Characterization of Abuse Properties of the Anesthetic Propofol

Using the Self-administration Paradigm in Rats

by

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University of Toronto

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Abstract

Propofol is a widely in use anesthetic drug. Propofol’s abuse liability has been supported by many case reports and a few animal studies. However, propofol’s reinforcing properties have not yet been investigated in-depth. In this study, multiple aspects of propofol’s abuse-related behaviour were investigated using the drug self-administration model in rats. METHODS: Rats were subjected to propofol self-administration under a fixed ratio 1 (FR1) schedule and different aspects of propofol self-administration behaviour including acquisition, maintenance of the behaviour under a higher ratio schedule, extinction and reinstatement were investigated. RESULTS: Rats acquired propofol self-administration under a FR1 schedule. The acquired behaviour was maintained under a FR2 schedule, showed a modest variation over a range of doses, and was extinguished upon substitution of vehicle for propofol, showing no reinstatement using a range of priming doses of propofol. CONCLUSION: Propofol has abuse potential showing modest reinforcing properties under our experimental conditions.
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Summary of Abbreviations

CYP   Cytochrome P450
CYP2B  Cytochrome P450 2B
CYP2B6 Cytochrome P450 2B6
DSM-IV Diagnostic and Statistical Manual of Mental Disorders (fourth edition)
FR Fixed ratio
GABA γ-aminobutyric acid
GABA_A γ-aminobutyric acid type A receptor
i.p. Intraperitoneal
i.v. Intravenous
mRNA Messenger RNA
PR Progressive ratio
s Second
UNODC The United Nations Office on Drugs and Crime
Section 1: Introduction

1.1 Substance use disorder

According to the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) “substance use disorder” consists of two main categories that are described as “substance abuse” and “substance addiction.” Both of these substance-related disorders are linked to a maladaptive pattern of substance use leading to clinically significant impairment. In addition to having a profound impact on an individual’s life, substance use disorders have enormous health, social and economic consequences.

The United Nations Office on Drugs and Crime (UNODC) has estimated that about 230 million people, or 5% of the world’s adult population, have used an illicit drug at least once in 2010 (UNODC 2012). According to the Canadian Alcohol and Drug use Monitoring Survey in 2011, the prevalence of past year alcohol and cannabis use was 78.0% and 9.1% respectively among Canadians 15 years and older (http://www.hc-sc.gc.ca/hc-ps/drugs-drogues/stat/_2011/summary-sommaire-eng.php).

The abuse of illicit drugs and alcohol claims the lives of more than 100,000 Americans each year, and tobacco use alone leads to an estimated 440,000 deaths per year (http://www.drugabuse.gov/publications/drugfacts/understanding-drug-abuse-addiction). From a global perspective, only one in five people who are in need of substance-related treatment actually receive it. It is anticipated that about US$200-250 billion would be needed to cover all costs of substance-related treatment worldwide (UNODC 2012).
1.2 Abuse liability of prescription drugs

In addition to tobacco, alcohol and illicit drugs (e.g. cannabinoids and stimulants), some prescription drugs are included as drugs of abuse because of their potential for non-medical (recreational) use. Non-medical use of prescription drugs is a health problem in many countries. For instance, in the United States, lifetime, annual and monthly prevalence of non-medical use of psychotherapeutics (mostly pain relievers) among persons 12 years and older was 20.4%, 6.3% and 2.7% in 2010, respectively (UNODC 2012). Recreational use of prescription drugs also occurs in combination. For example, opiate users experienced enhanced subjective effects with the combination of opiates and benzodiazepines, in comparison to when either drug was used alone (Lintzeris, Mitchell et al. 2007).

The commonly abused prescription drugs can be classified as depressants (e.g. barbiturates, benzodiazepines and sleep medications), opioids and morphine derivatives (e.g. codeine, morphine, methadone, fentanyl and oxycodone), stimulants (e.g. amphetamines, methylphenidate) and other compounds (e.g. dextromethorphan) (http://www.drugabuse.gov/publications/research-reports/prescription-drugs). In addition to these classes of prescription drugs, some anesthetic agents (e.g. isoflurane and ketamine) have also shown non-medical use and abuse potential. The abuse liability of drugs used in anesthetic practice will be discussed in the following section.
1.2.1 Abuse liability of drugs used in anesthetic practice

The anesthetic state consists of three components including unconsciousness, attenuation of autonomic responses to noxious stimuli and immobilization (Becker and Rosenberg 2008). Drugs in anesthetic practice can be divided into two main categories, preoperative drugs and anesthetic agents.

1.2.1A Preoperative drugs

Analgesics (e.g. \(\mu\) agonist opioids), anxiolytics (e.g. benzodiazepines) and anticholinergic agents (e.g. scopolamine) are the three main classes of drugs in the preoperative drug category (Zacny and Galinkin 1999). Preclinical and clinical data support the reinforcing effects and abuse liability of \(\mu\) agonist opioids such as fentanyl (Woods, Young et al. 1982; Hoehe, Duka et al. 1988), morphine and pethidine (Young, Swain et al. 1981; Lamb, Preston et al. 1991). Also, commonly used benzodiazepines such as diazepam (Griffiths, Lukas et al. 1981; de Wit and Griffiths 1991), lorazepam, alprazolam and triazolam (Funderburk, Griffiths et al. 1988; Evans, Funderburk et al. 1990; Griffiths, Lamb et al. 1991; Busto, Kaplan et al. 1994), as well as anticholinergic agents such as scopolamine (Glick and Guido 1982; Nuotto 1983) possess reinforcing effects and abuse potential.

1.2.1B Anesthetic agents

Inhalant (e.g. nitrous oxide, isoflurane, sevoflurane) and intravenous (e.g. methohexital, thiopental, pentobarbital, ketamine and propofol) anesthetics are used to induce and maintain unconsciousness during an operation. The abuse liability of some of these anesthetic agents has been documented by preclinical and clinical studies (Zacny and Galinkin 1999). The use of anesthetic agents is mostly limited to medical settings under the supervision of health
professionals. Therefore, health professionals are the main cohort for non-medical use of these drugs (Bell, McDonough et al. 1999; Luck and Hedrick 2004), although there are case reports outlining the abuse of anesthetic agents beyond medical settings by lay people (Schneider, Rada et al. 2001; Fritz and Niemczyk 2002).

1.2.1B1 Inhalant anesthetics with abuse potential

Since its discovery, nitrous oxide has been used as an inhalant anesthetic in both dentistry and medicine (Wilson, Kiselanova et al. 2008). Nitrous oxide has been self-administered by primates (Wood, Grubman et al. 1977) and has reinforcing effects in healthy volunteers, as well as moderate drinkers (Dohrn, Lichtor et al. 1993; Zacny, Cho et al. 1997; Zacny, Janiszewski et al. 1999; Walker and Zacny 2001; Wilson, Kiselanova et al. 2008).

The subjective effects of the volatile anesthetic isoflurane has been studied in non-drug abusing volunteers, showing sedative-like effects with substantial interindividual variability in drug liking (Zacny, Sparacino et al. 1994; Zacny, Janiszewski et al. 1999). Also, another volatile anesthetic, sevoflurane, has reinforcing effects in moderate drinkers, healthy volunteers, as well as healthy non-drug abusers (Zacny and Galinkin 1999; Zacny, Janiszewski et al. 1999; Walker, Beckman et al. 2004; Wilson, Kiselanova et al. 2008).

1.2.1B2 Intravenous anesthetics with abuse potential

Thiopental and methohexital are two short acting barbiturates commonly used in anesthesia (Zacny and Galinkin 1999). The reinforcing effects of thiopental have not been studied, but there is evidence that methohexital functions as a reinforcer in primates (Winger, Stitzer et al. 1975; Weerts, Ator et al. 1999) and rats (Pickens, Muchow et al. 1981). Although the information on the abuse liability of these two agents is limited, another short acting
barbiturate, pentobarbital, has abuse potential in rats (Denoble, Mele et al. 1985) and humans, with more prominent abuse liability in sedative abusers (Griffiths, Bigelow et al. 1980) than non-drug abusing volunteers (Cole-Harding and de Wit 1992).

Ketamine was introduced as a dissociative anesthetic having less toxic effects than phencyclidine (Jansen 2000). However, ketamine’s potential for abuse was noted less than a decade after its introduction (Jansen 2000; De Luca and Badiani 2011), and its misuse has been on the rise (Wolff and Winstock 2006; Wu, Schlenger et al. 2006; De Luca and Badiani 2011). Although ketamine is categorized as an intravenous agent in medical settings, it can be self-administered through different routes (e.g. intranasal and oral) and it is frequently used to augment other street drugs (De Luca and Badiani 2011). Consistent with data indicating the abuse potential of this anesthetic agent in humans, the intravenous self-administration of ketamine has been shown in rats (Collins, Weeks et al. 1984; Collins and Woods 2007; De Luca and Badiani 2011) and primates (Moreton, Meisch et al. 1977).

Propofol is another intravenous anesthetic agent with evidence indicating its reinforcing effects and abuse potential in humans. This anesthetic will soon be discussed from a broader perspective encompassing its therapeutic and abuse properties.

1.3 Propofol: an intravenous sedative, anesthetic drug

Propofol is a highly lipophilic intravenous anesthetic widely used in medical settings (Langley and Heel 1998). Propofol use has become widespread due to its rapid onset, short duration of action and fast recovery (Roussin, Montastruc et al. 2007). According to clinical surveys, propofol is the preferred anesthetic in operating rooms (Payne, Moore et al. 2003; Girard, Mauriat et al. 2004). A survey of urology and orthopedic day surgeries performed in 2000 in the UK has shown that propofol was the preferred anesthetic for induction of
anesthesia in 96.5% of cases (Payne, Moore et al. 2003); according to a French survey of cardiac surgeries conducted in 2001, 50% of patients underwent propofol-induced anesthesia (Girard, Mauriat et al. 2004).

Besides its well accepted use as an anesthetic agent, propofol is routinely used as a sedative in intensive care units (Abad-Santos, Galvez-Mugica et al. 2003). Other clinical indications of propofol include treatment of refractory seizure (status epilepticus), refractory migraine and tension headache, severe alcohol withdrawal and rapid opiate detoxification (Schneider, Rada et al. 2001; Marik 2004).

1.4 Propofol metabolism

1.4.1 Propofol metabolism in humans

The pharmacokinetics of propofol is described by a three-compartment model with compartments representing the plasma, rapidly equilibrating tissues such as the brain and slow equilibrating tissues such as adipose (Klausz, Rona et al. 2009). Upon intravenous administration, propofol displays an initial distribution half-life of 8 minutes, a slow distribution half-life ranging from 30-70 minutes and a terminal elimination half-life of 24 hours (Marik 2004). Propofol mainly undergoes direct glucuronidation which is mediated by the uridine diphosphate glucuronosyltransferase I family of enzymes (Vanlersberghe and Camu 2008). The oxidative hydroxylation of propofol is another metabolic pathway, in which propofol is hydroxylated, then subsequently conjugated to glucuronide or sulfate, before excretion in the urine (Marik 2004). The oxidative hydroxylation of propofol is mediated by cytochrome P450 enzymes (CYPs), with a prominent role for a member of the 2B subfamily, CYP2B6 (Court, Duan et al. 2001). Less than 3% of propofol is excreted unchanged in the urine and feces (Marik 2004).
1.4.2 Propofol metabolism in rats

In rats, CYP-mediated hydroxylation is the primary route of propofol metabolism (Le Guellec, Lacarelle et al. 1995). Direct propofol glucuronidation, measured in rat liver microsomes, revealed low activity in this species, which is in agreement with \textit{in vivo} data in rats showing the conjugated form of propofol’s hydroxylated metabolites (sulfate conjugates) as the main metabolites in urine (Le Guellec, Lacarelle et al. 1995).

1.4.3 Extrahepatic metabolism of propofol

In addition to hepatic metabolism of propofol, some extrahepatic sites are involved in propofol metabolism. Metabolism of propofol in extrahepatic sites has been confirmed in patients during the anhepatic phases of liver transplantation (Veroli, O’Kelly et al. 1992). In humans, the concentration of propofol in the central venous system was greater than in the radial artery, whereas the opposite was observed for propofol’s metabolite, 4-hydroxy propofol, indicating propofol metabolism in the lungs (Le Guellec, Lacarelle et al. 1995; Dawidowicz, Fornal et al. 2000). Also, kidney microsomes were able to glucuronidate propofol in rats and rabbits (Le Guellec, Lacarelle et al. 1995).

As the target organ for the sedative/anesthetic effects of propofol, the potential role of the brain in propofol metabolism has attracted some attention. In patients who underwent propofol-mediated anesthesia, blood samples from the radial artery showed higher levels of propofol and lower levels of propofol-glucuronide compared to blood samples from the internal jugular vein, suggesting brain-mediated metabolism of propofol (Zhang, Li et al. 2001). Experimental manipulations of brain CYP2B-mediated metabolism of propofol can alter the pharmacological effects of propofol. Inhibition of brain-expressed CYP2B activity by central administration of a mechanism-based inhibitor led to an increase in brain propofol
concentrations while plasma propofol levels remained unchanged, indicating no effect of the inhibitor on hepatic CYP2B activity (Khokhar and Tyndale 2011). Rats given this central administration of CYP2B inhibitor showed an increased propofol-induced sleep time which correlated with brain, but not plasma, propofol levels (Khokhar and Tyndale 2011).

1.5 Central mechanisms underlying propofol’s sedative and anesthetic effects

1.5.1 GABA-mediated transmission

GABA\textsubscript{A} receptors are ligand-gated chloride ion channels where receptor activation leads to an increase in chloride conductance and hyperpolarization of the postsynaptic membrane (Vanlersberghe and Camu 2008). Similar to other intravenous anesthetics, the sedative effects of propofol are mediated by a positive modulation of the inhibitory function of the neurotransmitter GABA through GABA\textsubscript{A} receptors.

Propofol affects GABA\textsubscript{A} receptor function in a dose-dependent manner. Within clinically relevant concentrations, low concentrations of propofol potentiated GABA-activated currents while moderate concentrations directly activated channel opening. However, at supratherapeutic concentrations, propofol suppressed the inhibitory system by desensitization of the GABA\textsubscript{A} receptors (Hara, Kai et al. 1993). Synaptic GABA accumulation due to inhibition of GABA uptake may also contribute to propofol-induced anesthesia (Mantz, Lecharny et al. 1995).
1.5.2 Other neurotransmission pathways

In addition to the GABA-ergic system, propofol interacts with other neurotransmitter receptors. Glycine receptors are ligand-gated chloride channels that mediate fast neuronal inhibition. Propofol, in a dose-dependent manner, enhanced strychnine-sensitive currents evoked by glycine in spinal neurons (Hales and Lambert 1991).

Tonic innervations by GABA-ergic input regulate the function of cholinergic neurons. GABA agonists such as muscimol exerted a marked inhibitory effect on basal acetylcholine release from the frontal cortex and hippocampus (Wood, Cheney et al. 1979). Similarly, propofol decreased basal acetylcholine release in the same cerebral regions (Kikuchi, Wang et al. 1998). Propofol dose-dependently inhibited the \( \alpha_{4}\beta_2 \) type of neuronal nicotinic acetylcholine receptors expressed in \textit{Xenopus laevis} oocytes (Flood, Ramirez-Latorre et al. 1997). Propofol was also able to inhibit the function of muscarinic acetylcholine type 1 receptors (Trapani, Altomare et al. 2000). These findings indicate that modulation of nicotinic acetylcholine receptors and interruption of central cholinergic muscarinic neurotransmission may contribute to propofol-induced unconsciousness. Propofol has noncompetitive inhibitory effects on the excitatory transmission of N-Methyl-D-aspartate receptors by modulating these receptors at a domain other than the agonist recognition sites (Orser, Bertlik et al. 1995).

1.6 Propofol: a drug with abuse potential

Propofol induces unconsciousness rapidly, is titrated easily and results in rapid awakening following discontinuation of administration (Abad-Santos, Galvez-Mugica et al. 2003). When administered by experienced and qualified clinicians, propofol is recognized to be safe for anesthesia and sedation (Levy 2011). Despite its well-described clinical and therapeutic properties, knowledge about propofol’s potential for abuse has been slow to
emerge, and the potential for its abuse, recreational use and dependency has recently raised some concerns (Wilson, Canning et al. 2010).

1.7 Potential mechanisms underlying propofol’s reinforcing effects

1.7.1 The mesolimbic dopamine system

The influence of motivational, emotional, contextual and affective information on behaviour is mediated by mesolimbic dopamine system (Pierce and Kumaresan 2006). The mesolimbic reward pathway is a collection of different brain regions and originates from ventral tegmental area. The dopaminergic projections of ventral tegmental area terminate primarily in nucleus accumbens but also innervate the amygdala, hippocampus, medial prefrontal cortex and ventral pallidum (Pierce and Kumaresan 2006).

Like natural rewards (e.g. food), most substances with abuse liability increase the extracellular levels of dopamine in mesolimbic system (Di Chiara and Bassareo 2007) and the critical role of this dopaminergic pathway in reinforcing properties of drugs has been documented. For instance, cocaine (McKinzie, Rodd-Henricks et al. 1999), d-amphetamine (Hoebel, Monaco et al. 1983) and opiates (Goeders, Lane et al. 1984) are self-administered directly into the rat nucleus accumbens and lesions of accumbal cell bodies disrupt cocaine and heroin self administration in rats (Zito, Vickers et al. 1985).

