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Effects of intermittent versus continuous access to nicotine self-administration in rats

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Effects of intermittent versus continuous access to nicotine self-administration in rats

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Résumé

Les fumeurs consomment des cigarettes de façon intermittente, inhalant chaque cigarette par intermittence, et consommant aussi des cigarettes par intermittence durant la journée. Toutefois, les modèles animaux utilisés pour étudier les effets de la nicotine impliquent un accès continu à la nicotine durant chaque session d'auto-administration. Ici, nous avons donné à des rats un accès intermittent versus continu à la nicotine durant des sessions d'auto-administration. Nous avons ensuite comparé les groupes sur des comportements de consommation caractéristiques de la toxicomanie.

Des rats mâles se sont auto-administrés de la nicotine (0.015 ou 0.03 mg/kg/infusion) durant 14 sessions (7h/session). Un groupe avait un accès continu à la nicotine durant chaque session (Long Access, LgA). L'autre groupe avait un accès intermittent (IntA; 12 minutes d'accès à la nicotine par heure). Nous avions donc 4 groupes; LgA-0.015, LgA-0.03, IntA-0.015 et IntA-0.03. Nous avons mesuré la consommation de nicotine et la motivation à consommer différentes doses de nicotine (0.0075, 0.015, 0.03, 0.06mg/kg/infusion), tel que mesuré par l'auto-administration de nicotine sous un horaire de ratio progressif. Après ~15–38 jours d'abstinence, nous avons aussi mesuré la rechute à la consommation induite par une réexposition soit à des stimuli distincts associés à la nicotine, soit à la nicotine elle-même (0.075, 0.15, 0.3 mg/kg, sous-cutané) ou du salin par injection sous-cutanée.

Durant les 14 sessions d'auto-administration, les rats LgA-0.03 ont consommé le plus de nicotine. Cependant, après ces 14 sessions, les groupes IntA ont démontré autant de motivation à obtenir la nicotine que les groupes LgA. Les deux groupes avaient aussi une vulnérabilité équivalente à la rechute après la période d'abstinence.

L'auto-administration de nicotine par intermittence chez le rat est davantage analogue au mode de consommation de la drogue chez l'humain. De plus, les rats consommant de la nicotine par intermittence prennent nettement moins de drogue que les rats consommant en continu, mais les deux procédures sont tout aussi efficaces pour produire des signes d'addiction à la nicotine. Ceci a des implications pour le raffinement des modèles animaux utilisés dans l'étude des changements neurobiologiques, psychologiques et comportementaux impliqués dans le tabagisme.

Mots-clés : Nicotine; addiction; auto-administration; consommation intermittente; consommation continue.

Abstract

Although cigarette smokers consume nicotine and other tobacco products found in cigarettes intermittently throughout each day, procedures to study nicotine effects in laboratory animals involve continuous nicotine access during self-administration sessions. Here, we compared the effects of intermittent versus continuous nicotine self-administration on behavioural features relevant to drug addiction, using an animal model.

Four groups of male rats self-administered different doses of nicotine (i.e., 0.015 or 0.03 mg/kg/infusion) during 14 sessions (7 h/session) either as continuous nicotine access (Long Access, or LgA) or intermittent access (IntA; 12 minutes of access to nicotine per hour). We assessed group differences in nicotine intake, as well as nicotine seeking under a progressive ratio schedule of reinforcement (a measure of incentive motivation for drugs). Following nicotine withdrawal, we also measured the susceptibility to cue (withdrawal days 15-30) as well as nicotine-primed (0.075–0.15–3 mg/kg, subcutaneous; withdrawal days 30–38) reinstatement of nicotine-seeking behaviour (measures of relapse).

LgA-0.03 rats consumed the most nicotine across the 14 self-administration sessions; however, despite taking much less nicotine than LgA groups, IntA groups showed similar levels of responding for nicotine under a progressive ratio schedule. After nicotine withdrawal, all groups also showed equivalent levels of cue and nicotine induced reinstatement behaviour.

IntA nicotine self-administration results in much less drug intake than LgA self-administration, yet the two procedures are equally effective in producing behavioural signs relevant to tobacco addiction. Also, IntA nicotine self-administration serves as a better model of the intermittency of cigarette smoking in humans. Our findings have important implications for preclinical studies investigating the behavioural, psychological, and neurobiological processes involved in tobacco addiction.

Keywords: Nicotine; addiction; self-adminsitration; intermittent consumption; continuous consumption.

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Acronyms and abbreviations

AMPA :	α-amino-3-hydroxy-5-methylisoazole-4-isoxazolepropionic acid
Ca^{2+}:	Calcium
DSM :	Diagnostic and Statistical Manual of Mental Disorders
GABA:	Gamma aminobutyric acid
IntA :	Intermittent access protocol of self-administration (7h, 12-min of access every hour,)
K:	Potassium
LgA:	Continuous access protocol of self-administration (7h *(long) access without interruption*)
Na^{+}:	Sodium
NMDA :	N-methyl-D-aspartate
ShA :	Short access protocol of self-administration (generaly 1–2h without interruption)

Notes on formatting

This master thesis has been written following the APA 6th edition formatting guidelines at the exception of themes and chapters headings which are personalized, and for the level 4 headings that are not indented for aesthetic and readability purposes. Double line spacing is used for the body of the text and single line spacing is used for the figure captions. For long figure captions, a line spacing of 1.15 is used to increase readability. Single line spacing is used for the subheadings with more than one line of text. The body of the text is written in Times New Roman with a font of 12, figures and legends captions are set at 10.5 in chapter 1, and the figures and legends captions of statistical results are written in Helvetica size 8 to 11, a sans-sherif font conforming to APA standards. Adobe Acrobat Reader kept the Helvetica format without converting it to Arial because the text is in an image format. Statistical figures are saved as pdf format or Adobe Illustrator format to keep a vectorial quality to preserve image quality at all viewing setting especially when inspecting details. Colors for the figures were selected in a list of web-safe colors; the colouring should be easy to visualise on any screen. Images used are either under creative common or public domain. Another derogation to the APA formatting is a modification of point 7.01 #2 of the APA guidelines; because this master thesis will not have a print version, as a sign of respect for the work of all individuals behind each scientific article, all authors are listed without the shortening of the list after seven authors. The additional bibliography at the end of the master thesis is a list of works that were consulted but not used for the content of the master thesis and are offered as suggested readings.

"Quitting heroin is like hell, but it's a short hell.
Cigarettes are just always there, and you've always done it.
I just pick 'em up, and light 'em up without thinking about it."
—Keith Richards, The Rolling Stones,
in a New Musical Express' interview 2019

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Chapter 1 :
General Introduction

Preamble

In the following introduction diverse subjects were chosen in order to better understand the importance of investigating an intermittent model of nicotine addiction that more closely resembles the changing pattern of consumption in humans.

The introduction is divided into three themes. The goal of the first theme is to sensitize and offer a general sense of inclusion to the knowledge of addiction as a broad topic and then more specifically to nicotine addiction. The goal of the second theme is to fully explore the factors that help explain the pharmacological mechanisms of nicotine addiction, provide some socio-historical background that helps explain the origin of tobacco and the evolution in the consumption patterns of humans and highlight the importance of modeling these changes. The third theme covers the various preclinical models that have been used to study the development of nicotine addiction and different phenotypic aspects of this disorder. The goal of this theme, conjointly with the second theme, is to position the usefulness and necessity of the new intermittent model of nicotine addiction presented in this thesis in relation to previous models reported in the literature.

Theme 1 : What is addiction and how do we define nicotine addiction?

1.1 What is addiction (*substance use disorder*)?

1.1.1 Elements of experts' consensuses on the definition of addiction.

Although there are subtle differences on how experts define addiction, most agree on key concepts. They agree that it is a disorder marked by chronic relapse where individuals have a loss of control in their capacity to limit their intake, that they keep using a substance in spite of the adverse consequences, and that they fall into a heuristically defined three stages cycle of binge/intoxication, withdrawal/negative affect and preoccupation/anticipation (craving). They also agree that substances interact with the brain, creating neuroplastic changes, and that each phase of addiction has underlying neurobiological mechanisms driving the effects (Camí, & Faré, 2003; Koob & Volkow, 2010, 2016; Smith, 2012; Wise & Koob, 2014). This includes changes in motivation stemming from alterations to the mesolimbic dopamine pathway (APA, 2013; Bonci, Bernardi, Grillner, & Mercuri, 2003; Camí, & Faré, 2003; Di Chiara & Imperato, 1988; Koob & Volkow, 2010, 2016; Nestler, 2005; Saal, Dong, Bonci, & Malenka, 2003; Wise, & Bozarth, 1987; Wise & Koob, 2014), although different drugs have this outcome also playing out through different mechanisms (Bonci et al., 2003; Koob & Le Moal, 2006, Koob & Volkow, 2010, Saal et al., 2003).

Historically, drug "addiction" and "dependence" have been used interchangeably. Contrary to popular beliefs, addiction is not a deficit of will, instead it is conceptualized as a mental illness that works against the individual (Leshner, 1997; Meyer, 1996; Miller, & Giannini, 1990; Wallace, 1999). However, this conceptualization may have adverse effects for a drug user, specifically removing a sense of agency and responsibility over their problematic use of a drug (Wiens, & Walker, 2015). For researchers and clinicians, the term "addiction" is useful as it more clearly identifies that the disorder is both physiological and behavioural (see Wise's definition in Wise &

Bozarth, 1987 and in Wise & Koob, 2014), whereas the term "dependence" which is often confused for the same meaning, refers to only the physiological dependence (Maddux, & Desmond, 2000; O'Brien, Volkow, & Li, 2006). Hereafter, references to "addiction" and "dependence" will have these meanings throughout the text.

1.1.2 The DSM's definition of addiction.

The diagnostis of an addiction disorder is often done with the Diagnostic and Statistical Manual of Mental Disorders (DSM; APA, 2013), a reference guide often considered as the gold standard (Kawa, & Giordano, 2012). The diagnosis threshold to diagnose the substance use disorder requires the observation of 2 criteria out of 11 (These criteria are detailed in **Table 1**, DSM-5 diagnostic criteria for substance use disorders). As the criteria cumulate into the diagnosis, the person suffering from addiction is classified according to the severity index found in table 1.

1.1.3 Transition to addiction.

Many people may experiment with drugs of abuse and may use them recreationally; however, only a small percentage of them develop an addiction (Anthony et al., 1994; APA, 2013; Lopez-Quintero et al., 2011). Researchers have sought to understand the factors that contribute to this change from recreational use to a problematic pattern of use.

Some fundamental research questions have been proposed to determine how the transition into addiction occurs. Is there a specific moment, or a threshold that an individual crosses before becoming addicted; or is it a continuum through which an individual progressively becomes addicted? Experts in the field agree that: (1) Addiction is a process that, initially, often involves impulsivity and positive reinforcement and then transitions into compulsivity (Everitt, 2014; Koob, & Le Moal, 1997; Volkow, & Morales, 2015; Wise, & Koob, 2014). (2) Addiction to certain

drugs may develop faster than others (Koob, & Le Moal, 1997; Volkow, & Morales, 2015; Wise, & Koob, 2014).

DSM-5 diagnostic criteria for substance use disorders	
Impaired control over substance use criteria	
1. Take the substance in larger amounts or over a longer period than was originally intended.	
2. Persistent desire to cut down or regulate the substance use and report multiple unsuccessful efforts to decrease or discontinue use.	
3. Spend a great deal of time obtaining the substance, using the substance, or recovering from its effects.	
4. Craving is manifested by an intense desire or urge for the substance.	
Social impairment criteria	
5. Recurrent substance use results in a failure to fulfill major role obligations at work, school, or home.	
6. Continued substance use despite having persistent or recurrent social or interpersonal problems caused or exacerbated by the effects of the substance (e.g., arguments with others about tobacco use).	
7. Important social, occupational, or recreational activities may be given up or reduced because of substance use.	
Risky use of substance criteria	
8. Use of the substance in physically hazardous situation.	
9. Continued substance use despite knowledge of having persistent or recurrent physical or psychological problem(s) that are likely to have been caused or exacerbated by the substance.	
Pharmacological criteria	
10. Tolerance as shown by either :	
a. A need for markedly increased amount of the substance to achieve the desired effect.	
b. A markedly diminished effect with continued use of the same amount of the substance.	
11. Withdrawal, as shown by either :	
a. The characteristic withdrawal syndrome of the substance.	
b. The substance is taken to relieve or avoid the withdrawal symptoms.	
Severity Index	
Mild	➔ Presence of two or three of the criteria.
Moderate	➔ Presence of four to five of the criteria.
Severe	➔ Presence of six or more symptoms.

***Table I*. Diagnostic criteria and severity Index for substance use disorder in the DSM-5.**
Reference : APA (2013)

(3) Repeated exposure to the drug produces neuroplastic changes that gradually contribute to the development of addiction (Everitt, 2014; Lüscher, & Malenka, 2011; Koob, & Le Moal, 1997; Volkow, & Morales, 2015; Wise, & Koob, 2014). There is no full agreement on the stage at which a person becomes addicted. Some argue that it is once the person begins to take the drug compulsively, while others argue that it is once an individual has reached the withdrawal/negative affect stage (Koob, & Le Moal, 1997; Wise, & Koob, 2014). As will be presented in section 1.5, individual differences and vulnerabilities also influence the transition to addiction (Volkow, & Morales, 2015).

Learning and habit formation is also essential to the transition to addiction. Such learning can occur through contingencies in the environment and aberant artificial learning from the effects of the drug. The neuroadaptation produced by the drug on the brain reward system creates associations between the rewarding effects of the drug and environmental stimuli present during the drug consumption. Because of these associations, environmental stimuli can later evoke memories, emotions and motivation related to drug use (Dani, & Balfour, 2011; Everitt, 2014; Torregrossa, Corlett, & Taylor, 2011).

It was long thought that escalation in drug consumption was the main contributor of the transition to addiction and that high drug intake is necessary for the resulting neurological, psychological and behavioural changes associated with a problematic use of a drug (Ahmed, 2012; Ahmed, & Koob, 1998; Edwards and Koob, 2013; George, Koob, & Vendruscolo, 2014). However, this idea has been challenged by preclinical studies comparing intermittent (drug available for ~5min/hour for ~6h) and continuous access (drug available for ~6h) to cocaine (Kawa et al., 2019a). These preclinical studies have shown that although less cocaine is consummed in an intermittent access model compared to a continuous long access model, the intermittent access

produces more significant phenotypic symptoms of addiction. These symptoms include: greater motivation to consumme the drug (Zimmer et al., 2012, Kawa et al., 2019c, Allain et al., 2018), longer lasting motivation to consumme the drug (e.g., Bentzley et al., 2014; James et al., 2018), an escalation of intake (Kawa et al., 2016, 2018; Pitchers et al., 2017a, b; Singer et al., 2018) in addition to other factors (see Kawa et al., 2019a). Such phenotypic symptoms were also found for intermittent access to cocaine with limited numbers of infusions possible to consume, although when limited, rats did not escalate their intake (Allain et al., 2018).

1.2 How does nicotine and tobacco addiction diagnosis differ from the DSM's general substance use disorder?

1.2.1 Brief history of nicotine addiction.

The first clues of the addictive properties of tobacco can be dated back to the first contacts between Europeans and Native Americans (Gately, 2002). Francis Bacon, one of the fathers of scientific empiricism, stated the following regarding the use of tobacco:

Quote 1:

> *In our time the use of tobacco is growing greatly and conquers men with a certain secret pleasure, so that those who have once become accustomed thereto can later hardly be restrained therefrom. (Francis Bacon, in Historia vitae Mortis, 1623, citation traced by Gately, 2002)*

Despite such observations, until a few decades ago, there was no agreement that nicotine and tobacco were addictive. It was not until the publication of the Surgeon-General 1988's report (US Department of Health and Human Services, 1988) that the public became aware of the potential dangers. Although there were still debates over the addictive properties of tobacco, the evolution of scientific techniques led to accumulating evidences of its addictiveness (Stolerman, & Jarvis, 1995; Dani, Balfour, 2011).

1.2.2 Differences in diagnosis.

There are three tools which are commonly used to provide diagnostic information about nicotine and tobacco addiction (Hasin, et al., 2013; Moolchan, 2002). These are the DSM-5 (APA, 2013). The Fagerström Test for Nicotine Dependence (original test by Heatherton, Kozlowski, Frecker & Fagerström, 1991; revised test by Korte, Capron, Zvolensky, & Normand, 2013; see **Table 2**) which has been cited 5500 times according to Wiley Online Library. And lastly, the Heaviness of Smoking Index (Heatherton et al., 1989), made of two criteria from the Fagerström Test for Nicotine Dependence (items 1 and 3 of the revised version).

The DSM-5 uses the generic substance use disorder criteria to diagnose tobacco use disorder; however, the DSM-5 includes specific indications for tobacco use disorder. For instance, (1) it is rare that a great deal of time is spent seeking tobacco or spent coping with negative consequenses of smoking since tobacco products are readily available and because individuals rarely intoxicate themselve, unless they are chain smokers. (2) Giving up on important social, occupational, or recreational activities also rarely occurs since smoke-free areas are practically the only reason why this criterion might be met. (3) Unless it stems from arguments or disapproval from others, users would rarely fail to fulfill important life roles and obligations (e.g., work, family, intimate relationship). (4) The use of tobacco in dangerous ways (e.g., smoking in situations that increase the risk of fire) is not prevalent. (5) The first use of tobacco usually occurs before the age of 21. (6) Finally, it is also included in the DSM that 80% of users attempt to quit smoking, that 60% of them relapse within a week and that less than 5% of those abstaining succeed in never smoking again.

Researchers have noted that there are several behavioural differences that distinguish those with nicotine and tobacco addiction, compared to those with other substance use disorders. Hughes

(2001, 2006) suggested: (1) that people with an addiction to nicotine seldom have behavioural problems (although rare it is not impossible to lose a job or get a divorce because of smoking). For example, taking nicotine doesn't make someone violent or susceptible to commit crimes or neglect their children. Moreover, because nicotine consumption does not affect behaviour negatively; it is hypothesised that users are not compelled to consume less since their daily activities are not affected. (2) Tobacco does not produce heavy intoxication or lead to mental health problems.

Fagerström Test for Nicotine Dependence — Revised Version		
Questions	**Answers**	**Points**
1.* How many cigarettes a day do you smoke?	10 or less ☐	0
	11–20 ☐	1
	21–30 ☐	2
	31 or more ☐	3
2. Do you smoke more in the morning than the rest of the day?	Never ☐	0
	Sometime ☐	1
	Most of the time ☐	2
	Always ☐	3
3. * How soon after you wake up do you have your first cigarette?	Within 5 minutes ☐	3
	6–30 minutes ☐	2
	31–60 minutes ☐	1
	After 60 minutes ☐	0
4. Cigarette most hate to give up	First in the morning ☐	1
	All other ☐	0
5. Do you find it difficult to refrain from smoking in places where it is forbidden, for example, in church, at the library, in the cinema, etc?	Never ☐	0
	Sometimes ☐	1
	Most of the time ☐	2
	Always ☐	3
6. Do you smoke if you are so ill that you are in bed most of the day?	Never ☐	0
	Sometimes ☐	1
	Most of the time ☐	2
	Always ☐	3
	Total score	

***Table 2*. Fagerström Test for Nicotine Dependence—revised version and severity score.** Items 1 and 4 are used to constitute the Heaviness of Smoking Index. *Reference* Korte, Capron, Zvolensky, & Normand, 2013.

These differences are reflected by their absence in the DSM. (3) Users of nicotine and tobacco products do not report using them for their euphoric properties but rather as a coping strategy to

decrease stress, anxiety, depression, anger, hunger, to reward themselves, or to improve their functioning. (4) Finally, tobacco products are consummed more often in comparison to other drugs. As of 2016, according to the Center for Disease Control and Prevention (Wang et al., 2017), the majority of users smoke every day throughout the year, smoking about 14 cigarettes per day for an average 140 nicotine puffs per day (in reference to Russel & Feyerabend, 1978 stating that an average of 10 puffs are inhaled for a cigarette) and 51100 puffs per year. Hughes (2006) mentions that with that many repititions, it is not surprising that nicotine is one of the most enduring and most habit forming substance use disorders. Moreover, Russel & Feyerabend (1978) suggested that each of these puffs reach the brain faster than an injection of heroin. This point will be further discussed in theme 2, section 2.2. Evidence of the addictive nature of smoking is corroborated by a report by Anthony and colleagues (1994) showing that 32% of tobacco users develop an addiction, which is a high percentage when compared to 23% for heroin, 17% for cocaine, 15% for alcohol, 11% for other stimulant drugs, 9% for cannabis, and 5% for hallucinogens.

1.2.3 Differences in stigmatization.

Compared to other drugs, the stigmatization of smoking is more accepted, likely due to the success of anti-smoking campaigns which use smoke-free policies, fear-arousing messages about the health consequences of tobacco and the 'denormalization' of smoking (Burns, 2014; Graham, 2012; Riley, Ulrich, Hamann, & Ostroff, 2017). Although the intention is not directly to stigmatize, it is an aftereffect of these campaigns (Riley, Ulrich, Hamann, & Ostroff, 2017). The effects of stigmatization may be favourable for some users, making them more likely to quit smoking; however, for other users, it may have the opposite effect (Castaldelli-Maia, Ventriglio, & Bhugra, 2016). These negative effects apply more to smokers from lower socioeconomic status (Farrimond,

& Joffe, 2006; Graham, 2012) and the societal stigmas may provoke smokers to self-stigmatize and lose their motivation to quit (Farrimond, & Joffe, 2006).

Finally, when compared to other drugs, the stigmas of tobacco smoking are less severe. Despite a general perception that people suffering from addiction are dangerous and have erratic behaviours (Crisp et al., 2000; Meltzer et al., 2013; Mannarini and Boffo, 2014), no study finds that this applies to tobacco addiction. As suggested by Hughes (2006), most will not asssociate smoking with the likelihood to commit crimes, or to misbehave. Moreover, it could be argued that less stigma is expected because tobacco is legal and widely distributed.

1.3 The impact of tobacco consumption on health and economy

Despite today's knowledge about its health impacts, tobacco smoking is still one of the leading causes of preventable diseases and deaths worldwide (Forouzanfar et al., 2015). According to the World Health Organization (WHO, 2017) smoking kills more than 7 million people and costs over 500 billion US dollars to the economy each year. A large review of smoking hazards estimates that up to a decade of an individual's life is lost due to smoking (Jha, 2013). Many medical problems are linked to smoking, including cancer, respiratory diseases, cardiovascular diseases, reproductive problems, among many other problems (National Center for Chronic Disease Prevention and Health Promotion, 2014).

1.4 General theories of addiction

Most theories of addiction come from theories of motivation. In their general sense, theories of motivation ask how animals set themselves in motion to seek what they need for survival and reproduction (Toates, 1986; Wong, 2000). In addition, humans also seek wellness (Csikszentmihalyi, 2007; Dweck, 2000; Maslow, 1943; McClelland, 1985; Murray, 1938; Ryan & Deci, 2017). The mesolimbic pathway is known to be implicated in engaging animals in

behaviours that preserve their life and the life of their offsprings (Newlin, 2002; Smith, 2012). It also drives humans to seek wellness (DeYoung, 2013; Di Domenico, & Ryan, 2017; Temnerud, 2018). Theories of addiction offer hypotheses on how an animal's motivation and its underlying neurobiological substrates get derailed into making actions without survival or reproduction goals to the point where it may cause harm to oneself or others.

1.4.1 Negative and positive reinforcement theories.

Positive reinforcement theory.

The dominating views on addiction prior to the positive reinforcement theory were physical dependence theories (Wise & Bozarth, 1987, Stewart and Wise, 1992). The main tenet of these theories is that the physical need for a drug drives the desire to continue drug taking. Such an idea mostly stemmed from Clark Hull's Drive Reduction Theory of motivation (1943). According to this theory, the intensity of the motivation to fulfill a specific need is proportional to the amount of time spent without attending to that need. In Hull's theory, when an animal engages in a behaviour that alleviates a deprived state, it forms a habit that strengthens over time, so that in subsequent situations where the animal feels that deprived state it will be more likely to exhibit that same behaviour.

The drive and dependence theories cannot account for the fact that although an animal deprived of water should be more energized to drink, it does so only if environmental stimuli signal that water is available (Campbell, 1960). Some findings on sexual behaviours also pose a challenge. First, although many individuals choose not to engage in sex for prolonged periods of time, longer intervals between sexual interactions does not necessarily increase sexual drive (Toates, 1986). Second, instead of reducing sexual drive, sexual stimulation increases sexual desire (Toates, 1986). Finally, findings about the neurobiological substrates of male sexual drive also pose a challenge; lesions of the medial preoptic area have shown to completely supress the sexual

drive of male rats as they ceased to copulate. Proponents of the drive theory associate need states with negative affects; thus, if activity in the medial preoptic area produces the need to copulate, it should also produce a bad feeling when stimulated. However, stimulation of the medial preoptic area facilitates copulatory behaviour in male rats and animals self-stimulate to trigger brain stimulations in that area (Malsbury, 1971).

Because of such opposing ideas and because of the challenges to the dependence theories, a positive reinforcement theory was proposed (Wise, & Bozarth, 1987, Stewart, & Wise, 1992). The "reinforcement" of a behaviour (*response*) is measured as an increase in the likelihood of the occurrence of that behaviour associated with a specific outcome. The "positive" in positive reinforcerment means that a pleasing (*appetitive/rewarding*) outcome occurred as a result of the behaviour. Because of the satisfying aspect of that outcome, the association with the behaviour is reinforced and the animal will be more likely to repeat that behaviour and will do so more often (Domjan, 2015).

Building on these ideas, Wise & Bozarth (1987) proposed a theory that could offer explanations to why some drugs are taken compulsively. Their psychomotor stimulant theory of addiction aimed to (1) provide a theory that found a *commonality* between all addictive drugs despite the diversity in drugs effects (e.g., depressant, excitatory, hallucinogen) to help find which drugs have addictive properties according to that commonality. (2) They aimed to compensate for the flaws of the previous dependence theories that only relied on explaining addiction by the physiological need for the drug.

Three elements of this theory provide testable hypotheses particularly relevant to this thesis. (1) All addictive drugs increase psychomotor activity. (2) Addictive drugs all produce changes in the mesolimbic dopamine pathway, which include the ventral tegmental area, the nucleus

accumbens, the amygdala, and the hippocampus (Stott, & Ang, 2013). (3) Finally, the biological effects of addictive drugs produces a compulsive desire to use them by the mechanisms of positive reinforcement.

In summary, the positive reinforcement theory offers the compelling idea that addictive drugs have a common biological mechanism, and proponents of this theory consider euphoria and positive affects produced by a drug as the key contributing factors to the development of addiction (Bijerot, 1980; McAuliffe, & Gordon, 1974; Stewart, de Wit, & Eikelboom, 1984). Nevertheless, proponents of the positive reinforcement theory do not consider addiction as being solely driven by positive reinforcements—negative reinforcements also contribute at a later stage and impact the severity and symptoms of addiction (Edward, 2016; Feltenstein, See, & Fuchs, 2020; Wise, & Koob, 2014).

Negative reinforcement and the allostatic theory.

In 1973, Solomon and Corbit published an *opponent process* theory of motivation that offered testable hypotheses based on a homeostatic mechanism of motivation and emotional regulation. In their approach, motivational and emotional phenomenons are governed by two brain processes that can be implicated in the motivation of drug consumption (**Fig. 1**). The A process—in the case of drug taking is the initial pleasurable effects of the drug—will reinforce drug-taking behaviours through Pavlovian conditioning. The brain, to maintain an homeostatic level of functioning will activate a B process, returning the brain back to a normal functioning level. Through multiple uses of the drug, the B process will be reinforced and last longer–this is equivalent to the development of drug tolerance; larger doses of the drug are required to produce the same pleasurable state that was initially induced due to the prolonged depressed states driven by the B process. This depressed state is aversive and associated with withdrawal symptoms. When sufficiently intense, this

negative state motivates the drug user to consume more to regain the positive effects of the A process and to reach homeostasis again.

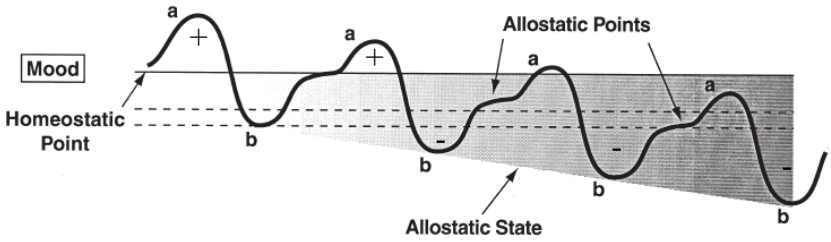

Figure 1. **Opponent process model of motivation.** Adapted from Koob and Le Moal (2001). Open access articles from Springer Nature are licensed under creative common (CC 2.0)[1] ©.

