrawal (PND53) reached a mean of 16 (PND33 < PND53). Interestingly, the overall mean levels (across all three ages) for the remaining groups ranged from 9 to 11. It seems as if alcohol consumption not only triggers heightened aggression during intoxication in adolescent rats but also produces intense withdrawal effects.

Nicotine Alone

Consumption of nicotine alone only affected aggressive behaviour at high doses. In contrast to the findings by Driscoll and Baettig (1981), higher doses of nicotine led to reductions in aggressive behaviour or to what the authors called, “no reaction”. These results are inconsistent with the current study, potentially due to methodological differences. Although the dose of 4mg/kg administered is relatively similar to “high” doses used in the current study, “high” dose and adult “fighting” males were used by Driscoll and Baettig (1981) where aggression was incited by a shock-induced fighting paradigm. The current study provided residents with a paradigm to freely engage in social interaction as opposed to social stress that may lead male subjects to reactive aggressively. Silverman in 1971 also reported reductions in aggressive behaviour of albino and hooded rats following high doses of nicotine. The dose of 25mg of nicotine per kilogram of body weight used by Silverman, is four times stronger than the “high” doses used in the current study. Given that high doses of nicotine in the current study (6mg/kg) increased aggression compared to low, the effects of quadrupling the dose levels of nicotine to 25mg/kg may have extended consequences. As reviewed earlier, nicotine is well known to have calming effects, and the current study supported this notion only at low doses. Consumption of low

dose nicotine neither reduced nor elevated aggression. Subjects given low doses of nicotine had similar frequency of aggressive behaviour towards intruders at all three ages. However, acute effects of high dose nicotine produced heightened aggression compared to baseline, before declining in withdrawal. Cherek (1981) and Johnson, et al. (2003) found the opposite effect where high doses of nicotine produced more of a "supressing effect", compared to low doses (1981). Although Cherek used humans, Johnson and colleagues (2003) showed that when rats were exposed to electric shocks (defensive aggression) at a fixed time of every 2 minutes, biting was at a high rate post-shock and moderate at inter-shock intervals. The same mice were used in a resident-intruder paradigm (also used in current study). Results showed a decrease in a dose-dependent manner where subjects showed reductions in post-shock biting, inter-shock interval biting, and biting in the resident-intruder paradigm. However, in the current study, the effects of high nicotine dose on aggression went the opposite direction. Rather than a free-roaming paradigm such as the resident-intruder paradigm, forced stress-induced situations may modify behavioural responses. This in turn can lead to adaptive and defensive behaviour aimed at adopting a submissive position which is a natural coping repertoire (Koolhaas, et al. 2013), rather than 'aggressive' behavioural by definition. Therefore based on dosage levels, the current study concludes that when the nicotine dose is high, aggression levels peak during chronic treatment compared to when nicotine doses are low, and that there were no withdrawal effects found.

Concurrent Alcohol and Nicotine

As mentioned earlier, when nicotine at both high and low doses was consumed concurrently with alcohol from PND35 – PND50, aggression levels were elevated during treatment compared to pre-treatment. The significant difference in aggression levels between pre- and during 'intoxication' that occurred in both concurrent groups was not surprising given that alcohol-induced aggression effects occurred. What is of most interest is that alcohol alone escalated aggression scores from 6 in pre-intoxication (PND33) to 27 during intoxication (PND43), which is an increase of more than four times the initial aggression levels. However, the average mean aggression scores for concurrent alcohol/ low nicotine and alcohol/ high nicotine increased from 4 and 5 during pre-intoxication (PND33), to 12 and 16 during intoxication (PND43). The overall increases in aggression levels from baseline to intoxication

was shown to be considerably less when nicotine at both doses was consumed concurrently with alcohol, as opposed to alcohol alone. These results suggest that nicotine may have an important protective function for chronic and acute alcohol consumption that may have reduced heightened aggression among the adolescent rats. Reasons for such results are not well documented in the literature on adolescent co-use and aggression. However possible explanations may be that nicotine has been shown by past scientific researchers to reduce the levels of blood alcohol concentration (BAC) when consumed concurrently (Pamell, et al. 2006). This may well lead to reductions in the pharmacological and biochemical effects of alcohol. If this is the case, reductions in alcohol-induced aggression are clearly influenced by nicotine. However, it may backfire in human co-users when alcohol's intoxication effect is weakened by nicotine, which may lead to individuals wanting to consume more alcohol to match the level of intoxication needed, thus making alcohol dependence more achievable.