Propofol at anesthetic (100 mg/kg i.p.) and sub anesthetic (60 mg/kg i.p.) doses increased the dopamine concentration in the rat nucleus accumbens which is one of the major components of the reward system (Pain, Gobaille et al. 2002). Application of nanomolar amounts of propofol to slices of rat brain ventral tegmental area enhanced the glutamatergic excitatory synaptic transmission and discharge of dopamine neurons (Li, Xiao et al. 2008). This effect of propofol was blocked by application of a dopamine type 1 receptor antagonist
suggesting the presynaptic dopamine (type 1) receptor-mediated facilitation of glutamatergic synaptic transmission and the excitability of ventral tegmental area dopamine neurons, probably by increasing extracellular dopamine levels (Li, Xiao et al. 2008). These changes in the ventral tegmental area, an addiction-related brain region, might contribute to the development of propofol abuse (Li, Xiao et al. 2008). Administration of a dopamine type 1 receptor antagonist, peripherally or directly into the nucleus accumbens, reduced propofol self-administration in rats indicating the role of dopaminergic reward system in mediating propofol’s reinforcing properties (Lian, Wang et al. 2013). Therefore, it can be concluded that the dopaminergic reward system likely also plays a central role in propofol’s reinforcing effects.

### 1.7.2 Other mechanisms involved in mediating propofol’s reinforcing effects

The role of GABA<sub>A</sub> receptors in the reinforcing effects of propofol has been suggested. Systemic administration of a low dose of bicuculline, an antagonist of GABA<sub>A</sub> receptors, before self-administration training significantly increased propofol self-administration in rats, suggesting a compensatory response (Yang, Wang et al. 2011).

The transcription factor DeltaFosB in the nucleus accumbens is a critical signaling molecule in drug abuse (Nestler 2008) and is induced by many drugs with abuse liability, including cocaine, morphine, ethanol and marijuana (Perrotti, Weaver et al. 2008). Intraperitoneal administration of 10 mg/kg propofol for 7 days resulted in a robust increase in DeltaFosB expression in rat nucleus accumbens at both protein and mRNA levels (Xiong, Li et al. 2011). This propofol-induced elevation in DeltaFosB expression was similar to that induced by nicotine and ethanol (Xiong, Li et al. 2011).
The endocannabinoid system may also be involved in mediating propofol’s pharmacological effects. *In vitro*, propofol is a competitive inhibitor of fatty acid amide hydrolase which catalyses the degradation of the endocannabinoid anandamide (Patel, Wohlfeil et al. 2003). In agreement with this finding, the brain content of anandamide was increased in mice during propofol-induced loss of the righting reflex (Patel, Wohlfeil et al. 2003). This effect may contribute to propofol’s sedative properties and abuse liability.

1.8 Evidence of propofol’s rewarding and subjective effects in humans

1.8.1 Case reports

A considerable number of case reports indicate the abuse potential of propofol, with an increase in recreational use of this drug among health professionals over recent years (Luck and Hedrick 2004; Wischmeyer, Johnson et al. 2007). A survey of anesthetist nurses revealed a changing trend in recreational drug use, suggesting propofol as the fourth most misused drug (Bell, McDonough et al. 1999). A survey including all 126 academic anesthesiology training programs in the United States, focusing on the prevalence of propofol abuse, found that one or more cases of propofol abuse were reported by 18% of these departments over the 10 year period from 1995 to 2005. This study showed a five-fold increase in propofol abuse, indicating an important and unrecognized problem (Wischmeyer, Johnson et al. 2007). Although the majority of case reports involve health professionals, propofol abuse among lay people has also been documented (Schneider, Rada et al. 2001; Fritz and Niemczyk 2002).
1.8.2 Evidence of propofol’s abuse-related deaths in humans

Propofol’s pharmacodynamic effects on the cardiovascular and respiratory systems may have potent side effects (Levy 2011). Propofol reduces systolic and diastolic blood pressure by 25-40% through diminished sympathetic vascular tone, effects on vascular smooth muscle calcium flux, and endothelial nitrous oxide release (Marik 2004). Propofol reduces respiratory drive and decreases protective airway reflexes; without proper supervision by an experienced provider, a single injection of propofol can result in apnea, respiratory arrest, hypoxia and death (Marik 2004).

As mentioned earlier, the recreational use of propofol is more abundant in health professionals. Of 45 reported cases of propofol abuse or dependency, 89% (40 of 45) involved health professionals, 40% (18 of 45) resulted in fatality and 83% (15 of 18) of these propofol abuse related deaths were of professionals (Wilson, Canning et al. 2010). In a survey of US anesthesia training programs, propofol abuse caused death in 38% of residents (6 of 16) who were reported of abusing the drug (Wischmeyer, Johnson et al. 2007). It seems that even among medical professionals familiar with propofol’s appropriate use and potential risks, propofol can be lethal when it is used as a recreational drug.

1.8.3 Human clinical data

The discrete-trial choice procedure is an experimental tool to assess the potential for positive reinforcement of drugs and has been used with many well-known drugs of abuse such as amphetamines (Lintzeris, Mitchell et al. 2007), nitrous oxide (Dohrn, Lichtor et al. 1993), caffeine (Stern, Chait et al. 1989), delta-9-tetrahydrocannabinol (Chait and Zacny 1992) and methylphenidate (Chait 1994). Nitrous oxide and caffeine were preferred over placebo by subjects in 41.6% and 42.6% of trials, respectively, which was not significantly different from
chance. However, in another study using the discrete-trial choice approach, all participants chose oral tetrahydrocannabinol and smoked marijuana over placebo (Chait and Zacny 1992).

The discrete-trial choice paradigm has been employed to evaluate propofol’s rewarding effects on healthy volunteers (Zacny, Lichtor et al. 1993). Two acute bolus injections of 0.6 mg/kg propofol and a similar volume of soy-based lipid emulsion control (intralipid) were given intravenously (i.v.) to 12 healthy volunteers in a blinded fashion. In the next session, subjects chose the drug they wished to receive. Propofol was chosen by half of the subjects based on its pleasant subjective effects, which were described as feeling high, light headed, spaced out and sedated. In contrast, increased dizziness and confusion or residual effects (e.g. fatigue) after the first session were described by the rest of subjects, who chose placebo (intralipid) over propofol. The results of this study suggested that propofol served as a reward in some individuals (Zacny, Lichtor et al. 1993) but not in others, similar to nitrous oxide and caffeine as mentioned earlier. In an attempt to explain the variability that was seen in this study, the authors hypothesized that a history of exposure to other drugs with abuse liability such as opiates (via prescribed use) could serve as a reason for choosing propofol over vehicle. However, some subjects without any past exposure to opiates also chose propofol, making it difficult to explain the interindividual differences in propofol preference in the latter study (Roussin, Montastruc et al. 2007).

In normal, healthy human volunteers, propofol, at a dose range of 0.2-0.6 mg/kg i.v., increased self reports of “drug liking” in a double blind cross over study (Zacny 1993). Likewise, propofol’s pleasant subjective effects, such as euphoria, sexual hallucinations and disinhibition, were described by patients after recovery from propofol anesthesia (Pakrashi and Park 2004), and 40% of 542 patients awoke after propofol anesthesia with feelings described as pleasurable (Brazolotto 1989).
1.9 Common animal models used in evaluating abuse liability of drugs

A critical component in preclinical assessment of abuse liability is to determine whether or not a drug of interest functions as a reinforcer (Ator and Griffiths 2003). To achieve this goal, different experimental approaches have been developed to assess the abuse liability of psychoactive drugs such as conditioned place preference, drug discrimination and self-administration paradigms.

1.9.1 Conditioned place preference

Conditioned place preference evaluates the motivational properties of a drug by means of Pavlovian conditioning, where the test drug and vehicle are administered to subjects in two distinct environments. After several pairings, the drug-paired environment (conditioned stimulus) becomes associated with the effects of the drug (unconditioned stimuli) and acquires incentive-motivational properties (O'Dell and Khroyan 2009). On the test day, during which there is no drug administration, animals are allowed to freely explore both environments. If the test drug has rewarding effects, the subjects are expected to spend more time in the drug-paired environment (O'Dell and Khroyan 2009; Lynch, Nicholson et al. 2010).

1.9.2 Drug discrimination

Drug discrimination is a way of examining the subjective properties of drugs (Balster 1991). Briefly, this procedure is based on state-dependent learning. During the training phase, repeated pretreatment with a training drug, such as a drug with abuse properties (e.g. cocaine), is paired with an operant response (e.g. lever pressing) which in turn results in the presentation of a reinforcer (e.g. food pellet). After completion of the training sessions, the drug of interest with unknown abuse potential is substituted for the training drug. If substitution leads to the
same operant response, this indicates that the training and test drugs have similar effects (Lynch, Nicholson et al. 2010).

Similar to conditioned place preference, drug discrimination is not a direct way to determine the reinforcing effects of a drug per se. However, drugs that share discriminative stimuli are likely to have a common pharmacological mechanism of action and similar reinforcing effects (Schuster and Johanson 1988). Drugs that show substitution for each other have shown similar subjective effects in humans (Schuster and Johanson 1988; Preston and Bigelow 1991).

1.9.3 Self-administration

The self-administration paradigm is a central approach in preclinical abuse liability assessment, providing a direct assessment of the reinforcing properties of a test drug (Ator and Griffiths 2003). The fundamental principle of self-administration is based on the strengthening of an operant behaviour by the presentation of a drug of interest after the operant response is performed (O'Dell and Khroyan 2009). By definition, an operant response is a purposeful and voluntarily emitted behaviour (e.g. lever press or nose poke) that can be modified by its consequences (e.g. food or drug delivery) (Lynch, Nicholson et al. 2010). The contingency between making a particular operant response and delivery of the putative reinforcer is specified by different schedules of reinforcement in to two main categories, fixed ratio and progressive ratio schedules (Ator and Griffiths 2003). In a fixed ratio (FR) schedule, a fixed number of operant responses, such as lever presses, is required for each drug delivery. For instance, on a FR5 schedule, responding is reinforced after every five lever presses. On FR schedules, especially FR1, animals learn more easily than on progressive ratio (PR) schedules, which makes the FR schedule an effective tool in the initial screening of a drug’s abuse
potential (Richardson and Roberts 1996). A progressive ratio schedule is a higher order schedule in which the response requirement increases systematically (e.g. 5, 10, 20, etc.) for each successive reinforcement. On a PR schedule, responding for the reinforcer will eventually cease when the response requirement becomes too great. This point is the break point and is a measure of a drug’s reinforcing efficacy (Richardson and Roberts 1996).

In addition to humans, the self-administration paradigm has been used to assess the reinforcing properties of drugs in different species including mice, rats and non-human primates. The close phylogenetic link and similarities in drug metabolism and other physiological characteristics with humans, make primates an appropriate choice for self-administration studies (Ator and Griffiths 2003). However, a recent review revealed that the results from primate self-administration studies were mostly mirrored by self-administration studies done in rats, with a few exceptions (O'Connor, Chapman et al. 2011). Haloperidol, imipramine and phenylpropanolamine are self-administered by rats, but not by monkeys, and there is no evidence of positive subjective effects of these drugs in humans. In contrast, self-administration behaviour was not established in rats given modafinil or hexobarbital, while the results from monkey studies were in line with the positive subjective effects of these drugs in humans (O'Connor, Chapman et al. 2011). For these few discrepant cases between rats and monkeys in self-administration, due to the limited data, it is not clear whether these cases truly reflect specific limitations of the rat for understanding the abuse liability of drugs (O'Connor, Chapman et al. 2011).

Rats offer distinct advantages over primates in terms of cost, care and availability, thereby providing a good alternative to primates in self-administration studies. Like in non-human primates, self-administration in rats is considered as an animal model with high predictive validity for the assessment of the abuse potential of drugs (O'Connor, Chapman et
al. 2011). The self-administration paradigm also has a high level of face validity because it mimics the voluntary consumption of drugs with abuse potential (O'Dell and Khroyan 2009). The most common routes of drug administration are intravenous and oral (Lynch, Nicholson et al. 2010). Generally, the route of drug delivery in self-administration studies is determined by the route used in humans for a particular drug. For example, studies with alcohol typically employ an oral route of administration, whereas for drugs such as cocaine and heroin, an intravenous route is the most appropriate route of self-administration to mimic the rapid onset produced by intravenous administration of these drugs in humans (Carroll and Mattox 1997; Lynch, Nicholson et al. 2010). In the following sections, different aspects of drug-induced self-administration behaviour including acquisition, extinction and reinstatement will be discussed.

1.9.3A Acquisition of drug self-administration behaviour

The acquisition phase of the self-administration paradigm functions as a powerful investigational tool to screen drugs with abuse potential (Ator and Griffiths 2003). The study of initiation of drug self-administration is ethically challenging in humans. Therefore, the acquisition phase of the self-administration paradigm serves as an investigational tool to study vulnerability to the reinforcing effects of drugs and to elucidate the underlying biological and behavioural factors involved in this vulnerability (Campbell and Carroll 2000; Lynch, Nicholson et al. 2010). The different phases of the self-administration paradigm, including acquisition, are conducted in an operant chamber in which two levers are assigned as active (drug-contingent) and inactive (non-drug contingent) levers. Although inactive lever responses are not contingent with drug delivery and do not have any programmed consequences, they are recorded and serve as a control condition to be compared with active lever responses. Usually, during training for acquisition, every delivery of the test drug upon active lever response is
paired with a visual (e.g. illumination of a light) and/or auditory cue (e.g. a beep sound) known as a conditioned stimulus (Cleva and Gass 2010). Acquisition is measured by certain criteria, including the level of drug intake (e.g. number of drug infusions) and the ratio of active to inactive lever responses. The focus in the acquisition phase is on the percentage of animals that acquire self-administration behaviour and the number of sessions needed to reach acquisition criteria (Lynch, Nicholson et al. 2010).

There are two common methods to facilitate the acquisition of drug self-administration behaviour. In the first approach, priming injections of the test drug are given to subjects to stimulate exploration on the response manipulandum (Campbell and Carroll 2000). In the second method, subjects are trained to respond for a natural reward (e.g. food) or a training drug with known abuse liability which strongly initiates and maintains self-administration behaviour (e.g. cocaine) (Carter and Griffiths 2009). Then the drug of interest with unknown abuse liability is substituted for the natural reward or the training drug. After acquisition of drug self-administration, the maintenance of the behaviour over time and under different schedules, including escalating fixed ratio or progressive ratio, can be studied. This latter phase of the self-administration model provides further information about a drug’s reinforcing efficacy and the motivational aspects of the drug self-administration behaviour.
1.9.3B Extinction of drug self-administration behaviour

Extinction is defined as “the removal of a reinforcer from a previously established stimulus relationship that leads to reduction in responding” (Widholm 2010). The extinction phase of the self-administration paradigm provides measures of incentive and motivational effects of drugs by assessing the persistence of drug self-administration behaviour in the absence of drug delivery contingent with an operant response (Koob 1995). The information provided by an extinction experiment includes the duration of extinction responding and the total number of responses during the extinction session (Koob 1995). Extinction sessions are often identical to training sessions for drug self-administration except that there is no drug delivery after completion of an operant response.

1.9.3C Reinstatement of drug self-administration behaviour

Reinstatement of extinguished drug self-administration behaviour is another aspect of drug-induced self-administration. Following extinction of self-administration behaviour, the ability of various stimuli to restore drug seeking behaviour is determined under conditions in which operant responses are no longer reinforced by drug delivery. A stimulus is considered capable of reinstating drug seeking behaviour if it causes an increase in responding that previously was reinforced by drug delivery (Lynch, Nicholson et al. 2010).

Preclinical studies using a reinstatement paradigm have shown that the conditions that reinstate drug seeking in animals are similar to those that precipitate drug craving and relapse in humans including exposure to stressors, reexposure to small priming doses of the drug and cues associated with the drug (Katz and Higgins 2003; Lynch, Nicholson et al. 2010).
1.10 Evidence of propofol’s abuse potential using animal models

Different experimental approaches have been used to assess the abuse liability of propofol, including conditioned place preference, drug discrimination and self-administration paradigms. Propofol induced place preference in rats at subanesthetic doses (30, 60 mg/kg i.p.), as well as during recovery from a propofol-induced anesthesia at a dose of 100 mg/kg, suggesting that contextual cues associated with propofol induce a motivational effect in rats (Pain, Oberling et al. 1996; Pain, Oberling et al. 1997).

Using the drug discrimination approach, propofol served as a discriminative stimulus and rats discriminated propofol (10 mg/kg, i.p.) from vehicle (2% methylcellulose). Propofol’s discriminative effect is similar to other compounds that act on GABA\(\alpha\) receptors (e.g. carisoprodol, a barbiturate-like compound and chlordiazepoxide, a benzodiazepine) (Gatch and Forster 2011).

Propofol’s reinforcing effects have been studied in different species using the self-administration paradigm. Using a drug-substitution strategy to facilitate the acquisition process, baboons self-administered propofol when it was substituted for cocaine. In this non-human primate study, propofol given at subanesthetic doses maintained a range of low to high levels of self-administration behaviour, suggesting interindividual differences in the response to propofol (Weerts, Ator et al. 1999).