Solomon and Corbit (1973) posit that returning to homeostasis creates Pavlovian associations and reinforces drug taking. This is reminiscent of Hull's (1943) assumptions that fulfilling a need associated with a negative state of deprivation reinforces the behaviour that provided for this need. Solomon and Corbit (1973) also state that neutral stimuli associated with the drug effects (the A process) become conditioned stimuli capable of eliciting the behaviour that produced the pleasurable effects of the drug. This theory incorporates the concepts in the positive reinforcement theory and combine them with a B process that becomes increasingly important as drug intake progresses.

Koob and Le Moal (1997) took the opponent process theory (Solomon, &Corbit, 1973) further by adding a *spiralling* progression component to the theory. They described the progression to addiction as a three-stage cycle consisting of a *binge/intoxication* stage, a *withdrawal/negative affect* stage, and a *preoccupation/anticipation* ("craving") stage. An individual first experiences the pleasurable effects of the drug and return to drug use if more pleasurable effects are desired. What differentiates a recreational use from a maladaptive use of the drug is the appearance of withdrawal symptoms. These withdrawal symptoms represent the opponent process described in the theory and oppose the pleasurable effects of the drug. For example, a psychostimulant drug

[1] For details on Springer Nature's copyright permissions, see "Open access publication (gold open access)" https://www.nature.com/npp/authors-and-referees/authors/

such as cocaine, in this second stage, comes with negative effects that produce an amotivational state. Because these withdrawal states are unpleasant, individuals seek the drug to counter the negative effects. By alleviating this unpleasant state, drug intake is strengthened by negative reinforcement (the behaviour is reinforced because it removes negative outcomes, Domjan, 2015). In Koob and Le Moal's theory (1997) negative reinforcement is the key to addiction.

As with dependence theories, there are some challenges to this theory. For instance, Stewart and Wise (1992) showed that the induction of negative effects of drugs do not make animals more likely to self-administer that same drug. More specifically, animals habituated to self-inject opioids did not exhibit more addictive behaviours when injected with naltrexone (an opioid antagonist). Another challenge to the theory is that animals can lever press for opioid injections without signs of physical dependence (Bozarth, & Wise, 1984). Furthermore, the likelihood to relapse remains high long after the withdrawal effects have ceased (Wise, & Koob, 2014). Self-reports from cocaine users also challenge this theory—their cravings often increase after using amounts that only produce pleasurable effects (Mahoney III, Kalechstein, De La Garza II, & Newton, 2007).

1.4.2 Incentive sensitization theory.

During the 1970s the drive and reinforcement-learning theories of motivation (i.e., Spencer, Thorndike, Hull, Skinner) began to be challenged by a collection of compelling evidences (Anselme, & Robinson, 2018; Berridge, 2000). The theories could not always account for the results (e.g., *avoidance learning*, Bolles, 1972a), and sometimes produced unexpected results such as the reinforcement of a different behaviour than the one being trained (e.g., *misbehavior*, Breland & Breland, 1961; *Autoshaping*, Brown & Jenkins, 1968; *supersitious behavior*, Staddon and Simmelhag, 1961; *polydipsia*, Falk, 1969). The particular details related to these challenges are

beyond the scope of this thesis, but an interested reader can refer to the articles of Bolles (1972b), Bindra (1978) and Toates (1986).

As the drive and reinforcement-learning theories were challenged, a new approach—the incentive motivation theory—was proposed by Robert Bolles (1972b), Dalbir Bindra (1978) and later by Frederick Toates (1986). Bolles (1972b) shifted the focus toward incentive cues (e.g., a bell sound) that increase motivation and create the expectancy of a stimulus (e.g., food) associated with the alleviation of an internal drive (e.g., hunger) (Anselme, & Robinson, 2018; Berridge, 2000). Bindra (1978) is considered the father of the incentive motivation theory (Anselme, & Robinson, 2018; Berridge, 2000; Toates, 1986), mainly due to his extensive early research (Bindra, 1968, 1969, 1974, 1976, 1978). He furthered the incentive theory of motivation by adding that incentive cues did not only produce expectancy but also became perceived as stimuli that satisfy a *central motivational state*. He advocates the importance of the relationship between internal motivation systems (e.g., hunger, thirst, etc) and external incentives. Further improvements to the incentive motivation theory were advanced by Toates (1986). He noted that in Bindra's model the presentation of an incentive cue always elicits a behaviour. In contrast, Toates thought internal states can modulate the value of external incentives and not always trigger a behavioural outcome. Toates supports his argument by building on Michel Cabanac's work (1979a, 1979b) on thermoregulation showing that a source of heat has a positive value to an individual if his body temperature is cold and a negative value if hot. Secondly, Toates claimed that internal states could be triggered by incentive cues without underlying physiological needs, an observation made by Weingarten (1983) in a study where cues associated with food could reinstate feeding even in sated rats. Finally, Toates also presented arguments that the motivation of behaviour requires both a

drive and incentives, and that behaviour itself is intrinsically rewarding (Toates, 1986; Berridge, 2000).

The application of the incentive motivation theory to drug addiction was first proposed by Stewart, de Wit, and Eikelboom (1984) and then expanded by Berridge and Robinson (1993) in what became known as the incentive sensitization theory of addiction. While their theory brings a new model of drug addiction, it also builds on the Bolles-Bindra-Toates incentive motivation theory with three significant additions. Berridge and Robinson (1993) distinguish wanting and liking as two different processes. They also posit that *liking* and *wanting* operate under different neurobiological processes. Finally, they add an "incentive sensitization" part to their model, whereby a positive feedback loop would perpetually increase wanting with each additional drug intake. The Bolles-Bindra-Toates incentive motivation theory and the addition by Berridge and Robinson (1993) offer explanations for the intense craving (wanting) of drug users, the pattern of chronic relapse despite efforts to abstain and the duality of an increase in wanting even as the liking wears off.

Berridge and Robinson (1993) state that sensitization to a drug occurs both at the neurobiological and behavioural levels. Behaviourally, the sensitization is manifest by increased responses to the cues associated with the drug and to the drug itself. Moreover, the drug and its associated cues become more salient in the environment, thus reducing the attention and motivation to go for non-drug incentives also present in the environment. At the neurobiological level, the mesolimbic dopamine system, implicated in the guidance to respond to the cues associated with the drug and the drug itself, is also sensitized. With repeating drug intake, more dopamine is released in response to the same amount of drug (e.g., Kalivas & Stewart, 1991) and incentive cues related to the drug also become increasingly efficient at triggering dopamine

release. These effects of the increased value of incentive cues on dopamine activity is also observed by Schultz (1998). In his experiments, increase in dopamine signaling first occurs at the consumption of a reward, but as a reward is repeatedly paired with a cue, an increase in dopamine activity starts appearing time-locked to that cue (Schultz, 1998).

Berridge and Robinson (1993) propose that such neuroadaptations are very long-lasting, thus offering a compelling explanation to the increased likelihood of relapse in people suffering from addiction. For instance, during abstinence, they propose that the saliency of drug-related stimuli remains sensitized. Many studies (e.g., Bossert, Marchant, Calu, & Shaham, 2013; Namba, Tomek, Olive, Beckmann, & Gipson, 2018; Shaham, Shalev, Lu, de Wit, & Stewart, 2002) since the seminal work of Grimm, Hope, Wise and Shaham (2001) have found that such sensitized neuroadaptions and behaviours *incubate* during a period of abstinence. Incubate means that the behavioural and neurological sensitization become more pronounced during abstinence, making individuals vulnerable to drug reinstatement.

In summary, Berridge and Robinson (1993) offer a compelling and different theory compared to the positive and negative reinforcement theories. The dissociation they make between wanting and liking is reminiscent of the distinction made between reinforcement and reward (White, 1989; Wise, & Rompré, 1989). Wanting is similar to a response elicited by reinforcement, while liking is similar to the rewarding and pleasurable effects of the drug. Finally, their theory is compatible with both the positive and negative reinforcement theories of addiction.

1.5 Risk factors involved in addiction with references to nicotine and tobacco addiction

1.5.1 Individual differences.

As mentioned before, not all individuals experimenting with a drug or using it recreatively develop an addiction. Two individuals may use the drug the same way, the same quantity, in the

same kind of environment and only one of them develops an addiction. In this case, individual factors are at play.

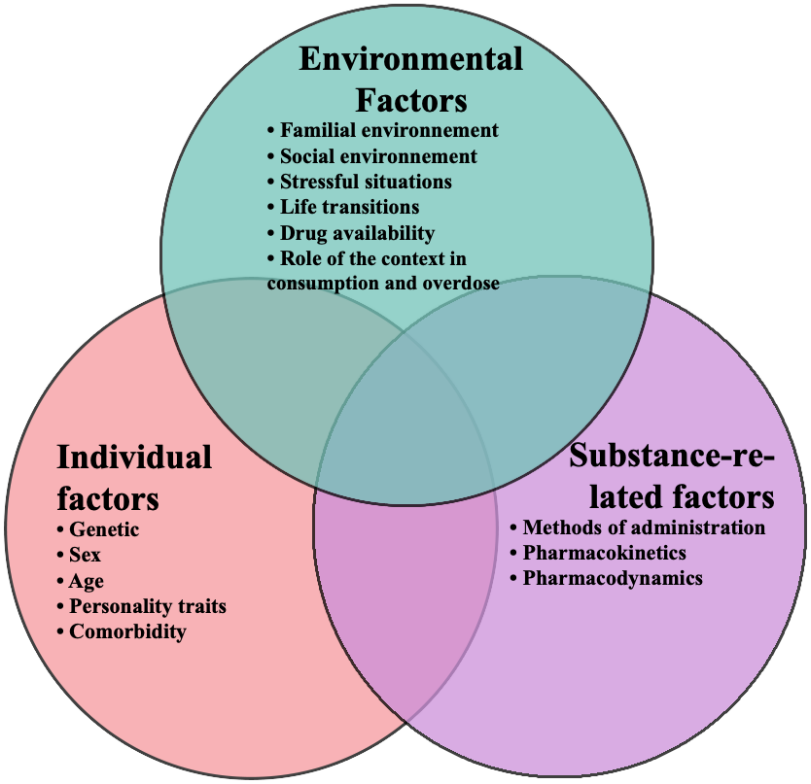

***Figure 2*. Risk factors associated with the development and maintenance of addiction.**

Genetic predisposition.

Some genetic predispositions related to addiction are well known. For instance, some genetic predispositions are drug-specific whereas other predispositions are shared by a subset of drugs (Volkow, 2012). Different stages and effects of addiction are also associated with varying genetic predispositions Also, an individual's genetics may help him or her to stay abstinent from a drug, while someone else's genetics may predispose him or her to develop the addiction faster (Koob, & Volkow, 2016).). Genetic predispositions to nicotine addiction have been reported in the literature and the exact mechanisms of such predispositions have just begun to be discovered (MacKillop, Basi, Amlung, McGeary, & Knopik, 2010). For example, the effect of genetic

variations on the CRHNA5 gene, a gene that encodes for the α5 nicotinic acetylcholine receptor subunit in the brain, has been associated with nicotine intake. The lack of expression of the α5 subunit in the habenula produces greater intakes of nicotine in mice. More specifically, the findings suggest that the absence of the subunit does not result in a more rewardinging experience but rather, disinhibit reward leading to the intake of higher doses of nicotine (Fowler, Lu, Johnson, Marks, & Kenny, 2011).

Age.

The first exposure to substance use is more likely to have occurred during adolescence, at the onset of puberty. This likelihood drastically decreases passed the age of 20 and rarely occurs after the age of 29. For cigarette use, the peak age for the first exposure is 16 and rarely happens in adults older than 20 (Chen, & Kandel, 1995). Once an individual begins to smoke, nicotine use persists long after adolescence and at a steady rate, unlike other drugs (Chen, & Kandel, 1995).

One concern with the use of drugs in younger people is the more dramatic impact they can have on developing brains (Arain et al., 2013; Casey, Jones, & Hare, 2008; Kolb, Mychasiuk, Muhammad, Li, Frost, & Gibb, 2012; Lydon-Staley, & Geier, 2018). The desire to seek sensations and take risks such as experimenting with drugs have been associated with neurodevelopmental stages in adolescence as inferred from human neuroimaging and animal studies (Casey, Jones, & Hare, 2008). For instance, the limbic system including the basal ganglia and amygdala—heavily implicated in motivation, engagement in activities and emotions—are generally well developed by the beginning of puberty. However, the frontal lobe—associated with reasoning, planning, impulse control and inhibition—reaches maturity around 25 years old (Arain et al., 2013; Casey, Jones, & Hare, 2008). The neurodevelopmental stage during adolescence and its relations to drug use are twofold (Casey, Jones, & Hare, 2008): (1) higher activity in the basal ganglia, the amygdala and

other limbic structures increase sensation seeking and risk taking and (2) diminished activity in the frontal lobe produce a lack of inhibition that further reinforces sensation seeking and risk taking. These opposing increase and decrease activities in the limbic structure and frontal lobe might be perceived as maladaptive. However, this hightened boldness can be considered critical for adolescents to be brave and experience with their environment to develop (Casey, Jones, & Hare, 2008). But this propensity to experiment comes with a price and, therefore, policies to sensitize youth to the negative effects of drugs are very important. Especially with tobacco, adolescents are more vulnerable because smoking cigarrettes has only mild immediate negative consequences and several positive effects that can be enjoyed throughout the day (Arain et al., 2013).

Personality traits.

Different individuals may justify their drug use differently due to their personality traits. Personality traits are ways of responding to one's environment based on previous experiences in the world and cognitive schemes that may or may not reach the awareness of the individual (Ryckman, 2014). Interestingly, some personality traits such as novelty-seeking and risk-taking are well-documented and have led to findings on the underlying neurobiological substrate associated with these traits and their relationship to drug use and maintenance. As explained above in the age subsection, activity in the basal ganglia and the frontal lobe are associated with novelty-seeking and risk-taking. Although adolescence is associated with a peak in exploration and risky behaviours, this peak is not experienced by every adolescent and certainly not to the same degree in all of them. In addition, these traits continue to shape behaviours throughout life (Casey, Jones, & Hare, 2008).

Comorbidities.

Comorbidity means the simultaneous presence of two or more disorders and such a condition can result in interacting symptoms and variations in the phenotype of these disorders (Feinstein, 1970). One effect of comorbidities that include drug addiction is the concept of "self-medication" —when drugs are used to cope with difficulties and thus alleviate negative states. This is similar to the theory of Koob and LeMoal (1997) that reducing the negative effects of drug abstinence is a critical element of addiction. When individuals suffer from more than the negative mental effects caused by drug intake, these other negative effects make the individual more likely to be entwined with drug addiction. Special care must be given to these individuals since other mental disorders contribute to their use of the substance. Indeed, depression, anxiety, post-traumatic stress disorder and other mood disorders have been associated with an increase in addictive behaviours (Quello, Brady, & Sonne, 2005; Turner, Mota, Bolton, & Sareen, 2018).

Sex differences.

Since 2016, the US National Institue of Health and the Canadian Institute of Health Research strongly recommend to include females in experimental designs (CIHR, 2018). This is promising because most studies have been conducted exclusively with males up to now. However, this can be double-edged, the media and even some researchers have a tendency to exagerate the size of sex differences. Briefly stated, one of the main problem is that too many researchers treat sex differences as a *yes or no* question (i.e., is the *p* value less than .05 ?), however, the most appropriate statistical method should aim to quantify *how much* of a sex difference there might be (i.e., effects size statistics; e.g., cohen's *d*, Eta square, etc.). Hence, reporting sex differences can provide meaningful and tailored clinical practices for both sexes, but it also has the potential to aggravate sex stereotypes (Maney, 2016). Sex and gender cannot be viewed as static variables

because their phenotypes are shaped by a combination of biological, sociocultural and environmental factors. Sex differences are not hardwired and can vary tremendously across contexts and experiments (Becker, McClellan, & Clover Reed, 2017).

In regards to biological sex differences in nicotine addiction, Flores and his colleagues (2019) recently conducted a meta-analysis of the pre-clinical litterature by evaluating the effect sizes of 20 self-administration studies that included both males and females rats. Such a meta-analysis is informative as it helps untangle conflicting results, for instance, in somes studies the nicotine consumption is higher in females (Grebenstein et al., 2013; Sanchez et al., 2014; Wang et al., 2014) while some studies find the opposite (Johnson et al., 2012; 30-Levin et al., 2011) or no difference (Feltenstein, Ghee, & See, 2012; Li et al., 2012; Pittenger et al., 2016; Swalve, Smethells, & Carroll, 2016a; 2016b). In summary, the meta-analysis found that female rats generally take an average of 0.18 standard deviations more nicotine than male rats and that variables that are likely to influence such increase nicotine intake are when the session last 23 hour, when the amount of lever presses required to acquire nicotine is more than one lever press, and finally when there is a light cue that is paired with the delivery of nicotine (Flores et al., 2019). These findings are consistent with some of the previous research done. For instance, the last point shows support for the study of Chaudhri and her colleagues (2005) that shows that female rats will inject themselves more than male rats when a cue light is combined to nicotine self-administration when the dose is 0.6mg/kg/inf (and not 0.3 or 0.15/mg/kg/inf).

1.5.2 Experiences and environment.

Role of environmental stimuli and contexts.

Multiple studies support the motivational effects of environmental stimuli on nicotine addiction in humans (Balfour et al., 2000; Brody et al., 2002; Due et al., 2002; Rose, 2005; Rose,

& Levin, 1991; Shiffman, Dunbar, Kirchner, Li, Tindle, Anderson, & Scholl, 2013) and for nicotine seeking in rats (Caggiula et al., 2001; Chaudhri et al., 2005; Cohen et al., 2005; Lesage et al., 2004; Paterson et al., 2005). For instance, pre-clinical research have shown that nicotine has a "dual reinforcing effect". This means (1) that nicotine has a *primary reinforcing* value and (2) that it also has a *reinforcement-enhancing effect*. The primary reinforcing value refers to the intrinsic rewarding effect of nicotine, which is reinforcing enough to stimulate motivation, but only a low level of motivation (Donny et al., 2003; Chaudhri et al., 2005), and that it also reinforces stimuli that are contingent (i.e., occuring in a proximal time and space) to nicotine self-administration in animals. Moreover, the presence of stimuli during the acquisition phase results in faster acquisition, more nicotine intake, enhanced motivation, and more persistent self-administration then in the absence of cues (Caggiula et al., 2001; 2002; Lesage et al., 2004; Chaudhri et al., 2005; 2006, 2007, Cohen et al., 2005; Paterson et al., 2005). The reinforcement enhancing effect refers to the reinforcement of stimuli even when they are not contingent on nicotine self-adminsitration (Chaudhri et al., 2006; 2007; Palmatier et al., 2006). Altogether, these findings on the role of environmental stimuli in nicotine addiction offer a crucial explanation to reconcile the intensity of addiction to nicotine despite the lack of strong intrinsic rewarding effects as is the case with other drugs such as cocaine and amphetamine (Perry, Zbukvic, Hyun Kim, & Lawrence, 2014).

Social and familial environments.

Physical, sexual, and emotional abuse, as well as witnessing domestic violence, are associated with increased risk of developing a substance use disorder in youth. The stress produced by these situations and the intense activation of the emotion and stress systems in the brain are often hypothesized as factors responsible for such an increase. Other social factors that increase the risk of substance use include having friends who use a substance, being peer-pressured, thinking that

using a substance will increase popularity and being bullied (Whitesell, Bachand, Peel, & Brown, 2013). A large meta-analysis revealed an increased risk of smoking when exposed to parents and/or siblings that smoke (Leonardi-Bee, Jere, & Britton, 2011). Another study had similar results and also found an increased risk associated with lower socioeconomic status (Tjora, Hetland, Aarø, & Øverland, 2011). In contrast, positive parent-child relationships are a protective factor.

Stress and life transitions.

As discussed in the previous section, stress can influence the risk of substance use. But stress can also influence the risk of relapse (Mantsch, Baker, Funk, D Lê, & Shaham, 2016). For instance, stress is associated with smoking relapse (Woodcock, Stanley, Diwadkar, Khatib, & Greenwald, 2019) and unsuccessful attempts to quit smoking (Kim, Chae, Park, Park, Park, & Jang, 2019). Moreover, after such failures, the stress linked to smoking cessation increases and enhances the difficulty in remaining abstinent (Skrubbeltrang Skov-Ettrup, Kejskov Egan, Dalum, & Schurmann Tolstrup, 2017). Furthermore, life transitions such as moving from adolescence to emerging adulthood with parenting responsibilities (Oesterle, Hawkins, & Hill, 2011) and breakups during emerging adulthood (Fleming, White, Oesterle, Haggerty, & Catalano, 2010) are associated with increased drinking and cigarette use.

1.5.3 Substance characteristics.

The characteristics of a substance also influences the risk of developing addiction. The effect of the drug is dependent on the pharmacokinetics—i.e., how the body interacts with it: (1) absorption, (2) distribution, (3) metabolism and (4) elimination of the drug (Hollinger, 2003). Each of these aspects influences the development of addiction for a given drug (Farré, & Camí, 1991). As this is a central topic to the present memoire, the detailed pharmacokinetics influences of nicotine to the developpement of nicotine and tobacco addiction are presented in section 2.2.

Theme 2 : Epistemology of nicotine and tobacco: What contributes to the development of nicotine and tobacco addiction and why is it so hard to quit?

2.1 Background on nicotine

2.1.1 Where does nicotine come from?

Nicotine comes from tobacco leaves found on Nicotiana plants. There are sixty-four different nicotiana plants and the two plants that have commonly been used by humans are the Nicotiana Rustica and the Nicotiana Tabacum (**Fig. 3**). The most consumed today is the Nicotiana Tabacum. The tobacco plants originated in the American continents and was discovered by humans over 18 000 years ago. Tobacco has insecticide properties and was used to protect corn and fruit trees, making it aversive the first times it is consumed. South Americans killed lice and other parasites by applying tobacco juice on their skin and treated their wounds and toothaches because of its analgesic and antiseptic properties.

***Figure 3*. Nicotiana Tabacum (left image) and Nicotiana Rustica (right image).** © Public domain: from Köhler, 1898. The Nicotiana plants are from the solanaceae family which is a family of nightshade plants that grow flowers. Both plants can grow from 1 to 2 meters.

Figure 4. A Mayan vase representing a spider monkey, likely depicting a theological figure. © Public domain.

One specific medical use of tobacco was to treat toothaches (Gately, 2002). The first civilisation that created historical artefacts representing tobacco were the Mayans, a civilisation that lived in Central America. The Mayans smoked tobacco. For them it was a source of pleasure, a medicine and a central element of rituals and religious practices. They often manifested their appreciation of tobacco by depicting their gods and other characters consuming tobacco (**Fig. 4**). Tobacco was also consumed as "snuff", a powdered form of tobacco that was dried, toasted and stored in white-flowered gourds or other bottle gourds. The name tobacco likely reflects the way European understood and wrote the word pronounced by the first nation of the Caribbean (Gately, 2002).

Figure 5. "Comme les Ameriques font feu". From André Thevet, "Les singularitez da la France Antarctique" (Paris, 1558), fol. 101 recto. The person represented in the middle of the image is an American native smoking tobacco through a pipe at the time where the European where discovering America. The image royalty is under public domain.

When Jean Nicot de Villemain brought back tobacco to Europe and heralded its medical properties, the plant gradually became known as a derivative of his name (i.e., *Nicotiana Tabaccum*) and eventually, as the alkaloid addictive chemical, nicotine (Dani, & Balfour, 2011). When

Europeans arrived on the American continent, the First Nations offered tobacco as a gift (Castaldelli-Maia, Ventriglio, & Bhugra, 2016). Rapidly, tobacco became an item of trade between Europeans and Native Americans. It was customary for Europeans to witness First Nations smoking tobacco (**Fig. 5**) and the colonialists rapidly adopted this habit. Interestingly, the use of many plants coming from First Nations was prohibited during the Spanish Inquisition; however, because tobacco was highly profitable it remained on the market (Gately, 2002).

2.1.2 Tobacco laws and changes in tobacco consummatory behaviour.

Statistics on smoking.

Several important events contributed to the decline of smoking in USA and Canada. The first most influential event was the 1964 Surgeon General's Report that claimed that tobacco causes cancer (Burns, 2014; Dani, & Balfour, 2011). It was then followed by the Federal Cigarette Labeling and Advertising Act in 1970 that forced companies to put health warnings on every pack of cigarettes. That same year, the USA government adopted the Public Health Smoking Act, forbidding all cigarette commercials on television and radio. In 1988, a second Surgeon General's Report further contributed to the decline of smoking by claiming that nicotine in tobacco is addictive.

With the accumulating evidences of the negative health consequences of smoking, multiple countries began to employ policies to diminish tobacco use, such as preventing smoking in public and some homes (Castaldelli-Maia, Ventriglio, & Bhugra, 2016). Each American state independently enacted smoking bans between 2002 and 2010[2]. In Canada smoking bans were enacted by provinces and most bans were passed between 2002 and 2006[3]. Each of these legislative

[2] See the following link to get information for the ban on smoking in each USA states. http://no-smoke.org/wp-content/uploads/pdf/SummaryUSPopList.pdf

[3] See the following link to get information for the ban on smoking in each Canadian provinces. https://www150.statcan.gc.ca/n1/pub/82-003-x/2006008/article/smoking-tabac/t/4060721-eng.htm

policies contributed to the substantial declines in tobacco smoking reached in the recent years (Burn, 2014). Of the USA population, ~42% were smokers in 1965, then ~36% in 1988, and 19.3% in 2017 (**Fig. 6A).** A similar decline happened in Canada, bringing the number of smokers down to 15.8% in 2018 (**Fig. 6B–C**).

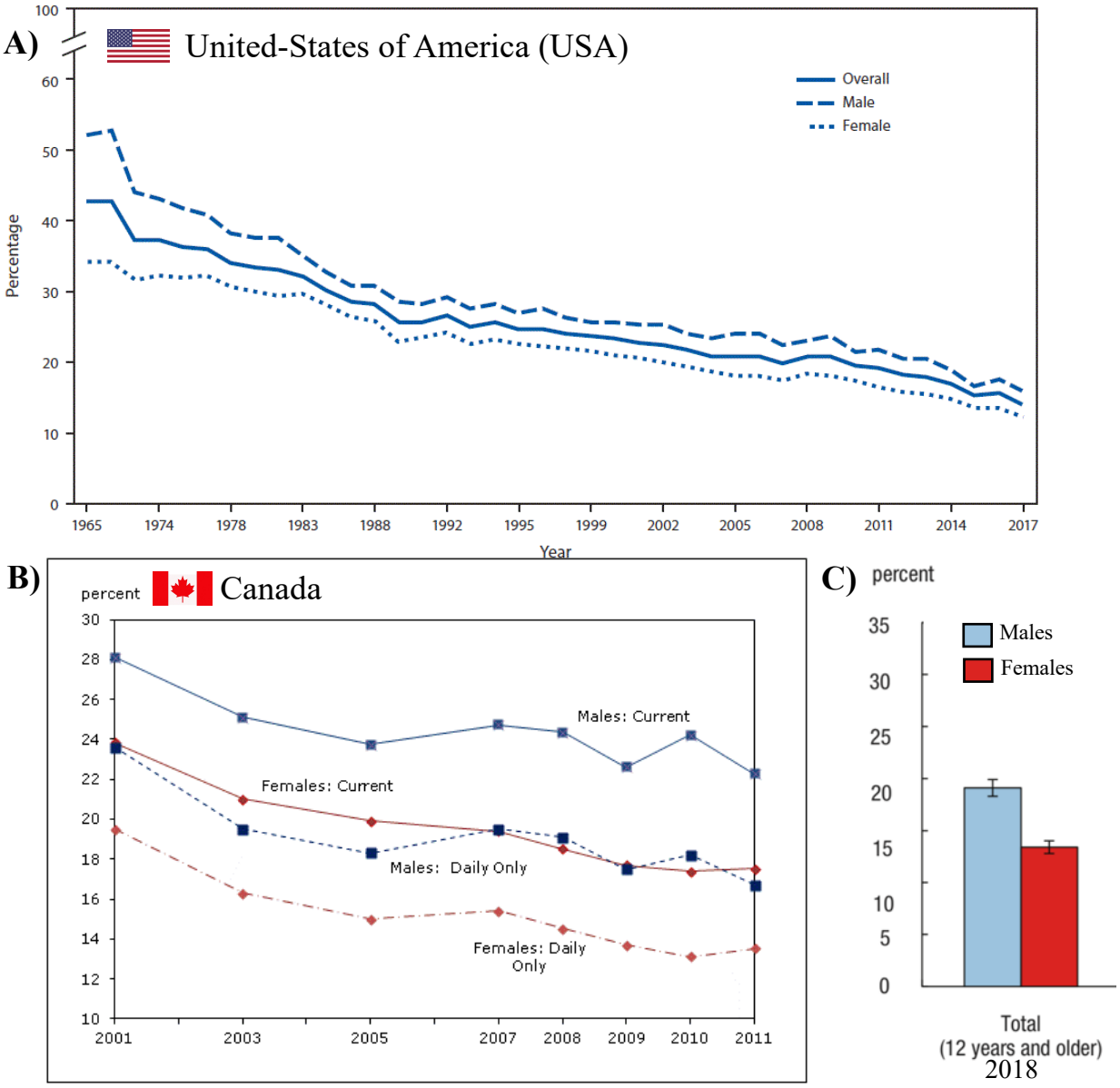

Figure 6. **Decreasing percentage of smokers**. **A)** Percentage of smoker in the USA (Wang et al., 2018). **B)** Percentage of smokers in Canada from 2001 to 2011 (Janz, & Statistics Canada, 2018). **C)** Percentage of smokers in Canada in 2018, adapted from Statistics Canada Catalogue no. 82-625-X chart 2 (2019).