To determine if the doses of nicotine affected the link between alcohol consumption and increased aggression, it was found that aggression levels during intoxication were considerably higher when alcohol was consumed with high doses of nicotine, compared to when it was consumed with low nicotine doses or water. In other words, adolescent male rats that were intoxicated by the co-use of alcohol and nicotine displayed more clinch attacks, pinning, boxing/ kicking and lateral threats towards intruders when the dose of nicotine was in the higher rather than lower range. Although this difference did not reach statistical significance, aggression levels of concurrent intoxication at both doses of nicotine almost matched that of the levels of nicotine alone at the equivalent doses. For example, during the intoxication period in PND43, low nicotine (m = 10.9) and concurrent ethanol/ low nicotine (m = 11.5) subjects had roughly similar levels of aggressive behaviour to one another and to controls (m = 10.5). High-dose nicotine subjects (m = 15.5) also showed identical mean levels to concurrent alcohol-high nicotine rats (15.5) during intoxication. However aggression were on average, more frequent than when low-doses of nicotine is consumed alone or concurrently with alcohol.

As mentioned earlier, the mean aggression levels during withdrawal for the concurrent alcohol-high nicotine and alcohol-low nicotine groups were very similar, sitting at a mean of 11.6 and 11.7 respectively. For high- and low-dose nicotine alone, aggression levels were also very similar. Which

suggest that the dose of nicotine is influential during treatment and not so much during withdrawal. No major difference occurred in aggressive responses in rats intoxicated by the combination of alcohol-low dose nicotine and the controls. However, compared to the baseline measures, aggression significantly increased when rats were given alcohol and low doses of nicotine, whereas controls had no significant increase. This supports the results by Matuszka et al. (2017) that co-use during adolescence is highly associated with physical aggression. On the other hand, Matuszka reported co-use to be the highest influencer of physical aggression compared to independent use and no-use, which is inconsistent with the current research. It would also seem that if nicotine alone influenced alcohol related aggression, it would make more sense for higher doses (or "more" nicotine) to be more effective in reducing aggression than less nicotine?

It is important to note that in the current research, alcohol did not modify levels of nicotine-induced aggression at both doses. However, three days after chronic treatment, alcohol seems to factor in its influence in conjunction with nicotine. For example, during withdrawal, aggression was more obvious in concurrent groups compared to nicotine alone groups, and less obvious compared to alcohol alone subjects. Average aggression levels of the concurrent groups were slightly higher than nicotine alone during withdrawal. The patterns of adolescent aggression over time demonstrated that nicotine seems to alleviate and minimize the level of alcohol-related aggression during the intoxication period and withdrawal. These results are inconsistent with the results found by Cherek (1981) for whom nicotine-withdrawal led to higher levels of aggression, as opposed to acute consumption. However, Cherek used human males who were addicted to nicotine and differed in levels of irritability. This may have affected aggression levels due to deprivation of nicotine and its negative affect experienced by the individual from not receiving the calming, stress-relief effects nicotine seems to produce. In conjunction to this, high levels of irritability is known to be strongly associated to aggression (Standford, et al. 1995). It is suggested that in line with the current study, nicotine's withdrawal effects are more noticeable when consumed with alcohol compared to when nicotine is consumed alone.

Summary of Hypothetical Predictions

Taken altogether, there are considerable variations in the relationship between the consumption of alcohol and/or nicotine and aggression. Firstly, in line with the first hypothesis that alcohol leads to heightened aggression, acute alcohol consumption increased aggressive behaviour in adolescent rats. Secondly, when alcohol is consumed simultaneously with nicotine, aggressive behaviour was significantly less frequent. Acute consumption of nicotine alone at higher doses led to increases in aggressive behaviour compared to acute nicotine consumption at lower doses. This was partially in line with our third hypothesis. Specifically, we assumed that higher doses of nicotine would produce more of a supressing effect on aggressive behaviour compared to lower. However results of the current study suggested the opposite effect. Therefore the third hypothesis was only supported when doses of nicotine were in the lower range. Acute consumption of alcohol and nicotine combined did produce heightened aggression compared to baseline, however levels of aggression were no different compared to acute nicotine consumption. Acute concurrent consumption and acute nicotine consumption produced similar frequencies of aggressive behaviour when the doses of nicotine were similar. This was not in line with our fourth predictions, because the addition of alcohol did not produce increases in nicotine-induced aggression. Nicotine withdrawal did not heighten levels of aggression which did not favour the fifth hypothesis. Instead, the levels of aggression were reduced. In line with the last hypothesis, alcohol withdrawal displayed decreases in aggression as predicted, but still at risk. Aggression levels during alcohol-withdrawal was still higher then withdrawal effects of nicotine and concurrent treatment. Therefore, overall it can be concluded that nicotine may be an effective way of reducing aggression of adolescent rats during alcohol intoxication and withdrawal, but more beneficial when doses of nicotine are in the lower ranges such as 3mg/kg.