Propofol self-administration was acquired and maintained in rats that were preexposed to methohexital, as well as in drug-naïve rats, under fixed ratio schedules (FR1 and FR5) of drug delivery (LeSage, Stafford et al. 2000). Contrary to the results in baboons and rats, in another study using a self-administration method, mice did not acquire propofol self-administration (Blokhina, Dravolina et al. 2004). In this mouse study, the duration of self-administration sessions was limited to 30 minutes, compared to 1-hour sessions in rats.
(LeSage, Stafford et al. 2000) and 5.5-hour sessions in baboons (Weerts, Ator et al. 1999). The shorter session duration might contribute to the lack of propofol self-administration in mice, although interspecies differences should be taken into account as well. Although studies in baboons and rats have provided some evidence indicating propofol’s liability for abuse, there are still some caveats that need to be addressed by further investigations.

First, propofol was able to maintain self-administration behaviour when it was substituted for cocaine and methohexital in baboons and rats respectively. By using a drug-substitution approach to facilitate the acquisition of a drug of interest with unknown abuse liability, it is not clear whether the observed behaviour was due to the reinforcing effects of the test drug or the result of extinction from the training drug (Carroll and Meisch 2011). Another disadvantage of the drug-substitution strategy is that the animals are not drug-naive. Drug history and preexposure to drugs with abuse properties affect drug self-administration outcomes. Preexposure to ethanol increased ethanol self-administration and total ethanol intake in both ethanol avoiding (DBA/2J) and ethanol preferring (C57BL/67) mice (Lintzeris, Mitchell et al. 2007). Also, rats with the highest alcohol intake in the operant self-administration procedure acquired cocaine self-administration more readily than rats with the lowest alcohol consumption (Mierzejewski, Rogowski et al. 2003). Thus, acquisition of a drug can be accelerated by preexposure to the same, or even a different, drug with abuse liability.

Methohexital is a short acting barbiturate with known reinforcing effects in monkeys and rats (Weerts, Ator et al. 1999; LeSage, Stafford et al. 2000). Methohexital acts on GABAA receptors and shows comparable pharmacological effects with propofol (Donny, Caggiula et al. 1998). Therefore, substitution of propofol for methohexital (a reinforcer with the same pharmacological effects) or cocaine (from a different class of reinforcers) could possibly function as a confounding factor in the propofol-induced self-administration behaviour.
reported in rats and monkeys. Using drug-naïve animals in self-administration experiments eliminates drug history as a confounding factor and will enhance interpretation of the data. Although propofol-induced self-administration in drug-naïve rats has been reported (LeSage, Stafford et al. 2000), no general conclusions were made as the results of this study were analyzed and presented individually, and the only group analysis was based on the pooled data from two groups of naïve and non-naïve rats (N=6 each) under two different schedules (FR1 and FR5). This grouped analysis showed that 9 of 12 rats worked harder for at least one dose of propofol than for vehicle (intralipid). Because of the small sample size there was not enough statistical power to study the impact of different conditions such as propofol dose, schedule and drug history and their interactions on the acquisition and maintenance of propofol self-administration. Therefore, conducting self-administration studies using a sufficient number of subjects will help to pull apart the effects of each experimental manipulation, such as propofol dose and the ratio of schedule, on different aspects of propofol self-administration behaviour including acquisition and maintenance. Second, in the self-administration paradigm under a FR schedule, the relationship between drug dose and rate of responding often generates an inverted U-shaped dose response curve. This form of dose response relationship has been shown in different species self-administering different classes of drugs, including stimulants and depressants (Lynch and Carroll 2001). At low doses on the ascending limb of a self-administration U-shaped dose response curve, responding rate increases as a function of increasing unit dose, which is attributed to an increase in reinforcing effect. However, the regulation of drug intake is apparent with the descending part of the dose response curve where the magnitude of response and the drug dose are negatively related (Lynch and Carroll 2001). In baboons, propofol self-administration at different doses generated an ascending curve (Weerts, Ator et al. 1999).
the contrary in rats, the propofol self-administration dose response curve was a descending curve on a FR1 schedule using a narrow range of propofol doses (LeSage, Stafford et al. 2000). Generating a self-administration dose response curve using a wider range of propofol doses will reveal the full shape of the curve, which in turn will provide more information regarding the changes in reinforcing effects and the regulation of drug intake as a function of dose.

Third, it is a common approach to have both drug-contingent and non-drug contingent operant responses (e.g. lever presses) in a self-administration study. Pressing on the drug-contingent lever (active lever) is an indicator of reinforcing effects of a test drug, while the performance of a subject on the non-drug contingent lever (inactive lever) serves as a control condition. The number of inactive lever presses also functions as an indicator of non-specific effects of a drug on locomotor activity. The reinforcing effect of a drug can also be assessed by comparing the drug-induced operant response with vehicle-induced responding.

In the study of propofol self-administration in rats (LeSage, Stafford et al. 2000), both levers in the experimental chambers were active and drug-contingent, and detection of propofol’s reinforcing effects was based primarily on comparing vehicle-maintained responding with propofol-maintained responding. In this rat study, some animals displayed high levels of operant responding for vehicle on a FR1 schedule which, according to the authors, complicated detection of propofol’s reinforcing effects. Therefore, propofol self-administration experiments using a “two lever discriminated responding paradigm” and assessing the preference for active over inactive lever will provide additional and reliable information regarding propofol’s reinforcing effects.

Fourth, craving and relapse are two important features of substance addiction. There is some evidence of psychological dependence on propofol, such as loss of control and continued use despite negative consequences, strong cravings and frequent relapses (Follette and Farley
Relapse to propofol use is an aspect of propofol-induced self-administration behaviour which has not yet been investigated experimentally. To determine the factors that promote drug-seeking behaviour and result in relapse after a period of abstinence (e.g. priming doses of the drug), reinstatement experiments may be a useful approach.
1.11 Statement of research problem

Substance abuse is a global problem with profound personal, social and economic consequences. Drug diversion and non-medical use of prescription drugs impose additional complexity to the substance abuse phenomenon, which has been a growing health problem in many countries (UNODC 2012). Thus, abuse liability studies are used in order to place a drug under the appropriate schedule in the Controlled Substances Act (Carter and Griffiths 2009).

Propofol is an intravenous sedative anesthetic agent that has been in use for decades. However, indications of propofol’s abuse liability have recently raised some concerns (Wilson, Canning et al. 2010). Human data, including case reports and a few clinical studies (Zacny, Lichtor et al. 1993), have shown that propofol has abuse potential.

Some preclinical behavioural procedures, such as conditioned place preference, drug discrimination and drug self-administration, have been employed to evaluate the reinforcing effects of propofol. In rats, propofol induced place preference at subanesthetic doses (30, 60 mg/kg i.p.) as well as after recovery from an anesthetic dose (100 mg/kg) (Pain, Oberling et al. 1996; Pain, Oberling et al. 1997). Propofol also served as a discriminative stimulus, whereby rats discriminated propofol (10 mg/kg, i.p.) from its vehicle (2% methylcellulose) (Gatch and Forster 2011). Unlike the self-administration paradigm, conditioned place preference and drug discrimination do not provide a direct assessment of the reinforcing effects of drugs. Therefore, drug self-administration is the “gold standard” of non-human abuse liability testing owing to its high level of face and predictive validity (Carter and Griffiths 2009).

To date, very few studies have employed a self-administration procedure to assess propofol’s reinforcing effects. While propofol-induced self-administration behaviour has been shown in baboons (Weerts, Ator et al. 1999) and rats (LeSage, Stafford et al. 2000), these studies have limitations, and many aspects of propofol self-administration behaviour such as
extinction and reinstatement (a model of relapse) have not been studied yet. Further investigations are required to extend our knowledge of propofol’s reinforcing properties and to provide comprehensive information on different aspects of propofol self-administration behaviour, including extinction and reinstatement.

1.12 Objectives and hypotheses

The main objectives of this study were to establish a model of propofol self-administration in drug-naïve rats, and to provide detailed information about different aspects of the reinforcing properties of propofol, including acquisition, extinction and reinstatement of self-administration behaviour.

We hypothesize that:

1) Rats with no drug history will acquire propofol self-administration behaviour.

2) Propofol-induced self-administration behaviour will be maintained over time and under different schedules.

3) Propofol self-administration behaviour will be dose sensitive.

4) Propofol self-administration behaviour will be extinguished in the absence of propofol.

5) Priming doses of propofol will restore the extinguished self-administration behaviour.
Section 2: Material and Methods

2.1 Animals

Adult male Wistar rats (225-250 g) were purchased from Charles River (St-Constant, QC, Canada). Rats were single housed and were maintained on a 12/12 hour reversed light/dark cycle (lights on at 19:00) and a restricted diet (five pellets of rat chow/day). Experimental procedures started after a 1-week acclimatization period. All procedures were approved by the Animal Care Committee at the University of Toronto and Center for Addiction and Mental Health (CAMH).

2.2 Apparatus

Propofol self-administration occurred in operant chambers operated by a computer-controlled interface system (Med Associates, St Albans, VT, United States). In each chamber, two levers were located 2.5 cm above a removable grid floor and were assigned as active and inactive levers. Depressing the active lever activated a microliter syringe pump (PHM-104, Med Associate) resulting in a delivery of 100 µl/kg of drug or vehicle over 1 s. Inactive lever presses were recorded but had no programmed consequences.

A white cue light was located 7.5 cm above the active lever and a tone generator was positioned above the cue light; both visual (20 s) and auditory (1 s) cues were turned on when the active lever was pressed, signaling the delivery of propofol. A house light was located on the opposite side of the chamber and signaled the onset of the self-administration session. A modified 22-gauge cannula, which was attached to the intravenous catheter on a daily basis, was connected to a fluid swivel with Tygon tubing protected by a metal spring. The swivel was attached to a 10 ml syringe with Tygon tubing.
2.3 Drugs

A clinically formulation of propofol (10 mg/ml Diprivan®; AstraZeneca, Mississauga, ON, Canada) was used in the first acquisition experiment (experiment 1). For the rest of the study, propofol (2, 6-diisopropylphenol (Sigma-Aldrich, Oakville, ON, Canada)) was dissolved in 20% intralipid (Sigma-Aldrich) that is a fat emulsion containing soybean oil, phospholipids from egg yolk and glycerin. The main reason for switching from one to another form of propofol was to enable more flexibility in adjusting the concentration of the solution to be injected, especially for doses higher than 1 mg/kg/100µl. It is worth mentioning that both sources of propofol have been used in previous propofol self-administration studies in baboons (Weerts, Ator et al. 1999) and rats (LeSage, Stafford et al. 2000).

2.4 Experimental procedures

2.4.1 Food training

After acclimatization and before conducting surgical procedures, rats were subjected to operant training for 45 mg sucrose pellets (Bioserv, Frenchtown, NJ, United States) under a fixed ratio 1 (FR1) schedule in operant chambers equipped with pellet magazines. Food training, as described previously (Shram, Funk et al. 2008), consisted of two 8-hour sessions in which rats could obtain up to 400 pellets. In the current study, 2-3 days prior to starting propofol self-administration sessions, rats underwent a short 1-hour consolidation session of food training during which they could earn up to 100 pellets (Le, A.D. and Coen, K. standard laboratory practice).
2.4.2 Jugular catheterization for intravenous self-administration of propofol

Rats were implanted with intravenous silastic catheters into the right jugular vein as described previously (Le, Li et al. 2006) under oxygen/isoflurane anesthesia. The catheter exited between scapulas and was connected to a catheter access port adjusted for a 22-gauge cannula. Catheters were flushed before and after daily propofol self-administration sessions with 0.1 ml of a sterile heparin-saline solution (50 Unit/ml) to maintain the patency of catheters.

The patency of catheters were verified by a Thiopental® test (Crombag, Badiani et al. 2001) at the end of each set of experiment. An injection of a short-acting barbiturate, Thiopental® (Vetoquinol, Lavaltrie, QC, Canada) (4 mg/0.1ml/rat), through a functioning, patent jugular vein catheter causes immediate ataxia and loss of muscle tone and righting response in rats. Only patent animals were included in data analyses.

2.4.3 Behavioural assessments

2.4.3A Acquisition of propofol self-administration behaviour

One week after i.v. catheter implantation into the jugular vein, rats were subjected to acquisition of propofol self-administration. In all acquisition experiments rats started self-administration of propofol on a FR1 schedule in 1-hour daily sessions. During self-administration sessions, after each propofol infusion, a 20 s time out period was applied during which active lever pressing was recorded but did not have any programmed consequences. Rats were tested at the same time daily until they reached acquisition criteria. In this study, acquisition was defined as a 2:1 ratio of active over inactive lever presses (Harris, Pentel et al. 2009) and ≥5 reinforcements per each 1-hour session for three consecutive days (Watkins, Epping-Jordan et al. 1999).
For the acquisition phase of our experiments we used a range of low and non-sedative doses of propofol (0.25, 0.56 and 1 mg/kg) (LeSage, Stafford et al. 2000). A bolus i.v. injection of 0.6 mg/kg of propofol served as a positive reinforcer in healthy volunteers (Zacny 1993) and consistent with this clinical finding propofol abusers have also reported bolus i.v. injections of 50 mg of propofol (~0.7 mg/kg for a 70 kg person). Therefore, the dose range of propofol used in our experiment is clinically relevant to the doses of propofol which are used for recreational purposes by humans.

2.4.3B Establishment of dose response curve

Generation of a dose response curve was conducted after five to six acquisition sessions, which is when the subjects met acquisition criteria for propofol self-administration behaviour and reached stable responding. In this phase of experimentation, a dose range of propofol (0.0625, 0.125, 0.25, 0.56, 1 and 1.7 mg/kg) was used to obtain a propofol self-administration dose response curve. Rats were allowed to self-administer propofol until they reached a stable level of responding (no more than 20% variation over three consecutive days) at different doses under different ratios (FR1 and FR2). After reaching stable responding at any given dose, based on the magnitude of responses (number of active lever presses and reinforcements), the next dose was chosen, thus there was not a predetermined order of doses during this part of experiment.
2.4.3C Extinction of propofol self-administration behaviour

During 1-hour extinction sessions, propofol was substituted with its vehicle (i.e. intralipid), the house light was continuously illuminated and the visual and auditory cues were removed. Rats were given extinction sessions until they reached the extinction criterion of less than 10 active lever presses in two consecutive days (Le, Quan et al. 1998).

2.4.3D Reinstatement of extinguished propofol self-administration behaviour

When rats achieved extinction criterion, an intraperitoneal (i.p.) injection of vehicle (i.e. intralipid) was administered 15 minutes prior to a session under the extinction condition. This final extinction session after i.p. intralipid injection served as a baseline to which propofol priming-induced reinstatement was compared (Le, Li et al. 2006). Following the baseline session, each priming dose of propofol was administered i.p. 15 minutes before testing for responding on the previously propofol-associated lever (active lever). During the 1-hour reinstatement sessions, pressing the active lever did not lead to drug or vehicle delivery, as previously described (Le, Li et al. 2006). It is common in reinstatement experiments for the priming doses of the drug of interest to be much higher than doses used for acquisition training and to be given to animals via a different route of administration. For instance, doses that were 16, 24 and 32 times higher than the training dose have been used for alcohol reinstatement (Le, Poulos et al. 1999). In a study of nicotine reinstatement, doses that were 25, 50 and 100 times higher than the nicotine training dose were used (Shaham, Adamson et al. 1997). In the current study, propofol priming doses were 15, 30 and 60 times higher than the propofol dose used during acquisition training, which resulted in a dose range of 3.75, 7.5 and 15 mg/kg of propofol which was administered via an i.p. route in an escalating order. Between priming
sessions, there was a number of extinction sessions (minimum one session) until the extinction criterion was regained (Le, Li et al. 2006).

2.4 Statistical analysis

The data from the different sets of experiment were analyzed with t-test (paired and unpaired) and ANOVAs (one-way ANOVA as well as one-way and two-way repeated measures ANOVAs). Significant effects using ANOVA (p<0.05) were followed by the appropriate post-hoc test (Bonferroni’s multiple comparison test). The Chi-Square test was used to compare the proportion of rats that acquired propofol self-administration across different doses.

2.5 Study design

The current study was carried out in four experiments using separate sets of animals. The behavioural assessments varied between experiments, which will be discussed in more detail later on. The time line of the experimental procedures is displayed in Table 1.