The hardening hypothesis and disadvantaged groups.

Although these declines are encouraging a large proportion of the population continue to smoke in USA (over 62.5 million) and Canada (over 4.9 million). The hardening hypothesis proposes that individuals who quit smoking due to the control policies and actions taken since 1964 might correspond to a category of smokers in a favorable position that facilitated quitting. On the other side, smokers who did not stop might have a different profile that makes it harder for them to quit[4] (Hughes, 2011; National Cancer Institute, 2003).

Consistent with the hardening hypothesis is that a large proportion of those who did not quit smoking are from lower socioeconomic status (SES) and/or from disadvantaged groups (Graham, 2012; Hugues, 2011). Individuals from lower SES are less likely to access smoking cessation programs. The numerous factors underlying this lack of access are still being researched today (Murray, Bauld, Hackshaw, & McNeill, 2009; van Wijk, Landais, & Harting, 2019). Hardened smokers may need different interventions to stop smoking (Burn, 2014).

Changes in temporal patterns of consumption.

Most smoking bans prevent smoking at work, indoors in public places (e.g., restaurants, schools, universities, hospitals, bars), in public transit (e.g., taxi, bus, aircrafts) and even some homes. With the interventions prescribed by the smoking policies and the smoking bans, temporal patterns of nicotine consumption changed in two central ways. (1) With increased stigmas and negativity associated with smoking, a proportion of users switched to non-daily smoking (Castaldelli-Maia, Ventriglio, & Bhugra, 2016; Shiffman, Dunbar, Scholl, & Tindle 2012; Shiffman et al., 2014). The non-daily smokers pose a challenge to conventional theories of nicotine

[4] A full report on the hardening hypothesis is available in the 15th monograph of the National Cancer Institute (2003). https://cancercontrol.cancer.gov/brp/tcrb/monographs/15/index.html

addiction as they do not consume in a way to maintain a homeostatic, steady plasma nicotine level (Benowitz, Kyut, & Jacaob, 1982a; Russel, 1978) compared to daily smokers (i.e., higher amount

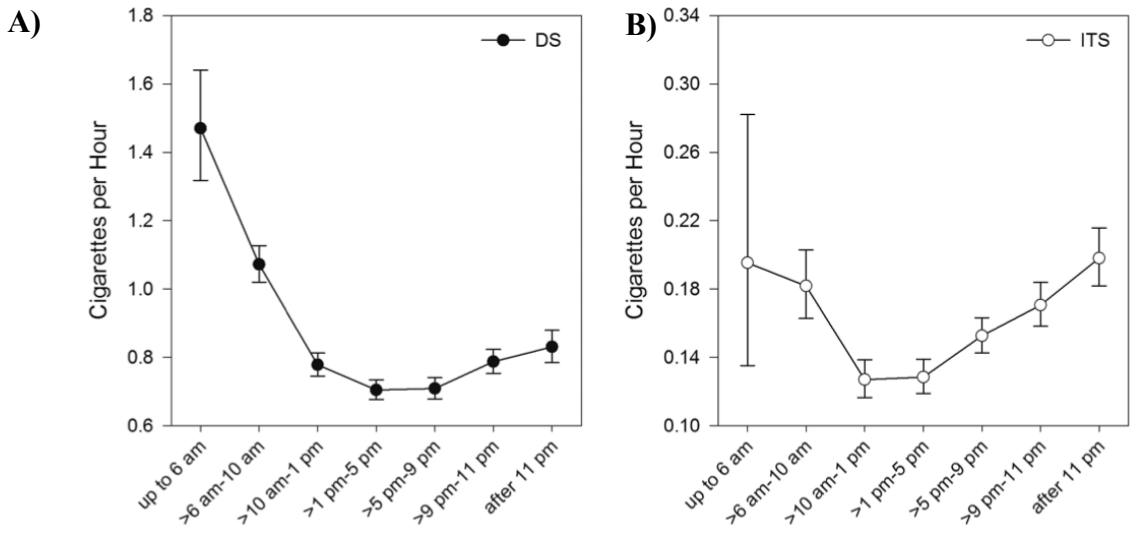

Figure 7. **Cigarette consumption by time of day for A) daily smokers (DS) and B) non-daily smokers (identified as intermittent smokers in this study; ITS).** Cigarettes consumed within each time block are averaged across all days of the week. The non-daily smokers smoked 4–27 days per month. Data represented as mean ± SEM. Figure adapted from Shiffman et al., 2014), PLOS One articles © are under creative commons (CC 2.0). See this link for details about permissions: https://journals.plos.org/plosone/s/licenses-and-copyright

during the morning and the end of the day with a dip in between; **Fig. 9**) but they have as much difficulty to quit smoking (Shiffman et al., 2014). (2) Even for those who continuous with daily smoking, restrictions on smoking areas, also dirupted their temporal pattern of consumption. Having to use an outdoor space authorised for smoking forced smoking to take place during allocated time by workplaces which limited the duration and frequency of smoking. Chandra and colleagues (2007) have compared the within-day pattern of nicotine consumption of individuals in work environments with and without smoking restrictions (**Fig. 10**). Overall, smoking restrictions

diminished smoking during work time. Importantly, Chandra and colleagues (2007) identified that different clusters of individuals had different smoking patterns throughout the day regardless of smoking restrictions in work environments. Some individuals experience small dips in their consumption within a day at work which produces small mood-related withdrawal symptoms (negative affect and restlessness), negative states that were good predictors of later cigarette consumption during the day (Chandra, Scharf, & Shiffman, 2011). Although smokers generally reduced their smoking due to smoking restrictions, they used their breaks to slightly compensate their smoking before and after work. Some individuals even quit their job to preserve their smoking pattern, a maladaptive behaviour measured by question 5 of the revised Fagerström questionnaire (see **Table 2**; Borland, Cappiello, & Owen, 1997; Chandra, Scharf, & Shiffman, 2011; Owen, & Borland, 1997).

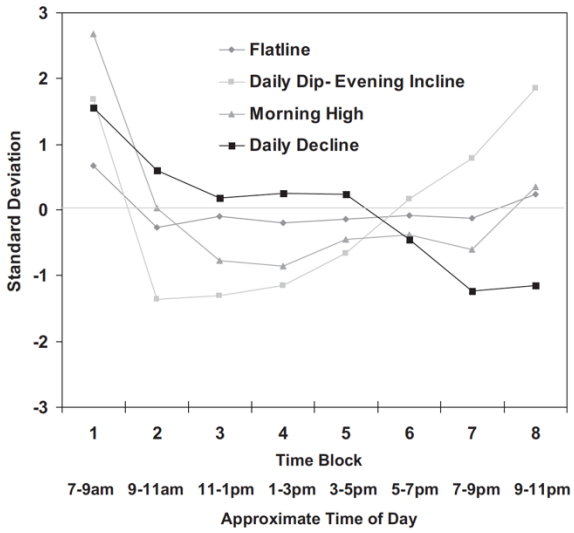

Figure 8. **Patterns of consumption in smokers where there are smoking bans.** Image from Chanda et al., 2007. The American Psychological Association approve the use of their material as long as no more than three figure/tables are used. See https://www.apa.org/about/contact/copyright/#not-required.

2.2 Phamacokinetic characteristics of nicotine

2.2.1 General information on pharmacokinetics.

The absorption of a drug is related to the amount of drug taken, the route of administration (for the pharmacokinetic profile & data of the different forms of nicotine administration, see **Table 3** & **Fig. 9** for humans, and **Table 4** & **Fig. 10** for rats), its solubility, its molecular size and the level of ionization of the drug. More significant ionization helps the drug to cross the blood-brain

barrier. Higher solubility allows a more rapid absorption and increases the bioavailability. Finally, the following last two factors were often thought of as less important in earlier studies, but are now major variables to be considered. These are: (A) the speed at which a drug reaches the brain to produce its effects, and (B) the temporal pattern of the drug intake (Allain, Minnogianis, Roberts, & Samaha, 2015). Each of these factors will be examined in detail as they motivated the development of the intermittent access model of self-administration presented in this master thesis.

Speed of action.

The speed at which the drug reaches the brain depends, in part, on the route of administration (see **Table 3** & **Fig. 9** for humans, and **Table 4** & **Fig. 10** for rats). Drugs injected intravenously have more significant absorption and more rapid effect. The effect is even faster when the drug is smoked (e.g., crack cocaine, nicotine; Farré, & Camí, 1991) as it goes from the lungs to the heart and then to the brain without having to travel from to blood to the heart to reach the lung as is the case with intravenous intake. For instance, the time until the maximal concentration (i.e., *C*max) of nicotine is reached (i.e., *t*max) when smoking is 4 to 10 minutes, whereas the time to reach maximal concentration with an intravenous injection of nicotine is between 30 to 35 minutes (see **Table 3**). The importance of such rapid effect is supported by findings from Farré and Camí (1991), in that they found that greater self-administration occurred when the effects of the drug are felt more quickly after consuming the drug. Such increase self-administration was also observed in studies using more rapid intravenous infusion of cocaine and nicotine in rats (Samaha & Robinson, 2005; Samaha, Yau, Yang, & Robinson, 2005; Allain, Minnogianis, Roberts, & Samaha, 2015). To the opposite, slow rising in plasma concentration of nicotine by nicotine patches produces less potent effects, and are therefore used to temper cravings and to help wean off from smoking tobacco (Allain, Minnogianis, Roberts, & Samaha, 2015). Because of these

motivational effects of the speed of action of different routes of administration, it is important to accurately model the speed of action in animal models of nicotine addiction.

Temporality of action.

The temporal pattern of drug intake also matters. Studies of cocaine self-administration in rats have shown that, when small amount of the drug is taken in an intermittent compared to a continuous manner, the phenotypic characteristics of addiction (e.g., psychomotor activation, increased motivation) are largely increased (Allain, Minnogianis, Roberts, & Samaha, 2015). Although there has been a rising interest in using such models, most studies examined the effects of intermittency in cocaine consumption, while no published work exists, to the best knowledge here, for nicotine consumption (Kawa et al., 2019).

2.2.2 Absorption of nicotine.

Nicotine is a weak base with a pK_a of 8.0 and is the main alkaloid of tobacco. Nicotine is absorbed as a function of its pH and the biological membrane it crosses. In most cigarette the pH is 5.5–6. When smoked, nicotine is rapidly absorbed through the lung by a dissolution to a pH of 7.4 (Benowitz, Hukkamen, & Jacob III, 2009; Woodward, & Tunstall-Pedoe, 1993). With an average of 17 cigarettes per day, a smoker absorb approximately 0.3 mg/kg^{-1}/day (Matta et al., 2006).

2.2.3 Distribution of nicotine.

After passing through the lungs, nicotine enters the bloodstream at a pH of 7.4 which makes it 69% ionized. When reaching the heart, nicotine rapidly goes to the left ventricle to the arterial circulation and then into the brain (Benowitz, Hukkamen, & Jacob III, 2009). On average the half-life for nicotine's distribution is 8 minutes (Hukkamen, Jacob III, Benowitz, 2005).

2.2.4 Metabolism, excretion and clearance of nicotine.

The liver metabolizes nicotine into six main metabolites. The most abundant, cotinine, represents up to 70 to 80% of metabolized nicotine (Matta et al., 2006). The metabolism of nicotine is influenced by several factors including meals, age, sex, chronopharmacokinetics, kidney diseases (Benowitz, Hukkamen, & Jacob III, 2009) and ethnicity (Matta et al., 2006). For example, the clearance slowers down in older people, women tend to have a faster metabolism and asians have higher nicotine metabolism because of higher levels of CYP2A6 enzymes compared to Caucasian, African American. Rats do not have the same nicotine metabolism as do human, their cytochrome P450 that metabolise nicotine is the CYP1B1/2 (Matta et al., 2006).

Nicotine is excreted through urine depending on its pH and through the kidneys. Total cleareance of nicotine from the plasma takes about 1200 minutes on average, although this number differs for chronic smokers. In average, the elimination half-life ($t_{1/2}$) for nicotine is 2 hours in humans (Russel, & Feyerabend, 1978). The terminal $t_{1/2}$ can be slower in chronic smoker reaching up to 20 hours. (Matta et al., 2006). In rats, the average $t_{1/2}$ in the brain is 52 minutes (Ghosheh, Dowskin, Li, & Crooks, 1999; but see also Schechter, & Jellinek, 1975) and 54 minutes in the plasma (Kyerematen, Taylor, de Bethizy, & Vessel, 1988).

2.2.5 The bolus and titration theories of nicotine addiction.

The two mains pharmacokinetics theories of nicotine addiction are the bolus and titration theories. Supporters of the bolus theory (Russel, & Feyerabend, 1978) state that one of the main factors that make tobacco so addictive is the fast peaking transition of nicotine from the lungs to the brain (within 5 to 10 seconds after each inhalation of tobacco smoke). Conversely, supporters of the titration theory (Ashton, Stepney, Thompson, 1979; Gritz, Baer-Weiss, & Jarvik, 1976; Stolerman, Goldfarb, Find, & Jarvik, 1973) state that smokers can titrate their consumption to their

preferred level of nicotine by the number of puffs, the strength of inhalation, and the number of cigarettes smoked, as a function of the "strength" (i.e., the tar yield; light/regular) of the cigarette. As such, smokers have a level of control that makes the self-administration more reinforcing and more likely to develop into an addiction (Ashton et al., 1979; Benowitz, Hukkamen, & Jacob III, 2009; Stolerman et al., 1973; Gritz et al., 1976; Woodward, & Tunstall-Pedoe, 1993).

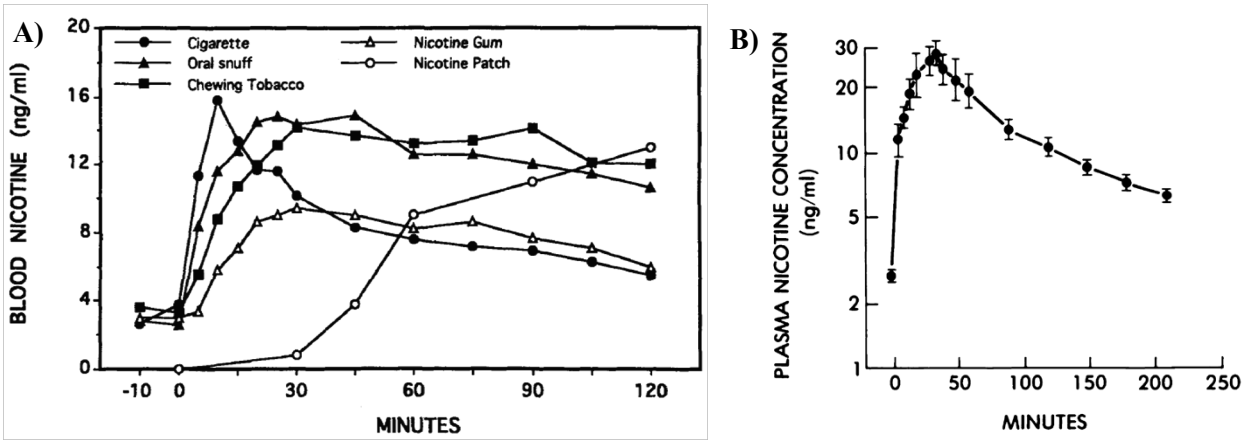

Figure 9. **Pharmacokinetic profile of plasma nicotine levels in humans as a function of the forms of drug administration. A)** The cigarettes smoked were the usual brands of cigarettes smoked by the participants (US Federal Trade Commission yields: tar average 18.0 mg [range 11.7–23.1]; nicotine average 1.1 mg [range 0.8–1.31] and were smoked for 9 minutes, one puff every 45 seconds for a total of 12 puffs. The oral snuff (2.5g) was a typical dose reported by the participants. The chewing tobacco (average 7.9g) was chewed for 30 minutes. The nicotine gums were two 2mg pieces of Nicorette®, chewed for 30 minutes. Source: Original data from Benowitz, Porchet, Sheiner, & Jacob III (1988), and nicotine patch data from Benowitz, Chan, Denaro, & Jacob III (1991), permission to reuse the figures were obtained from the Clinical Pharmacology & Therapeutics journal. **B)** An intravenous injection of 2µg/kg/min nicotine for 30 min, from Benowitz, Jacob III, Jones, & Rosenberg, (1982b).

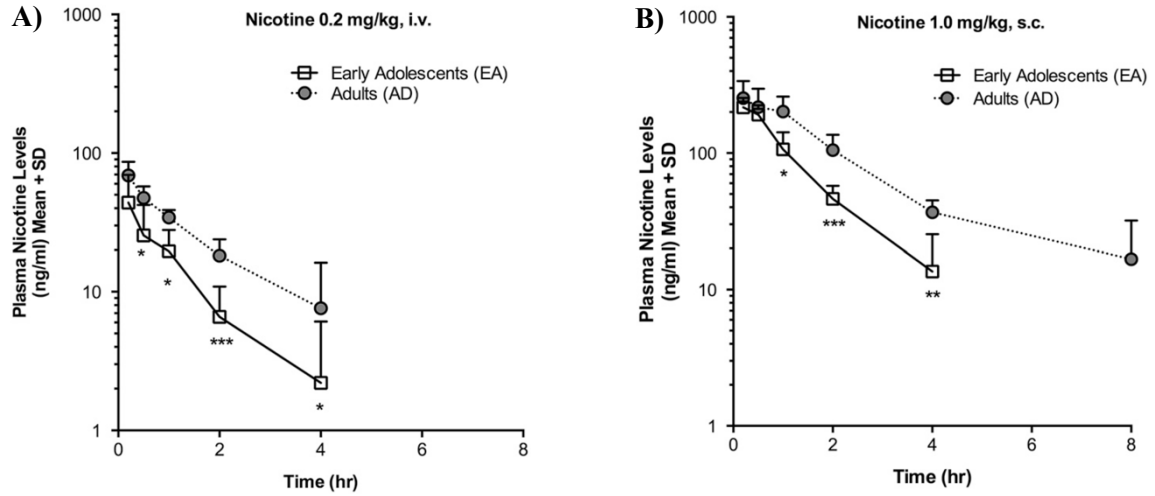

Figure 10. **Pharmacokinetic profile of plasma and brain nicotine levels in rats as a function of the forms of drug administration. A)** Intravenous injection of 0.2mg/kg nicotine in early adolescent and adult rats. (2014). **B)** Subcutaneous injection of 1.0 mg/kg nicotine in early adolescent and adult rats. For both graph A) and B), since the rats were decapitated at 2 hours to measure brain nicotine pharmacokinetics, the pharmacokinetic data at 4h were taken from the trunk of the animal. Source for both graphs: Craig and colleagues (2014), permission to reuse the figures were obtained from the American Society for Pharmacology and Experimental Therapeutics.

Table 3.
Nicotine pharmacokinetics in humans plasma through different forms of administration

	$AUC_{0-\infty}$ (ng/min/ml)	CL_T (ml/min)	$t_{1/2}$ (min)	t_{max} (min)	C_{max} (ng/ml)	*F*(%)	VD (L)	Ke	References
Intravenous nicotine									
0.5ng/kg/min over 30 min	N/A	1085 (282)	157 (79)	~30	~9	100	202 (57)	0.0231	Benowitz, & Jacob III, 1993
1ng/kg/min over 30 min	N/A	1277 (444)	98 (33)	~35	N/A	100	176 (72)	0.0198	Benowitz et al., 1982b
1.5ng/kg/min over 30 min	N/A	1304 (315)	142 (58)	~35	N/A	100	186 (41)	0.0198	Benowitz et al., 1982b
2ng/kg/min over 30 min	N/A	1291 (178)	114 (33)	~30	~32	100	185 (46)	0.0231	Benowitz, et al., 1982b; 1993; 1999; Pérez-stable et al., 1998
21mg over 24 hour	290 (4)	N/A	168 (58)	1038	14 (4.07)	100	211 (42)	0.0456	Benowitz et al., 1991
0.7mg over 2 min	15.4	N/A	135	~10	14.1	100	N/A	0.0693	Johansson et al., 1991
Smoking									See also Benowitz et al., 2006
~1.5mg in 1.5–2 cigarette over 5–10 min (9–12 puffs)	543 (93)	1232 (242)	154 (64)	4–10	39.8 (13; arterial), 18.6 (6.1; veinous)	80–90	N/A	0.0045	Benowitz et al., 2000; Hukkanen et al., 2005; Lunell et al., 2000; Gourlay & Benowitz, 1997
Transdermal patch									
14mg/24h (Nicoderm®)	256 (53)	N/A	192 (60)	264	12.2 (2.9)	N/A	N/A	0.237 (0.072)	Gupta et al., 1993a; Prather et al., 1993
36mg/24h (Nicoderm®)	300 (112)	N/A	168 (54)	372 (354)	20.4 (6.6)	68	N/A	0.245 (0.084)	Gupta et al., 1993b
23.2mg/24h (Custom patch)	N/A	N/A	258	708	12.6	N/A	N/A	N/A	Benowitz, Chan, Denaro, & Jacob III, 1991
Nasal Spray									
0.5mg/0.1ml (Nicotrol)	N/A	N/A	N/A	4.7 (1.6; arterial), 24.8 (23.8; venous)	10.4 (3.8; arterial), 5.4 (3.3; veinous)	N/A	N/A	N/A	Gourblay & Benowitz, 1997
1mg/0.1ml (Custom Spray)	9.1 (4.1)	N/A	139 (51)	11.5 (11.8)	8.1 (5)	58 (17)	N/A	0.005 (0.014)	Johansson et al., 1991
Gum									
2mg	11.49 (4.22)	N/A	127.2 (84–204)*	45 (30–60)*	3–9	78	N/A	0.33 (0.2–0.5)*	Benowitz et al., 1988; Hukkamen et al., 2005; Du 2018
4mg	24.6 (7.85)	N/A	120.6 (78–246)*	45 (30–90)*	10–17	55	N/A	0.34 (0.2–0.5)*	Steven, 1994; Hukkamen et al., 2005; Du, 2018
Chewing tobacco									Benowitz et al., 1988
~7.9g over 30 min	N/A	N/A	N/A	30	11–15	N/A	N/A	N/A	Benowitz et al., 1988
Snuff tobacco									Benowitz et al., 1988
2.5g	N/A	N/A	N/A	15–50	9–18	N/A	N/A	N/A	Benowitz et al., 1988
Subcutaneous nicotine									
0.8 mg/kg/ml	N/A	0.79 (0.15)†	229 (86)	19 (7)	5.2	~100	202 (43)	0.08 (0.02)	Le Houezec et al., 1993
1.2 mg/kg/ml	N/A	0.88 (0.15)†	231 (62)	19 (5)	7.6	~100	216 (51)	0.07 (0.03)	Le Houezec et al., 1993
2.4 mg/kg/ml	N/A	0.98 (0.16)†	195 (33)	25 (9)	14.8	~100	212 (27)	0.06 (0.01)	Le Houezec et al., 1993

Note. The most representative data are presented according to the reference on the right side of the table. Data are represented as mean and the standard deviation are presented in parentheses. En-dash signifies that there is a range of means or range of standard deviation across experiments. * = This signifies that pharmacokinetic data for nicotine gum coming from the article of Du (2018) are expressed as median and the min–max range is presented in parentheses. † = Clearance for Le Houezec et al., 1993 are expressed as ml/min/kg. $AUC_{0-\infty}$ = area under curve 0 to infinite. CL_T = Total Clearance. $t_{1/2}$ = half-life. *t*max = time until maximal concentration. Cmax = maximal concentration. *F* = Bioavailability. VD = Volume of distribution. Ke = Elimination constant.

Table 4.
Nicotine pharmacokinetics in rats plasma and brain through different forms of nicotine administration

	$AUC_{0-\infty}$ (ng/min/ml)	CL_T (ml/min)	$t_{1/2}$ (min)	t_{max} (min)	C_{max} (ng/ml)	*F*(%)	VD (L)	Ke	References
Plasma									
Intravenous nicotine									
0.1mg/kg	32 (3)	N/A	54 (6)	~1–3	6 dpm/ml	100	4.7–5.7	0.0128	Kyeremathen, Taylor, deBethizy, & Vesell, 1988
0.2mg/kg	92 (12)	1.4	92	~1–10	69 (18)	100	3.2	0.0075	Craig et al., 2014
0.5mg/kg	174 (9)	N/A	66 (6)	~1–3	40 dpm/ml	100	4.7–5.7	0.0105	Kyeremathen, Taylor, deBethizy, & Vesell, 1988
1.0mg/kg	267 (29)	N/A	60 (10)	~1–3	70 dpm/ml	100	4.7–5.7	0.0115	Kyeremathen, Taylor, deBethizy, & Vesell, 1988
Subcutaneous nicotine									
1mg/kg	479 (56)	N/A	74	~5–15	253 (65)	N/A	N/A	0.0094	Craig et al., 2014
2mg/kg	103 (4.5)	N/A	63 (11.4)	12–16	122 (17.2)	N/A	N/A	0.011	Onoue, Yamamoto, Seta, & Yamada, 2011
Intraperitoneal nicotine									
2mg/kg	97	20	69.5	45	1134.8	N/A	N/A	0.0099	Xu et al., 2019
Brain									
Intravenous nicotine									
0.2mg/kg	~130(25)*	†	†	N/A	~60 (4; at 2 hour)*	83.2–88.4*	†	†	Craig et al., 2014
Subcutaneous nicotine									See also Katner et al., 2015
0.8mg/kg	N/A	N/A	52	5	2000 pmol/g of brain	N/A	N/A	0.0133	Ghosheh, Dwoskin, Li, & Crook, 1999
1mg/kg	534 (59)*	N/A	97*	N/A*	~250 (50; At 2 hour)	83.2–88.4*	N/A	0.0071*	Craig et al., 2014
Intraperitoneal nicotine									See also Schechter, & Jellinek, 1975
2mg/kg	124.6	15	97.2	45	1639.6	N/A	N/A	0.0071	Xu et al., 2019

Note. In the experiment of Kyeremathen and colleagues (1988) the term "dpm" refers to disintegrations per minute, a measure of the activity of the source of radioactivity. *= Brain nicotine pharmacokinetics following subcutaneous and intravenous injections in the experiment of Craig and colleagues (2014) were taken 2 hours after each injection. The specific reason for this delayed pharmacokinetic measurement is that rats were sacrificed just before 2 hours to collect their brain and study the brain nicotine pharmacokinetics, while the trunk was kept to keep studying its plasma pharmacokinetics, see Craig and colleagues (2014). † = See the plasma pharmacokinetic of 0.2mg/kg intravenous nicotine for a proxy value. $AUC_{0-\infty}$ = area under curve 0 to infinite. CL_T = Total Clearance. $t_{1/2}$ = half-life. *t*max = time until maximal concentration. Cmax = maximal concentration. *F* = Bioavailability. VD = Volume of distribution. Ke = Elimination constant.