Importance of Adolescent Research

The fundamental importance of targeting adolescents arise from the risk factors that can predict and influence alcohol and/or nicotine abuse during this phase of development. These risk factors include parents and sibling smokers, peer pressure, personality factors (e.g. impulsivity, risk-taking, sensation seeking), parental monitoring, deprived socioeconomic environments and more (Patrick &

Schulenberg. 2014; Kendler, et al. 2008). This inevitably leads to initial use among the young population. Initial use is at peak during early to mid-adolescence and then further use and abuse increases through to late adolescence and early adulthood. The current study supported this time sequence by sowing that there were increases in alcohol and/ or nicotine consumption as adolescent rats got older. Another scientific issue as mentioned earlier, is the biological properties of the adolescent brain. Unlike the adult brain, a teenage brain is still in the process of development. The two most important events that occur during adolescent brain development includes myelination of axons (i.e. insulates and increases in the speed of neural transmission which strengthens connectivity within brain) and synaptic pruning (e.g. eliminating of irrelevant connections to allow more efficient transmission between neurons). The frontal cortex is also an important portion of the brain that deals with higher cognitive processes, planning, inhibitory control, and where most "thinking" occurs. This area is still under the process of maturing during adolescence (Witt. 2010). Mixing toxic substances such as nicotine and alcohol may worsen and interfere with these processes leading to long-term deficits including memory impairments, learning difficulties, cognitive deficits, drug-specific dependency, mood disorders, inter-personal and social problems.

Strengths and Limitations

Although the present study used disciplined methodological procedures that include following behavioural testing protocols, strict dosage measurements, weaning criteria, home cage maintenance and so forth, there are a few limitations to consider. Although the results in the current study was statistically significant, sample size was relatively small with 10 subjects per group. More significant results and greater power could have been produced from a larger sample size. Additionally, removing adolescent rats at a very young age from their natural habitual settings may have interfered with their natural development affecting their behaviour and interactions with peers. Physical isolation (as mentioned earlier) was accounted for to some degree in the current study. However each experimental subject was still physically isolated from their neighbours, parents and natural habitat. This reason alone may potentially influence lead to unusual behaviour, including increased aggression.

The resident intruder paradigm has been incorporated successfully by many animal researchers and has shown reliable results when studying aggression using male subjects. There are several setbacks to the resident-intruder paradigm procedure used in the current experiment. This model provides that the subject termed 'the resident' is encouraged naturally by the physical setting and time spent within the specific environment to adopt a sense of 'territoriality' or 'home'. Based on the typical resident-intruder paradigm studied in the past, developing territoriality tends to enhance where there are unique physical features (Clapperton, 2006) and being caged with a female rat (Olivier & Young, 2002; Koolhaas, et al. 2013). Before behavioural testing begins in a typical resident-intruder paradigm, the female resident is removed and replaced with an 'intruder' rat. However, in the current study, no female partner resided with each resident subject. The majority of scientific researchers use adult rats, where the female companion resembles more of a natural setting for breeding and mating, in which animals protect and establish territories. Additionally, the current study used adolescents that, before weaning, lived and interacted with siblings and a mother. Placing weaned subjects into 'home' cages without including their pre-weaning natural setting, or a mirror of their natural environment, could minimize their tendencies to develop 'territoriality' when an intruder is introduced.

Thirdly, subjects were not given a free-choice paradigm used by many researchers (Zou, et al. 2009; Ferris, et al. 1997; Tuominen, et al. 1990) in the administration of alcohol and nicotine. This free choice paradigm method allows subjects to consume voluntarily and realistically corresponds to consumption of alcohol and nicotine in humans. Furthermore, when subjects are given concurrent alcohol and nicotine, we cannot distinguish how much nicotine and alcohol is being consumed independently to make more accurate assumptions about the behavioural differences that have occurred between co-use and single-use. The quality of the intruder (i.e. size) has shown to be a significant influence on interactions between the resident and intruder (Olivier & Young, 2002). Smaller animals are encouraged to be used as intruders in the resident-intruder model. Unfortunately, intruders in the current experiment were similar in age and size to residents. Using smaller intruders could have brought more significant results in the current study, however results remained favourable.