<table>
<thead>
<tr>
<th>Week 1</th>
<th>Week 2</th>
<th>Week 3</th>
<th>Week 4</th>
<th>Starting at week 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acclimatization</td>
<td>Food training</td>
<td>i.v. surgeries</td>
<td>Recovery</td>
<td>Behavioural assessments</td>
</tr>
</tbody>
</table>

*Table 1: Time line of experimental procedures*
2.6 Behavioural assessments

2.6.1 Experiment 1

In a previous propofol self-administration study in rats (LeSage, Stafford et al. 2000) the acquisition of propofol self-administration was conducted at a subanesthetic dose of 1.7 mg/kg of propofol. To assess the reinforcing efficacy of a lower dose of propofol in establishing self-administration behaviour we used a dose of 1 mg/kg of propofol in the acquisition phase of experiment 1. The dose response curve of propofol self-administration primarily was generated in our first experiment using a narrow range of subanesthetic doses of propofol (0.56, 1 and 1.7 mg/kg) previously tested by others (LeSage, Stafford et al. 2000). Therefore, in experiment 1, all rats (N=22) were subjected to the following behavioural assessments:

- Acquisition training at 1 mg/kg propofol on a FR1 schedule
- Maintenance of the self-administration behaviour on a higher ratio schedule (FR2)
- Generating a dose response curve on a FR2 schedule using different doses of propofol with the following order: 1, 0.56 and 1.7 mg/kg

For the assessment of acquisition and maintenance of self-administration behaviour, all rats were included in the data analyses. However, only patent rats which successfully completed the entire dose response curve experiment were included in data analyses of dose response curve experiments. This strategy was used in all experiments of the current study.
2.6.2 Experiment 2

To further characterize the reinforcing efficacy of lower doses of propofol in inducing self-administration behaviour two lower doses of propofol (0.25 and 0.56 mg/kg) were used as acquisition training doses in experiment 2A and 2B. With respect to the fact that 1.7 mg/kg was on descending limb of dose response curve (generated in experiment 1) showing the lowest magnitude of responding, we extended the dose range to lower doses to generate a full dose response curve with more precise ascending section of the dose response curve. Thus, in experiment 2, two groups of rats (N=17 each) were assigned to two different behavioural studies as follows:

Group A

- Acquisition training at 0.25 mg/kg propofol on a FR1 schedule
- Generating a dose response curve on a FR1 schedule using different doses of propofol in the following order: 0.25, 0.56, 1, 0.125 and 1.7 mg/kg

Group B

- Acquisition training at 0.56 mg/kg propofol on a FR1 schedule
- Maintenance of the self-administration behaviour on a higher ratio schedule (FR2)
- Generating a dose response curve on a FR2 schedule using different doses of propofol in the following order: 0.56, 1, 0.25 and 1.7 mg/kg

2.6.3 Experiment 3

In experiment 3, all rats (N=17) underwent the following behavioural assessments:

- Acquisition training at 0.56 mg/kg propofol on a FR1 schedule
- Generating a dose response curve on a FR1 schedule using different doses of propofol in the following order: 0.56, 1, 0.25, 0.125 and 0.0625 mg/kg
2.6.4 Experiment 4

To study the extinction and reinstatement of propofol self-administration behaviour we chose 0.25 mg/kg of propofol as an acquisition training dose because based on our results from experiments 1-3 this dose was able to induce self-administration behaviour in the majority of rats with a high magnitude of responding. Therefore, in experiment 4, all rats (N=19) were assigned for the following behavioural assessments:

- Acquisition training at 0.25 mg/kg propofol on a FR1 schedule
- Maintenance of the self-administration behaviour at 0.56 mg/kg propofol for six sessions on a FR1 schedule
- Extinction of the propofol self-administration behaviour
- Reinstatement of the extinguished behaviour using different priming doses of propofol in an escalating order (3.75, 7.5 and 15 mg/kg) which were respectively 15, 30 and 60 times higher than the training dose of 0.25 mg/kg.

The base of catheters (on the back of the animals), as well as the drug lines in the operant chambers, were constantly chewed by five of the rats in this set of experiments. These rats were excluded from the data analyses. Therefore, 14 of 19 rats were included in the acquisition analyses. Of these 14 rats, only five rats were subjected to extinction and reinstatement experiments due to very low levels of responding in the other rats just prior to starting extinction sessions.
Section 3: Results

3.1 Experiment 1

3.1.1 Rats acquired propofol self-administration behaviour at a training dose of 1 mg/kg

Acquisition of propofol self-administration behaviour was tested at a training dose of 1 mg/kg under a fixed ratio (FR) 1 schedule in 22 rats. The majority of rats, 81% (18 of 22), acquired propofol self-administration behaviour and met the acquisition criteria, as assessed in the last three sessions of a five-day acquisition training phase.

Analyzing the active and inactive lever presses as a percentage of total lever responses in the total group of rats (N=22) using two-way repeated measures ANOVA, there was a significant effect of lever (F (1,168)=315.01; p<0.0001) with no significant effect of session (F (4,168)=0.32; p=0.86) or session by lever interaction (F (4,168)=1.02; p=0.39). Bonferroni’s multiple comparison test showed a significant difference between the active and inactive lever presses in all acquisition training sessions (p<0.001; Figure 1A). These results indicate that the active lever response was significantly higher than the response on the inactive lever and that this difference remained over the five acquisition sessions.

Similar analyses using the number of active and inactive lever presses showed a significant effect of lever (F (1,168)=57.43; p<0.0001), session (F (4,168)=46.34; p<0.0001) and session by lever interaction (F (4,168)=12.47; p<0.0001). The *post-hoc* test showed a significant difference between the number of active and inactive lever presses in all sessions (p<0.01, p<0.001; Figure 1B). These findings indicate that the number of active lever presses was significantly higher than the number of inactive lever presses and that this difference varied across different sessions.
Figure 1: Acquisition of propofol self-administration at 1 mg/kg
A) and C) illustrate the active versus inactive lever presses as a percentage of total lever presses at a training dose of 1 mg/kg under a FR1 schedule in all and in acquired rats, respectively. There was a significant difference between active and inactive lever presses in all sessions.
B) and D) display a comparison between the actual number of active and inactive lever presses at the same dose and schedule in all and in acquired rats, respectively. There was a significant difference between the number of active and inactive lever presses in all sessions (**p<0.01, ***p<0.001 compared to the inactive response in the same session).
In rats that met the acquisition criteria (N=18), statistical analyses showed similar results as those seen in the total group (N=22). Analysis of lever press data as a percentage of total lever presses showed a significant effect of lever ($F_{(1,136)}=315.88; p<0.0001$) with no significant effect of session ($F_{(4,136)}=0.32; p=0.83$) or session by lever interaction ($F_{(4,136)}=1.38; p=0.24$). The post-hoc test showed significantly higher responding on the active lever in comparison with inactive lever in all acquisition training sessions ($p<0.001$; Figure 1C). Analysis of the number of active and inactive lever presses showed a significant effect of lever ($F_{(1,136)}=54.71; p<0.0001$), session ($F_{(4,136)}=38.98; p<0.0001$) and session by lever interaction ($F_{(4,136)}=8.65; p<0.0001$). Using the post-hoc test we found that there was a significant higher number of active lever presses compared to inactive lever presses in all sessions ($p<0.01, p<0.001$; Figure 1D). The comparison between acquisition sessions in the number of reinforcements and the total intake showed a significant difference between the first and the last three sessions in all (N=22) and in acquired rats (N=18) showing the highest magnitude in the first session (one-way repeated measures ANOVA, followed by Bonferroni’s post-hoc test: $p<0.05$; Figure 2 (A-D)). Analysis of propofol self-administration behaviour individually revealed intersubject variations in the number of active and inactive lever presses as well as the number of reinforcements and the total propofol intake among acquired and not acquired rats (Figure 3 (A-C)) which is in agreement with the data was reported previously in propofol self-administration studies in rat and monkey (Weerts, Ator et al. 1999; LeSage, Stafford et al. 2000). Comparing the self-administration behaviours in acquired and not acquired rats as two independent groups showed no significant difference between acquired and not acquired rats in the number of active lever presses (unpaired t-test: $p=0.05$), inactive lever presses (unpaired t-test: $p>0.05$) or reinforcements (unpaired t-test: $p=0.2$), or the total propofol intake (unpaired t-test: $p=0.058$) (Figure 3 (A-C)).
Figure 2: The number of reinforcements and the total propofol intake across the acquisition sessions at 1 mg/kg

A) and B) show the number of reinforcements and the total propofol intake in the total group of rats (N=22) on a FR1 schedule at 1 mg/kg, respectively. There were significant differences between the first and the last three acquisition sessions in the number of reinforcements and the total propofol intake.

C) and D) display, respectively, the number of reinforcements and the total propofol intake in acquired rats (N=18) on the same schedule and dose. There were significant differences between the first and the last three acquisition sessions in the number of reinforcements and the total propofol intake (#p<0.05 compared to the last three sessions).
Figure 3: Interindividual variation in propofol self-administration behaviour during the acquisition phase at 1 mg/kg

A)-C) left side graphs display the number of lever presses, the number of reinforcements and the total propofol intake in the last three acquisition sessions for each individual rat, respectively.

A)-C) right side graphs show a comparison between acquired (N=18) and not acquired rats (N=4) in the number of lever presses, the number of reinforcements and the total propofol intake in the last three acquisition sessions, respectively. Analyzing acquired and not acquired rats as two groups, there was no significant difference between these two groups in the number of active lever presses (p=0.05), inactive lever presses (p>0.05) or reinforcements (p=0.2), or the total propofol intake (p=0.058).
3.1.2 Rats maintained propofol self-administration behaviour under a FR2 schedule at 1mg/kg

After animals acquired propofol self-administration at 1mg/kg on a FR1 schedule, the ratio was escalated and the maintenance of the behaviour was studied under a FR2 schedule. Analyzing the active and inactive lever presses as a percentage of total lever responses in the total group of rats (N=22) using two-way repeated measures ANOVA, there was a significant effect of lever ($F_{(1,168)}=370.3; p<0.0001$) with no significant effect of session ($F_{(4,168)}=0.45; p=0.45$) or session by lever interaction ($F_{(4,168)}=2.03; p=0.09$). Bonferroni’s multiple comparison test showed significantly higher responding on the active lever in comparison with inactive lever in all self-administration sessions ($p<0.001$; **Figure 4A**). These results indicate that under a higher demanding ratio propofol at a dose of 1 mg/kg had sufficient reinforcing effects to maintain the self-administration behaviour. Similar analyses using the number of active and inactive lever presses showed a significant effect of lever ($F_{(1,168)}=25.29; p<0.0001$) with no significant effect of session ($F_{(4,168)}=2.0; p=0.09$) or session by lever interaction ($F_{(4,168)}=1.29; p=0.27$). The post-hoc test showed significantly higher number of active lever presses compared to inactive lever presses in all sessions ($p<0.01, p<0.001$; **Figure 4B**).

Analysis of the active and inactive lever presses as a percentage of total lever presses in rats that acquired self-administration behaviour (N=18) showed a significant effect of lever ($F_{(1,136)}=268.70; p<0.0001$) with no significant effect of session ($F_{(4,136)}=0.56; p=0.68$) or session by lever interaction ($F_{(4,136)}=3.23; p=0.014$). Responding on the active lever was significantly higher than responding on the inactive lever in all self-administration sessions (Bonferroni’s post-hoc test: $p<0.001$; **Figure 4C**).
Figure 4: Maintenance of propofol self-administration behaviour under a FR2 schedule at 1 mg/kg

A) and C) illustrate the active versus inactive lever presses as a percentage of total lever presses at the dose of 1 mg/kg under a FR2 schedule in all and in acquired rats, respectively. There was a significant difference between active and inactive lever presses in all sessions. B) and D) display a comparison between the actual number of active and inactive lever presses at the same dose and schedule in all and in acquired rats, respectively. There was a significant difference between the number of active and inactive lever presses in all sessions (*p<0.05, **p<0.01, ***p<0.001 compared to the inactive response in the same session).
Analysis of the data using the number of active and inactive lever presses in acquired rats showed a significant effect of lever (F(1,136)=27.73; p<0.0001) with no significant effect of session (F(4,136)=1.25; p=0.29) or session by lever interaction (F(4,136)=1.25; p=0.29). The post-hoc test showed that the number of active lever presses was significantly higher than inactive lever presses in all self-administration sessions (p<0.05, p<0.01, p<0.001; Figure 4D).

The comparison of the number of reinforcemements and the total intake across different sessions on the FR2 schedule showed no significant difference between sessions (one-way repeated measures ANOVA: p>0.05; Figure 5 (A-D)). Figure 6 (A-C) shows different aspects of self-administration behaviour in acquired rats during the transition from FR1 to FR2 schedule. The comparison of the means of the last three sessions under the two schedules showed that the number of active lever presses (paired t-test: p<0.02), number of reinforcemements and the total propofol intake (paired t-test: p<0.001) were significantly higher under the FR1 schedule (Figure 6 (A-C)).
Figure 5: The number of reinforcements and the total propofol intake across self-administration sessions under a FR2 schedule at 1 mg/kg

A) and B) show the number of reinforcements and the total propofol intake in the total group of rats (N=22) on a FR2 schedule at 1 mg/kg, respectively.

C) and D) display, respectively, the number of reinforcements and the total propofol intake in acquired rats (N=18) on the same schedule and dose. In all and in acquired rats, there were no significant differences across the sessions in the number of reinforcements or the total propofol intake.
Figure 6: The comparison of propofol self-administration behaviour between FR1 and FR2 schedules at 1 mg/kg

A)-C) show the number of lever presses, the number of reinforcements and the total intake under FR1 and FR2 schedules in acquired rats (N=18), respectively. The comparison of the means of the last three sessions under the two schedules showed a significant difference between FR1 and FR2 schedules in the number of active lever presses (p<0.02) and reinforcements and the total propofol intake (p<0.001).
3.1.3 Dose response curve of propofol self-administration on a FR2 schedule

Following the maintenance of propofol self-administration behaviour at 1 mg/kg on a FR2 schedule, when animals reached stable responding at this dose, the unit dose of propofol was altered. A dose response curve of propofol self-administration was generated using doses of 0.56, 1 and 1.7 mg/kg of propofol. Self-administration of each dose was conducted for four or five sessions or until the rats reached stable responding (no more than 20% variation) for three consecutive days. After reaching stable responding at any given dose, based on the magnitude of responses (number of active lever presses and reinforcements) the next dose was determined, resulting in a propofol dose order of 1, 0.56 and 1.7 mg/kg. This approach was used to determine the order of doses in all dose response curve experiments. Of the total number of rats, only those that successfully finished the entire dose response curve phase, and that were still patent, were included in the data analyses of the dose response curve experiments. Therefore, 12 of 22 rats were included in the dose response curve analyses in experiment 1. Of these 12 rats, only 10 had acquired the behaviour. When analyzing the number of reinforcements and the total propofol intake in all rats (N=12), there were no significant differences between different doses (one-way repeated measures ANOVA, p>0.05; Figure 7 (A and B)). In acquired rats (N=10), there was a significant difference between 1 and 1.7 mg/kg in the number of reinforcements showing higher magnitude of responding at 1 mg/kg. The total propofol intake was significantly higher at 1 mg/kg compared to 0.56 mg/kg (one-way repeated measures ANOVA, followed by Bonferroni’s post-hoc test, p<0.05; Figure 7 (C and D)).
Figure 7: Dose response curve of propofol self-administration on a FR2 schedule

A) and C) show the number of reinforcements as a function of propofol dose for the last three sessions at each dose in all and in acquired rats, respectively. B) and D) show the total intake as a function of dose for the last three sessions at each dose in all and in acquired rats, respectively.

In acquired rats, there was a significant difference between 1 and 1.7 mg/kg in the number of reinforcements. There was a significant difference in the total propofol intake between 1 and 0.56 mg/kg ($p<0.05$).
3.2 Experiment 2

Acquisition of propofol self-administration behaviour was examined at two lower doses, 0.25 mg/kg (experiment 2A) and 0.56 mg/kg (experiment 2B), in two groups of rats (N=17 each). In addition, two dose response curves were generated under FR1 and FR2 schedules using a wider range of propofol doses.

3.2.1 Experiment 2A

3.2.1A Rats acquired propofol self-administration behaviour at a training dose of 0.25 mg/kg

Under a FR1 schedule and at a dose of 0.25 mg/kg, 76% of rats (13 of 17) acquired propofol self-administration and met the acquisition criteria, as assessed in the last three sessions of a six-day acquisition training phase. In all rats (N=17) two-way repeated measures ANOVA showed a significant effect of lever (F\( _{1,160} =417.59; \ p<0.0001 \)) with no significant effect of session (F\( _{5,160} =0.31; \ p=0.90 \)) or session by lever interaction (F\( _{5,160} =1.30; \ p=0.26 \)). Responding on the active lever was significantly higher than responding on the inactive lever in all sessions (Bonferroni’s post-hoc test: p<0.001; Figure 8A). Analyzing the number of lever presses, there was a significant effect of lever (F\( _{1,160} =38.08; \ p<0.0001 \)), session (F\( _{5,160} =19.45; \ p<0.0001 \)) and session by lever interaction (F\( _{5,160} =8.71; \ p<0.0001 \)). Bonferroni’s multiple comparison test showed that the number of active lever presses was significantly higher than inactive lever presses in all sessions (p<0.01, p<0.001; Figure 8B). In rats that met the acquisition criteria (N=13) analysis of the active and inactive lever presses as a percentage of total lever presses showed a significant effect of lever (F\( _{1,120} =678.66; \ p<0.0001 \)) with no significant effect of session (F\( _{5,120} =0; \ p=1 \)) or session by lever interaction (F\( _{5,120} =2.26; \ p=0.05 \)). Responding on the active lever was significantly higher than the inactive lever response in all acquisition training sessions (post-hoc test p<0.001; Figure 8C).
Figure 8: Acquisition of propofol self-administration at 0.25 mg/kg
A) and C) illustrate the active versus inactive lever presses as a percentage of total lever presses at an acquisition training dose of 0.25 mg/kg under a FR1 schedule in all and in acquired rats, respectively. There was a significant difference between active and inactive lever presses in all sessions.
B) and D) show a comparison between the actual number of active and inactive lever presses at the same dose and schedule in all and in acquired rats, respectively. There was a significant difference between the number of active and inactive lever presses in all sessions. 

(**p<0.01, ***p<0.001 compared to inactive response in the same session).
Analysis of the data using the number of active and inactive lever presses showed a significant effect of lever (F (1,120)=46.74; p<0.0001), session (F (5,120)=13.61; p<0.0001) and session by lever interaction (F (5,120)=5.78; p<0.0001). The post-hoc test showed that the number of active lever presses was significantly higher compared to the number of inactive lever presses in all sessions (p<0.01, p<0.001; Figure 8D).