Experimental evidences from Rose and colleagues (2010) oppose the bolus theory and provides important pharmacokinetic information on nicotine absorption and distribution. They have shown that nicotine reaches the brain in an average of 7 seconds, but that it takes about 3 to 5 minutes before the brain reaches its maximal value of nicotine (*Cmax*; 4.7 ± 0.5% in dependent smokers and 6.3 ± 0.7% in non-dependent smokers). Moreover, their work shows that the accumulation of nicotine is slower for dependent smokers than for non-dependent smokers. Their

experiment also indicates that a slower washout of nicotine can in part, account for this slower accumulation of brain nicotine in the lungs of dependent smokers compared to non-dependent smokers. Finally, Rose and colleagues (2010) also show that the volume of the puff of non-dependent smokers is 1.4 times smaller than dependent smokers, this is consistent with the titration theory considering that nicotine accumulates faster in non-dependent smokers.

2.3 Overview of the mains brain areas involved in nicotine addiction

2.3.1 Ventral tegmental area.

Since the early 1980s, it was well established that the ventral tegmental area (**Fig. 11**) is involved in the initiation of motivated action and in the learning process of positive reinforcement (Fields, Hjelmstad, Margolis, & Nicola, 2007). Interest for this region sparked when Olds & Milner (1954) showed electrical self-stimulation of the medial forebrain bundle in rats (Fields et al., 2007). Despite the focus on dopamine function in the ventral tegmental area, the brain region contains less than 60% of dopaminergic neurons (Margolis et al., 2006). Theses neurons project to cortical and limbic structures and are called the mesocorticolimbic pathway (Stott, & Ang, 2013). The structures receiving projections from the ventral tegmental area include the amygdala, the nucleus accumbens (ventral striatum), the prefrontal cortex, the hippocampus, the lateral hypothalamus, the enthorinal cortex, the lateral septal area, and the ventral pallidum. The ventral tegmental area also projects to the prefrontal cortex and the hippocampus (Fields et al., 2007). The ventral tegmental area receives inputs from the prefrontal cortex, the bednucleus of the stria terminalis, the lateral hypothalamus, the dorsal raphe nucleus, the pedunculopontine tegmental nucleus and the laterodorsal tegmental nucleus (Fields et al., 2007).

2.3.2 The nucleus accumbens and the dorsal striatum.

The nucleus accumbens is a ventral structure of the striatum. The majority of accumbal cells are medium spiny neurons, which are GABAergic and constitute up to 90 to 95% of the neurons in that region. The other 5–10% of cells are interneurons. There are four different types of interneurons, which include acetylcholine interneurons and three different types of GABAergic interneurons (Scofield et al., 2016).

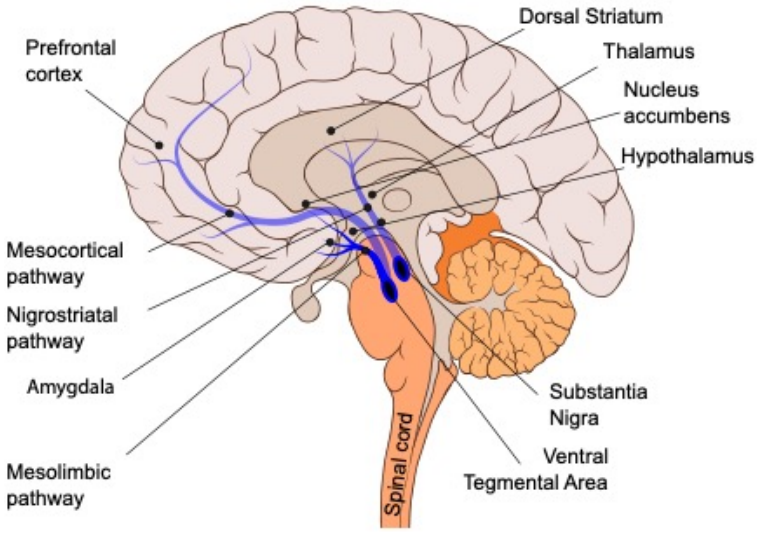

Figure 11. **Brain executive, motivational and emotional systems**. Adaptated from an image created by Patrick J. Lynch (2015), licensed under Creative Common (BY-SA 4.0).

The nucleus accumbens is further subdivided into the core and the shell. These subregions receive different afferents from other brain areas. Both receive projections from the ventral tegmental area, amygdala, hippocampus, prefrontal cortex, pallidum and the thalamus but the shell specifically receives inputs from the lateral hypothalamus and the locus coeruleus, whereas the core gets inputs from the substantia nigra. Efferent inputs from the core go to other basal ganglia nuclei, to neurons in the hypothalamus and in the ventral tegmental area. The shell projets to the hypothalamus and to the ventromedial pallidum, which further projects to the thalamus and ventral

tegmental area (Scofield et al., 2016). The accumbal projections to the basal ganglia are associated with motor outputs that support motivated actions (Floresco, 2015).

The activity of the striatum is implicated in the initiation of behaviours and in associative learning between stimuli or actions that can predict a rewarding outcome. In other words, its activity is linked to the incentive salience of reward-related cues (Floresco, 2015). Throughout associative learning between a reward (in this case nicotine) and its predictive cues, there is a shift in the region of the nucleus accumbens most engaged during incentive motivation. When first exposed to a stimulus paired with a reward, the shell is recruited, but after multiple conditioning, the core also become recruited. When an animal's response has become habitual and automatic, the dorsal striatum becomes recruited (Everitt, & Robbins, 2013; Scofield et al., 2016; Voorn, Vanderschuren, Groenewegen, Robbins, & Pennartz, 2004).

2.3.3 Amygdala.

The amygdala complex is a brain structure of the limbic system. It is made of 4 groups of substructures, which include (1) the basolateral amygdala, further divided into the lateral and basal nucleus, (2) the centro-medial amygdala, also divided into a lateral and medial part, (3) the cortical-like group, subdivided in 5 structures, (4) as well as two types of intercalated cells (Sah, Faber, Lopez de Armentia, & Power, 2003; Rudy, 2014; Janak, & Tye, 2015). The basolateral amygdala is mostly composed of glutamatergic neurons and inhibitory interneurons, while projections from the centro-medial amygdala are mostly GABAergic. The efferent inputs from the amygdala can both inhibit or excite and the structures that receive projections from the amygdala include the nucleus accumbens, the medial prefrontal cortex, the infralimbic cortex, the prelimbic area, the ventral hippocampus, the bednucleus of the stria terminalis, among other areas (see Sah et al., 2003; and Janak, & Tye, 2015). The amygdala is often associated with fear responses, but it has

many other functions, such as attributing emotional valence (i.e., good or bad, negative or positive) to environmental stimuli or internal states (Balleine, & Killcross, 2006; Janak, & Tye, 2015). It is also involved in other process including: anxiety, feeding, social behaviour (Janak, & Tye, 2015), sexuality (Everitt, 1990), appetitive conditioning, instrumental responses for reward cues (Servonnet, Giovanni, El Hage, Rompré, & Samaha, 2020), stress, and has implications in processing pain (Veinante, Yalcin, & Barrot, 2013). Some functions of the amygdala are especially relevant to addiction. The amygdala mediates associative learning between a reward and predictive cues, a necessary process for the cues to gain incentive value. For instance, basolateral amygdala lesions disrupt the acquisition of cocaine-induced conditioned place preference by impairing the assignment of incentive value (Fuchs et al., 2002). Also, AMPA glutamate receptors inactivation in the basolateral amygdala abolishes the selective excitation produced by reward-predictive cues during reward-seeking (Malvaez et al., 2012). The amygdala is also implicated in the reinstatement of drug-seeking during withdrawal. The negative affect produced by stimulation of the amygdala may lead to the desire to consume the drug to alleviate these unpleasant states (Koob & LeMoal, 2008; Zorilla, & Koob, 2019).

2.3.4 Prefrontal cortex.

The prefrontal cortex is a large brain area of the frontal lobe involved in executive and cognitive functions such as impulse control, emotion regulation, inhibition, decision making, reasoning, planning, self-awareness and working memory (Kolb, & Whishaw, 2015). The prefrontal cortex is involved in craving (Koob, & Volkow, 2016). Moreover, dysfunction of the prefrontal cortex lowers inhibition and impairs salience attribution, consequences that predispose to the reinstatement of drug seeking after a period of abstinence (Goldstein, Volkow, 2002; 2011). Furthermore, stress may weaken the prefrontal cortex network (Arnsten, 2009; 2015), with cortisol

levels rising during withdrawal (Koob, 2017), adding concerns when addicts deal with life stressors during treatment. Different drugs have different effects on the frontal lobe activity and these effects reverse when an animal experiences withdrawal. (Goldstein, Volkow, 2002; 2011).

2.4 Nicotine pharmacodynamic characteristics and influences

Nicotine, like other addictive substances, engages the mesolimbic dopamine pathway to create an exagerated and narrowing motivation to seek and consume the drug. The main motivational effect of nicotine is produced by increasing midbrain dopamine activity by recruiting dopaminergic and GABAergic neurons of the ventral tegmental area (**Fig. 12**) (Korpi et al., 2015).

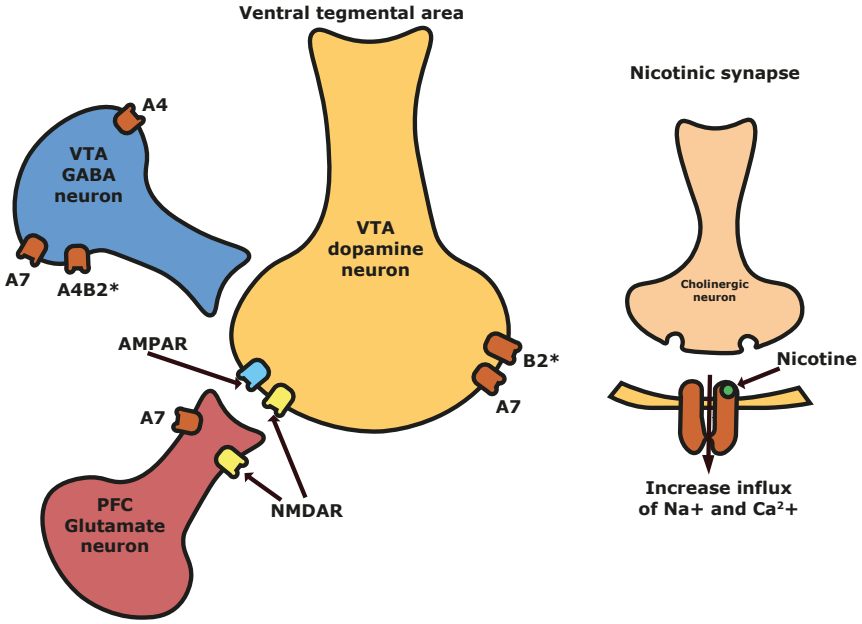

Figure 12. **Mechanisms of action involved in nicotine addiction.** The ventral tegmental area is an important area of the brain involved in motivation. Different nicotinic acetylcholine receptors are located on dopamine and GABA neurons of the ventral tegmental area. A neuron from the prefrontal cortex is shown to project to the ventral tegmental area and to send glutamate.

Nicotine acts on nicotinic acetylcholine receptors, an ionotropic receptor that lets sodium (Na^+) and calcium (Ca^{2+}) flow inside the cell and lets potassium (K^+) flow out (Dani, 2015). The nicotinic acetylcholine receptors are pentameric (5 subunits). In total, sixteen subunits have been identified and their different combinations can form either a homomeric (made of only one type

of subunit) or heteromeric nicotinic acetylcholine receptors. Only the $\alpha 7$, $\alpha 8$ and $\alpha 9$ can form homomeric pentamers (Millar & Gotti, 2009).

The diverse effects of nicotine can be accounted for by the diversity of subunits and the spread of nicotinic receptors throughout brain areas involved in motivation including the ventral and dorsal striatum, the prefrontal cortex and the ventral tegmental area. The receptor types expressed by the ventral tegmental area and substantia nigra are the $\alpha 4\beta 2$, $\alpha 4\alpha 5\beta 2$, $\alpha 3\beta 4$*, $\alpha 6\beta 2\beta 3$, $\beta 2$* and $\alpha 7$ (Korpi et al., 2015). The stars mean that the abbreviation includes pentamers with other subunits that can be added to the subunit preceding the star (e.g., $\beta 2$* is equivalent to $\beta 2$-containing nicotinic acetylcholine receptors).

The ventral tegmental area receives glutamatergic projections from the prefrontal cortex. The action of nicotine on the presynaptic $\alpha 7$ nicotinic acetylcholine receptors of these projections are thought to produce activation of NMDA receptors on dopamine cells of the ventral tegmental area (Mansvelder, & McGehee, 2000; Jones, & Wonnacott, 2004) inducing burst firing (Livingstone, & Wonnacott, 2009), a type of firing time-locked to rewards and reward predictions in the ventral tegmental area (Schultz, 1998). Glutamatergic neurotransmission and receptors are responsible for long-term potentiation, a long-lasting process that strengthens the connections between two neurons by facilitating the excitability of the post-synaptic cell through an increase in AMPA over NMDA receptors at the synapse (Park et al., 2018). The activation of $\alpha 7$ increases the AMPA/NMDA ratio (Gao, Jin, Yang, Zhang, Lukas, & Wu, 2010). It offers a mechanism by which glutamate and acetylcholine neurotransmission can interact to facilitate dopamine cell depolarization and increase activity in dopaminergic neurons of the ventral tegmental area projecting to the nucleus accumbens.

The upregulation of nicotinic acetylcholine receptors is also implicated in drug sensitization. Studies have consistently shown that of all nicotinic acetylcholine receptor subtypes, the most sensitive and thus more prone to upregulation is the $\alpha 4\beta 2$ nicotinic acetylcholine receptor (Korpi et al., 2015). Upregulation of the nicotinic acetylcholine receptors on dopamine cells in the ventral tegmental area contributes in exciting these cells and increases the likelihood that long-term potentiation at synapses with AMPA and NMDA would occur. This increase enhances the probability of dopamine firing and thus favors drug addiction (Korpi et al., 2015).

Nicotine also binds directly to $\alpha 7$ and $\beta 2^*$ nicotinic acetylcholine receptors on dopamine cell bodies (Korpi et al., 2015). This mechanism further increases the frequency of cell depolarization and increases the likelihood of creating association with other firing neurons (Hebb, 1949).

Adding to the complexity, GABAergic interneurons within the ventral tegmental area also express $\alpha 7$, $\alpha 4\beta 2$ and $\alpha 4^*$ nicotinic acetylcholine receptors. It is hypothesized that multiple nicotine intakes desensitize the $\alpha 4\beta 2$ nicotinic acetylcholine receptors, reduce activity in GABAergic interneurons which produces disinhibition of dopaminergic cells and facilitates dopamine firing (Livingstone, & Wonnacott, 2009; Korpi et al., 2015).

Theme 3 : Animal models of nicotine addiction

3.1 Why do we need animal models to study addiction and what is the self-administration model?

3.1.1 Description of the model and reasons for its widespread use.

From Thordike's puzzle box to Skinner's operant box that both formed the basis of operant conditioning theories (Domjan, 2015), the drug self-administration model provided an intuitive extension of these early experimental designs to study the effects of stimuli, contexts and drugs on animals in a controlled environment (Panlilio, & Goldberg, 2007). The first most convincing demonstration of drug self-administration in rodents comes from Weeks' study (1962). He showed rats pressing a lever to obtain intravenous injections of morphine (Katz, 2016). Until today, this paradigm has spurred an incredible amount of research. For example, just typing "drug self-administration" or "self-administration" on PubMed yield from over 200 000 to 350 000 research entries.

Initially, rats have a surgical intervention to implant a catheter that is attached to the jugular vein on one end and goes to the back of the animal on the other end. This procedure allows to connect the rats to an infusion pump with a cable. As the animal explores its environment in the operant box it eventually presses a lever that is programmed to delivers the infusion of a drug. A second lever is present and does not deliver any drug. Two elements confirm that a drug is reinforcing, firstly the animal should not ignore the levers, and secondly, the animal should press the active lever more than the inactive lever (O'Connor, Chapman, Butler, & Mead, 2011).

Two compelling reasons to use animal models of drug addiction are the rigorous control the experimenter has and the model offers the possibility to investigate many other symptoms of addiction beyond the evaluation of only the reinforcing properties. For instance, an experimenter can manipulate: the number of sessions and days of self-administration, the duration of the session,

the state of the animal (e.g., stressed or not), the genetics and neurobiology of the animals, the number of responses required to obtain the drug, the environmental cues (e.g., house light, cue light, sound, etc.) the dose of the drug, the speed of administration and the temporal frequency at which the drug is given (Allain, Minnogianis, Roberts, & Samaha, 2015; Chaudhri, Caggiula, Donny, Palmatier, Liu, & Sved, 2006; García-Pardo, Roger-Sánchez, de la Rubia Ortí and Asunción Aguilar Calpe, 2017; Kawa et al., 2019).

By manipulating these variables, it is possible to assess their effects on the development of phenotypic symptoms of addiction, such as the acquisition of self-administration, the motivation to seek the drug at various doses, the resistance to extinction, the susceptibility to relapse and the effects of different environmental or internal stimuli on the renewals of the drug-seeking behaviours during access to self-administration and during abstinence (Koob, & Volkow, 2016).

The motivation for the drug is assessed through a progressive ratio of responding. Established by Richardson and Roberts (1996), the basic idea of progressive ratio is that after each acquired reward through sufficient amount of active lever press, the subsequent lever press requires a greater amount of responding to acquire the reward, e.g., 1, 2, 4, 6, 9, 12, 15, 20, etc. The last required amount of responding that is reached by the animal is called the breakpoint. By comparing breakpoints it is possible to evaluate the influence of diverse variables on the strength of the motivation of the animal.

3.1.2 Strengths of the self-administration models and differences with non-contingent models.

The self-administration model is also reffered to as a contingent model because the animal make a behavioural response to inject the drug itself and receive the injection upon that behaviour. In contrast, when the animal gets the drug through other means than by its own behaviour it is referred as a non-contingent drug administration. By using these two differents approach, different

knowledge can be gained. For instance the comparison of different group of rats that were exposed to either contingent or non-contingent nicotine helped shape the dual-reinforcement model (Caggiula et al., 2009). In one of the critical experiments that shaped the nicotine dual-reinforcement model, Donny and his colleagues (2003) compared rats that received nicotine either through self-administration (contingent nicotine) or through a yoke protocol (non-contingent)—a protocol whereby rats receive nicotine when a rat from the other group self-administer nicotine, this ensure that they receive the same pattern and amount of nicotine injections as the contingent group. Additional control groups were included, see Donny et al. (2003). Specifically, since the yoked group received nicotine independent of their behaviour, the prediction at the time would have been that the light visual cues occuring as the lever is pressed would not be reinforced (Meisch & Lemaire, 1993). Still, to the contrary, rats pressed more on the active lever producing the visual cues in the absence of a contingent nicotine delivery, this so at a statistically significant level above contingent saline combined to visual stimuli. This led support to the hypothesis that nicotine enhances the reinforcement of other incentives. Contingent and non-contingent administration of food also helped gain valuable insights to the dual-reinforcement model of nicotine. For instance, Chaudhri and her colleagues (2006a) found that by previously increasing the value of a visual stimuli such as pairing (contingent) food to lever presses with a light would produce greater amount of lever presses for stimuli that would later be reinforced through non-contingent nicotine, whereas if food delivery was unpaired (non-contingent) the light stimuli would be less reinforced by non-contingent nicotine. Contingent and non-contignent nicotine studies also helped assess the value of non-pharmacoogical stimuli for nicotine addiction in human, as non-contingent intravenous delivery of nicotine will not satisfy a smoker and will keep them craving, unless they have at least a denicotinized cigarette that they can smoke at the time of

the non-contingent delivery of nicotine (Rose et al., 2000). Finally, contingent and non-contingent models help elucidate the neurophysiological phenomenons that underlie the pharmacological effect of a drug on the brain (non-contingent) and the phenomenon that are tied to the non-pharmacological effect of drug taking (contingent). For example, Lominac, Sacramento, Szumlinski, and Kippin (2012) compared the neurobiological effect of contingent and non-contingent amphetamine on the brain and found that the neuroadaptation that occurred with contingent amphetamine were not the same in the nucleus accumben as with non-contingent amphetamine. Specifically, greater dopamine and glutamate sensitization occurred in rats from the contingent amphetamine group in comparison to the non-contingent one.

3.2 Evolution of the parameters used in animal models to study nicotine addiction

Nicotine was not always regarded as an addictive drug, even after the Surgeon General's 1988 report asserted the addictiveness of nicotine, some disagreements persisted for years. However, thanks to the advances of the self-administration models its reinforcing properties and its capacity to produce physiological dependence have been asserted (Stolerman, & Jarvis, 1995). Later it was shown that nicotine seeking could be reinstated by the drug itself (Shaham, Adamson, Grocki, & Corrigal, 1997), by stress (Buczek, Lê, Wang, Stewart, Shaham, 1999), and by environmental cues associated with its consumption (LeSage, Burroughs, Dufek, Keyler, & Pentel, 2004; Liu, 2006). Other researcher also found that past use of nicotine impact the motivation consume it again as assessed with a progressive ratio (Donny et al., 1999) and that environmental stimuli associated with its consumption also affect future self-administration (Chaudhri et al., 2006a; 2006b).

Table 5

Overview of the different preclinical models of nicotine addiction with rat subjects

	Escalation of intake	Somatic sign of withdrawal	Motivation at progressive ratio	Cues reinstatement	Nicotine reinstatement	Stress/yohimbine reinstatement
ShA (1h/2h)	No[1, 2]	No[1]	**[3]	Yes[2]	It depends[2]	Yes[2]
LgA (6h)	No[1]	Yes[1]	N/A	N/A	N/A	N/A
LgA (23h)	No [4, 5. 6, But yes[7]	N/A	N/A	N/A	N/A	N/A
LgA (12h)	No[8]	N/A	N/A	N/A	N/A	N/A
Weekly IntA	Somewhat [9]	N/A	N/A	N/A	N/A	N/A
Daily IntA	Yes[10]	Yes[10]	*[10]	N/A	N/A	N/A
Minutes IntA	No[11]	N/A	‡	N/A	N/A	N/A
Hourly IntA	No[12]	N/A	****[12]	Yes[12]	N/A	N/A
Combined daily & hourly IntA	Yes[13] †	N/A	***[13]	Yes[13]	It depends[13]	It depends[13]

Note. Models are ordered from oldest to newest, from top to bottom. ShA = continuous short-access; LgA = continuous long-access; IntA = intermittent-access; **ShA (1h/2h) and LgA (6h)** studies include Paterson & Marksou (2004)[1]; Feltenstein et al., (2012)[2]; Donny et al., (1999)[3]. **LgA (23h)** studies include Brower et al., (2002)[4]; Fu et al., (2001)[5]; LeSage et al., (2002)[6]; Valentine et al., (1997)[7]. **LgA (12h)** is the study of Kenny, & Markou (2006)[8]. **Weekly-IntA** is the study from O'Dell, & Koob (2007)[9], which correspond to 4 days access and 3 days of no-access for 4 week. **Daily-IntA** studies include Cohen, Koob, & George (2012)[10]; O'Dell, & Koob (2007)[9]. **Minutes-IntA** is the study of Holmes et al., (2018)[11], with minutes meaning changing inter-infusion no-access range of time between 20 and 300 seconds. This study used nose-pokes instead of lever presses. **Hourly IntA** is unpublished data currently in work from Gueye, Allain and Samaha (in work, 2020)[12]. **Daily + Hourly IntA (DH-IntA)** is the present study[13], with daily meaning a day is skipped between each self-administration session and hourly meaning that every hour the rats have access for 12 minutes. || For the mean motivation measured on progressive ratio across all doses * = obtained at least the 5th injections break point, ** = obtained at least the 8th injection break point, *** = obtained at least the 12th injection break point, **** = obtained at least the 15th injection break point. † = there is an escalation of intake, but it specifically occurs in the first 3 minutes of the first access cycle. ‡ = the Holmes et al., (2018) study is very particular, the measure that slightly resembles a progressive ratio went from a fixed-ratio 1 to a fixed-ratio 5, and as the fixed-ratio increased rats selected a nicotine port—among 5 ports—that delivered the highest amount of nicotine.

3.2.1 Effects of the session duration on the likelihood of rats to show symptoms of addiction to nicotine.

Most early studies of nicotine self-administration used a limited or a short access to the drug (ShA; **Table 5**). Sessions duration tended to vary from 1 to 2 hours and were carried out 5 days per week (Paterson, & Markou, 2004). In later experiments, session durations were increased to 23 hours (LgA-23h) mostly with the intent to better mimick nicotine availability in humans (Brower, Fu, Matta, &, Sharp, 2002; Fu, Matta, Brower, & Sharp, 2001; LeSage, Keyler, Shoeman, Raphael, Collins, & Pentel, 2002; Valentine Hokanson, Matta, & Sharp, 1997, for female rats see also Cox, Goldstein, & Nelson, 1984). The animals in these 23-hour access models were housed in the operant boxes and were removed after the 23rd hour to provide them cares, to refill the drug solutions and to clean the boxes.

Nicotine self-administration models have often been compared to models of cocaine and heroin administration, as a large body of literature existed for these drugs by the end of the 1980s compared to nicotine self-administration, even if a first study can be traced back to Clark (1969) (Hanson, Ivester, & Morton, 1979). Even up to 1989, few self-administration studies using nicotine were published (Corrigal, & Coen, 1989). Without surprise, methods used for other drugs heavily influenced the perspectives taken by researchers conducting nicotine self-administration studies. One of the most enduring views borrowed from results obtained with other drugs, is the idea that the escalation of drug intake over multiple sessions is a key marker of the transition into addiction (Ahmed, & Koob, 1998) as it represents the loss of control over the intake similar to what is observed in humans. However this escalation over multiple sessions is not found during nicotine self-administration using ShA, LgA-6h and LgA-23h (Paterson, & Markou, 2004).

3.2.2 Effects of the frequency of nicotine consumption over days on the likelihood of rats to show symptoms of addiction to nicotine.

In a sustained attempt to observe intake escalation for nicotine as for other drugs, new models were developed by O'Dell and Koob (2007; Weekly-IntA) and Cohen, Koob, & George (2012; Daily-IntA). The rationale to adjust models was that not all users are daily smokers (non-daily smokers, sometimes referred as chippers; Shiffman, Kassel, Paty, Gny, & Zettler-Segal, 1994), and especially adolescents and young adults. Roughly 30–50% of them gradually increase their smoking from non-daily smoking to more regular smoking during the first four years (Doubeni, Reed, & Difranza, 2010; Kim, Fleming, & Catalano, 2009). In a first experiment O'Dell and Koob (2007) had the animals on long access sessions, 23 hours of self-administration per session over four days followed by a three days of abstinence, repeating this cycle for four weeks. They found a form of dose-dependent escalation. In a second experiment, Cohen, Koob and George (2012) gave rats a 21-hour access to self-administration followed by 24-hour without access. Their results also supported the theory of escalation through multiple sessions, but a replication study is awaited.

3.2.3. Effects of the frequency of nicotine consumption within a day on the likelihood of rats to show symptoms of addiction to nicotine.

As discussed previously, smoking restrictions have changed when and where people can smoke. The temporal pattern of consumption within a day, related to "when" people can consume their drug (i.e., intermittent or continuous), matters drastically for cocaine addiction. Currently, there is limited knowledge of how intermittent consumption of nicotine within a day affects the developpement and symptoms of nicotine addiction. Holmes and colleagues (2018) as well as Gueye, Allain and Samaha (in work, 2020) developped the first two models to investigate these effects. Both models differ substantially. The Holmes and colleagues' (2018) model is an intermittent model that uses 20 to 300 seconds of no-drug periods. During access periods the rats

nosepoked for nicotine. There were five different nose poke holes associated with various doses of nicotine. They showed that increasing the duration of no-drug intervals, rats were more likely to compensate by choosing the highest nicotine dose over other doses. One intermittent model from Samaha's laboratory (Gueye, Allain and Samaha; in preparation, 2020) consists of 12-minutes of access to nicotine self-administration every hour during each session that lasts 7 hours. The second model developped by Samaha's laboratory is presented in this thesis. The same paradigm is used (12-minutes of access, every hour, during 7-hour session) but each self-adminsitration day is interleaved with a day of abstinence. This experimental design was based on similar intermittent models conducted with cocaine self-administration (Allain, Roberts, Lévesque, & Samaha, 2017; Allain et al., 2015; Calipari, Ferris, Zimmer, Roberts, & Jones, 2013; Calipari, Ferris, Siciliano, Zimmer, & Jones, 2014; Calipari, Siciliano, Zimmer, & Jones, 2015; Gueye, Allain, & Samaha, 2015; Kawa et al., 2019; Zimmer, & Jones, 2015; Zimmer, Dobrin, & Roberts, 2011).

Goals and rationale of the study.