Although there is more research needed to examine the combined effects of nicotine and alcohol during adolescence, the current study had a number of advantages. It was the first to examine both of the most concurrently abused drugs in the world collectively and independently in adolescent rats and its behavioural consequences. Secondly, the current study tested for aggressive behavioural changes over time during adolescence. This long-term method in animals allows us to monitor not just the outcome of intoxicated behaviour but also its comparison to pre-treatment and the impacts of chronic consumption during withdrawal. Empirical evidence was reviewed that suggest a strong impact of alcohol intoxication on aggression which was consistent with the current study. The home cages of each resident were not large enough for intruders to escape, which may have increased the probability of an interaction between the resident and intruder. This is favourable in the current study because increasing interaction brings out more responses from the resident towards the intruder. Larger cages may create more distraction for both animals and produces more space to flee.

The adoption of a video-recording method to analyse the behaviours of each subject was another advantage which enabled a more accurate examination of the specific behaviours and postures each subject performed. Such recordings are useful for re-evaluating any scores that might be inaccurate. As mentioned before, prior to testing, subjects in the current study were housed in pairs in a single cage with a wire mesh barrier separating the two halves of each cage. As isolation is a significant predictor of aggressive behaviour (Olivier and Young, 2002), the current study controlled for this to a certain extent by enabling each rat to see, smell and hear its neighbour. Although the majority of scientific animal research on aggression involves adult subjects, the effects of isolation may be greater during adolescence. Consequently the caging arrangement in the present study enabled adolescent isolation effects to be minimised. Finally, the testing time periods used in the current study remained equal at all three ages. Each subject was tested at the exact same time-period at all three ages.

Future Recommendations

The research described in this thesis has identified some important effects of chronically consuming nicotine and alcohol together or alone on aggressive behaviour. Future research could aim to enhance our understanding of the underlying pharmacological, behavioural, social and bio-chemical aspects of

the combination of these drugs among adolescent animals and humans. Specifically, future studies might focus on comparisons of concurrent alcohol and nicotine consumption in adults and adolescents separately, or provide a theoretical model for prevention or reduction of alcohol-induced aggression via the inclusion of nicotine. Another approach would be to analyse aggressive behaviour using multiple behavioural models that may further account for specific social interactions, responding and postures displayed in different settings, in which the resident-intruder model does not account for. For example, shock-induced models may force subjects to reactive defensively as opposed to 'defending' its territory. Future research could also investigate potential influences of confounding factors (familiar vs unfamiliar intruder, aggressive vs non-aggressive subjects, group vs. singly caged, the impacts of provoking vs non-provoking situations, etc.). Furthermore, it would be interesting to consider interactions between early and late onset of initial concurrent alcohol and nicotine consumption.

In addition to further research ideas, interventions could focus on educating children and school teachers about the negative implications of drug use, specifically nicotine and alcohol. This could raise awareness from a very young age before children reach the age at which exposure to these widely abused drugs becomes most risky. Interventions should also include education on this topic in the workforce aimed at parents, siblings, mentors, coaches and more. This method not only targets the younger population directly, but could also be effective through a different or meaningful route. Adults in the workforce can also learn about the consequences and can go home to educate their own children, siblings, partners and friends about this issue. In terms of a legal standpoint, laws and policy makers may consider raising the legal drinking age, reduce the percentage of alcohol in beverages sold or increase the legal age of buying specific products (e.g. 21+ legally allowed to purchase straight spirits, under 21 can only purchase mild cigarettes, etc.).

Conclusion

In summary, the current study provides support for the effects of chronic alcohol, high dose nicotine and the concurrent consumption of both on heightened aggression. Nicotine is suggested to alleviate the alcohol-induced aggression and reduce such behaviours in a dose-dependent manner. We ought to encourage future scientific research targeting drug abuse during adolescence, otherwise its serious

consequences will affect the community surrounding young individuals as well as being a significant risk to the individuals themselves.

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Appendix A

ANIMAL ETHICS COMMITTEE

Secretary, Rebecca Robinson
Telephone: +64 03 369 4588, Extn 94588
Email: animal-ethics@canterbury.ac.nz

Ref: 2017/16R

29 September 2017

George Leafa

Psychology UNIVERSITY OF CANTERBURY

Dear George

I am pleased to inform you that the Animal Ethics Committee (AEC) has approved your application entitled: "The Effects of Alcohol and/or Nicotine Exposure During Adolescence, and its Behavioural Outcomes; Anxiety and Aggression".

Approval has been granted:

(a) for the use of 126 male adolescent rats (90 experimental, 36 intruders).

(b) for your research project to be undertaken from 29th September 2017 to 31st March 2018. 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 MPI 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 MPI Animal Manipulation Statistical form 30 days after the completion of this project, or once every three years, whichever 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

James Briskie

Professor Jim Briskie
Chair
University of Canterbury Animal Ethics Committee