The comparison between acquisition sessions in the number of reinforcements and the total propofol intake showed a significant difference between the first and the last three sessions in all and in acquired rats with the highest magnitude in the first session (one-way repeated measures ANOVA, followed by Bonferroni’s post-hoc test: p<0.05; Figure 9 (A-D)). Analysis of acquired and not acquired rats as two groups showed that the number of active lever presses (unpaired t-test: p=0.02) and the number of reinforcements (unpaired t-test: p=0.01) and the total propofol intake (unpaired t-test: p=0.01) were significantly higher in acquired rats compared to not acquired rats (Figure 10 (A-C)). There were interanimal differences in the number of active and inactive lever presses as well as the number of reinforcements and the total propofol intake among acquired and not acquired rats (Figure 10 (A-C)).
Figure 9: The number of reinforcements and the total propofol intake across the acquisition sessions at 0.25 mg/kg

A) and B) show the number of reinforcements and the total propofol intake in the total group of rats (N=17) on a FR1 schedule at 0.25 mg/kg, respectively. There were significant differences between the first and the last three sessions in the number of reinforcements and the total propofol intake.

C) and D) display, respectively, the number of reinforcements and the total propofol intake in acquired rats (N=13) on the same schedule and dose. There were significant differences between the first and the last three sessions in the number of reinforcements and the total propofol intake (#p<0.05 compared to the last three sessions).
Figure 10: Interindividual variation in propofol self-administration behaviour during the acquisition phase at 0.25 mg/kg
A)-C) left side graphs display the number of lever presses, the number of reinforcements and the total propofol intake in the last three acquisition sessions for each individual rat, respectively.
A)-C) right side graphs show a comparison between acquired (N=13) and not acquired rats (N=4) in the number of lever presses, the number of reinforcements and the total propofol intake in the last three acquisition sessions, respectively. Analyzing acquired and not acquired rats as two groups there was a significant difference between acquired and not acquired rats in the number of active lever presses (p=0.02) and the number of reinforcements (p=0.01) and the total propofol intake (p=0.01).
3.2.1B Dose response curve of propofol self-administration on a FR1 schedule

After establishment of propofol self-administration behaviour at a training dose of 0.25 mg/kg on a FR1 schedule, all rats (N=17) were subjected to a dose response curve experiment on the same schedule (FR1). A dose response curve of propofol self-administration behaviour was generated using a broad range of propofol doses (0.125-1.7 mg/kg); the order of doses was 0.25, 0.56, 1, 0.125 and 1.7 mg/kg. Self-administration of each dose was conducted for three to six sessions or until the rats reached stable responding (no more than 20% variation) for three consecutive days. The dose response curve analyses were conducted only on those animals that went through the entire of dose response curve experiment, and that were still patent. Therefore, in experiment 2A, only five of 17 rats were included in the dose response curve analyses. One-way repeated measures ANOVA, followed by Bonferroni’s post-hoc test revealed that the number of reinforcements at the dose of 0.125 mg/kg was significantly higher compared to two highest doses 1 and 1.7 mg/kg (p<0.05; Figure 11A). However, there were no significant differences between different doses in the total propofol intake (one-way repeated measures ANOVA: p=0.1; Figure 11B).
Figure 11: Dose response curve of propofol self-administration on a FR1 schedule
A) and B) show the number of reinforcements and the total propofol intake as a function of propofol dose for the last three sessions at each dose, respectively. There was a significant difference in the number of reinforcements between 0.125 mg/kg and two highest doses (p<0.05). There were no significant differences between different doses in the total propofol intake.
3.2.2 Experiment 2B

3.2.2A Rats acquired propofol self-administration behaviour at a training dose of 0.56 mg/kg

Propofol self-administration behaviour was established using 0.56 mg/kg as a training dose and 65% of rats (11 of 17) acquired propofol self-administration behaviour and reached the acquisition criteria, as evaluated in the last three sessions of a six-day acquisition training phase under a FR1 schedule. In all rats (N=17) analyzing the active and inactive lever presses as a percentage of total lever presses using two-way repeated measures ANOVA, there was a significant effect of lever (F (1,160)=127.0; p<0.0001) with no significant effect of session (F (5,160)=0.0; p=1.0) and a significant effect of session by lever interaction (F (5,160)=2.75; p=0.02). Bonferroni’s multiple comparison test showed that responding on the active lever was significantly higher than responding on the inactive lever in all sessions (p<0.001; Figure 12A). Analysis of the number of lever presses showed a significant effect of lever (F (1,160)=44.43; p<0.0001), session (F (5,160)=29.51; p<0.0001) and session by lever interaction (F (5,160)=7.29; p<0.0001). The post-hoc test showed significantly higher number of active lever presses in comparison with inactive lever in all sessions (p<0.05, p<0.01, p<0.001; Figure 12B). In acquired rats (N=11) statistical analyses showed the same results as those seen in all rats. Analysis of the percentage of total lever presses showed a significant effect of lever (F (1,100)=554.87; p<0.0001) with no significant effect of session (F (5,100)=0; p=1.0) and a significant effect of session by lever interaction (F (5,100)=3.32; p=0.008). The post-hoc test showed a significant difference between the active and inactive lever presses in all acquisition training sessions displaying a higher magnitude of responding on the active lever (p<0.001; Figure 12C).
Figure 12: Acquisition of propofol self-administration at 0.56 mg/kg
A) and C) show the active versus inactive lever presses as a percentage of total lever presses at an acquisition training dose of 0.56 mg/kg under a FR1 schedule in all and in acquired rats, respectively. There was a significant difference between active and inactive lever presses in all sessions.
B) and D) display a comparison between the actual number of active and inactive lever presses at the same dose and schedule in all and in acquired rats, respectively. There was a significant difference between the number of active and inactive lever presses in all sessions (*p<0.05, **p<0.01, ***p<0.001 compared to inactive response in the same session).
Using the number of active and inactive lever presses in data analyses, there was a significant effect of lever (F (1,100)=42.43; p<0.0001), session (F (5,100)=12.50; p<0.0001) and session by lever interaction (F (5,100)=2.73; p=0.02). The post-hoc test showed significantly higher magnitude of responding on the active lever compared to inactive lever in all sessions (p<0.01, p<0.001; Figure 12D).

In all and in acquired rats the number of reinforcements and the total propofol intake were significantly higher in the first session compared to the last three sessions (one-way repeated measures ANOVA, followed by Bonferroni’s post-hoc test: p<0.05; Figure 13 (A-D)). Analyzing acquired and not acquired rats as two groups, there was a significant difference in the number of active lever presses (unpaired t-test: p<0.02) and the number of reinforcements (unpaired t-test: p=0.01) and the total propofol intake (unpaired t-test: p=0.01) between these two groups showing higher magnitude in acquired rats, while there was no significant difference between acquired and not acquired rats in the number of inactive lever presses (unpaired t-test: p=0.05; Figure 14 (A-C)). There were interanimal differences in the number of active and inactive lever presses as well as the number of reinforcements and the total propofol intake within acquired and not acquired rats (Figure 14 (A-C)).
Figure 13: The number of reinforcements and the total propofol intake across the acquisition sessions at 0.56 mg/kg
A) and B) show the number of reinforcements and the total propofol intake in the total group of rats (N=17) on a FR1 schedule at 0.56 mg/kg, respectively. There were significant differences between the first and the last three sessions in the number of reinforcements and the total propofol intake.
C) and D) illustrate the number of reinforcements and the total propofol intake in acquired rats (N=11) on the same schedule and dose, respectively. There were significant differences between the first and the last three sessions in the number of reinforcements and the total propofol intake (#p<0.05 compared to the last three sessions).
Figure 14: Interindividual variation in propofol self-administration behaviour during the acquisition phase at 0.56 mg/kg

A)-C) left side graphs display the number of lever presses, the number of reinforcements and the total propofol intake in the last three acquisition sessions for each individual rat, respectively.

A)-C) right side graphs show a comparison between acquired (N=11) and not acquired (N=6) rats in the number of lever presses, the number of reinforcements and the total propofol intake in the last three acquisition sessions, respectively. There was a significant difference between acquired and not acquired rats in the number of active lever presses (p<0.02) and reinforcements (p=0.01) and the total propofol intake (p=0.01) while there was no significant difference between groups in the number of inactive lever presses (p=0.05).
3.2.2B Rats maintained propofol self-administration behaviour under a FR2 schedule at 0.56 mg/kg

After animals acquired propofol self-administration behaviour at 0.56 mg/kg on a FR1 schedule, the ratio was escalated and the maintenance of the behaviour was studied under a FR2 schedule. Analysis of the active and inactive lever presses as a percentage of total lever presses in all rats (N=17) showed that there was a significant effect of lever (F (1,128)=35.16; p<0.0001) with no significant effect of session (F (4,128)=1.05; p=0.3) and a significant effect of session by lever interaction (F (4,128)=2.50; p=0.04). The post-hoc test showed significantly higher responding on the active lever in comparison with inactive lever in the majority of self-administration sessions (p<0.01, p<0.001; Figure 15A). Analysis of the data using the number of active and inactive lever presses showed a significant effect of lever (F (1,128)=9.09; p=0.005) and session (F (4,128)=3.62; p=0.007) with no significant effect of session by lever interaction (F (4,128)=1.95; p=0.1). The number of active lever presses was significantly higher than the number of inactive lever presses in session one and three (Bonferroni’s post-hoc test p<0.05, p<0.001; Figure 15B).

Analysis of the active and inactive lever presses as a percentage of total lever presses in acquired rats (N=11) showed a significant effect of lever (F (1,80)=57.03; p<0.0001) with no significant effect of session (F (4,80)=0.73; p=0.5) or session by lever interaction (F (4,80)=0.99; p=0.4). The post-hoc test showed significantly higher responding on the active lever compared to inactive lever in all self-administration sessions (p<0.001; Figure 15C).
Figure 15: Maintenance of propofol self-administration behaviour under a FR2 schedule at 0.56 mg/kg

A) and C) illustrate the active versus inactive lever presses as a percentage of total lever presses at 0.56 mg/kg under a FR2 schedule in all and in acquired rats, respectively. In all rats, there was a significant difference between active and inactive lever presses in all sessions except session three.

B) and D) show a comparison between the actual number of active and inactive lever presses at the same dose and schedule in all and in acquired rats, respectively. There was a significant difference between the number of active and inactive lever presses in session one and three in all rats and in sessions one, three and four in acquired rats (*p<0.05, **p<0.01, ***p<0.001 compared to inactive response in the same session).
Analysis of the data using the number of active and inactive lever presses in acquired rats (N=11) showed a significant effect of lever (F (1,80)=12.21; p=0.002) and session (F (4,80)=3.44; p=0.01) with no significant effect of session by lever interaction (F (4,80)=1.9 5; p=0.1). Using the post-hoc test we found that the number of active lever presses was significantly higher than inactive lever presses in sessions one, three and four (p<0.05, p<0.01, p<0.001; Figure 15D). The number of reinforcements and the total propofol intake were significantly higher in the first session compared to the last three sessions (one-way repeated measures ANOVA, followed by Bonferroni’s post-hoc test: p<0.05; Figure 16 (A-D)).

Figure 17 (A-C) shows different aspects of self-administration behaviour in acquired rats during the transition from FR1 to FR2 schedule. The comparison of the means of the last three sessions under the two schedules showed that the number of reinforcements (paired t-test: p<0.01) and the total propofol intake (paired t-test: p<0.01) were significantly higher under the FR1 schedule. There was no difference between the two schedules in the number of lever presses.
Figure 16: The number of reinforcements and the total propofol intake across self-administration sessions under a FR2 schedule at 0.56 mg/kg

A) and B) show the number of reinforcements and the total propofol intake in the total group of rats (N=17) on a FR2 schedule at 0.56 mg/kg, respectively. There were significant differences between the first and the last self-administration sessions in the number of reinforcements and the total propofol intake.

C) and D) illustrate the number of reinforcements and the total propofol intake in acquired rats (N=11) on the same schedule and dose, respectively. There were significant differences between the first and the last self-administration sessions in the number of reinforcements and the total propofol intake (#p<0.05 compared to the last three sessions).
Figure 17: The comparison of propofol self-administration behaviour between FR1 and FR2 schedules at 0.56 mg/kg

A)-C) show the number of lever presses, the number of reinforcements and the total propofol intake under FR1 and FR2 schedules in acquired rats (N=11), respectively. The comparison of the means of the last three sessions under the two schedules showed a significant difference between FR1 and FR2 schedules in the number of reinforcements and the total propofol intake (p<0.01).
A comparison of efficacies between different doses of propofol under the FR2 schedule (experiments 1 and 2B) showed no significant difference in the number of reinforcements between 1 and 0.56 mg/kg in all and in acquired rats, indicating that these two tested doses had the same reinforcing efficacy (unpaired t-test: p=0.4, p=0.9) (Figure 18).

Figure 18: Different doses of propofol had the same efficacy under the FR2 schedule
This figure shows a comparison of the number of reinforcements between two different doses of propofol under the FR2 schedule. There was no significant difference in the number of reinforcements between the two doses of propofol (1 and 0.56 mg/kg) on the FR2 schedule in experiment 1 and 2B (p=0.4 in all rats, p=0.9 in acquired rats). The numbers under each column represents the number of rats.
3.2.2C Dose response curve of propofol self-administration on a FR2 schedule

Following the maintenance of propofol self-administration behaviour at 0.56 mg/kg on a FR2 schedule, when animals reached stable responding at this dose, the unit dose of propofol was changed. A broad dose range of propofol (0.125-1.7 mg/kg) was used to generate a dose response curve of propofol. Self-administration of each dose was conducted for four to six sessions or until the rats reached a stable responding for three consecutive days (no more than 20% variation). The order of doses in this dose response curve experiment was 0.56, 1, 0.25 and 1.7 mg/kg. The dose response curve analyses were conducted on those rats that successfully finished the entire of dose response curve experiment, and that were still patent. Therefore, six of 17 rats were included in the dose response curve analyses in experiment 2B. Of these six rats only four had acquired the behaviour. When analyzing the number of reinforcements there were no significant differences between different doses in all rats (one-way repeated measures ANOVA: p>0.05; Figure 19A). However, there was a significant difference between 1.7 mg/kg and all lower doses in the total intake showing the highest intake at 1.7 mg/kg (one-way repeated measures ANOVA, followed by Bonferroni’s post-hoc test: p<0.05; Figure 19B). In acquired rats, there were no differences in the number of reinforcements between different doses but the total propofol intake was significantly higher at 1.7 mg/kg compared to other low doses (one-way repeated measures ANOVA, followed by Bonferroni’s post-hoc test: p<0.05; Figure 19 (C and D)).
Figure 19: Dose response curve of propofol self-administration on a FR2 schedule
A) and C) show the number of reinforcements as a function of propofol dose for the last three sessions at each dose in all and in acquired rats, respectively.
B) and D) show the total intake as a function of dose for the last three sessions at each dose in all and in acquired rats, respectively. There was a significant difference in the total propofol intake between 1.7 mg/kg and all lower doses in acquired and in not acquired rats (\( p < 0.05 \)).
3.3 Experiment 3

3.3.1 Acquisition of propofol self-administration at a training dose of 0.56 mg/kg

A group of 17 rats underwent training for acquisition of propofol self-administration at 0.56 mg/kg. The majority of rats, 88% (15 of 17), acquired self-administration behaviour and met the acquisition criteria under a FR1 schedule. The interindividual differences in the number of active and inactive lever presses as well as the number of reinforcements and the total propofol intake are shown in Figure 20 (A-C). Analyzing acquired and not acquired rats as two groups, the number of reinforcements and the total intake were significantly higher in acquired rats in comparison with not acquired rats (unpaired t-test: p=0.016). However, there was no significant difference between the two groups in the number of active (unpaired t-test: p=0.05) or inactive lever presses (unpaired t-test: p=0.1) Figure 20 (A-C).
Figure 20: Interindividual variation in propofol self-administration behaviour during the acquisition phase at 0.56 mg/kg
A)-C) left side graphs display the number of lever presses, the number of reinforcements and the total propofol intake in the last three acquisition sessions for each individual rat, respectively.
A)-C) right side graphs show a comparison between acquired (N=15) and not acquired rats (N=2) in the number of lever presses, the number of reinforcements and the total propofol intake in the last three acquisition sessions, respectively. Analyzing acquired and not acquired rats as two groups, there was a significant difference between acquired and not acquired rats in the number of reinforcements and the total propofol intake (p=0.016). There was no significant difference between these two groups in the number of active (p=0.05) or inactive lever presses (p=0.1).
3.3.2 Dose response curve of propofol self-administration on a FR1 schedule

After acquisition training on a FR1 schedule, all rats (N=17) were subjected to a dose response curve experiment on the same ratio (FR1) using a wide range of propofol doses (0.0625-1 mg/kg). Self-administration of each dose was continued for five to seven sessions or until the rats reached a stable responding for three consecutive days (no more than 20% variation). After reaching stable responding at any given dose, depending on the magnitude of responses (number of active lever presses and reinforcements) the next dose was determined, resulting in a propofol dose order of 0.56, 1, 0.25, 0.125 and 0.0625 mg/kg in this experiment. The data analyses in the dose response curve experiments were performed on those rats that successfully completed the entire dose response curve phase, and that were still patent. Therefore, 12 of 17 rats were included in the dose response curve analyses in experiment 3. The number of rats reduced to 10 when we analyzed the data in rats that had acquired the behaviour. One-way repeated measures ANOVA showed no significant difference in the number of reinforcements between different doses in all or in acquired rats (P>0.05; Figure 21 (A and C)). The total propofol intake at 0.56 and 1 mg/kg was significantly higher than the three lowest doses (0.0625, 0.125 and 0.25 mg/kg) in all and in acquired rats (P<0.05; Figure 21 (B and D)).
Figure 21: Dose response curve of propofol self-administration on a FR1 schedule
A) and C) show the number of reinforcements as a function of propofol dose for the last three sessions at each dose in all and in acquired rats, respectively.
B) and D) show the total intake as a function of dose for the last three sessions at each dose in all and in acquired rats, respectively.
There were no significant differences in the number of reinforcements between different doses in all or in acquired rats. There were significant differences in the total propofol intake between 0.56 and 1 mg/kg and the three lowest doses (0.0625, 0.125 and 0.25 mg/kg) in all and in acquired rats (p<0.05).
3.3.3 The temporal pattern of propofol self-administration behaviour

The pattern of self-administration behaviour is similar among different drugs of abuse when access is limited to short daily sessions. To find out whether propofol shows the same temporal pattern as other drugs of abuse, we studied the pattern of propofol self-administration behaviour during self-administration sessions. We analyzed 1-hour sessions in six 10-minute time blocks at a low and high dose of propofol.