What most defines smoking addiction is the failure to quit despite known health risks. Smoking has decreased substantially from 1960 until now (Fig. 8; Janz, & Statistics Canada, 2018; Wang et al., 2018), mainly due to bans and policies restricting opportunities and areas allowing smoking (Shiffman et al., 2014; Barnett, Moon, Pearce, Thompson, & Twigg, 2017; CDC, 1999; U.S. Department of Health and Human Services, 2014; Wilson et al., 2012). However, many nicotine users continue to struggle, experiencing repeated failures to stop their smoking habits. The hardening hypothesis proposes that these smokers might belong to a subcategory of individuals more prone to relapse. They might be differently affected by nicotine addiction and thus have more difficulties in stopping smoking and in staying abstinent (Hughes, 2011; National Cancer Institute, 2003). A large body of literature has linked several individual differences to increase in likelihood of relapse, identifying important factors to consider in cessation smoking programs. However, other factors could interact with these individual differences and exacerbate the difficulties experienced by this subcategory of smokers.

With smoking restrictions and stigmas came important changes in the pattern of nicotine smoking: smokers cannot consumme nicotine as often as they previously did. As a consequence, several individuals transition from continuous to intermittent consumption. The effects of this pattern of nicotine consumption in humans remains unknown. Preclinical studies on patterns of cocaine use have shown that, although a lower amount of drugs is consumed in an intermittent access compared to a continuous access model, the intermittent access produces more significant phenotypic symptoms typical of addiction including greater motivation to consume the drug (Zimmer 2012, Kawa et al., 2019c, Allain et al., 2018), more lasting motivation to consume the drug (e.g., Bentzley et al., 2014; James et al., 2018) and, in some cases, escalation of drug intake.

The knowledge of the effects of intermittent consumption of nicotine within a day are minimal. Current models have sessions with continuous access to nicotine lasting for 1 to 2 hours (short-access model), ~6 hours (long-access model) (Paterson, & Markou, 2004) or 23 hours that are interleaved with a day without nicotine (daily intermittent; Cohen, Koob, & George, 2012). These models do not mimic the new day-to-day reality of humans since smoking restrictions have been enforced. Data from studies using other drugs, however, suggest that intermittent access to an addictive substance such as nicotine increase motivation to seek the drug more persistently than continuous access. These results provide a rationale to hypothesize that intermittent access to nicotine increases susceptibility to relapse. If that were true, this susceptibility, when compounded to individual predispositions to nicotine addiction, could explain why smoking cessations have been particularly challenging for a subset of individuals despite smoking restrictions and stigmas.

This thesis tested this hypothesis, in rats, using an intermittent and a continuous access model of nicotine self-administration, characterized patterns of nicotine intake in that model, measured motivation to seek nicotine using progressive ratio tests and operationalized susceptibility to relapse by examining data from extinction and reinstatement procedures. Under the hypothesis that intermittent access increases motivation to seek nicotine persistently, rats assigned to intermittent access to nicotine were expected to lever press more during progressive ratio tests, to take longer to extinguish self-administration and to lever press more in reinstatement tests.

Overall, rats having intermittent access to nicotine during the self-administration phase performed similarly to the ones with continuous access during the progressive ratio, extinction, and reinstatement tests. Nevertheless, even if there were no evidence that intermittent access produces a greater susceptibility to reinstate nicotine, the results do not exclude the possibility that intermittent access could interact with individual differences to increase susceptibility to reinstate.

Chapter 2 :
Materials and Methods

1. Animals

1.1 General procedure.

Male Wistar rats (*N* = 29, 225g–250g; Charles River Laboratories, St Constant, Canada, QC) were housed individually in polycarbonate shoebox cages (19" long x 10½' wide x 8" deep, #18780) with stainless steel wire bar lids (#20421) in a temperature (23.5ºC ± 1.5) and humidity-controlled (50% ± 10) room and under a 12-hour reverse light cycle (light off at 8h30 am). All experiments began at 9h AM, during the dark phase of the rats' circadian cycle. After three days of acclimation to the home environment rats were handled by the experimenter for 3 days to habituate them to him. One of the LgA-0.015 rats died during a reimplantation surgery before finishing his 14 self-administration sessions. The Université de Montréal ethical committee has approved all procedures involving rats, and the Canadian Council on Animal Care (CCAC) guidelines were followed.

1.2 Food restriction.

During all phases of the protocol—except for the food training and surgery—rats had an *ad libitum* access to water and were rationed to 25g/day of food (Charles River standard rat chow) given between 16h and 20h and at least 1h after finishing a session in an operant box. For the first three days at their arrival food is delivered *ad libitum*. At the 4th day after their arrival, rats began to be food rationed to 25g/day. During food training in operant boxes and the day before, rats are food rationed at 15g/day in order to increase their exploratory motivation, enabling them to more successfully activate the active lever and learn the self-deliver food pellet. The day of the surgery, the day before, and the day after, rats are food rationed to 35g/day so that they have a sufficient

diet for the catheter implantation surgical procedure. The food restriction used throughout the experiment is a moderate regiment commonly used in self-administration studies and a range of 80 to 85% of free-feeding body weight is maintained (Bongiovanni and See, 2008; Ferrario et al., 2005; Figueroa-Guzman et al., 2011; McFarland et al., 2003).

2. Surgery

2.1 Catheter implantation.

The intravenous catheters are crafted by the experimenter according to a standard protocol of the laboratory (see Samaha, Minogianis, & Nachar, 2011). The catheters ressemble the Med Associates' PHM-131 catheters, to the exception that the base of the catheters are made from the base of a cutted 200µl pipette tip and that the base, the soft surgical polypropylene mesh, the canulla and the heat shrink tubing around the Tygon® catheters' tubing are all solidly fixed together with dental cement (A-M Systems, powder #525000, solvant #52600).

The surgical procedure is performed as follows: 1) First, two incisions are made on the rats' back with a 5" staight-&-curved operating cisor (side to side direction), one on the middle of the back (~2.2 cm, large enough to pass the catheter and the surgical mesh attached to it) and a second at the bottom level of the scapula (~55 mm, just large enough to pass only the base of the catheter). 2) With a 4½" straight iris cisor a third incision (head to toes direction) ~1.5 cm long is made at the location of the right jugular vein. 3) The catheter tubing is then passed from the larger back incision to the frontal incision by passing it under the skin with a 5.5" curved hemostatic forcep. 4) The jugular vein is then located inside the animal and then isolated from the surrounding tissue with openings of the iris cisor, a surgical probe rod and a straight dressing forcep. 5) The jugular vein is gently stretched using a straight dressing forcep and the upper part of the jugular is clamped with hemostatic mosquito forceps. 6) The vein is then kept stretched by holding the clamp and a

round tip probe with one hand 7) While the vein is extended, the other hand uses a black 22 gauge (0.7mm x 32mm) needle fixed to a surgical wooden cotton swab to pierce the upper top part of the jugular vein and go through to make some space inside. 8) The Tygon® tubing of the catheter is then inserted into the jugular vein using a curved iris forcep. 9) Using two straight dressing forceps the sillicone ball of the catheter tubing that touches the newly made entrance to the vein is fixated to the vein by two 4-0 non-absorbable black silk suture threads (SP-104), one below the jugular vein, and one above, each tied two times. Each of the four hanging ends of thread (top and bottom) are tied together two times. 10) During all the tying steps and at every step until the end of the surgery, a 1ml syringe connected to the catheter is used to see if blood can be drawn (~0.01ml pull, exchanged with a ~0.01ml push of saline) to ensure the catheter is functioning correctly. 11) While carefully ensuring that the catherer curves in a way that it does not get blocked or get uncomfortable for the animal, the second sillicon ball of the catheter is fixated to the chest muscles with two 4-0 silk threads with the same threading technique described at point nine above. 12) The hemostatic mosquito forceps clamping the jugular vein is removed and the incision in the skin above the jugular vein is sutured with simple interrupted percutaneous stitches using 5-0 non-absorbable black silk suture threads (SP-115), the hemostatic mosquito forceps to hold the suture needle (15.5 mm 3/8 curve) and a straight dressing forcep. 13) The rat is then turned, belly on the table, and the catheter is slided though the skin using the hemostatic mosquito forceps to be taken out through the incision at the scapula. 14) The incision at the middle of the back of the rat is sutured the same way as point twelve, and if necessary, a stitch is also made to tigthen the incision around the catheter at the level of the scapula. 15) A laboratory-made polyethylene cap is fixated on the catheter to isolate it from the exterior when not in use. With the catheter, the rats can be

securely tethered to the infusion apparatus placed on top of the operant cage and self-administer nicotine by pressing the active lever.

2.2 Perioperative surgery care.

A few minutes before the surgery, rats are anesthetized with 5% isoflurane and maintained at 2% isoflurane during surgery. At the beginning of surgery each rat receive an intramuscular injection of 0.02ml of penicilin-G (antibiotics, Derapen, 300 mg/ml; CDMV, Saint-Hyacinthe, Canada, QC) and a 0.03ml subcutaneous injection of Rymadil (Carprofen, Zoetis). At the end of the surgery, rats are injected with 0.1 ml of 0.9% saline water through the catheter to ensure that there is no resistance in the catheter, followed by a 0.1ml mix of saline water, heparin (Sigma-Aldrich, Inc., Oakville, Canada, ON) and enrofloxacin (Baytril, 10mg/kg, CDMV, St-Hyacinthe, Canada, QC), which prevent blood clotting (the heparinized saline water mix is 1mg of heparin/5ml 0.9% saline water/1ml of enroflaxin) and flamazine (cream 1% w/w, silver sulfadiazine 10 mg/g, Smith & Nephew) a topical antibacterian cream is applied on all three incisions. After surgery, rats are given 7 days of rest before the experimental procedure begins. During this rest period, they are closely monitored to ensure that they stay healthy: surgical scars are verified so that the stitches are still holding the scar, all skin is verified so that no infections or œdemas occur, weight and behaviours of the rats are also examined. For the first two days, chlorexidine gluconate (2% w/w), a disinfectant for veterinary use, and flamazine are applied on the scars to prevent infections and help recovery. Chlorexidine is again applied at the third day, but not flamazine since too much flamazine moisturizes the skin too much and hinders the recovery.

2.3 Catheter patency follow up.

2.3.1 Catheter flushing.

Each day the rats' catheter were flushed with daily alternating 0.1ml of saline or the 0.1 heparinized saline mix. The days where rats are placed in operant boxes, their catheters were flushed with saline before entering the operant boxes and when they came out it was flushed with either saline or the heparinized saline in concordance with the daily alternance. The days where rats were not tested, they were still flushed according to the daily alternating solutions but in their home cage.

2.3.2 Propofol test.

After the 7h self-administration phase, and after the progressive ratio, 0.01 ml/kg of propofol (CDMV, St-Hyacinthe, QC)—an intravenous anesthetic used for procedural sedation (Folino, Muco, & Parks, 2020)—was injected through the catheter. A 0.1 ml injection of saline is injected through the catheter before and after the propofol injection. Common symptoms produced by injection of propofol in rats are general ataxia and diskinesia of the jaw and sometimes protrusion of the tongue. A propofol test that does not produce symptoms in the rat is indicative that the catheter is non-functional and needs to be replaced by reimplantation of a new catheter in the left jugular vein instead of the right one.

3. Apparatus

All experiments were done by placing the rats in a standard operant box (23.5"w x 22"h x 16"d; Med Associate Inc., St. Albans, VT, model MED-008-CT-B2) itself contained in a sound attenuating (0.75" wide pannels) box (25"w x 24"h x 17.5"d) equipped with a fan making background noise. Operant boxes were located in a testing room separated from the room where animals were housed and were equipped with the central light (*house light*; hooded, 100 ma, 28 V

DC, Med Associate Inc., St. Albans, VT, model ENV-215M) located in the top-back-center of the boxes. The boxes also had two retractable levers (Med Associate Inc., St. Albans, VT, model ENV-112CM), one active (on the side far from the box door) and one inactive (close to the box door). The food cup is hollowed in the wall between the two levers. A white light (*cue light*; 100 mA, Med Associate Inc., St. Albans, VT, model ENV-221M) is placed above each lever. Throughout each phase of the protocol (except for extinction and nicotine/saline induced reinstatement), the light above the active lever lit up for 25s whenever a fixed ratio was accomplished. A sound device was also equipped in the operant boxes (sonalert tone generator, 2900 Hz, 85-dB tone, model ENV-223 AM) and emitted a high pitch sound for 5s when a fixed ratio was accomplished. The cue light and the sound stimulus together form the cues (*nicotine-paired* cues) that are paired with nicotine self-adminsitration. Med-PC software (v.4, Med Associate) was used to run programs controlling the operant boxes and to collect data.

Nicotine solutions were contained in a 20-ml syringe placed in a 3.33 RPM syringe pump (flow rate 0.016 ml/h–24.122 ml/h, Med Associates Inc., St. Albans, VT, model PHM-100 single speed) and was delivered over 5 seconds upon three active lever presses in the corresponding phases of the experiment. Polyethylene tubing was used to attach the syringe to a fluid swivel (Lomir, Notre-Dame-de l'Ile-Perrot, QC) and attached to a freely moving arm (PHM-110-SAI, Med Associates Inc., St. Albans VT) to allow the rats to move freely without making tension on the tubing system. A metal spring with a polyethylene tubing inside was fixated to the fluid swivel and suspended into the cage to connect to the catheter at the back of the rats in sessions where they could self-administer nicotine.

4. Nicotine

4.1 Type of nicotine.

Nicotine bitartrate dihydrate was used ($C_{10}H_{14}N_2$ • $2C_4H_6O_6$ • 2H2O; molecular weight 498.44g/mol; distributed by MP Biomedicals). It is a "salt" composed of tartaric acid and nicotine extracted from the nicotiana tobaccum plant. Nicotine extracted from the nicotiana plant is naturally found in the form of salt, which signify that what is extracted is not purely nicotine.

4.2 Nicotine doses calculation.

Since nicotine bitartrate dihydrate is a salt, two important elements had to be considered. First, to ensure that the nicotine bitartrate dihydrate salt composite contained the desired quantity of nicotine, a calculated correction to the molecular weight has to be done. The formula with the correction for doses at the self-administration phase at fixed and progressive ratio is the following :

Table 1
***Nicotine doses calculations*.**

1	Desired nicotine dose (e.g., 0.03mg)/kg • mean weight of the group of rats in kg • (498.44/162) = X
2	X / mean volume of infusion of the operant boxes = Nicotine concentration per infusion
3	Nicotine concentration per infusion • desired nicotine volume (e.g., 500 ml of the dose 0.03mg/kg/infusion) = Desired volume of nicotine that contains the nicotine concentration per infusion.

Note. The formula for the nicotine doses calculations was retrieved from Matta et al., 2006.

For the nicotine reinstatement tests, the calculation is the same to the exception that 1ml replaces the infusion volume in the formula since rats are injected subcutaneously with 0.1ml of nicotine per 10g of their weight (i.e., desired nicotine dose/kg/ml).

4.3 Nicotine pH adjustments.

In its salt form, nicotine does not readily cross the blood-brain barrier. To bypass this, Philip Morris and other tobacco companies manipulate the ionisation of nicotine by using ammoniac (Stevenson, & Proctor, 2008). With a pH closer to the blood pH (pH 7.2–7.4), nicotine readily

crosses the blood-brain barrier and produces more significant action in the brain. For nicotine to have these same characteristics of action as those used by tobacco companies, it was ensured in this experiment that the pH of nicotine used was between 7.2 and 7.4. On average the pH of the nicotine bitartrate dihydrate used was of 3.3. Sodium hydroxide (NaOH) was used to set the pH at the physiological blood level. Nicotine solutions were renewed every 48h to ensure they kept the same capacity of action across time.

4.4 Nicotine doses.

Two doses of nicotine were selected for this experiment (0.015 and 0.03mg/kg/infusion) and were compared between the two consumption patterns (IntA and LgA). The 0.015mg/kg/infusion dose was selected, since in rats, it is a dose that is more proportional to what is used in humans (Sorge, & Clarke, 2009). The 0.03mg/kg/infusion dose was also selected since it was often used in the literature, thus allowing the possibility of comparisons (Flores, Uribe, Swalve, & O'Dell, 2019).

4.5 Speed of infusion.

The selection of a 5s speed of infusion was based on unpublished results from Gueye, Allain, & Samaha (in work, 2020) as it produced the highest persistence for the strength of responding to cue-induced reinstatement in comparison to a 90s infusion speed. When a variable increases the strength of a behaviour it is suggestive of a preference, hence a preferred speed may more closely represent the titration that would occur in human smokers. Moreover, as stated in the introduction, how fast a drug reaches the brain is an important factor as it influences the addictive potential of a substance (Allain et al., 2015). A second reason to choose the 5s speed of infusion was that compared to other infusion rate (25s and 100s) it produces greater activity in the dorsal striatum, an important brain area for motivation (Samaha, Yau, Yang, & Robinson, 2005).

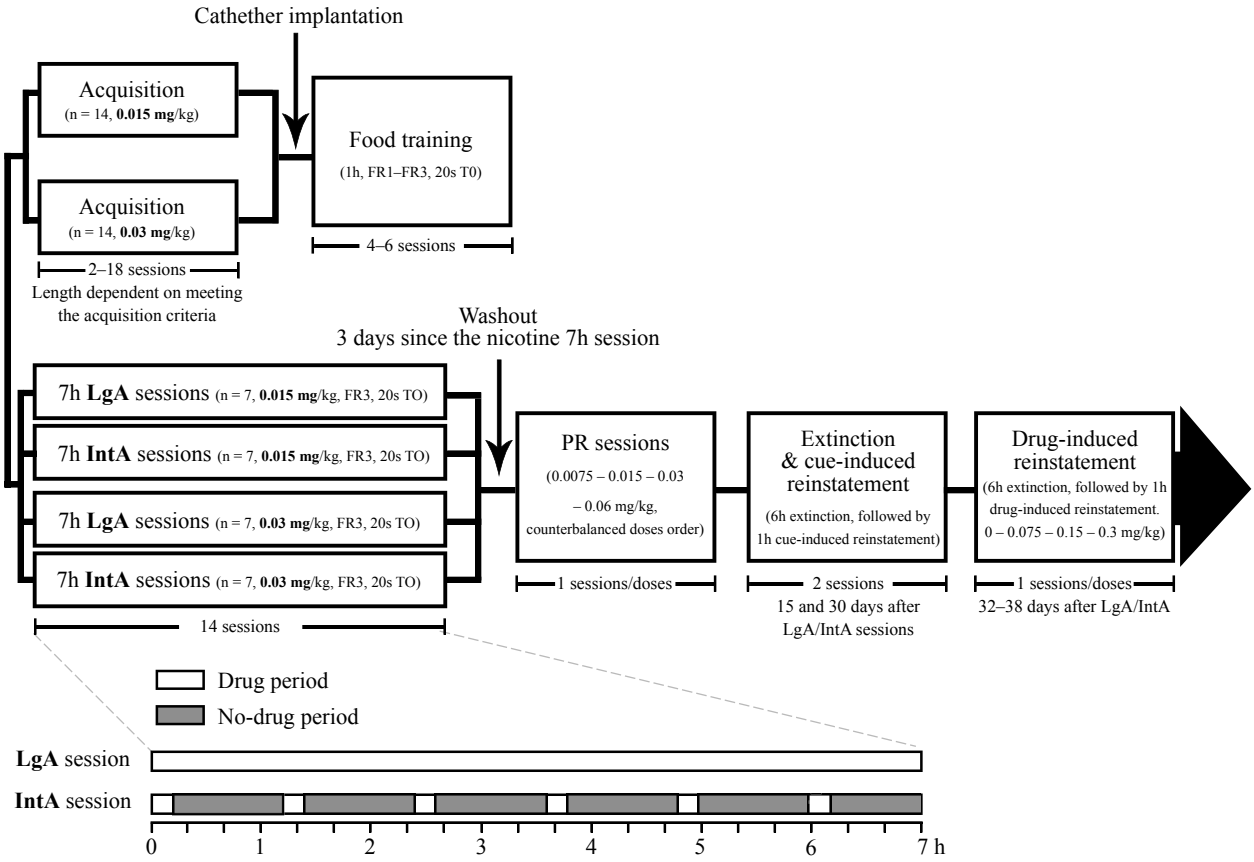

Figure 1. **Sequence of experimental events.** Rats (n = 28) were put in operant boxes to learn to make operant responses on the active lever and obtain food pellets (4–6 sessions of 1h, based on success criteria). Once acquired and with a catheter implanted, the rats were separated into two groups based on nicotine dose (0.015mg/kg/inf, n = 14; and 0.03mg/kg/inf, n = 15) and were then put in the operant boxes to acquire the nicotine self-administration behaviour by pressing the lever that was previously associated with food delivery (2–18 sessions of 1h, based on success criteria). Once nicotine self-administration behaviour was acquired rats were then separated again: each initial group based on different nicotine dose were separated into long-access sessions (LgA; 7h session, continuous access to nicotine) and intermittent-access (IntA; 7h session, 12 minutes of access to nicotine each hour), making four groups (LgA-0.015mg/kg/inf, n = 7; LgA-0.03mg/kg/inf, n = 7; IntA-0.015mg/kg/inf, n = 7; and IntA-0.03mg/kg/inf, n = 7). The four groups were then tested every 2 days for a total of 14 sessions of 7h with their respective dose and access to nicotine. Starting at the 4th day after the 14 sessions, all rats were tested every 2 days on progressive ratio sessions (1h–5h, session stop when no more infusion are obtained for 1h) and were all tested at four different doses (0.0075–0.015–0.03–0.06mg/kg/inf) for one session each. At the 15th and 30th day after the 14 nicotine self-administration sessions, the rats were put in the operant boxes for a 6h extinction session (pressing lever = no cues, no reward) immediately followed by a 1h cue-induced reinstatement test (relapse test with exposure to cue). At the 32nd, 34th, 36th, and 38th days after the 14 nicotine self-administration sessions, the rats had again 4 extinction sessions (6h) immedietely followed by a 1h nicotine-induced reinstatement test and saline-induced reinstatement test at three different doses (0.075–0.15–0.3mg/kg/ml and "0"/saline 1ml/kg) for one session each.

4.6 Calibration.

The infusion volume produced by the syringe pumps were measured and corrected to ensure that they each delivered the same mean volume. As such, the mean volume across all syringe pumps was used in the calculation of the nicotine doses concentration for the self-administration and progressive ratio phase (i.e., desired nicotine quantity in mg/kg/infusion).

5. Acquisition of food self-administration

5.1 General procedures.

Rats were placed in an operant box for 1h where they learned to self-administer banana-flavoured food pellets (45mg, grain-based, VWR, Montréal). The house light is activated during all phases of the experiment, and two retractable levers are presented. One lever is called active, as it delivers a reward—in this case, a food pellet—and the other lever is called inactive, as it produces no consequences. Both lever presses are recorded in all phases of the experiment. When a rat has pressed the required number of presses—either one time (FR1) or three times (FR3)—both levers become unavailable for 25 seconds ("time out"). When the active levers retract, a light gets activated above it for 25 seconds and for 5 seconds a slight high pitch sound (2900 Hz, 85-dB) get activated. For the food training as well as the nicotine self-administration training rats are tested every day.

5.2 Success criteria.

Rats had 2 to 3 days at FR1, then 2 to 3 days at FR3, the number of days are based on meeting the success criteria. To fill the criteria, a rat needed to do enough active lever presses to obtain 20 food pellets per day for two consecutive days (a maximum of 100 pellets per day may be obtained). If a rat did not meet the success criteria, he was not fed at the end of the day and was placed in the operant box all night with water and an unlimited quantity of food pellets that could be acquired

by pressing the active lever. The day after the success criteria were met, the rats were brought for surgery for the catheter implantation.

6. Acquisition of nicotine self-administration

6.1 General procedures.

After seven days of recovery from the catheter implantation surgery, rats were separated into two groups of different nicotine dose (0.015mg/kg/inf, n = 14; and 0.03mg/kg/inf, n = 15) and were then placed in the operant boxes so that they learned to self-administer nicotine intravenously under an FR3. The tone (5s) and light (25s) activate, and both levers retract (time out) when a successful number of responses were made on the lever. Retracting the levers for a time-out period prevents rats from injecting too much nicotine since large amount may produce aversive effects—these acquisition sessions last 1h. The rats were divided into two groups, one group had access to the 0.015mg/kg/infusion dose and the other had the 0.03mg/kg/infusion dose. The parameter for nicotine self-administration were selected to replicate and compare the results of Gueye and colleagues (in work, 2020) with the present study.

6.2 Success criteria.

The rats had to meet three success criteria to pass to the next phase. First (1), rats needed to press on the active lever for twice the number of inactive lever presses for two consecutive days. Second (2), rats needed to self-administer a minimum of 6 infusions for the two consecutive sessions. Finally (3), a regularity in the pattern of consumption was to be observed with the SoftCR software (Med Associates Inc., St. Albans, VT). The score for the first sessions was not taken into account since rats were transitioning from the food training. The minimum number of days that was allowed to meet these criteria was 18 days, no rat went over that number of days (see **Fig. 1A** in the results section for individual results).

7. Intermittent and continuous access nicotine self-administration sessions

Each of the two groups of rats (i.e., 0.015 and 0.03 mg/kg/infusion) were further divided by being put through a continuous access to nicotine (7h of unlimited access; LgA-0.015mg/kg/inf; n = 7; and LgA-0.03mg/kg/inf, n = 8) or an intermittent access to nicotine (12 minutes of access to nicotine every hour for 7h; IntA-0.015mg/kg/inf, n = 7; IntA-0.03mg/kg/inf, n = 8,) during 14 sessions of 7h each. The groups were balanced by taking into account the individual scores obtained for the two sessions meeting the acquisition of nicotine self-administration success criteria. The variables taken into account to constitute the group were the mean number of infusion and the number of days to arrive at the success criteria. In both the IntA and LgA session, the tone (5s) and light (25s) activate, and both levers retract when a successful number of responses are made on the lever (FR3). For these 7h session, rats were tested every two days, in other words, after a self-administration session, a day was skipped before the next 7h session. Following the 14 self-administration sessions, rats were left in their home cage and were handled for three days. For the IntA rats, the 1 hour time between each access cycle was determined based on nicotine's half-life in rats (52 min in the brain; Ghosheh, Dowskin, Li, & Crooks, 1999**)**. The choice of 6 access cycles was made based on how long the session would last with the no-access period, and to ensure that sufficent data would be collected with each session to desribe a pattern within session. This combined to the half-life requirement led to the selection of 7 hours sessions. The sessions length of the LgA rats was set to 7 hours so that they have the same session length as the IntA rats. Finally, the selection of the criteria follows what was done in Gueye and colleagues (in work, 2020), allowing for comparisons and replications of the results.

8. Nicotine self-administration under progressive ratio

On the 4th day after the 7h self-administration sessions, the rats' motivation to work and obtain nicotine was tested during progressive ratio (PR) sessions that could last from 1h to 5h depending on the rats' response on the active lever. The session stopped if no nicotine was self-administered for 1h since the last nicotine infusion was obtained. The rats were tested at 4 nicotine doses (i.e., 0.0075 – 0.015 – 0.03 – 0.06mg/kg/infusion) according to a progressive ratio—an exponentional increase in the number of lever presses for each subsequent infusion [Based on Richardson & Roberts's formula (1996) : 5 x e (number of injection x 0.2) – 5; e.g., 1, 2, 4, 6, 9, 12, 15, 20, 25, 32, etc]. The nicotine doses order was counterbalanced among each of the 4 groups of rats. Like the 7h self-administration sessions, the rats were tested every two days for the progressive-ratio sessions. The selection of the dependent session length (1h–5h dependent on responding) was selected to replicate and compare the results of Gueye and colleagues (in work, 2020) with the present study. However, to obtain a complete dose-response curve, all rats were exposed to all doses of the progressive ratio instead of being only exposed to three doses (i.e., the dose that they used to self-administer during the 7h sessions and the doses below and above that dose).

9. Extinction and reinstatement of nicotine seeking induced by nicotine-associated cues

On the 15th and 30th day after their last 7h nicotine self-administration session, rats were placed in operant boxes for a 6h extinction session. During the extinction session, three active lever presses did not deliver nicotine, and did not activate the light and sound, but retracted the levers for 25 seconds. The rats were not cabled during extinction and reinstatement sessions.