3.3.3A The pattern of responding on the active and inactive levers during self-administration sessions

At 0.25 mg/kg of propofol, there was a significant difference in the number of active lever presses between the first 10-minute time block and the second, fourth and fifth blocks showing the highest magnitude of responding during the first time block (one-way repeated measures ANOVA, followed by Bonferroni’s post-hoc test: p<0.05). Inactive lever pressing was consistent throughout the sessions with no significant differences between 10-minute time blocks (Figure 22A).

At the dose of 1 mg/kg, the number of active lever presses in the first block was significantly higher compared to the number of active lever presses in the rest of the blocks (one-way repeated measures ANOVA, followed by Bonferroni’s post-hoc test: p<0.05). Also, the number of inactive lever presses was significantly higher in the first block compared to the third, forth and fifth time block (one-way repeated measures ANOVA, followed by Bonferroni’s post-hoc test: p<0.05) (Figure 22B).
Figure 22: The temporal pattern of active and inactive lever responses in 1-hour self-administration sessions

A) and B) display the temporal pattern of lever presses in 1-hour sessions at 0.25 and 1 mg/kg of propofol, respectively. At 0.25 mg/kg (A), there was a significant difference in the number of active lever presses between the first and the second, fourth and fifth time block while the number of inactive lever presses was consistent across the time blocks.

At 1 mg/kg (B), there was a significant difference in the number of active lever presses between the first and the rest of the time blocks. There was also a significant difference in the number of inactive lever presses between the first time block and the third, fourth and the fifth time block (**p<0.05; compared to the same response in other time blocks). Data is shown as the mean of the last three self-administration sessions + SEM.
3.3.3B The temporal pattern of propofol infusions (reinforcements) and total propofol intake during self-administration sessions

At 0.25 mg/kg of propofol the number of reinforcements in the first time block was significantly higher than the second and third time block, and the total propofol intake was significantly higher in the first time block compared to the second block (one-way repeated measures ANOVA, followed by Bonferroni’s multiple comparison tests: p<0.05; Figure 23A). At 1 mg/kg of propofol, statistical analyses showed that the number of reinforcements and the total propofol intake were significantly higher in the first time block compared to each other 10-minute time block (p< 0.05; Figure 23B).
Figure 23: The temporal pattern of propofol infusions (reinforcements) and total propofol intake during self-administration sessions

A) 0.25 mg/kg

B) 1 mg/kg

Figure 23: The temporal pattern of propofol infusions (reinforcements) and total propofol intake during self-administration sessions
A) and B) show the temporal pattern of reinforcements (propofol infusions) and total propofol intake in 1-hour self-administration sessions at 0.25 and 1 mg/kg, respectively. At the dose of 0.25 mg/kg of propofol (A), there was a significant difference in the number of reinforcements between the first and the second and third time block, and there was a significant difference in the total propofol intake between the first and the second block. At the dose of 1 mg/kg of propofol (B), there was a significant difference in the number of reinforcements and the total propofol intake between the first block and each other time block (p<0.05; compared to the same response in other time blocks). Data is shown as the mean of the last three sessions at each dose + SEM.
3.4 Experiment 4

3.4.1 Extinction of propofol self-administration behaviour

To further characterize the propofol self-administration behaviour profile, an extinction experiment was performed. In the acquisition phase of this experiment, the majority of rats (78%; 11 of 14) acquired self-administration behaviour at 0.25 mg/kg. After acquisition of propofol self-administration at 0.25 mg/kg and reaching stable responding at the dose of 0.56 mg/kg, propofol’s vehicle (intralipid) was substituted for propofol.

After two to five extinction sessions (with an average of 3.2 sessions), propofol self-administration behaviour was extinguished in all subjects (Figure 24). In the current study the extinction criterion was defined as less than 10 active lever presses during a 1-hour extinction session for two consecutive days. In the last two extinction sessions, the number of responses on the previously drug contingent lever was significantly lower than the number of active lever presses in the last three self-administration sessions (paired t-test; p<0.05; Figure 25). Responding on the active lever was highest in the first day of the extinction phase (one-way repeated measures ANOVA, followed by Bonferroni’s post-hoc test: p<0.05; Figure 26). The number of rats included in the analyses dropped to five because the rest of the animals showed a very low magnitude of response (<10 active lever presses) before starting the extinction sessions. Of the five rats included in data analyses, four rats had met acquisition criteria.
Figure 24: Extinction time course
A) and B) display the time to reach extinction criteria in individual rats and the mean time to reach extinction in all rats, respectively; N=5.

Figure 25: Extinction of propofol self-administration behaviour
This figure is a comparison between the last two extinction sessions and the last three self-administration sessions in the magnitude of active lever responses. The number of responses on the previously active (drug-contingent) lever in the last two extinction sessions was significantly different from the number of active lever presses in the last three sessions of self-administration (p<0.05); N=5.
Figure 26: The pattern of responding across extinction sessions
This figure represents the magnitude of responding on the previously active (drug-contingent) lever across extinction sessions. Responding on the previously active lever was higher in the first session of the extinction phase than in other sessions (\( p<0.05 \) compared to the rest of extinction sessions); \( N=5 \).
3.4.2 Reinstatement experiment using a range of propofol priming doses

Following meeting extinction criterion, during an additional extinction session rats were administered an i.p. injection of propofol vehicle (intra lipid) 15 minutes prior to the extinction session. This extinction session served as a baseline to which propofol priming-induced reinstatement was compared. The priming doses of propofol were 15, 30 and 60 times higher than the training dose used in the acquisition phase (0.25 mg/kg), which resulted in the following doses of propofol: 3.75, 7.5 and 15 mg/kg i.p., respectively. None of the propofol priming doses were able to increase propofol self-administration behaviour above that of vehicle baseline (one-way repeated measures ANOVA: p>0.05; Figure 27).

![Figure 27: Priming doses of propofol were unable to reinstate propofol self-administration behaviour](image)

This figure displays the magnitude of responding on the previously drug-contingent (active) lever after administration of different priming doses of propofol. There was no significant difference in the number of responses on the previously active lever after administration of priming doses of propofol (3.75, 7.5 and 15 mg/kg i.p.) compared to vehicle baseline (p>0.05); N=5.
3.5 An overall analysis of propofol self-administration results across different sets of experiments

3.5.1 A comparison of the acquisition training phases across different experiments

In the current series of experiments we conducted five acquisition experiments using different doses of propofol. We used a training dose of 1 mg/kg in the first experiment, 0.56 mg/kg in experiments 2B and 3, and 0.25 mg/kg in experiments 2A and 4. In all acquisition experiments, the majority of rats (65-88%) acquired propofol self-administration behaviour (Figure 28A). The comparison of the proportions of rats that acquired self-administration behaviour at different doses across different experiments revealed no significant difference between different doses (Chi-Square test: $\chi^2 (2)= 0.43$, $p=0.8$ (Figure 28B)).
Figure 28: A comparison of the outcomes of the acquisition phase across different sets of experiments
A) displays the proportion of rats that acquired self-administration behaviour in different acquisition experiments in the current study.
B) illustrates a comparison of the proportion of rats that acquired self-administration behaviour across different training doses. The first two columns represent the mean of two experiments at 0.25 and 0.56 mg/kg, respectively. There was no significant difference in the proportion of rats that acquired the behaviour between different training doses.
3.5.2 A comparison of self-administration behaviours across different acquisition experiments using different doses of propofol

We compared self-administration behaviour in all acquisition experiments at different training doses across different experiments. For the number of active and inactive lever presses and the number of reinforcements there were no significant differences between different training doses. However, the total propofol intake was significantly higher in the experiment using 1 mg/kg compared to each of the experiments using 0.56 and 0.25 mg/kg as training doses. Also, the total propofol intake was significantly higher in one of the experiments using 0.56 mg/kg compared to the experiments using 0.25 mg/kg (one-way ANOVA, followed by Bonferroni’s *post-hoc* test: p<0.05; Figure 29 (A-C)).
Figure 29: A comparison of self-administration behaviours across different acquisition experiments using different doses of propofol

A)–C) show, respectively, the number of lever presses, the number of reinforcements and the total propofol intake for the last three acquisition sessions across different acquisition experiments using different training doses of propofol. The number of active and inactive lever presses and the number of reinforcements were not significantly different across different experiments. There were significant differences in total intake between the experiment using 1 mg/kg and four other experiments using 0.25 and 0.56 mg/kg of propofol as training doses. The total propofol intake was higher in one of the experiments using 0.56 compared to the experiments using 0.25 mg/kg as a training dose (\(p<0.05\)). The numbers under each bar represent the number of rats that acquired propofol self-administration behaviour.
3.5.3 Dose response curve of propofol self-administration under different schedules using pooled data

To further assess the dose response curve experiments, we generated a dose response curve under FR1 and FR2 schedules regardless of the acquisition training dose. We pooled data at each dose from experiments 1 and 2B to generate a pooled dose response curve under the FR2 schedule (Figure 30 (A-D)). Also, we used pooled data of experiments 2A, 3 and 4 to generate a combined dose response curve under the FR1 schedule (Figure 31 (A-D)). One-way ANOVA, followed by Bonferroni’s post-hoc test was used for statistical analyses in this section of the study.
Figure 30: Dose response curve of propofol self-administration under the FR2 schedule using pooled data
A)-D) display the number of reinforcements and the total propofol intake as a function of dose for the last three sessions at each dose in all and in acquired rats. In all rats there was a significant difference in the total propofol intake between 1.7 mg/kg and 0.25 and 0.56 mg/kg ($p<0.05$).
Figure 31: Dose response curve of propofol self-administration under the FR1 schedule using pooled data

A)-D) display the number of reinforcements and the total propofol intake as a function of dose for the last three sessions at each dose in all and in acquired rats. There was a significant difference in the total propofol intake between 1 and 0.56 mg/kg in all and in acquired rats. There were significant differences in the total propofol intake between 0.56, 1 and 1.7 and three lowest doses (0.0625, 0.125 and 0.25 mg/kg) ($p<0.05$).
3.5.4 Evaluation of interindividual differences in body weight and weight gain across different experiments

Environmental stress facilitates acquisition of drug self-administration and it is possible that the food restriction used in the current study served as a source of stress. Therefore, we were interested to assess whether there was a correlation between body weight (weight gain/loss) and magnitude of self-administration behaviour during the acquisition phase at different doses. At any given acquisition training dose used in this study, all animals showed a stable body weight. There was no weight loss, nor was there a significant difference between acquired and not acquired rats in weight gain during the acquisition time course (5-6 days) (unpaired t-test: p>0.05; Figure 32-34).
Figure 32: The assessment of body weight during propofol acquisition training at 0.25 mg/kg
A) shows a comparison between acquired and not acquired rats in body weight across different sessions of the acquisition phase. There was no difference between the body weight of acquired and not acquired rats during acquisition training.
B) illustrates the interindividual variation in body weight in acquired and not acquired rats. Animals had similar body weight in both acquired and not acquired groups. Data is shown as the mean of all acquisition sessions for each rat+SD.
C) displays a comparison between acquired and not acquired rats in weight gain during the acquisition phase. There was no significant difference between acquired and not acquired rats in weight gain.
Figure 33: The assessment of body weight during propofol acquisition training at 0.56 mg/kg

A) shows a comparison between acquired and not acquired rats in body weight across different sessions of the acquisition phase. There was no difference between the body weight of acquired and not acquired rats during acquisition training.

B) illustrates the interindividual variation in body weight in acquired and not acquired rats. Animals had similar body weight in both acquired and not acquired groups. Data is shown as the mean of all acquisition sessions for each rat+SD.

C) displays a comparison between acquired and not acquired rats in weight gain during the acquisition phase. There was no significant difference between acquired and not acquired rats in weight gain.
Figure 34: The assessment of body weight during propofol acquisition training at 1mg/kg

A) shows a comparison between acquired and not acquired rats in body weight across different sessions of the acquisition phase. There was no difference between the body weight of acquired and not acquired rats during acquisition training.

B) illustrates the interindividual variation in body weight in acquired and not acquired rats. Animals had similar body weight in both acquired and not acquired groups. Data is shown as the mean of all acquisition sessions for each rat+SD.

C) displays a comparison between acquired and not acquired rats in weight gain during the acquisition phase. There was no significant difference between acquired and not acquired rats in weight gain.
Section 4: Discussion

4.1 General discussion

4.1.1 Propofol is a drug with reinforcing properties

In the context of the self-administration paradigm, the acquisition of drug self-administration behaviour is an important indicator of the reinforcing effects of drugs and serves as a screening tool to identify psychoactive drugs with abuse potential (Richardson and Roberts 1996). The abuse liability of a test drug is determined by measuring its reinforcing effects; a drug is considered a reinforcer if responding for the drug is maintained above responding for the inactive lever or other control conditions. We studied the reinforcing effect of propofol by conducting self-administration experiments in rats. In the current study, independent groups of animals were tested for the acquisition of propofol self-administration behaviour at different propofol infusion doses.

The design of a self-administration study and the decision of using facilitated or spontaneous acquisition should be based on the main questions that the study intends to answer. Facilitated acquisition will serve as a helpful strategy if 1) the main objective of the study is an initial screening investigation aiming to show the reinforcing effects of a test drug and 2) the main emphasis of the study is on aspects of self-administration behaviour other than acquisition, such as maintaining the behaviour or extinction and reinstatement. Taking the technical limitations of intravenous self-administration into consideration, the advantages of facilitated acquisition become more apparent. One of the major limitations of intravenous self-administration is that the maintenance of patent catheter preparations in rodents can be poor, especially over periods of 6 weeks or more (Koob 1995). Therefore, establishment of drug self-administration behaviour in the majority of subjects over a shorter period of time by using
facilitated acquisition strategies is useful in intravenous self-administration studies, as it makes the experiments more efficient, while also reducing the number of animals sacrificed. Thus, using facilitated acquisition is a common approach in self-administration studies to assist with and shorten the acquisition phase. Different methods including drug-substitution and food training have been developed to facilitate the acquisition of drug self-administration behaviour (Carter and Griffiths 2009). Using a drug-substitution strategy may introduce uncertainty into the results and make the data difficult to interpret, as it may be unclear whether the observed reinforced behaviour after substitution is due to the reinforcing effects of the test drug (a drug with unknown abuse potential) or is the result of extinction from the training drug (a drug with known abuse liability, e.g. cocaine or methohexitol) (Carroll and Meisch 2011). This may be considered a disadvantage of the drug-substitution method since extinction can take two weeks or more (Carroll and Meisch 2011). Another disadvantage of the drug-substitution approach is that the training drug itself can influence the amount of the test drug that is subsequently self-administered (Mierzejewski, Rogowski et al. 2003).

To avoid the potential confounding factors associated with the drug-substitution strategy, we used a food training procedure to facilitate the acquisition of propofol self-administration. In the current study, drug-naïve, food-restricted animals were trained to respond for food (sucrose pellets) as a natural reward under a FR schedule prior to propofol self-administration sessions. Fixed ratio schedules play a key role in drug self-administration research. Animals learn more easily on a FR schedule, especially FR1, making this schedule an effective approach in the initial screening of a test drug with abuse potential (Richardson and Roberts 1996). Thus, a FR1 schedule was used in food training and in acquisition experiments in this study.
Our results showed that propofol at all doses tested in this experiment (0.25, 0.56 and 1 mg/kg) supported the transition from food-reinforced to drug-reinforced responding, indicating propofol’s reinforcing properties. Two main end points of an acquisition experiment are: 1) the percentage of animals that acquire drug self-administration behaviour and meet the acquisition criteria, and 2) the latency to acquire the self-administration behaviour, which is defined as the number of sessions needed to reach the acquisition criteria (Carroll and Lac 1997). In our study, at all propofol doses the majority of rats (65-88%) acquired self-administration behaviour (Figure 28A) and met the acquisition criteria in the last three sessions of the acquisition training phase (5-6 days). There were no significant differences in the proportion of rats that acquired self-administration behaviour across the different doses of propofol (Figure 28B).