The extinction sessions were immediately followed by a 1h session of cue-induced reinstatement, with the light and sound that were paired with the nicotine self-administration in previous phases. As soon as the extinction session finished, the cue-induced reinstatement session

started with a free presentation of the regular tone (5s) and light (25s) stimuli with the levers retracting (25s). During the session, three active lever presses activated the tone (5s), and light (25s) and both levers retracted, but no nicotine was delivered. The duration of the extinction sessions and cue-induced reinstatement tests, as well as the selection of the 15th and 30th day after the last self-administration sessions were selected to replicate and compare the results of Gueye and colleagues (in work, 2020) with the present study.

10. Extinction and reinstatement of nicotine seeking induced by subcutaneous injections of nicotine or saline

On the 32nd, 34th, 36th, and 38th day after the last 7h nicotine self-administration session, rats underwent other extinction sessions followed by 1h nicotine/saline-induced reinstatement tests. Nicotine (0.075–0.15–0.3mg/ml/kg) and saline were injected subcutaneously as soon as the 6h extinction finished. Saline and the nicotine doses were selected to follow these respective reinstatement tests from previous studies (Shaham, Adamson, Grocki, & Corrigall, 1997; Shram, Funk, Li and Lê (2008). Saline and each nicotine doses were tested once, each after an extinction session. The rats were not habituated to subcutaneous injection prior to their first injection. The nicotine doses and saline order were counterbalanced among each of the 4 groups of rats. During the nicotine/saline-induced reinstatement test 3 active lever presses did not deliver nicotine, and did not activate the light and sound, but retracted the levers for 25 seconds.

11. Statistics and data integrity

The alpha (α) was fixed at 0.05 (i.e., $p < .05$) for statistical significance tests, since for this type of experiment with non-human animals it offers a good exchange between the types 1 and 2 errors (alpha justification based on Laken et al., 2018). The a priori powers were not calculated because the literature did not offer sufficient effect size statistical information to make the calculation. For these same reasons, the type 2 errors (β) could not be calculated. The sample sizes

were determined according to the number of operant boxes available and according to the peer knowledge regarding the sample size capability to meet parametric statistical assumptions for this type of experiment.

Partial eta-squared (η_p^2) was selected to represent the effect size of main effects and interaction when doing analysis of variance (ANOVA) statistical tests. This variable represents the percentage of variance that only one designated independent variable can explain about the dependent variable when controlling for the variance of all other independent variables and the error variance. An advantage of this effect size is that it is intuitive since it represents the percentage of explained variance as the size of the effect (Norouzian, & Plonsky, 2017). The use of effect sizes ease future studies to conduct meta-analysis and sample size calculations. The Tukey's HSD test for multiple comparisons was used for post-hoc tests.

When comparing two levels of an independent variable, student t-tests were used and standard eta-squared (η^2) were used as effect sizes instead of Cohen's *d*. This was done to remain constant with the way of interpreting the effect sizes and because effect size that are expressed as the percentage of an effect (e.g., eta-square) are easier to understand than non-percentage effect size (e.g., Cohen's *d*) (Brooks, Dalal, & Nolan, 2014). The standard eta-squared (η^2) is different from partial eta-squared (η_p^2) in that it doesn't control for the variance of other independent variables. This may seem less advantageous than the partial eta square but the two are the same when there is only one independent variable tested, hence reporting eta-squared is the proper way when comparing the two levels of only one independent variable (Norouzian, & Plonsky, 2017).

The validity of the parametric statistical assumptions were verified for all tests. The normality criterion could be judged as violated if the skew of the distribution was above three standard deviations, if the kurtosis of the distribution was above ten standard deviations, or if the

distribution standardized on a Q-Q plot indicated that the data had a pattern that excessively departed from the line of normality. The homogeneity of variance criteria (homescedasticity and sphericity) were evaluated with the Levene's test. The variance was considered heterogeneous if the *p*-value of the Levene's test was below 0.05 or—to preserve an effect size perspective—if the *F*-value of the test was superior to 10. Outliers more extreme than 3 standard deviations were removed from the analysis and the presentation of the data. The variance was heterogeneous for the mean number of infusions taken during the acquisition phase and required the use of the Welch t-test as a correction.

To maximize the readability of the result section, unless non-significance support a claim, only results with either $p < .05$ or $\eta_p^2 > .15$ are reported, the former is based on the alpha value set for statistical significance and the later has been decided to keep a meaningful effect size approach on the reporting of the results. Analysis and data presentation were done with GraphPad Prism (v. 8.2.1 for Mac). However, analysis of variance with three independent variables (3-way ANOVA) that had two independent variables with more than two levels were done with SPSS (v.24 for Mac, IBM) since Prism does not yet support 3-way ANOVA above that threshold (see chapter 3 Fig. 3).

Chapter 3 :
Results

1. Acquisition of food and nicotine self-administration at different doses

Before self-administration of nicotine, rats were put in operant boxes to learn to press an active lever to obtain food. All rats readily learned to seek food within an average of 4.19 days (*SD* = 0.65). Food training helps rats to learn to make responses on the active lever to facilitate the acquisition of nicotine self-administration. This method is widely used in self-administration studies and is known not to interfere with subsequent self-administration within experimental designs (Clemens, Caillé, & Cador, 2010).

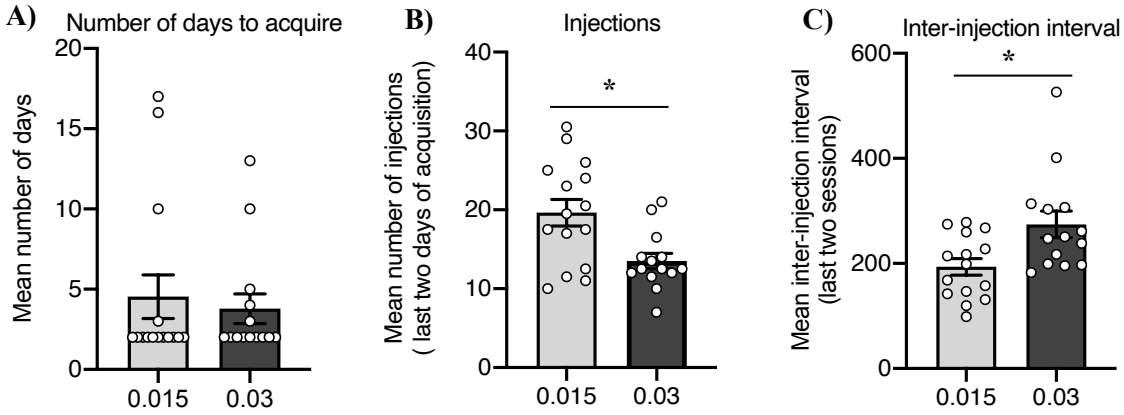

***Figure 1*. Acquisition of nicotine self-administration**. **A)** Mean number of days to acquire the nicotine self-administration behaviour. **B)** Mean number of injections for the last two days of nicotine acquisition. **C)** Mean inter-injection interval for the last two days of nicotine acquisition. * = $p < .05$. Data are represented as Mean ± SEM.

The majority of rats readily learned to self-administer nicotine (**Fig. 1A**) with no significant difference between the two doses. There was a significant difference, however, for the mean number of injections between the rats assigned to the 0.015mg/kg/inf dose ($M = 19.63$, $SD = 6.58$) and the rats assigned to the 0.03/kg/inf dose ($M = 13.5$, SD = 3.66), Welch $t(22.18) = 3.128$, $p = 0.005$, $\eta^2 = 0.306$ (**Fig. 1B)**. There was also a significant difference for the mean inter-injection interval (III) between the rats assigned to the 0.015mg/kg/infusion dose ($M = 193.5$, $SD = 60.25$) and the rats assigned to the 0.03/kg/infusion dose ($M = 274.4$, $SD = 94.14$), $t(27) = 2.774$, $p = 0.01$,

$\eta^2 = 0.222$ (**Fig. C**). These results show that rats self-administering a lower dose of nicotine obtain more infusions over a session, separated by shorter intervals compared to rats self-administering a higher dose.

2. Nicotine intake at different doses under different access conditions (intermittent or continuous)

To evaluate group differences in cumulative intake of nicotine over the 14 self-administration sessions between all four groups, a two-way ANOVA (2-Dose x 2-Access) was conducted (**Fig. 2)**. The analysis revealed a trend for a two-way interaction between dose and access, $F(1, 24) = 3.4$, $p = 0.078$, $\eta_p^2 = .124$, as well as a main effect of access $F(1, 24) = 33.7$, $p < .001$, $\eta_p^2 = .584$, and a main effect of dose, $F(1, 24) = 27.65$, $p < .001$, $\eta_p^2 = .535$. To look further into the nature of the interaction, post-hoc tests were conducted using Tukeys's correction for multiple comparisons. The LgA-0.03 group ($M = 14.96$, $SD = 2.47$) was found to have consumed a statistically greater amount of nicotine in comparison to the IntA-0.15 ($M = 4.21$, $SD = 1.83$), IntA-0.03 ($M = 7.53$, $SD = 3.45$) and LgA-0.015 ($M = 8.06$, $SD = 2.26$), all $p < .001$. This analysis indicates that the continuous access to the higher dose of nicotine (LgA-0.03) resulted in the highest nicotine intake over the 14 nicotine self-administration sessions. The continuous access to the lower dose of nicotine (LgA-0.015) also resulted in a higher nicotine intake compared to the intermittent access but only if comparing with the lower dose of nicotine (IntA-0.015) ($p < 0.046$).

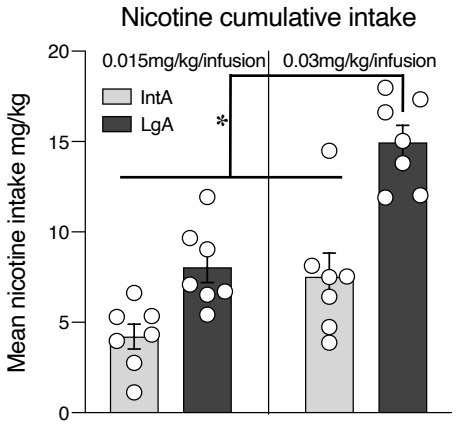

Figure 2. Cumulative intake of nicotine over the 14 self-administration sessions. The doses of nicotine are taken into account in the accumulation. Data are represented as Mean ± SEM. * = $p < .05$.

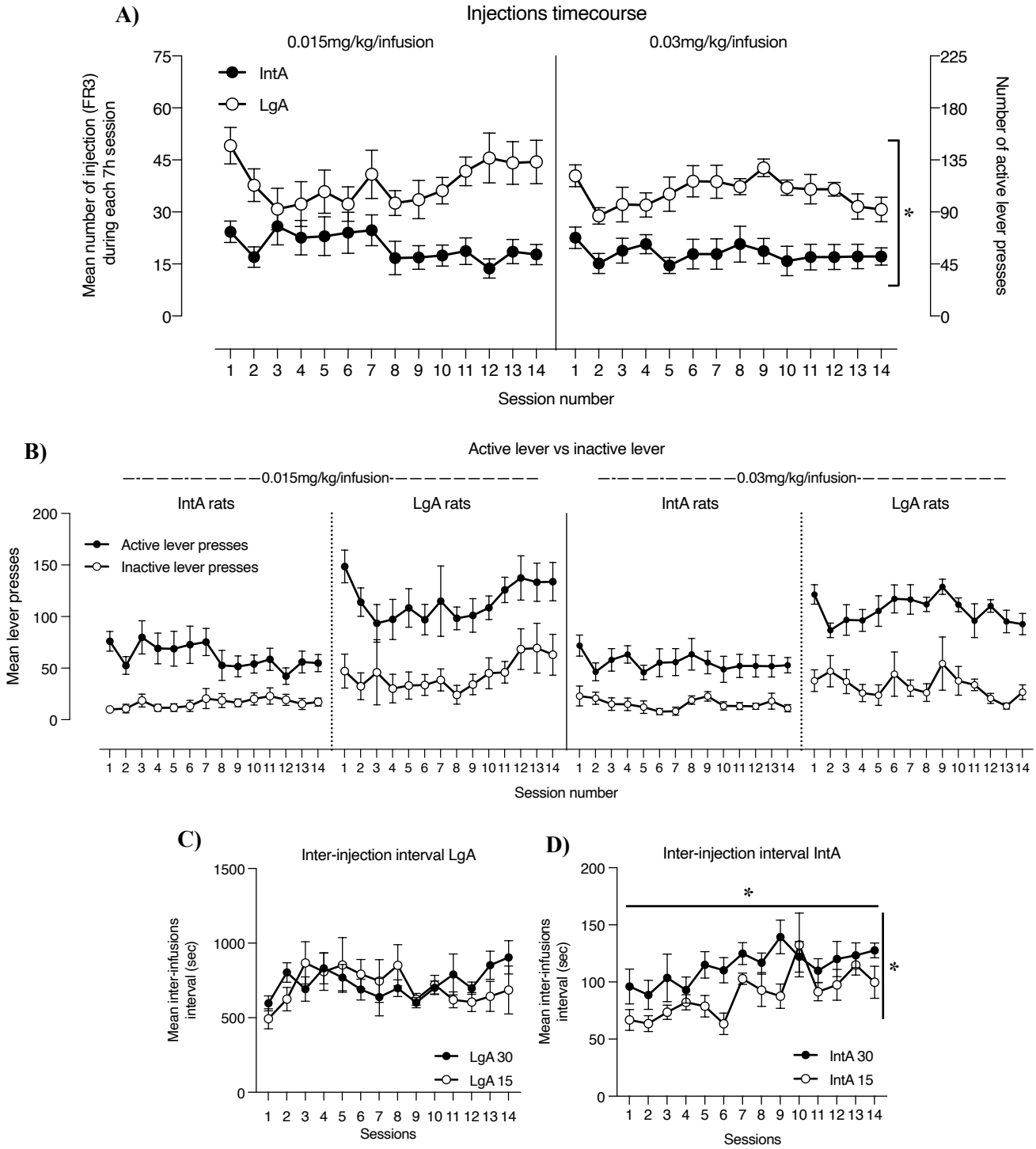

***Figure 3*. Nicotine consumption, inter-injection interval and lever presses during the 14 self-administration session (7h)**. **A)** Mean number of injections obtained (FR3; left y axis) and mean number of active lever presses (right y axis) over the 14 sessions of 7h for each of the 4 groups of rats. **B)** Mean number of active and inactive lever presses for each of the 4 groups of rats. **C)** Inter-injection intervals of the long access group. **D)** Inter-injection intervals of the intermittent-access group. Data are represented as Mean ± SEM. * = $p < .05$.

3. Patterns of nicotine intake within sessions at different nicotine doses and under different access conditions

To examine the pattern of nicotine intake between doses and access types over the 14 sessions of nicotine self-administration, a three-way ANOVA was conducted (2-Access x 2-Doses x 14-sessions; **Fig. 3A**). The analysis revealed a significant three-way interaction, $F(12, 312) = 1.989$, $p = 0.021$, $\eta_p^2 = 0.071$, a significant two-way interaction (2-Access x 14-Sessions), $F(13, 312) = 2.157$, $p = 0.011$, $\eta_p^2 = 0.082$, a main effect of session, $F(13, 312) = 2.214$, $p = 0.013$, $\eta_p^2 = 0.085$, and a main effect of access, $F(1, 24) = 30.74$, $p < 0.001$, $\eta_p^2 = .562$. The large main effect of access types is largely due to the fewer opportunities to obtain nicotine in the intermittent access. The 3-way interaction suggests that nicotine intake changes over sessions and that this effect is greater for rats self-administering with a continuous access and even more so if the dose is low. Importantly, all 4 groups successfully discriminated the active lever from the inactive lever (**Fig. 3B**).

To examine the inter-infusion intervals at the different doses over the 14 nicotine self-administration sessions separately for the continuous and the intermittent access groups, two two-way ANOVAs (2x-Doses x 14-Sessions) were conducted (**Fig. 3C–D**). The two-way ANOVAs for the IntA groups revealed a main effect of session, $F(3.63, 41.59) = 3.468$, $p = 0.018$, $\eta_p^2 = 0.232$, and a main effect of dose, $F(1, 12) = 10.26$, $p = 0.008$, $\eta_p^2 = 0.461$. Independently of doses, rats with intermittent access to nicotine took a longer amount of time between nicotine injections as the session progressed but overall, the 0.03mg/kg/inf group took the longest between each injection. No significant effect was found in the two-way ANOVA for the LgA groups.

To further examine the pattern of nicotine intake, data were analysed within session at differences doses, and under different access, from the 1st, 7th, and 14th sessions for all 4 groups. A separate three-way ANOVA for each access type was conducted [IntA: (3-Days x 6 Cycles x 2

Doses) LgA: (3-Days x 7 hours x 2 Doses)] (**Fig. 4A–B**). The analysis for the IntA groups revealed a day per access cycle interaction, $F(10, 120) = 2.028$, $p = 0.036$, $\eta_p^2 = 0.145$, and a main

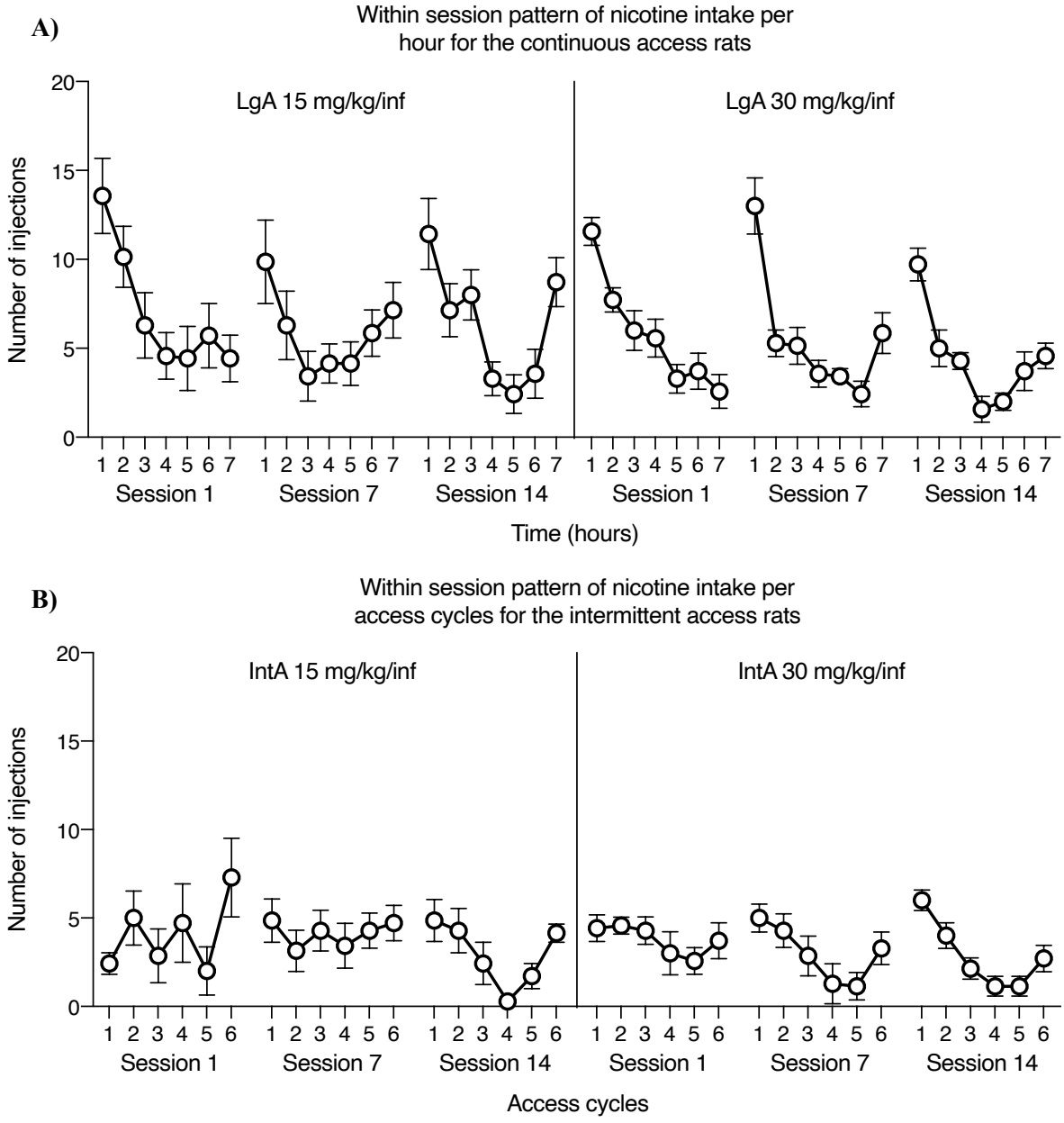

***Figure 4*. The pattern of intake throughout a session change from the 1st to the 7th and to the 14th sessions of nicotine self-administration. A)** Within session pattern of nicotine intake per hour for the continuous access rats. **B)** Within session pattern of nicotine intake per access cycles for the intermittent access rats. An access cycle is given each hour, with the first starting at the onset of the session. Both the A and B graph represent the 1st, 7th and 14th session of the 7h sessions. In both IntA and LgA a change in the pattern of nicotine consumption within sessions occur as the rats go through the first to the last sessions. Data are represented as Mean ± SEM.

effect for the access cycle, $F(5, 60) = 5.533$, $p < .001$, $\eta_p^2 = .316$. The analysis for the LgA groups revealed a day per hours interaction, $F(10, 120) = 2.028$, $p = 0.036$, $\eta_p^2 = 0.145$ and a strong main effect of hours, $F(6, 72) = 40.012$, $p < .001$, $\eta_p^2 = 0.769$. For both access types, nicotine intake varied within and between sessions and this pattern was not affected nicotine doses.

The qualitative analysis provides some insight into these interactions and main effects, both the IntA and LgA develop a specific pattern of consumption within their session as they progress through the 14 sessions of nicotine self-administration. Specifically, as the IntA progress, they tend toward the following pattern: a high intake at the beginning and the end of the session, but a large dip in their consumption at the 4th and 5th cycles. Interestingly the LgA rats manifest the same progression toward that pattern within their sessions with higher nicotine intake at the beginning and end of session, and with a dip at the 4th and 5th hours.

A minute-by-minute pattern of intake is represented in **Figure S1 (A–F)** for the whole 7h session on the 1st, 7th, and 14th sessions for the 3 most representative long-access rats at each nicotine doses. The consumption patterns of these rats shows that even by selecting the three most representative rats for each dose there is still a certain amount of qualitative difference in the pattern of intake between these rats. A more detailed look at the nicotine consumption of intermittent access rats within each access cycle at both nicotine doses for the 1st, 7th and 14th sessions is also presented in **Figure S2 (A–B)**. The IntA-0.03 rats show more consumption around the first cycle and at the beginning of the cycle when they consume, whereas the IntA-0.015 appear to spread their injections a bit more over each cycle and throughout the time without these cycles.

Additionally, a closer look was taken at the nicotine consumption within each access cycle (12 min) of the IntA rats by dividing the first and last access cycles each into four bins of 180 seconds (3min) and by conducting two-way ANOVAs (2-Doses x 14-Sessions) to see if there was

a significant difference within each bins (**Fig. 5 A–B**). For the first cycle, the analysis of the first 180 seconds revealed a main effect of sessions, $F(13, 156) = 2.675$, $p = .002$, $\eta_p^2 = .182$, and a main effect of doses, $F(1, 12) = 8.367$, $p = 0.014$, $\eta_p^2 = .411$. The session main effect represents an

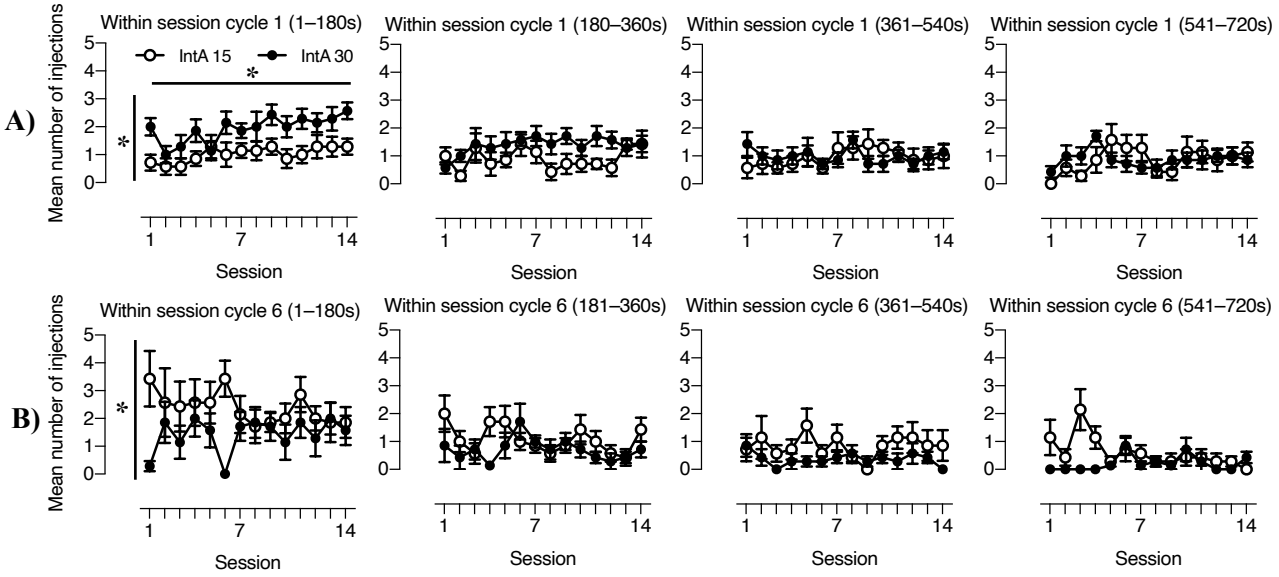

***Figure 5*. Within session of IntA rats mean consumption per 12 min nicotine cycles divided in time bins of 180s and plotted over the 14 sessions of 7h self-administration**. The number of injections taken (Y axis) are plotted over the 14 self-administration sessions in 4 different graph of 3 minutes each. **A)** First 12 min cycle within a session separated in 4 bins of 3 min . **B)** Last 12 min cycle within a session separated in 4 bins of 3 min. * = $p < .05$. Data are represented as Mean ± SEM.

escalation of intake during the first 180 seconds of the first cycle over the 14 nicotine self-administration session. The absence of an interaction effect between the doses and sessions, while there is a dose main effect, means that rats at both doses escalated their intake as they were progressing throughout the 14 nicotine self-administration sessions, but it also means that in the first 3 mins of access—while controlling for other factors—41% of the variance of how many infusions are taken can be accounted for by which nicotine dose the rats received. More precisely, in this first 3 min bin, the IntA-0.03 rats took on average 0.908 (*Mean difference*, *SD* = .395) more infusions than the IntA-0.015. In sum, over the the 14 self-adminsitration sessions both IntA 0.015

and 0.03 rats took more and more infusions during the first 3 minutes when the first cycle started, but on average, the IntA took more infusions.

The analysis of the last cycle reveals a statistically significant interaction between the doses and the sessions during the first 180 seconds of that cycle, $F(12, 156) = 2.721$, $p = 0.012$, $\eta_p^2 = .185$. This interaction can be seen as the number of injections from both the 0.015 and 0.03 rats converge together over the 14 nicotine self-administration sessions, but there were no main effect of session, $F(13, 156) = 0.557$, $p = 0.885$, $\eta_p^2 = .044$, and no main effect of dose, $F(1, 12) = 1.972$, $p = 0.186$, $\eta_p^2 = .141$. In sum, the results for the last access cycle suggests that at the beginning of the last access cycle, the IntA rats do not take the same amount of nicotine but over time their number of nicotine infusions converges together.