Together, the acquisition experiments in our study did not show a relationship between the acquisition training dose and the proportion of rats that acquired self-administration behaviour, nor the latency to acquire the behaviour. However, the initial available dose of a drug is thought to be an important determinant of the rate and probability that acquisition will occur. The acquisition of drug self-administration displays dose dependency for different types of drugs with abuse liability including cocaine, amphetamine and heroin. When three groups of rats were subjected to acquisition at three doses of cocaine (0.125, 0.25 and 0.5 mg/kg), although similar percentages (60-80%) of rats in each group acquired self-administration behaviour, the latency to acquire the behaviour was shorter at higher doses (Schenk, Valadez et al. 1993). It has also been found that the percentage of rats that acquired amphetamine and cocaine self-administration behaviour increased, and the mean number of days to meet the acquisition criteria declined, as a function of increasing dose for each respective drug (Carroll and Lac 1997). Increasing the dose of heroin from 0.082 to 0.188 to 0.375 mg/kg also resulted
in an increase in the proportion of rats that acquired self-administration behaviour (van Ree, Slangen et al. 1978).

There are a number of factors that could explain the results of propofol acquisition experiments in the current study. One possibility would be that the acquisition of propofol self-administration is not sensitive to variation in training dose, as even the lowest dose tested in this study (0.25 mg/kg) had sufficient reinforcing effects to induce the acquisition of self-administration behaviour in the majority of rats. In contrast to this notion, in a very recent study using a spontaneous acquisition approach with no prior food training, propofol was not self-administered at a dose of 0.56 mg/kg, but was self-administered at two higher doses (1 and 1.7 mg/kg) by rats with ad lib access to food (Lian, Wang et al. 2013). In addition to food training, which facilitates the learning process of operant responding, the feeding condition of animals also influences the acquisition of drug self-administration. For instance, rats on a restricted diet (10 g/day) acquired cocaine-reinforced behaviour faster than rats with ad lib access to food (6 versus 16 days) (Carroll and Lac 1993). Thus, using food training and food restriction in our study may have facilitated acquisition, allowing it to occur even at the lowest training dose tested (0.25 mg/kg), whereas a dose of 0.56 mg/kg could not support self-administration in untrained rats which had free access to food (Lian, Wang et al. 2013). In the current study, the facilitating effects of food training and/or food restriction may have resulted in a basement effect, since the percentage of rats that acquired the behaviour and the latency of acquisition were the same regardless of the training dose.

Although food training and food restriction play a facilitating role in the acquisition of drug self-administration, these facilitating conditions are neither necessary nor sufficient to establish drug self-administration behaviour. Food-deprived rats and monkeys showed higher levels of nicotine and phencyclidine self-administration, however, food deprivation was not
necessary to establish self-administration (Carroll 1982; Donny, Caggiula et al. 1998). As has been recently observed, food restriction and food training are not critical in establishing propofol self-administration. Rats with ad lib access to food and no food training acquired propofol self-administration at higher doses of propofol (1 and 1.7 mg/kg) (Lian, Wang et al. 2013). Propofol self-administration behaviour was also established at 1.7 mg/kg in food-restricted rats with no food training (LeSage, Stafford et al. 2000). Therefore, food training and food restriction provide optimal conditions to establish propofol self-administration for subsequent assessments of other phases of propofol self-administration behaviour including maintenance, extinction and reinstatement. However, employing food training and food restriction as facilitating factors are not central requirements for the acquisition of propofol self-administration in rats, as is also seen with other drugs of abuse.

It is also possible that the dose range of propofol that we used in our facilitated acquisition experiments was not broad enough to identify the doses of propofol that are unable to support the acquisition of self-administration behaviour. The facilitated acquisition strategy, as a general technique, allows sufficient sensitivity to determine doses of a drug that are unable to induce acquisition. For instance, food-trained, food-restricted rats did not acquire nicotine self-administration at a dose of 0.01 mg/kg, but at two higher doses, 0.03 and 0.06 mg/kg, nicotine self-administration behaviour was established (Donny, Caggiula et al. 1998). Therefore, it is likely that even with the relatively low dose of propofol (0.25 mg/kg), we did not test a dose that was unable to support self-administration behaviour in rats.

In conclusion, we found that different doses of propofol, as low as 0.25 mg/kg, supported rapid acquisition of self-administration behaviour in the majority of animals tested, indicating the abuse liability of propofol at the tested range of doses. The acquisition phase of the self-administration paradigm is a powerful and validated animal model to study the
reinforcing properties of drugs. Also, the predisposing factors involved in vulnerability to initiation of drug use can be addressed in acquisition experiments. The identification of behavioural and biological factors involved in vulnerability to the reinforcing effects of a drug with abuse liability is an important aspect of addiction studies using animal models. A better understanding of these predisposing factors might be applied to prevention efforts in humans (Campbell and Carroll 2000). One future direction in characterizing propofol self-administration might be to examine a number of factors which influence the acquisition of drug self-administration behaviour, including environmental factors (e.g. feeding condition and stress) and individual differences (e.g. age and sex differences).

4.1.2 Interindividual differences in propofol’s reinforcing effects

Only a proportion of individuals who engaged in non-medical or illicit drug use at some point in their lives continue on to become a substance abuser or substance dependent (Grant, Dawson et al. 2004) and there is an interindividual variation in the vulnerability to substance use disorders. Individual differences in personality traits (e.g. novelty and sensation-seeking) (Wills, Vaccaro et al. 1994), comorbid psychiatric disorders (e.g. mood and anxiety disorders) (Glantz and Hartel 1999) and genetic factors (Tyndale and Sellers 2002) are major factors that are involved in the interindividual variation in vulnerability to substance abuse and substance dependence as two main categories of substance disorder.

Clinical (Zacny 1993) and preclinical (Weerts, Ator et al. 1999; LeSage, Stafford et al. 2000) studies in monkeys and rats have shown interindividual differences in propofol’s reinforcing properties. Consistent with previously reported data, propofol self-administration behaviour was not acquired by all subjects tested in the current study and even among those rats that acquired self-administration behaviour, the magnitude of responding showed
intersubject variations (Figure 3, 10, 14 A-C). As mentioned earlier in this section anxiety disorder, as a consequence of repeated exposure to stress, is considered as a comorbid disorder that influences the vulnerability to substance use disorders. In the next section the potential role of anxiety in the intersubject differences seen in propofol’s reinforcing properties will be discussed in more details.

4.1.3 Potential effects of propofol’s anxiolytic properties on propofol self-administration

It is a frequent finding of human laboratory studies that benzodiazepine-like drugs display their reinforcing effects primarily in subjects with a history of drug or alcohol abuse, in anxious subjects and in patients with sleep disorders (Griffiths and Weerts 1997). Benzodiazepines, unlike other drugs of abuse, do not function consistently as reinforcers in subjects without the above mentioned predisposing conditions (Licata and Rowlett 2008). It can be hypothesized that individuals who suffer from anxiety self-administer a benzodiazepine-like drug to alleviate this anxiety (Helmus, Tancer et al. 2005), and thus anxious individuals would find the drug more reinforcing than people without anxiety, as a result of the therapeutic effects of the drug in reducing the underlying neuropsychological problem.

Like benzodiazepines, propofol is a positive modulator of GABA<sub>A</sub> receptors, suggesting that these drugs might show some common therapeutic effects (e.g. anxiolytic effects) as well as reinforcing properties. Propofol at subanesthetic doses has shown anxiolytic effects in humans. Continuous infusion of propofol (1mg/kg/h) in cancer patients four hours (i.e. 4 mg/kg total) prior to chemotherapy markedly reduced anxiety levels (Borgeat and Forni 1992). In patients that underwent endoscopy procedures, propofol (0.95 mg/kg i.v.) showed
equivalent anxiolytic effects to a benzodiazepine drug, midazolam (0.08 mg/kg i.v.) (Patterson and Cunningham 1989).

Consistent with these human data, preclinical studies in rats support propofol’s anxiolytic properties. Subanesthetic doses of propofol (1, 3 and 9 mg/kg i.p.) increased the number of entries and the time spent in the open arms of an elevated plus maze compared to vehicle (Pain, Oberling et al. 1999). Also, using an elevated plus maze strategy in mice, a non-anesthetic dose range of propofol (20, 40 and 60 mg/kg i.p.) showed anxiolytic effects (Kurt, Bilge et al. 2003). It can be argued that the results of these studies were due to a decline in the perception of anxiogenic situations because of the sedative effects of propofol rather than to its anxiolytic properties. However, in these studies there were no significant changes in the locomotor activity of animals, indicating that propofol functions as an anxiolytic drug at doses that do not cause notable sedation or sleep (Pain, Oberling et al. 1999; Kurt, Bilge et al. 2003).

Wide use of serotonin reuptake inhibitors and serotonin receptor agonists in anxiety disorders indicates a central role of serotonin as a modulatory neurotransmitter in anxiety (Gordon and Hen 2004). Conflict behaviour is one of the predictive anxiety-related behaviours in rats. A conflict situation increases serotonin release in the dorsal hippocampus, and this elevated serotonin release can be inhibited by midazolam (Matsuo, Kataoka et al. 1996). Like the benzodiazepine midazolam, propofol at subanesthetic doses (20 and 40 mg/kg i.p.), showed anti-anxiety effects in a conflict test by inhibiting serotonin activity in rat dorsal hippocampus (Matsuo, Ayuse et al. 1997). Therefore, propofol is an anxiolytic drug with a mechanism of action similar to that of the benzodiazepine midazolam. In agreement with the anxiolytic effects of propofol, the subjective effects associated with propofol abuse have been described by propofol abusers as relief from anxiety, stress alleviation, calm feelings and restful sleep (Roussin, Montastruc et al. 2007; Levy 2011).
Many propofol abusers suffer from post-traumatic stress disorder (Welliver and Baker 2012). Refractory and persistent insomnia is one of the debilitating symptoms of post-traumatic stress disorder. Insomnia is treated effectively by propofol, and animal data show that propofol-induced sleep is restorative and mimics some natural sleep stages (Tung, Lynch et al. 2001). Thus, it can be hypothesized that, like benzodiazepines, propofol might be more reinforcing in individuals with predisposing factors such as insomnia and anxiety, which may have arisen as a consequence of exposure to stressors. Consistent with this assumption a recent study of 22 cases of propofol abuse has revealed that insomnia and anxiety were the main reasons for abuse propofol in 41% and 21% of cases and only 18% of propofol abusers were seeking euphoria (Earley and Finver 2013).

Different forms of stressors, both physical and social, have shown promoting effects on the initiation of drug self-administration behaviour in animal studies (Campbell and Carroll 2000). It has been suggested that restricted diet can function as a stressor leading to an increase in acquisition of drug self-administration. Both female and male food-restricted rats showed a nearly 96% increase in the number of heroin infusions relative to food-satiated rats. This effect was antagonized by an inhibitor of corticosterone synthesis, ketoconazole (Carroll, Campbell et al. 2001). Corticosterone reversed the effect of ketoconazole in female rats, indicating that increases in heroin self-administration in female food-restricted rats were due to a stress response (Carroll, Campbell et al. 2001). Therefore, the high acquisition rate observed in our study could also have been the result of food restriction functioning as a stressor. Due to the impact of stressors on subsequent behaviours, such as anxiety-like behaviours, it could be hypothesized that propofol-induced self-administration behaviour is due, at least in part, to propofol’s anxiolytic properties when food restriction functions as a stressor. The variability between animals in risk for acquisition, and even interindividual differences in the levels of
responding (i.e. active lever press) among the rats that acquired the self-administration behaviour, might be due to variability in anxiety levels.

To find the potential relationship between food restriction and propofol self-administration behaviour, we analyzed the animals’ weight data to assess possible correlations between weight loss (an indicator of stress) and the magnitude of self-administration behaviour during the acquisition phase. Animals did not have any weight loss during the acquisition time course (5-6 days). At any given acquisition training dose there was no difference in weight or weight gain between the rats that acquired and those that did not acquire self-administration behaviour (Figures 32-34 (A and C)). In addition, all acquired and not acquired animals had a stable body weight with no interindividual variation during the acquisition phase (Figures 32-34 (B)). One possible conclusion may be that food restriction had no influence on stress levels. However, an alternative conclusion could be that weight is not a sensitive indicator of changes in anxiety levels of animals under food restriction.

Overall, propofol’s reinforcing effects could possibly be augmented by the anxiolytic effects of propofol, thus it would be interesting to look at the effects of stress and anxiety on propofol self-administration behaviour in future studies. For instance, it would be possible to expose animals to another source of environmental stress, such as restraining the rats for a period of time, prior to acquisition sessions and assess the effect of stress on the outcomes of acquisition training. Another way to study this could be to measure the anxiety levels of rats by measuring the plasma levels of corticosterone, or by using an animal model of anxiety-like behaviour (e.g. elevated plus maze), prior to propofol acquisition training.
4.1.4 Propofol self-administration behaviour was established in rats with no drug history

A history of drug or alcohol abuse could lead to increased reinforcing effects of subsequent drugs with abuse liability such as benzodiazepines (Griffiths and Weerts 1997). Our results showed that prior exposure to a drug with abuse liability is not essential for establishing propofol self-administration behaviour in rats. Our findings are in agreement with a previous study in rats showing no difference between drug-naïve and methohexital treated rats with regards to acquisition of propofol self-administration behaviour (LeSage, Stafford et al. 2000). Our results suggest that propofol has sufficient reinforcing effects to serve as an initial drug of abuse in humans without any past experience of drug abuse. This conclusion is in agreement with case reports of propofol abuse by individuals with no history of drug abuse (Welliver and Baker 2012).

4.1.5 Propofol self-administration was maintained under a more demanding fixed ratio schedule

The relative reinforcing efficacy of the different unit doses of drugs with potential abuse liability can be studied using escalating FR as a type of response cost procedure (Marquis, Webb et al. 1989). The interaction of varying the unit dose and the fixed ratio size has been interpreted as a way to measure the reinforcing efficacy of different unit doses of a drug with abuse potential (Meisch 2000).

In independent cohorts of animals, we studied the ability of two different doses of propofol to maintain self-administration behaviour under higher fixed ratio schedules. After rats acquired propofol self-administration and reached stable responding at training doses of 1 and 0.56 mg/kg in experiments 1 and 2B respectively, the schedule was increased from FR1 to FR2. Rats showed a significant preference for active over inactive lever pressing and
maintained their responding for propofol at a steady rate when the fixed ratio was increased. We observed similar response rates (i.e. total number of active lever presses) under FR1 and FR2 schedules, resulting in a lower number of propofol injections under the higher ratio (FR2). Consistent with our observations, raising the ratio from FR1 to FR5 led to a downward shift in the number of propofol injections, although propofol still maintained a higher number of infusions compared to vehicle in the majority of rats (LeSage, Stafford et al. 2000). The inverse relationship between the FR value and the number of reinforcements has also been shown for other drugs of abuse. For both pentobarbital (Lamaire and Meisch 1984) and phencyclidine (Marquis, Webb et al. 1989), as the FR value was increased, a decrease in the number of reinforcements occurred, which was less prominent at high unit doses than at lower unit doses. Unlike with other drugs of abuse (Lamaire and Meisch 1984; Marquis, Webb et al. 1989), the results of our maintenance experiments under an escalating ratio were not affected by dose, showing no significant differences in the number of reinforcements between the two doses of propofol (0.56 and 1 mg/kg) under the FR2 schedule (Figure 18). This indicates that these two tested doses had the same reinforcing efficacy.

Together our data revealed that under escalating FR schedules, which are representative of increasing response cost, rats maintained propofol self-administration behaviour, suggesting that the reinforcing properties of propofol are sustained under more demanding requirements for drug delivery. There was no difference in the reinforcing efficacy of the two doses tested in the maintenance phase of self-administration behaviour. Using a wider range of propofol doses under a broader range of FR values, or a progressive schedule, might provide us with more information regarding the relative reinforcing efficacy of the different unit doses of propofol and the motivational aspects of propofol self-administration behaviour.
4.1.6 Dose response curve of propofol-induced behaviours

A dose dependent change in self-administration behaviour is another property supporting the fact that self-administration behaviour is controlled by response-contingent drug deliveries rather than other factors such as conditioned reinforcing effects of environmental cues. A typical dose response curve in self-administration studies is an inverted U-shaped curve where the number of reinforcements is a function of dose. This form of dose response relationship has been reported in different species, including monkeys and rats, for the self-administration of stimulants, depressants, dissociative anesthetics, barbiturates and opioids (Lynch and Carroll 2001). At low doses, on the ascending part of the dose response curve, the response rate is directly related to the unit dose of the drug, showing a peak response at moderate doses. However, at higher doses there is an inverse relationship between responding and drug dose, generating the descending limb of the dose response curve. The compensatory relationship between the unit drug dose and the magnitude of responding in the descending limb of the curve tends to maintain similar levels of total drug intake as the dose increases. In this study, we generated propofol self-administration dose response curves under both FR1 and FR2 schedules. Our results showed that propofol-induced self-administration behaviour, represented by the number of propofol infusions, is dose dependent and is affected by response-contingent drug delivery, which in turn indicates propofol’s reinforcing properties. However, in some cases (Figure 19 (A and C) and 21 (A and C)), general rates of responding were low and did not significantly change with dose.