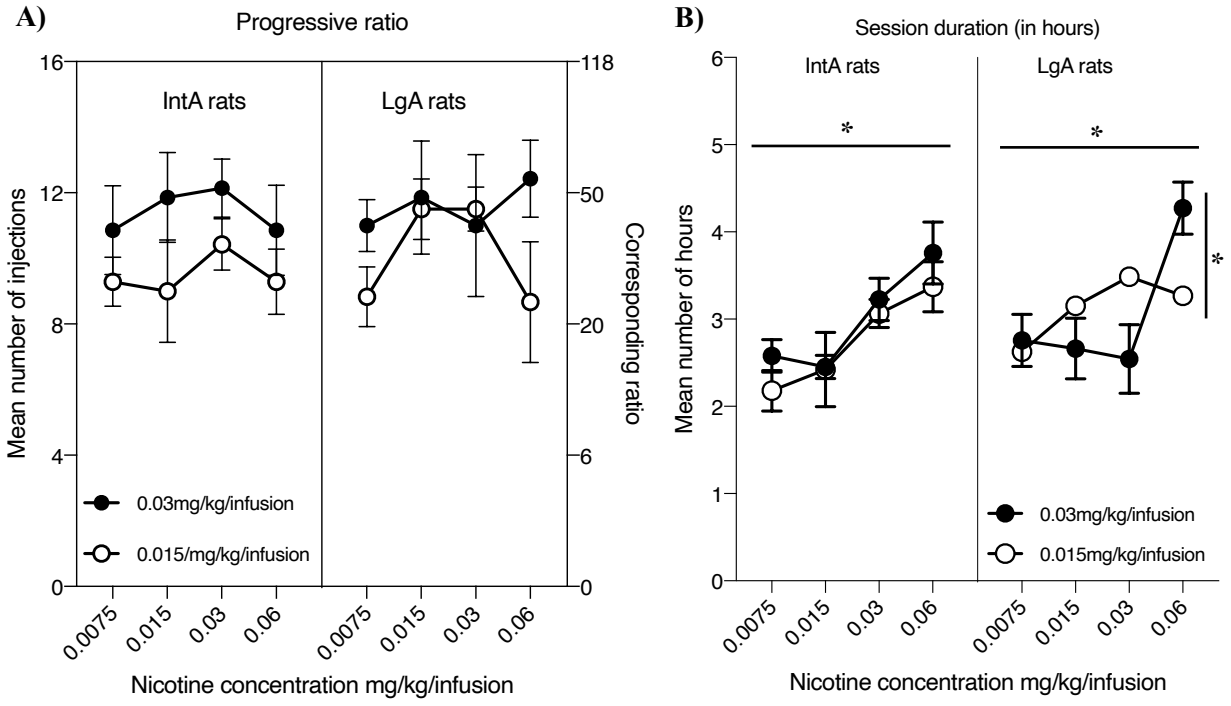

***Figure 6*. Progressive ratio**. **A)** The four groups of rats were tested with four different doses of nicotine under a progressive ratio to see if past history of doses, or access type, had an influence on the motivation of the rats. **B)** The sessions' lengths were shown to increase as a function of the doses that the rats were tested at—larger doses resulted in longer session. Sessions' lengths were influenced by the fact that the progressive ratio program was dictated to stop if the animal did not self-administer within an hour after its previous injection. Data are represented as Mean ± SEM. * = $p < .05$.

4. The effects of a history of intermittent or continuous acess to nicotine on motivation to self-administer

To evaluate differences in motivation to work for nicotine, rats were allowed to self-administer the drug under a progressive ratio schedule of reinforcement (**Fig. 6A**). To determine if there were differences in motivation based on the doses of nicotine previously used during the 14 nicotine self-administration sessions or access types (i.e., IntA or LgA) and in relationship to the different doses tested at the progressive ratio (0.0075–0.015–0.03–0.06mg/kg/inf), a 3-way ANOVA was done (2-Access x 2-7h self-administration doses x 4-progressive-ratio doses). No interactions or main effects turned out statistically significant. This means that no matter the doses that rats were previously exposed to, or the type of access, both LgA and IntA rats were equally motivated regardless at which doses they were tested during the progressive ratio tests.

To determine if the doses and type of access that the rats were previously exposed to had an effect on the progressive ratio session length for each of the four doses tested, another 3-way ANOVA was done (**Fig. 6B**). The analysis revealed a statistically significant 2-way interaction (2-progressive-ratio doses x 2-7h nicotine self-administration doses), $F(3, 69) = 2.85$, $p = 0.044$, $\eta_p^2 = .111$, and a main effect for the progressive-ratio doses, $F(3, 69) = 12.12$, $p < 0.001$, $\eta_p^2 = .345$. As shown in Figure 5B, the effect of the progressive-ratio doses is that the sessions lasted longer when the rats were tested with higher doses; this happens because progressive ratio tests stopped when a rat stopped taking nicotine for more than 1 hour.

5. Drug intake and the motivation to self-administer

To investigate if the cumulative quantity of nicotine consumed during the progressive ratio tests correlated with previously administered doses or access types, Pearson *r* correlations were computed (see **Table 1** below). These analyses show that, overall, when rats had a higher cumulative quantity of nicotine during progressive ratio tests, they also had higher cumulative

quantity of nicotine during their self-administration phase. However, this trend was statistically significant only in few cases.

Pearson *r* values				
	IntA-0.015	IntA-0.03	LgA-0.015	LgA-0.03
PR-0.0075	0.6854	0.7724 *	0.6260	0.1660
PR-0.015	0.8996 **	0.5612	0.8255 *	0.5155
PR-0.03	0.3458	0.5558	0.7155	0.7253
PR-0.06	0.4665	0.7530	0.7002	0.5861

***Table 1*. Correlation matrix between the cumulative intake of nicotine over the 14 sessions of nicotine self-administration and the number of injections taken during the progressive ratio test.** The left side of the figure refers to the doses of nicotine that were used during the progressive ratio. The top of the figure refers to the four different groups of rats based on their dose and access types. * = $p < .05$, ** = $p < .01$.

6. The effect of a history of intermittent or continuous access to nicotine on cue-induced reinstatement

To evaluate the influence of the doses and access history of nicotine self-administration on the susceptibility to relapse, the nicotine-seeking behaviour of the rats was first extinguished in all four groups at the 15th and 30th day after the last 7h session (**Fig. 7A & D**). Their susceptibility to relapse was then tested immediately after in a 1h cue-induced reinstatement test. Two 3-way ANOVAs revealed that none of the four groups of rats were more resistant to extinction from each other both at the 15th day and 30th day, as there were no 3-way interactions (6-Time x 2-Access x 2-Doses) for the extinction at the 15th day $F(5, 120) = 0.426$, $p = .830$, $\eta_p^2 = .017$, there were no 3-way interactions for the extinction at the 30th day, $F(5, 120) = 0.719$, $p = .610$, $\eta_p^2 = .029$, and there were no 2-way interactions and no doses or access main effects for both the 15th and 30th day, all p values were above .05 and all η_p^2 were below .15.

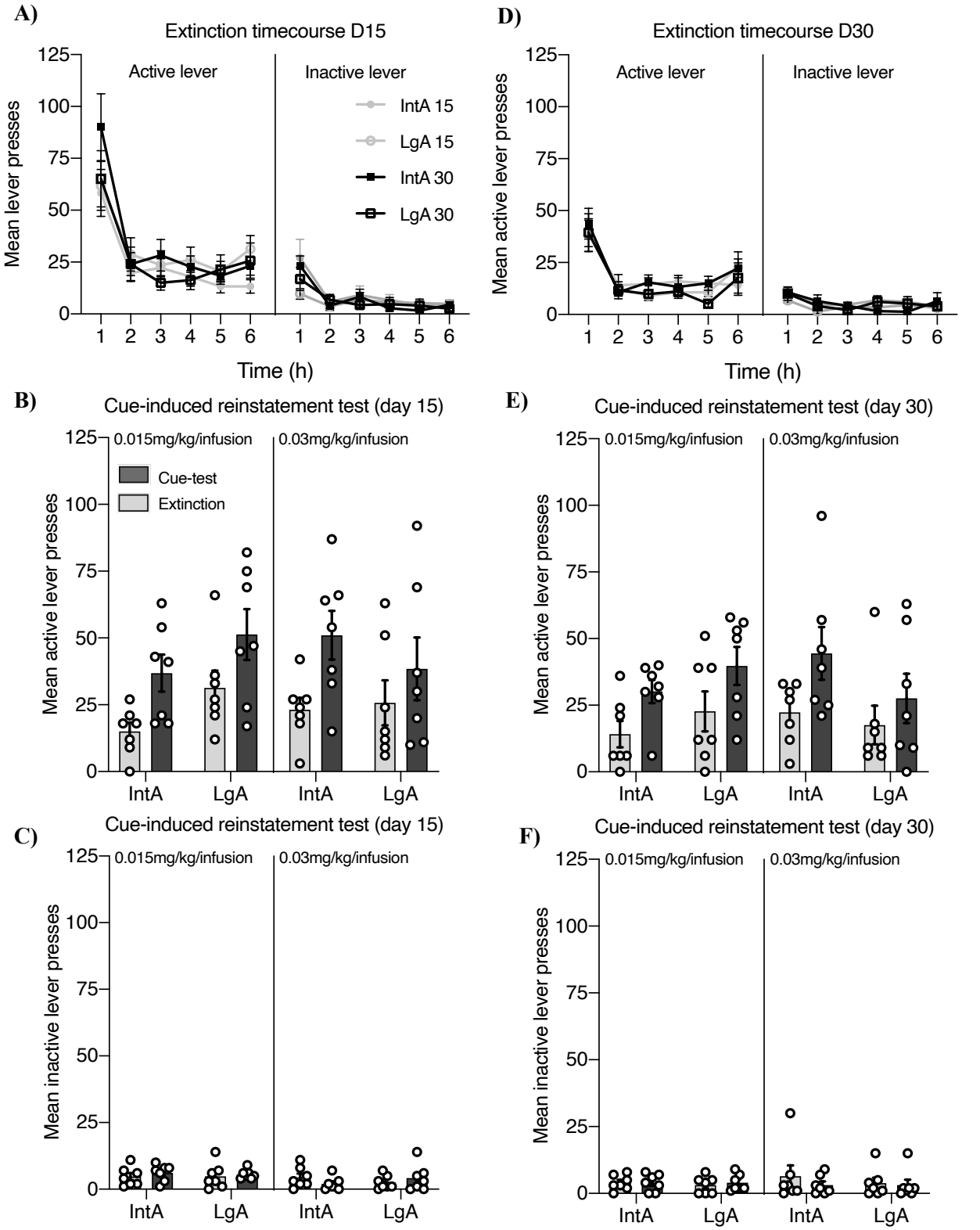

Figure 7. **Extinction and cue-induced reinstatement at the 15th and 30th day after the last 7h self-administration session**. Data are represented as Mean ± SEM. **A & D)** Extinction session before the cue induced reinstatement test plotted over the 6 hour of extinction. **B & E)** Active lever presses for the last cue-induced reinstatement test and for the last hour of the extinction session preceding the cue-induced reinstatement test. **C & F)** Inactive lever presses during the cue-induced reinstatement test and at the last hour of the extinction session.

To evaluate if the reinstatement of nicotine seeking occurred following the presentation of cues that were previously paired with nicotine self-administration two 3-way ANOVAs (2-Doses x 2-Access x 2-Conditions) were conducted. The presentation of the nicotine paired cues induced a reinstatement of nicotine seeking in all four groups of rats at both the 15th (**Fig. B & C**) and 30th day (**Fig. 7E & F**) tests as shown by the main effects of condition (extinction last hour x cues induced reinstatement test); a main effect that is a statistically significant difference between the number of lever presses during the extinction last hour and the cue-induced reinstatement test. This is shown by the ANOVAs' main effect results of condition for both 15th day test, $F(1, 24) = 49.06$, $p < .001$, $\eta_p^2 = .672$, and for 30th day test, $F(1, 24) = 24.97$, $p < .001$, $\eta_p^2 = .510$. There were no statistically significant 3-way interactions for both the 15th day test, $F(1, 24) = 1.274$, $p = .270$, $\eta_p^2 = .05$, or for the 30th day test, $F(1, 24) = 1.016$, $p = .323$, $\eta_p^2 = .040$, there were no statistically significant 2-way interations, and also no main effect for access and doses, all p values were above .05 and all η_p^2 were below .15.

7. Drug intake and susceptibility to cue-induced reinstatement

Pearson correlations were computed between the cumulative intake of the 14 nicotine self-administration sessions for every four groups and the susceptibility to reinstate nicotine seeking behaviour following the presentation of stimuli paired with nicotine consumption. No statistically significant correlations were found (see **Table. S1**).

8. The effect of a history of intermittent or continuous access to nicotine on nicotine- and saline-induced reinstatement

The influence of the doses and access types on the susceptibility to relapse following re-exposure to nicotine itself and to a minor stressor (saline) were also assessed. This was done by giving the rats other extinction tests (**Fig. 8**) at 32nd, 34th, 36th and 38th day after the last 7h session, which were immediately followed by a reinstatement test of subcutaneous injection of saline or

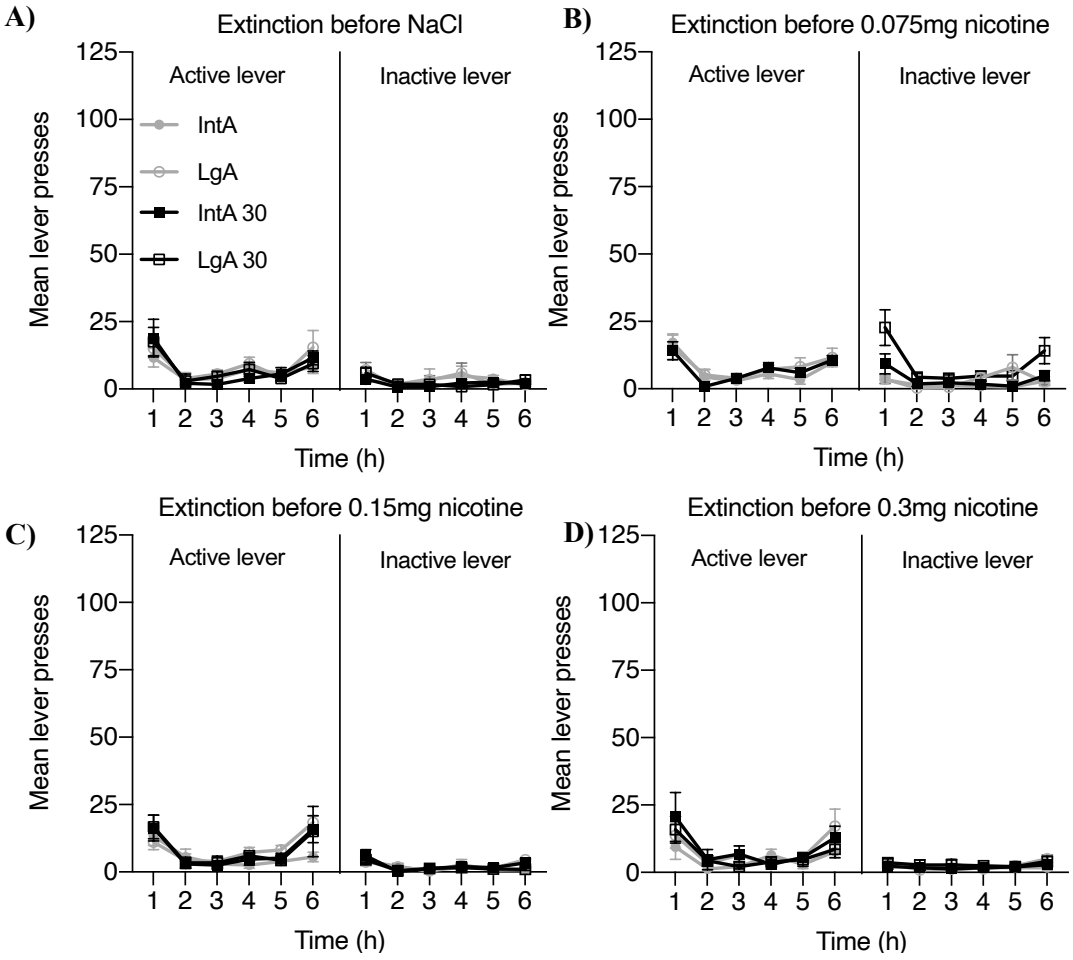

***Figure 8*. Extinction sessions before each nicotine/saline induced reinstatement tests.** Extinction tests conducted at the 32nd, 34th, 36th and 38th day after the last 7h session before the saline and nicotine induced reinstatement tests.

one of the three doses of nicotine. The active lever presses during the last hour of the extinction preceding each respective dose of nicotine and saline were compared to the active lever presses during the 1h nicotine/saline reinstatement tests to determine if reinstatement of nicotine seeking occurred (**Fig. 9**). A 3-way ANOVA was conducted for each of these tests. All four ANOVAs had a statistically significant main effect of condition (extinction last hour x nicotine/saline induced reinstatement test), which meant that all injections reinstated the nicotine-seeking behaviour as shown by the superior amount of active lever presses during the reinstatement tests in comparison to the extinction last hour—for saline, $F(1, 24) = 8.139$, $p = .009$, $\eta_p^2 = .253$, for the 0.075mg/kg/ml

nicotine subcutaneous dose, $F(1, 23) = 23.55$, $p < 0.001$, $\eta_p^2 = 506$, for the 0.15mg/kg/ml nicotine subcutaneous dose, $F(1, 23) = 16.57$, $p < .001$, $\eta_p^2 = .419$, and for the 0.03mg/kg/ml nicotine subcutaneous dose, $F(1, 21) = 34.36$, $p < .001$, $\eta_p^2 = .621$. There were no statistically significant 3-way interactions for the reinstatement test with saline, $F(1, 24) = 0.653$, $p = .427$, $\eta_p^2 = .026$, no 3-

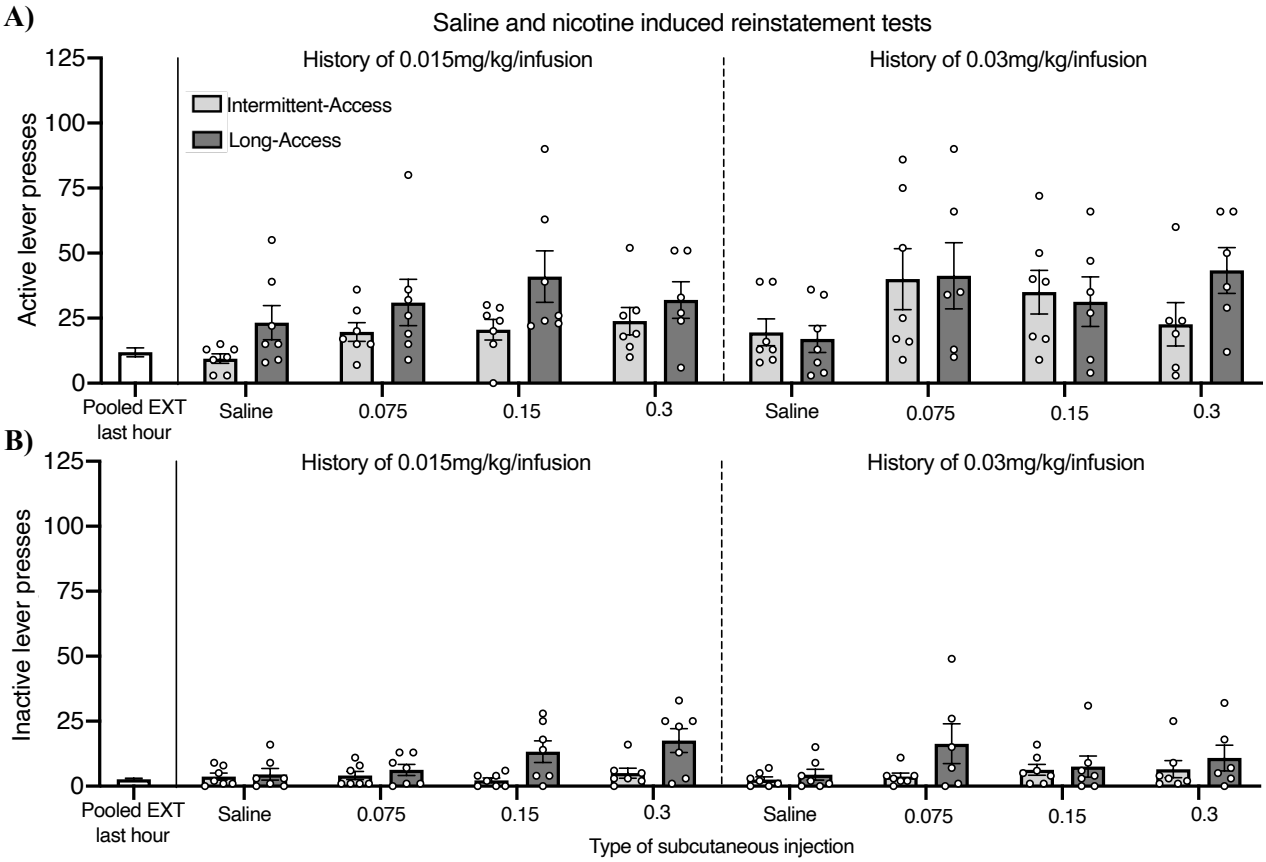

***Figure 9*. Nicotine and saline induced reinstatement**. The injections (e.g., saline & 0.075, 0.15, 0.3mg/kg/ml nicotine doses) and the test days (day 32, 34, 36, and 38 after the last self-administration session) were counterbalanced. The active lever presses of the extinction's (EXT) last hour of all 4 groups of rats (IntA-0.015, IntA-0.03, LgA-0.015 & LgA-0.03) across the extinction's last hour preceding the saline and the 3 nicotine induced reinstatement tests were pooled into one column. The appropriateness of pooling this data was evaluated with a 3-way ANOVA (2-Access x 2-Doses-7h-SA x 4-Subcutaneous-Doses), there were no statistically significant 3-way interaction, $F(3, 72) = 0.959$, $p = .417$, $\eta_p^2 = .038$, and there were no statistically significant 2-way interactions or main effect across the tests and between the group of rats. The same was done for the inactive lever presses as there were also no significant interactions or main effects across the tests and between the group of rats. The saline and nicotine induced reinstatement tests are done with a single subcutaneous injections just before the test. **A)** Pooled active lever presses for the last hour of extinction and active lever presses during the saline and nicotine (0.075, 0.15, & 0.3/mg/kg/ml) induced reinstatement tests. **B)** Pooled inactive lever presses for the last hour of extinction and inactive lever presses during the saline and nicotine induced reinstatement tests. Data are represented as Mean ± SEM.

way interaction for the reinstatement test with the 0.075mg/kg/ml nicotine, $F(1, 23) = 0.261$, $p = .614$, $\eta_p^2 = .011$, no 3-way interaction for the reinstatement test with the 0.15mg/kg/ml nicotine, $F(1, 23) = 0.250$, $p = .622$, $\eta_p^2 = .011$, and no 3-way interaction for the reinstatement test with the 0.3/kg/ml nicotine, $F(1, 22) = 4.281$, $p = 0.97$, $\eta_p^2 = .125$, these ANOVA did not yield statistically significant 2-way interations, and also no main effects for access and doses, all p values were above .05 and all η_p^2 were below .15.

9. Nicotine intake and susceptibility to nicotine- and saline-induced reinstatement

Pearson *r* were computed to investigate if the history of previous nicotine self-administration correlates with the degree to which rats reinstate their seeking behaviour after saline or nicotine injections. To represent the degree of reinstatement, the difference between the active lever presses during reinstatement tests and during the last hour of extinction that preceded the reinstatement were converted in difference scores (reinstatement - extinction, **Fig. S4**). The correlations were made between these scores and the cumulative intake of each group during their 14 self-administration sessions.

Pearson *r* values				
	IntA-0.015	IntA-0.03	LgA-0.015	LgA-0.03
Saline I-R	0.2302	0.6084	-0.7346	0.6623
0.075 I-R	0.4361	0.6337	0.8187 *	0.7699 *
0.15 I-R	-0.1715	0.6428	0.5600	0.6868
0.3 I-R	0.5235	0.8677 *	0.6258	0.7659 *

***Table 2*. Correlation matrix between the differences scores at the nicotine/saline induced reinstatement test and the cumulative nicotine intake of the four doses-access group during the 14 self-administration session (IntA-0.015, IntA-0.03, LgA-0.015, LgA-0.03)**. The "I-R" on the left end of the table stand for induced reinstatement test, for instance saline I-R mean saline induced reinstatement test. Doses are represented as mg/kg/ml, except for saline which is kg/ml. * = $p < .05$.

Table 2 shows that, overall, when rats had higher cumulative intake during their self-administration phase, they also reinstated to a greater degree. However, this trend was significant only in few cases and some correlations are negative.

Chapter 4 :
Discussion

1. Research aims, objectives and summary of the results

Although the proportion of people smoking has largely gone down in Canada and in the USA (Janz, & Statistics Canada, 2018; Wang et al., 2018) there are still over 68 million people smoking in these two countries. The decline of smoking is a complex process that has been influenced by the efforts of many scientists and policy makers and involve multiple factors (see CDC, 1999), including an increase in the knowledge of the health risks of tobacco (Burn, 2014; CDC, 1999; U.S. Department of Health and Human Services, 2014), smoking policies (Shiffman et al., 2014; Barnett, Moon, Pearce, Thompson, & Twigg, 2017; CDC, 1999; U.S. Department of Health and Human Services, 2014; Wilson et al., 2012), as well as to some extent the stigmatization of smoking (Bernat et al., 2010; Evan-Polce, Castadelli-Maia, Schomerus, & Evans-Lacko, 2015; Wilson et al., 2012; but see Hemsing et al., 2012; and Helweg-Larsen, & Sorgen, 2019) among other factors (see CDC, 1999). These factors were advanced by some key events such as the 1964 Surgeon General Report backed with over 7000 research articles about tobacco smoking and diseases, as well as the 1988 Surgeon General Report that acknowledged the addictiveness of tobacco and nicotine and the 1998 *Tobacco Master Settlement Agreement* that forced the tobacco companies to makes all their private documents available to the public (CDC, 1999). The declines are very encouraging but the struggle against tobacco and nicotine are not yet finished, there are still over 62.5 million smokers in the USA (Wang et al., 2018) and over 4.9 million in Canada (Janz, & Statistics Canada, 2018). Moreover, as stated by the hardening hypothesis, those that are still smoking may be different from the smokers who stopped smoking (Hughes, 2011; National Cancer Institute, 2003). The individuals still smoking may not be influenced as much by health

risk advertisements, they may be more motivated to consume tobacco/nicotine, they may also have more difficulty abstaining and may be more likely to relapse. To help these smokers it is important to understand the context (zeitgeist and ortgeist) in which smoking occurs—because of smoking bans in public areas, and even in individual homes, as well as because of the stigma associated with tobacco, the pattern of smoking has become more restricted. Because smokers have to wait until they are in an appropriate context (e.g., not near a child, not near pregnant women) and environment (e.g., not at work, not in public area, not in public transport) the pattern of consumption becomes more intermittent. In other words, there are larger gaps of time within a day between each moment where an individual can smoke.

Despite the intuitive idea that less of a drug produces fewer phenotypic symptoms of addiction, this is not necessarily always the case. Other factors such as an intermittent temporal pattern of consumption of a drug can have important implications in the development and maintenance of addiction (Kawa et al., 2019). The aim of this study was to evaluate if such effects are found for nicotine addiction as well as to provide a better model of humans' smoking. To do so, the results of the temporality of consumption were evaluated by comparing rats that were subjected to 14 nicotine self-administration sessions of 7h each with either a continuous access to nicotine or an intermittent access, each at two different doses of nicotine—0.015 and 0.03mg/kg/infusion. The objectives were to compare the patterns of nicotine intake and to evaluate the effects of these different doses and access on the motivation to consume nicotine and the susceptibility to relapse when exposed to stimuli associated with nicotine consumption as well as the susceptibility to relapse when re-exposed to the nicotine itself and a mild stressor.

Rats were put in operant boxes where they had to learn at first to press on an active lever to obtain food pellets. Rats are first trained with food to facilitate their learning of nicotine self-

administration (Clemens, Caillé, & Cador, 2010). After food training, rats were given 1h sessions to acquire the behaviour of self-administering nicotine intravenously at either a 0.015 or 0.03mg/kg/infusion dose—a behaviour that was rapidly acquired. Although, one specific comment posited by Wise and Koob (2014) regarding the number of days to acquire is that it should not be generalized to humans and especially more so for tobacco smoking. Because humans are capable of understanding and anticipating the health consequences of smoking (Wise, & Koob, 2014) just as it is seen in the large decrease in smoking following raising awareness of the association between tobacco smoking and cancer among the public (Wang, et al., 2018; Janz, & Statistics Canada, 2018; Statistics Canada, 2019; figure 8 in chapter 1).

After acquiring the behaviour to self-administer nicotine at two different doses (0.015 and 0.03mg/kg/inf) the two groups of rats were further separated by being subjected to a continuous (LgA) or intermittent access (IntA) to nicotine during 14 self-administration sessions of 7h. Rats in the continuous access to nicotine had an unlimited access to nicotine during 7h while the intermittent access rats can only self-administer for 12 minutes every hour during the 7h sessions. Without a doubt, because the continuous access rats have more time to consume nicotine, they do consume more nicotine. The LgA rats that had the largest dose of nicotine (0.03mg/kg/inf) consumed the greatest amount among all four groups because the dose is double the amount of the other dose. In regards to the quantity consumed, extended access model (i.e., access that are at least greater than 3 hours) to drugs has led to an enduring thought about the amount consumed, which is that quantity has a significant influence on the development of addiction (Ahmed, 2012; Ahmed, & Koob, 1998). In this line of thought a prediction would be that the LgA rats should have greater phenotypic symptoms of addiction. However, the present study points to the contrary. Even though the IntA rats consumed far less nicotine, they showed similar levels of motivation to self-

administer nicotine as shown under a progressive ratio. During abstinence they also demonstrated a similar susceptibility to the reinstatement of their nicotine seeking behaviour when re-exposed to stimuli previously associated with nicotine consumption. They also showed a susceptibility to reinstate when exposed to subcutaneous injections of nicotine or saline. Clues as to why such results occur can be found in recent studies of intermittent model of cocaine consumption. In spite of lower intake of a drug, the intermittent model of drug use is accounted to produce spikes of the drug in the brain throughout a session (Robinson & Berridge, 1993) and since the experiment of Zimmer, Dobrin and Roberts (2011) on intermittent cocaine self-administration, other studies have shown that intermittent drug spiking produce even stronger phenotypic symptoms of addiction (Allain, Roberts, Lévesque, & Samaha, 2017; Allain & Samaha, 2018).

2. Comparisons of the nicotine induced reinstatement with other studies

In some experiments, researchers find that a re-exposure to nicotine can reinstate the nicotine-seeking behaviour (Shaham et al., 1997). Still, not all experiments can detect such reinstatement following a nicotine re-exposure. For instance, Clemens, Caillé and Cador (2010) were only able to achieve a nicotine-induced reinstatement when the nicotine paired cues were present during the reinstatement test. Similarly, Feltenstein, Ghee and See (2012) also did not see a reinstatement of nicotine seeking, unless they injected the rats with yohimbine, a pharmacological agent that is used to produce stress in the animal. In line with this last idea, the present study did not habituate the rats to subcutaneous injections prior to the reinstatement test. By counterbalancing saline and all nicotine doses, it was ensured that a certain number of animals were first experiencing subcutaneous injections for the first time in each of the group. As such, a little amount of stress among the animal is represented in the data of the reinstatement test. This can be seen as the saline subcutaneous injection also produced a reinstatement of nicotine seeking.

3. Comparisons with other similar studies

The three studies that have similarities to the model of the present study are the daily intermittent model from Cohen, Koob, and George (2012), the minutes-intermittent model by Holmes and colleagues (2018) and hourly-intermittent model from the unpublished study of the Samaha laboratory (Gueye, Allain, & Samaha, 2020). The present study used a combination of both a daily- and hourly-intermittent access models with the access cycle lasting 12 minutes and the no-access lasting 1 hour. The "daily" part means that a day is skipped between each self-administration session. It is for this daily component that the present study resembles the study by Cohen and colleagues (2012). One specific difference between the present study and the study of Cohen is that their rats escalated their nicotine intake over the multiple nicotine self-administration sessions. Whereas here, this did not occur. This difference may be accounted because of another difference between their study and the present study, namely, rats in their study had session lengths of 21 hours, while here, rats had session lengths of 7 hours. For the study by Holmes and colleagues, one interesting finding that they show is that as they increase the time out period (no-access to nicotine) between each lever presses, the rats begin to develop a preference for higher doses of nicotine. Although this is interesting, the present study did not offer multiple choices of doses to the rats. Finally, the other researchers from the Samaha laboratory (Gueye et al., 2020) had compared the effect of different speeds of infusion in the hourly-intermittent model with the two same doses (i.e., 0.015 and 0.03mg/kg/inf) and the same number of 7h self-administration session (14 days). When comparing the results of the present study to those with the same speed of infusion from Gueye and colleagues (2020) some similarities and some differences appear. In terms of similarities, all rats in both studies reinstated their nicotine seeking behaviour when exposed to stimuli previously associated with nicotine consumption at the 15th day after the last 7h

nicotine self-administration session. Another similarity is that the 0.03-IntA group on the 30th day after the last 7h nicotine self-administration session reinstated their nicotine seeking behaviour following the presentation of nicotine associated cues. In terms of differences, the 0.015-IntA group in their study did not reinstate at the 30th day when exposed to cues associated with nicotine consumption, whereas it reinstated here. Finally, one difference between the two studies is that here a combination of daily and hourly intermittence was used whereas Gueye and colleagues (2020) used an hourly-IntA model.

4. Comparisons with intermittent models of other drugs

While intermittent access to nicotine here is shown to produce similarity to a continuous access, studies of intermittent access for cocaine addiction found that the intermittent model produces stronger phenotypic symptoms of addiction, such as greater escalation of intake, greater motivation (Kawa, allain, Robinson & Samaha, 2019) and sensitization of cocaine-induced dopamine overflow (Kawa, Valenta, Kenedy, & Robinson, 2019). The differences between cocaine and nicotine can be used to gain some knowledge on the mechanisms to which intermittent access has an effect. For instance, IntA cocaine produces escalating dose and some stronger addictive symptoms than LgA. In contrast the data here show that IntA nicotine does not produce stronger addictive symptoms than LgA nicotine. Such differences point to the diverse pharmacological effects of these drugs. In other words, there are differences between the effect of the IntA for these two psychostimulants because their pharmacological effects are different. It also means that the stronger addictive symptoms seen with IntA cocaine compared to LgA cocaine, or the equivalent addictive symptoms seen with IntA nicotine compared to LgA nicotine, are not only due to intrinsic ways of the brain to respond to the intermittency of a reward. Yet, it is possible

that the brain's natural functioning is influenced by the intermittency of natural reward encoding (Berridge and Robinson, 1993).

5. Neurobiological mechanism

The intermittent nicotine self-administration in comparison to continuous access self-administration may involve different effects of sensitization and desensitization of nicotinic acetylcholine receptors. For instance, several studies have shown that some of the nicotinic acetylcholine receptors become desensitized after chronic exposure to nicotine leading to an increase in the expression of these receptors (Changeux et al., 1984; Mansvelder et al., 2002; Marks et al., 1983; Schartz & Kellar, 1983; Wonnacott, 1990; Flores et al., 1999;) however, others have also found the opposite (Gentry et al., 2003; Marks et al., 1993). Recent research from Semenova and colleagues (2018) address this question by comparing intermittent and continuous non-contingent nicotine effects on the brain. For instance, non-contingent intermittent nicotine produces upregulation of α4β2* nicotinic acetylcholine receptors in the ventral tegmental area and the prelimbic cortex, but not in the nucleus accumbens. A finding that they were able to replicate from Baker and colleagues (2013). They also find that compared to continuous non-contingent infusion of nicotine, non-contingent intermittent nicotine does not produce somatic sign of withdrawal. These findings and the findings of the present study warrant the need to investigate the neurobiological mechanisms of contingent intermittent access to nicotine.

6. The role of environmental stimuli

The pharmacokinetics of nicotine are not the only factor involved in the present model. For instance, nicotine seeking behaviour in rats is influenced by environmental stimuli (Caggiula et al., 2001; Chaudhri et al., 2005; Cohen et al., 2005; Lesage et al., 2004; Paterson et al., 2005). When stimuli are paired with self-administration, rats will acquire nicotine self-administration

more rapidly, consume more nicotine, and will be more motivated to self-administer nicotine and for a longer period of time (Caggiula et al., 2001; 2002; Lesage et al., 2004; Chaudhri et al., 2005; 2006, 2007, Cohen et al., 2005; Paterson et al., 2005). The intermittent access rats may be differently affected by environmental cues. For instance, the reinforcing value of the cues was assessed at the cues induced reinstatement tests. Despite being exposed to fewer cues and consuming a decreased amount of nicotine, the intermittent access rats also reinstated their nicotine seeking behaviour both at 15 and 30 days after their last 7-hour self-administration session. One possible factor that could explain the reinstament of the intermittent access rats at the cue-induced reinstatement test, would be that the environmental cues were intrinsically reinforcing and that even if these rats would have no previous history of nicotine self-administration they would have pressed the lever because it is a pleasing stimulus to them (Caggiula et al., 2001; Rose, & Corrigall, 1997). However, when paired together, nicotine and nicotine-paired cues have a synergestic effect—their combination produces greater degrees of response than if the amount of response for each are added together (Caggiula et al., 2002). Therefore, it can be at least assumed that the results seen are due to both nicotine and the nicotine-paired stimuli. Future studies could investigate the possible differences in the role of cues on nicotine self-administration behaviour of intermittent access rats. This is important since cues produce high level of responding compared to nicotine alone (Caggiula et al., 2002).

7. Comparisons with the nicotine intake in humans.

Previous intravenous self-administration studies have shown that the nicotine intake increases the dopamine overflow in the nucleus accumbens (Pontieri et al., 1996) and is reduced by systemic dopaminergic antagonists and by dopaminergic-depleting lesions, which suggests that dopaminergic transmission is implicated in the reinforcing effects of nicotine in rats (Corrigall and

Coen, 1991; Corrigall et al., 1992). However, these studies used high unit doses of nicotine (30 – 50 μg/kg i.v.), which are very different from those that cigarette smokers would be exposed to. Indeed, cigarette smokers extract 1 to 3 μg/kg nicotine per puff, which equals to 10 to 30 μg/kg per cigarette (Matta et al., 2007). Therefore, a 15 μg/kg dose of nicotine per infusion was selected for the present experiment, which allows rats to self-administer nicotine infusions that mimic more closely the pharmacokinetics of cigarette smoking in humans (Sorge & Clarke, 2009). A higher unit dose of nicotine (30 μg/kg/infusion) often used in several previous animal studies was also selected because it generates maximal responses, thus allowing the possibility of comparisons and evaluations of the effects of varying the infusion dose on the reinforcing value of nicotine (Flores, Uribe, Swalve, & O'Dell, 2019).

8. Applicability of the model to vaping products

As the problem of cigarette smoking declined considerably over the last four decades, another problem appeared—vaping. The use of e-cigarettes is becoming more popular among adolescents (**Fig. 1**, Surgeon General report, 2016) and will require specific attention. Although it is possible

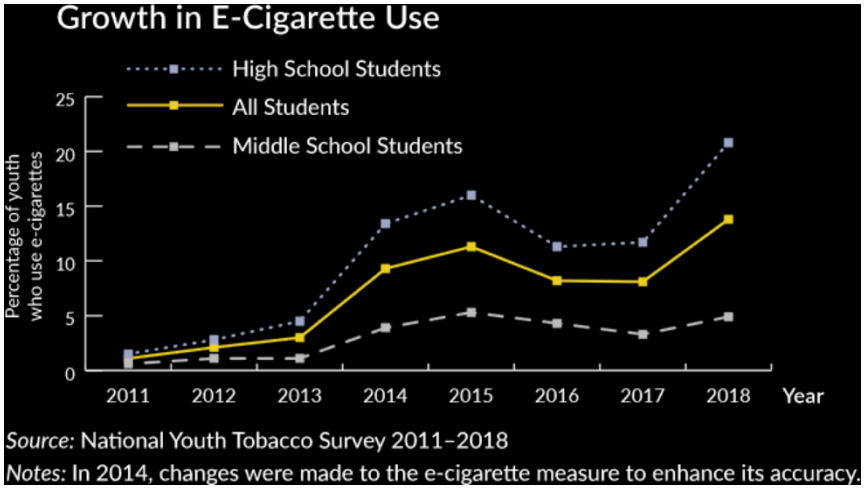

***Figure 1*. Rise in the proportion of youth who use E-Cigarettes.** Figure from the 2016 Surgeon General's report on e-cigarette.

that the pattern of use in vaping may be different from cigarrette, vaping is also banned from public areas in Canada and vaping was taken seriously by the Canadian government as in 2018 the tobacco act was reformed to be named the Tobacco and Vaping Products Act (TVPA). Hence, e-cigarette users—just like cigarette users—are limited in when and where they can get their nicotine. This inability to smoke wherever and whenever they want brings similarities to the present intermittent access model.

9. If the IntA rats express just as much phenotypic aspects of nicotine addiction as the LgA rats, does that mean that the intermittent model is not necessary?

At first glance, it may seem that no difference means that nothing interesting is happening and that the two models produce the same phenotypic aspect of nicotine addiction. However, this is not the case. The two models are different in fundamental elements. One of these elements is that, just as it was observed by Kawa, Allain, Robinson and Samaha (2019), the temporal pattern of intake poses a challenge to the idea that the consumption of large amounts of a drug is necessary for the development of the phenotypic aspects of the addiction. Here, it was shown that despite consuming up to 72% less nicotine than LgA rats, the IntA rats were just as much motivated to obtain nicotine and susceptible to relapse induced by re-exposure to cues associated with their nicotine consumption or by unhabituated saline and nicotine re-exposure.

10. Since the results between IntA and LgA are similar, does that mean that it doesn't matter whether an intermittent model or a continuous long-access model is used?

Although the two models do not differ in the phenotypic symptoms of addiction evaluated here, the IntA model may have different impacts in the brain (Kawa et al., 2019a). Such differences are seen in non-contingent nicotine administration of intermittent nicotine in comparison to continuous nicotine (Brynildsen et al., 2016; Ghoshesh, Dwoskin, Miller, & Crooks, 2001). On the contrary, the continuous access model is still valuable since not all countries have the same

smoking policies that ban smoking from public places and thus these countries' tobacco policies do not influence the pattern of nicotine intake into a more intermittent one.

11. Implications for nicotine addiction in humans

There are multiple implications to this model. First, by using a model that more closely imitates the pattern of consumption in humans, it will be more appropriate to use preclinical findings from such a model in clinical help for smokers. Second, because the model used a combination of daily and hourly intermittent access, some statements can be made for specific individuals; smokers who try to wean out of smoking may not be so successful since from the present results their motivation for nicotine and their susceptibility to relapse are no different than someone who can continuously use nicotine. Moreover, individuals who start smoking, mostly adolescents or young adults, will likely start by smoking in a daily intermittent pattern (sometimes referred to as non-daily; Cohen,, Koob and George, 2012). The present results suggest that although it may seem less perilous to smoke less, adolescents would transition to the addiction of nicotine just as fast and strong as someone that would be able to smoke more often.

12. Limitations of the present study and future directions

Male rats were used to study the differences between the intermittent access and continuous access to nicotine. However, this research must also be investigated in females. Several sex differences are found in preclinical studies of nicotine addiction. Females will self-administer more nicotine in the presence of a light cue (Chaudhri et al., 2005), when the number of lever presses to self-administer nicotine is higher than one lever press, or when self-administration sessions last 23 hours (Flores et al., 2019). Therefore, because the current model uses a fixed-ratio of three lever presses to self-administer nicotine, the present model may produce greater self-administration in female rats.

Additionally, the cues used in the present experiment were a combination of a light and sound stimulus and findings from the meta-analysis of Flores and colleagues (2019) suggests that such combination stimuli do not produce an increase in self-administration in female. As Wise & Bozarth (1987) proposed in their psychomotor stimulant theory of addiction, drugs of abuse including nicotine (Schoffelmeer, De Vries, van de Ven, & Vanderschuren, 2002) influence the mesolimbic dopamine pathway (Di Chiara, & Imperato, 1988). Thus, the influence of drugs on this pathway can be observed by the psychomotor sensitization that occurs when stimulant drugs are taken. However, in the present experiment, locomotion was not measured, and it is an important variable that requires further investigation. For instance, Baker and colleagues (2013) found that injecting a nicotine dose of 0.4 mg/kg/ml intraperitoneally four times a day for over seven days produced a sensitized locomotor activity for the drug. This suggests that a locomotor sensitization would occur in intermittent access rats. However, it does not present a comparison with long access rats. Moreover, non-contingent administration of drugs does not produce the same effects as contingent administration.

Conclusion

In the last four decades, large decreases in tobacco smoking have occurred. The adoption of several policies such as bans of tobacco marketing and smoking bans are accounted for such success. One important effect of smoking bans is a change in the pattern of nicotine consumption, since smokers are now limited in the environment and context where they can smoke (Chandra et al., 2007). The aim of this study was to provide a rat model that better fits the current cultural context of smoking and to evaluate if the change in pattern produces differences in the symptoms associated with nicotine addiction. The results here show that rats that were subjected either to continuous access to nicotine or an intermittent access over multiple sessions were similar in their motivation to self-administer nicotine and also similar in their susceptibility to relapse following re-exposure to stimuli associated with their nicotine consumption, as well as their susceptibility to relapse when re-exposed to nicotine or a saline subcutaneous injection. Such similarity can be surprising when considering that rats with the intermittent access take far less nicotine in comparison to the continuous access rats. To conclude, this research benefits scientific literature as it is the first model to utilize an intermittent access to nicotine self-administration.

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Appendix

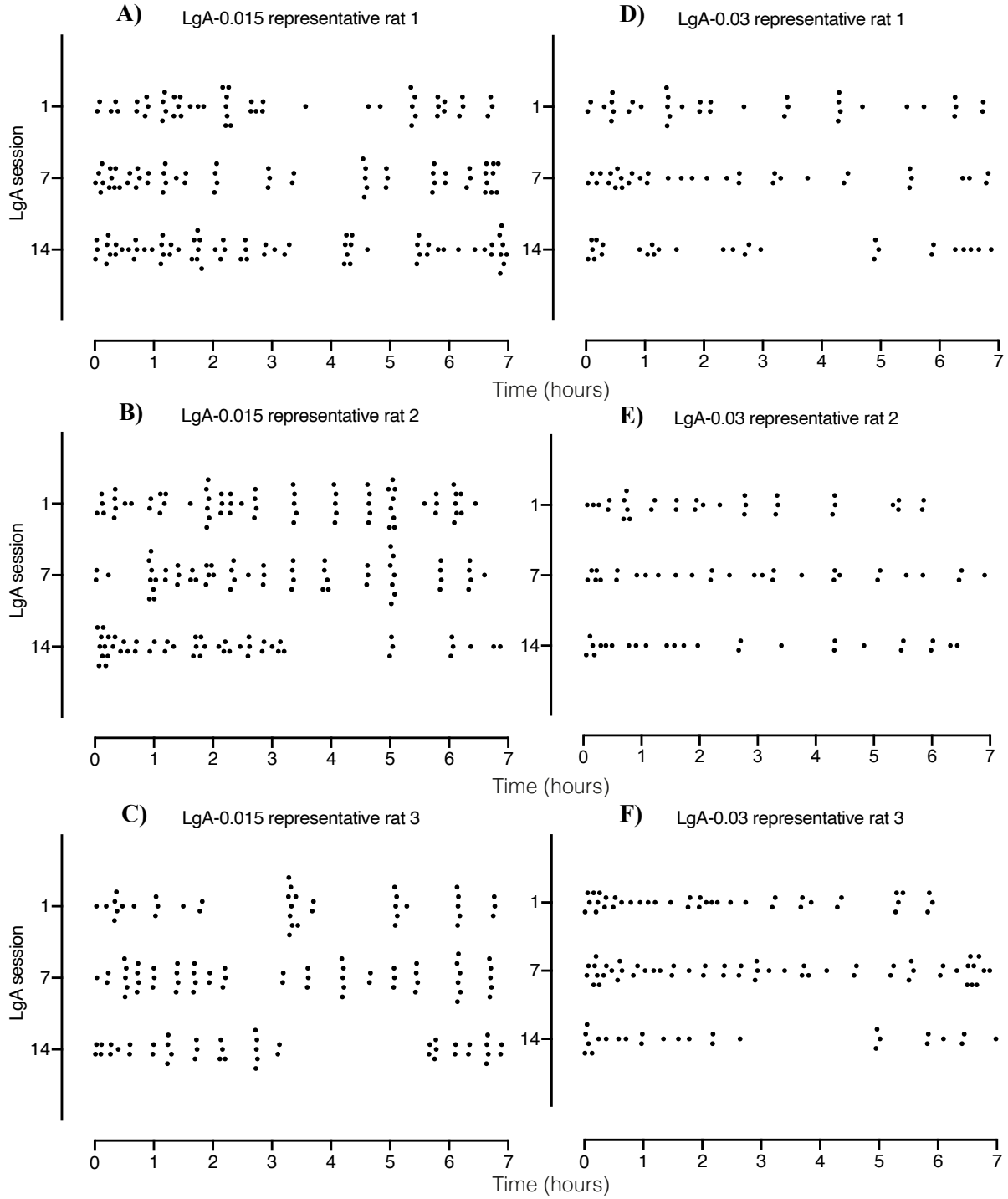

***Figure S1*. Within session detailed consumption patterns from representative long access rats**. Three representative LgA rats per dose of nicotine are shown to depict the precise intake pattern of nicotine intake during the 1st, 7th and 14th nicotine self-administration sessions. The Y axis represents the 1st, 7th and 14th sessions. The X axis has major tick at each hour but individual data points are plotted at the precision of minutes (420 minutes in total; 7h*60min).

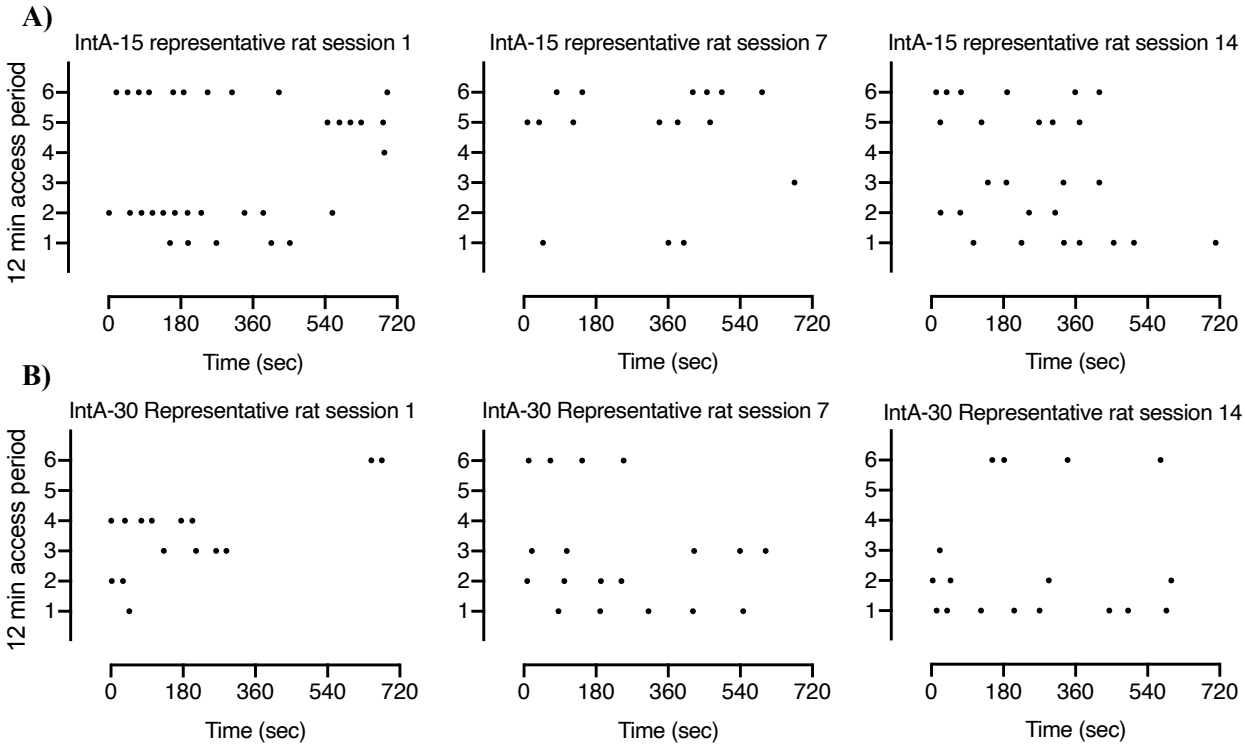

***Figure S2*. Representative rats detailed within session consumption per 12 min nicotine access cycles divided in time bins of 180s from a representative rat for session 1, 7 and 14 of the 7h self-administration phase**. Two representative IntA rats one for each dose of nicotine (**A**, IntA-0.015; and **B**, IntA-0.03) were selected to depict the average pattern of intake during each of the 6 cycle of the 1st, 7th and 14th session. The Y axis on each graph represent the 12 minutes access cycle. The X axis represents the time of the cycle. Each graph from left to right represent the 1st 7th and 14th sessions.

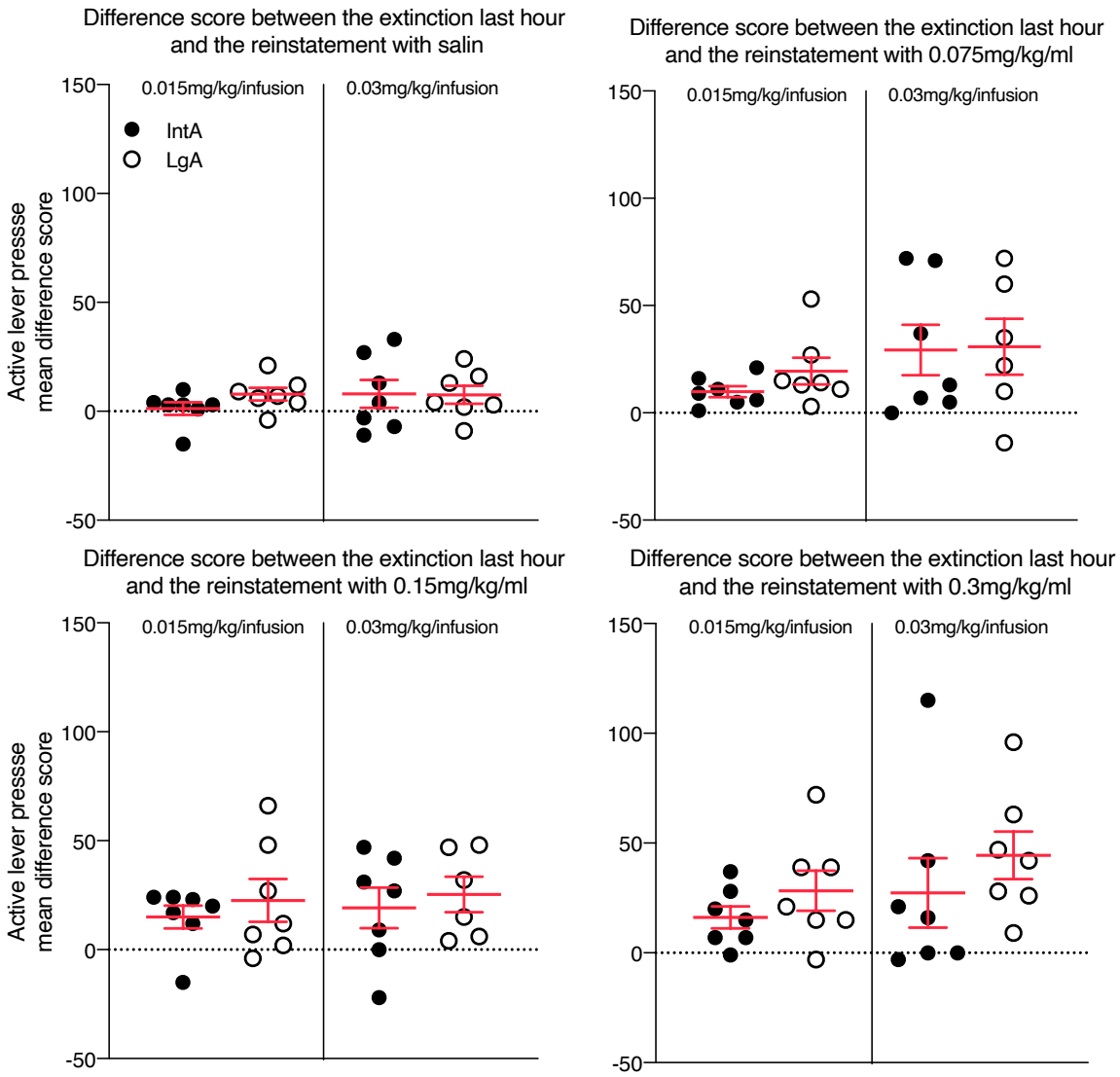

***Figure S3*. Difference-scores between the number of active lever presses during the extinction period and the subsequent nicotine- or saline-induced reinstatement test.** Scores above zero demonstrate an increase in active lever presses at the reinstatement test induced by either saline or nicotine relative to the number of active lever presses during the last hour of the extinction session that preceded each test.

Pearson *r* values				
	IntA-0.015	IntA-0.03	LgA-0.015	LgA-0.03
W-Day 15	0.5913	0.5811	-0.4558	0.5853
W-Day 30	-0.305	0.6614	0.4822	0.1197

***Table S1*. Correlation matrix between the difference score obtained in the cue-induced reinstatement and the cumulative nicotine intake over the 14 sessions of nicotine self-administration**. W-Day stand for withdrawal day.