In the current study, there was some variation across different parts of the study regarding the shape of the propofol self-administration dose response curve. Under a FR1 schedule, response was an inverse function of dose, with the highest number of propofol infusions at the lowest dose of 0.125 mg/kg (Figure 11A). This finding is in agreement with a
previous propofol study in rats which generated a descending dose response curve under a FR1 schedule (LeSage, Stafford et al. 2000). Also, a very recent study showed the same descending dose response curve for propofol self-administration (Lian, Wang et al. 2013). However, in another experiment (Figure 21 (A and C)) in the current study, a shallow inverted U-shaped dose response curve was generated with 0.56 mg/kg as the optimum dose under the same low ratio schedule (FR1). Under the FR2 schedule the dose response curve was a shallow inverted U-shaped (Figure 7 A and C) or flat curve (Figure 19 A and C). Despite the differences in the shape of the curves, the position of the highest tested doses of propofol (1 and 1.7 mg/kg) on the descending limb of the curves was a consistent finding in the majority of the dose response curve experiments. To further analyze the dose response curves across the experiments, we generated a combined dose response curve at each FR schedule, merging data from different acquisition training doses. We pooled our data at each dose tested from experiments 1 and 2B to generate a combined dose response curve under FR2 and from experiments 2A, 3 and 4 to generate a pooled dose response curve under FR1. The combination of data sets from different experiments resulted in a flat dose response curve on the FR2 (Figure 30 A and C) and a descending curve on the FR1 schedule (Figure 31 A and C).

The observed inter-experiment variation in the shape of the dose response curves might be rooted in the different experimental conditions among the different experiments. When animals reached stable responding at any given dose, based on the magnitude of responding (number of active lever presses and reinforcements), the next dose was determined, and thus there was no fixed order of doses between experiments. It might be possible that the order of presentation of propofol doses had an effect on the dose response function. However, this possibility is not supported by other studies. For instance, there was no difference in the shape of dose response curves of ethanol oral self-administration in rats when different orders of
alcohol’s concentrations (e.g. escalating vs. unsystematic) were presented (Carnicella, Yowell et al. 2011). Alternatively, in the present study, acquisition experiments were performed using different training doses of propofol, which might also contribute to variation in the shape of dose response curves.

Regulation of drug intake is achieved by changing response to reach a relatively constant level of drug intake over a specified time period. This regulation of drug intake is thought to contribute to the descending part of an inverted U-shaped dose response curve, where increasing unit doses result in lower response rates, but similar total drug intake; while this occurs to some degree with all classes of drugs of abuse, there is variation in the precision of this regulation (Lynch and Carroll 2001). A greater than 12-fold increase in cocaine dose resulted in a less than two-fold increase in cocaine total intake in rhesus monkeys (Wilson, Hitomi et al. 1971). The total intake of barbiturates is regulated relatively precisely in rhesus monkeys. Eight-fold or higher increases in the unit dose of barbital, amobarbital, pentobarbital, methohexital and thiopental result in less than two-fold increases in total intake (Goldberg, Hoffmeister et al. 1971; Winger, Stitzer et al. 1975). A four-fold increase in dose led to a less than two-fold increase in ethanol total intake in monkeys (Karoly, Winger et al. 1978). In one of our dose response curve experiments, as the propofol dose increased along the descending limb of the dose response curve, the total intake remained constant as a result of decreases in the magnitude of responding (Figure 7 (B and D)); however, this tight regulation was not a common feature across the different dose response curve experiments in the current study. When examining the effects of increasing dose on the descending limb of the dose response curve, in some cases there were no robust changes in the magnitude of responding, and thus the propofol total intake increased as the dose escalated (Figure 11 (B) and 21 (B and D)). The descending limb of a dose response curve in the self-administration studies, and the associated
regulation of intake, is thought to be a consequence of the adverse effects of drugs (e.g. sedative effects) and an attempt to keep drug intake at a desired level. Our results suggest that even at the highest doses tested in this study, propofol might not have sufficient adverse effects, thus reducing the need to regulate propofol total intake. Overall, we found that there was only a modest sensitivity to propofol dose, leading to flattened dose response curves and increasing intake with escalating unit dose.

4.1.7 The temporal pattern of propofol self-administration behaviour was similar to that of other drugs with abuse liability

Our results showed a burst of responding on the active lever, and the highest number of propofol infusions, at the beginning of the daily session followed by a lower and consistent rate of responding and infusions during the rest of each session (Figure 22 and 23). For relatively short daily sessions with unrestricted access to the drug (using small ratio schedules and short timeouts), as we used in this study, this pattern of responding is a common feature for many different classes of drugs with abuse liability. Stimulants such as cocaine (Ettenberg, Pettit et al. 1982) and nicotine (Corrigall and Coen 1989), depressants such as pentobarbital and methohexital (Winger, Stitzer et al. 1975), ethanol (Karoly, Winger et al. 1978), opioids (Downs and Woods 1974) and dissociative anesthetics such as ketamine (Moreton, Meisch et al. 1977) have shown the same temporal pattern of responding as we saw with propofol. Thus, propofol shares this property with other drugs of abuse. It has been suggested that the animals respond at relatively high magnitudes during the initial few minutes of the self-administration session to increase blood levels quickly to preferred values (Pickens, Meisch et al. 1978).
4.1.8 Propofol-induced self-administration behaviour was extinguished upon substitution of vehicle for propofol

To confirm that drug presentation is the event that maintains self-administration behaviour, it is important to ensure that responding eventually ceases when the operant response does not produce a drug infusion (Koob 1995). The previously drug-contingent operant response displayed during extinction sessions is referred to as drug seeking behaviour because the response is no longer reinforced by drug (Cleva and Gass 2010).

In the last set of our experiments (experiment 4), once rats acquired propofol self-administration at 0.25 mg/kg and reached stable self-administration behaviour at 0.56 mg/kg, they were then subjected to an extinction procedure. During the 1-hour extinction sessions, propofol’s vehicle (intralipid) was substituted for propofol and animals were given an injection of vehicle upon pressing the active lever, without any injection-contingent cues. Under our experimental conditions propofol self-administration behaviour was extinguished in all rats. Animals met the extinction criteria (less than 10 active lever presses for 2 consecutive days) after 2-5 extinction sessions with an average of 3.2 sessions (Figure 24 (A and B)). Contrary to our results, rats showed high levels of responding for vehicle under a FR1 schedule in a previous study (LeSage, Stafford et al. 2000). This discrepancy between these two studies could be due to the differences in the design of our extinction study from the LeSage study, in which different doses of propofol and the vehicle (intralipid) were available for at least five sessions in a mixed order which varied across animals, and infusions were signaled by the offset of stimulus lights and house light which remained the same during both propofol and vehicle deliveries (LeSage, Stafford et al. 2000).
The extinction procedure can provide measures of the incentive or motivational properties of drugs by assessing the persistence of drug seeking behaviour in the absence of response-contingent drug availability (Koob 1995), and it has been suggested that greater resistance to extinction is related to greater reinforcing efficacy (Shram, Funk et al. 2008). Thus, the fast extinction of propofol self-administration behaviour in our study suggests that propofol is likely a drug with a low reinforcing efficacy. However, other interpretations can be made as follows. First, it is possible that the drug-paired cues became conditioned motivational stimuli with reinforcing properties. Therefore, the short duration of extinction could be due to the facilitation of extinction by the removal of injection-contingent cues. Second, in a self-administration study using extended access to nicotine in rats (23 hours per day for 40 days), the rate of extinction was dose-dependent (O'Dell, Chen et al. 2007). Rats receiving higher doses of nicotine exhibited more lever pressing during extinction compared to rats receiving lower doses of nicotine. Rats receiving higher doses of nicotine also showed higher levels of dependence measured by mecamylamine precipitated withdrawal, and the authors suggested that resistance to extinction can be used as a marker for nicotine dependence (O'Dell, Chen et al. 2007). This finding is consistent with the slower extinction of drug seeking behaviour in cocaine and heroin dependent rats (Shalev, Grimm et al. 2002). Thus, the relatively fast extinction in our study may be due to the lower doses of propofol used, the shorter duration of access and maintenance, and/or the fact that the animals were not dependent on propofol.

To summarize, we found that the contingency of propofol delivery on operant response is an important factor in maintaining self-administration behaviour, providing more evidence to support propofol’s reinforcing properties. The short latency to reach extinction criteria could be due to propofol’s low reinforcing efficacy. However, the role of conditioned motivational stimuli, such as environmental cues, in the extinction of propofol self-administration
behaviour, as well as the low dose, should be taken into account. The important role of environmental context, including drug-paired cues, in drug seeking behaviour has been documented for many drugs of abuse including nicotine and alcohol. Conducting extinction experiments using a range of doses and durations of prior maintenance, and in the presence of drug-paired cues may provide more comprehensive data on the role of dose, duration of access and environmental context (as a conditioned reinforcing stimulus) in propofol seeking behaviour, respectively.

4.1.9 Priming doses of propofol did not reinstate extinguished self-administration behaviour

Reinstatement experiments in animals after extinction of drug self-administration behaviour are used to model relapse to recurrent drug use in humans after a period of abstinence. There are three types of triggers used to restore extinguished self-administration behaviour in animals which are analogous to the conditions that lead to relapse in humans: re-exposure to priming doses of the self-administered drug, exposure to drug-associated environmental cues, and stress (Lynch, Nicholson et al. 2010).

None of the three tested priming doses of propofol were able to reinstate drug seeking behaviour under our experimental conditions (Figure 27). Our results might suggest that propofol is a drug with low reinforcing effects. However, there are other possible explanations for our findings. First, the reinforcing efficacy of propofol doses tested in this experiment was not large enough to reinstate the extinguished self-administration behaviour; it might be possible to restore the extinguished behaviour by using higher doses of propofol. Second, in this study rats had a short 1-hour access to propofol in daily sessions, and there is some evidence showing the effect of length of training sessions on reinstatement outcomes. For
example, rats with long access to cocaine (6-hour session/day) responded to a priming dose of cocaine while rats with short access (1-hour session/day) were not responsive (Ahmed and Cador 2006). Likewise, priming doses of heroin were able to reinstate drug seeking behaviour in rats with long access (6-hour session/day) to heroin self-administration while rats with short access (1-hour session/day) did not respond to heroin priming doses (Lenoir and Ahmed 2007). Third, the lack of response-contingent cues during reinstatement testing abolished stress-induced reinstatement of cocaine seeking behaviour in rats with limited access to the drug (2h/day) (Beardsley, Howard et al. 2005). However, it is unknown whether the facilitating effect of cues on reinstatement depends on sensory reinforcement and/or drug conditioning (Lenoir and Ahmed 2007). Extinguished nicotine self-administration behaviour in rats was reinstated by nicotine-paired cues and treatment with an anxiogenic drug, yohimbine, but not with a nicotine priming dose alone (Feltenstein, Ghee et al. 2012). When combined with nicotine-paired cues both yohimbine and nicotine-prime resulted in enhanced reinstatement (Feltenstein, Ghee et al. 2012). Therefore, in the present study, the omission of programmed response-contingent cues during reinstatement testing could contribute to the lack of reinstatement of propofol self-administration behaviour.

In conclusion, we showed that in the absence of previous propofol-paired cues, priming doses of propofol used in this study were not sufficiently reinforcing to reinstate the extinguished propofol self-administration behaviour. With regards to the possible incentive and motivational properties of conditioned stimuli (drug-paired cues), further reinstatement experiments using cues alone, and in combination with propofol priming doses, will help to distinguish the effects of each factor alone or in combination on restoring propofol seeking behaviour.
4.1.10 A comparison between propofol and benzodiazepines as regulated drugs

Propofol, like benzodiazepines, is a positive modulator of GABA\(\Lambda\) receptors and bears some similarities with this class of drugs. Propofol produced anxiolytic effects similar to midazolam (Patterson and Cunningham 1989) and the discriminative effect of propofol was comparable with that of the benzodiazepine chlordiazepoxide (Gatch and Forster 2011). Benzodiazepines and propofol have shown some common features regarding their reinforcing properties that will be discussed later on.

In the current study we provided some evidence indicating propofol’s reinforcing effects, although there were some suggestions that propofol is a relatively weak reinforcer. For instance, propofol showed 1) a modest magnitude of responding under FR1 and FR2 schedules (i.e. number of active lever presses or number of reinforcements per 1-hour sessions), 2) a modest sensitivity to changes in unit dose, resulting in flattened dose response curves and 3) a rapid extinction with no reinstatement of the extinguished self-administration behaviour using a range of priming doses. Similar to our results, intravenous self-administration of the benzodiazepine diazepam in rats showed a fast extinction upon substitution of vehicle for the drug (Pierce and Kumaresan 2006). In the diazepam self-administration study the magnitude of responding (i.e. number of reinforcements) was about 14 infusions/hour at an optimum dose (Pierce and Kumaresan 2006) which is comparable with our results (Figure 11 and 21).

Using a progressive ratio strategy makes it possible to compare the relative reinforcing strength of benzodiazepines with that of other drugs of abuse such as stimulants and opioids. Under similar experimental conditions the break point maintained by the benzodiazepine midazolam was markedly lower than break points maintained by cocaine and alfentanil (a selective mu opioid receptor agonist), indicating the low reinforcing strength of midazolam compared to cocaine and alfentanil (Di Chiara and Bassareo 2007). Although there is no
evidence addressing the relative reinforcing strength of propofol compared to other drugs of abuse, considering the similarities between propofol and benzodiazepines, it might be concluded that propofol is a weak reinforcer compared to other drugs of abuse such as stimulants and opioids.

The reinforcing effect of benzodiazepines is prominent in individuals with predisposing factors such as a history of drug or alcohol abuse, anxiety and sleep disorders (Griffiths and Weerts 1997). Therefore, poly-drug users and people suffering from anxiety face a higher risk for the abuse of benzodiazepines (Licata and Rowlett 2008). The widespread use of benzodiazepines as effective anxiolytic drugs led to growing evidence of their abuse liability; in 1975, despite their relatively weak reinforcing properties, diazepam and many other benzodiazepines were regulated as schedule IV drugs (Licata and Rowlett 2008).

Unlike benzodiazepines, propofol is not a prescription drug per se and its medical use is essentially limited to hospitals and medical settings. Propofol is commonly used as an intravenous anesthetic and is also used routinely as a sedative in critically ill patients in intensive care units (Levy 2011). Therefore, medical professionals are the main group in which propofol abuse has been observed, likely due to easy access to the drug (Welliver and Baker 2012).

In light of growing evidence indicating propofol’s abuse potential among health professionals (Wischmeyer, Johnson et al. 2007), the placement of propofol under schedule IV of the “Controlled Substances Act” has been proposed by the Drug Enforcement Agency in the United States. This schedule includes benzodiazepines such as diazepam, alprazolam and clonazepam. The Drug Enforcement Agency has expressed concerns regarding the diversion and abuse of propofol and has suggested the need for higher level of restrictions to reduce the ease of access to the drug and consequently reduce the diversion and abuse of propofol.
Despite the proposal for the placement of propofol under schedule IV, propofol is currently not a controlled drug in the US or Canada. The lack of consistent accountability and the loosely monitored inventories of propofol allow for easy access to the drug in medical settings which may reduce the full disclosure of diversion (Welliver and Baker 2012). Thus, some hospitals in the US have taken action by regulating propofol availability. A recent study found that 29% of academic anesthesia departments in the US had some form of regulation of access to propofol leading to less propofol abuse in these departments (Wischmeyer, Johnson et al. 2007).

In conclusion, although propofol might function as a weak reinforcer with modest abuse liability, effective regulation of propofol’s availability in medical settings (e.g. applying security measures and record keeping policies) may be beneficial while not hindering its effective medical use.
4.2 Summary of findings

The current study aimed to investigate propofol’s reinforcing efficacy and provide a more detailed profile of propofol as a drug with potential abuse liability. We showed that propofol has reinforcing properties and that propofol-induced self-administration behaviour can be reliably established in rats.

In the acquisition phase propofol’s reinforcing effects were manifested by a preference for the drug contingent response over the control response (not contingent with drug delivery) where the majority of rats met the acquisition criteria in a relatively short time frame. For the first time we demonstrated that even low doses of propofol (0.25 and 0.56 mg/kg) had sufficient reinforcing efficacy to establish self-administration behaviour in the majority of drug-naïve, food trained rats. Propofol’s reinforcing properties were further supported as propofol self-administration behaviour was maintained over a period of time and under a more demanding ratio. In addition, propofol-induced responding was modestly sensitive to the dose of the drug, although there was some variation in the shape of the dose response curve across the different experiments. We did not see a robust dose dependent adjustment in the number of propofol infusions self-administered, suggesting that under the conditions tested, total intake was not tightly regulated. The study of temporal pattern of propofol self-administration behaviour throughout each session led us to another novel finding in our study. We showed that like other drugs of abuse, propofol caused the highest magnitude of responding at the beginning of the session (first time block), which dropped considerably in the second time block followed by a constant level of responding for the rest of the session. Lastly, for the first time the extinction and reinstatement of propofol self-administration were investigated. We showed that propofol self-administration behaviour was extinguished upon substitution of vehicle for the drug, suggesting that drug-contingency is an important factor for the self-
administration behaviour. Under our experimental conditions, different priming doses of propofol were unable to reinstate the extinguished self-administration behaviour.

4.3 Conclusions

Using a well-defined and widely used animal model of addiction, we found that propofol is a positive reinforcer in rats, indicating its abuse potential. Rats acquired propofol self-administration behaviour at different training doses under a FR1 schedule and maintained the behaviour under a more demanding fixed ratio (FR2). Our findings support emerging evidence suggesting the abuse potential of propofol while providing a more comprehensive profile of propofol as a drug with abuse potential.

The modest variation in responding as a function of changing dose, the fast extinction of the acquired self-administration behaviour upon substitution of vehicle for propofol, and the lack of reinstatement of the extinguished behaviour using priming doses of propofol, may all considered indications of propofol’s modest reinforcing properties.
References


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