

**Strategies to Improve Opioid Analgesia:  
Nociceptin Receptor Agonists and Intranasal Delivery  
in Monkeys**

**by**

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## **DEDICATION**

For Joanna, Phil, and Tony who have been educating me since birth.

And to a lifetime of mentors whose training, patience, and faith have brought to me this point: Marlene Clary, John K. Robinson, Sandra D. Comer, Gail D. Winger, and James H. Woods.

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## TABLE OF CONTENTS

DEDICATION.....	ii
ACKNOWLEDGEMENTS.....	iii
LIST OF FIGURES.....	vii
LIST OF ABBREVIATIONS.....	ix

### CHAPTER

<b>ONE. General Introduction.....</b>	<b>1</b>
A brief history of opioid receptor pharmacology.....	3
The <i>Holy Grail</i> and less <i>Holy</i> alternatives.....	7
Once more into the breach in the quest for the <i>Holy Grail</i> :	
NOP receptor agonists.....	10
And another less <i>Holy</i> alternative: Intranasal opioid administration.....	12
Specific Aims.....	18
References.....	20
<b>TWO. Characterization of the Discriminative Stimulus Effects of a NOP</b>	
<b>Receptor Agonist Ro 64-6198 in Rhesus Monkeys.....</b>	<b>28</b>
Introduction.....	28
Methods.....	31
Results.....	36

Discussion.....	39
References.....	52

**THREE. A Model to Measure the Analgesic Effects of Intranasally**

<b>Administered Opioids in Rhesus Monkeys.....</b>	<b>55</b>
Introduction.....	55
Methods.....	58
Results.....	62
Discussion.....	65
References.....	77

**FOUR. Intranasal versus Intravenous Naloxone: Behavioral Effects and**

<b>Receptor Occupancy Studies Using PET Imaging.....</b>	<b>81</b>
Introduction.....	81
Methods.....	84
Results.....	89
Discussion.....	91
References.....	103

**FIVE. General Discussion.....**

Overview.....	107
Characterizing the discriminative stimulus effects of Ro 64-6198.....	109
Intranasal drug administration: opioid agonists.....	112

Comparison of intranasal and intravenous naloxone in behavioral measures and receptor occupancy.....	115
References.....	118

## LIST OF FIGURES

### Figures

1.1	Discriminative stimulus effects Ro 64-6198 in Ro 64-6198-trained monkeys.....	46
1.2	Discriminative stimulus effects of Ro 64-6198 in fentanyl-trained monkeys.....	47
1.3	The effects of selective antagonists in Ro 64-6198 and fentanyl-trained monkeys.....	48
1.4	Drug substitution studies in Ro 64-6198 and fentanyl-trained monkeys.....	49
1.5	Diazepam substitution studies in Ro 64-6198-trained animals.....	50
1.6	Antinociception studies with Ro 64-6198 and fentanyl.....	51
2.1	Time course of intranasal and intramuscular fentanyl in the warm water tail-withdrawal assay.....	72
2.2	Peak effects of intranasal and intramuscular fentanyl in the warm water tail-withdrawal assay.....	73
2.3	Time course of intranasal and intramuscular buprenorphine in the warm water tail-withdrawal assay.....	74

2.4	Peak effects of intranasal and intramuscular buprenorphine in the warm water tail-withdrawal assay.....	75
2.5	Studies of MMP 2200 in capsaicin-induced allodynia.....	76
3.1	Time course of intranasal and intravenous naloxone antagonism of fentanyl-induced antinociception.....	98
3.2	The effects of intranasal and intravenous naloxone on tail-withdrawal latency averaged across the 90-minute session.....	99
3.3	Brain tissue time-radioactivity curves for [ <sup>11</sup> C]carfentanil alone, and in the presence of intranasal and intravenous naloxone.....	100
3.4	Receptor occupancy estimates for intranasal and intravenous naloxone .....	101
3.5	microPET Images.....	102



## LIST OF ABBREVIATIONS

blood brain barrier (BBB)

benzodiazepine (BZD)

carfentanil (CFN)

central nervous system (CNS)

distribution volume ratio (DVR)

fixed ratio (FR)

Food and Drug Administration (FDA)

gama aminobutyric acid (GABA)

intramuscular (IM)

intranasal (IN)

intrathecal (IT)

intravenous (IV)

Intravenous drug users (IDUs)

per os, (by mouth), (PO)

phencyclidine (PCP)

pharmacokinetic (PK)

pharmacodynamic (PD)

positron emission tomography (PET)

maximum percent effect (MPE)

naloxone (NLX)

non-human primates (NHPs)

nociceptin (NOP)

nociceptin/orphanin FQ receptor (NOPr)

receptor occupancy (RO)

subcutaneous (SC)

time out (TO)

transient receptor potential-receptor (TRP)

volumes of interest (VOI)

# CHAPTER ONE

## GENERAL INTRODUCTION

*“This marriage of good science and good therapeutics with all its attending benefits should give heart, confidence, persistence, and dedication to those who travel the road of experimental therapeutics.”*

- William R. Martin, 1979  
*British Journal of Clinical  
Pharmacology*

The medicinal and troublesome properties of opium and opium alkaloids have been known for thousands of years (Levinthal, 1985). More than 160 years of research following the isolation of morphine, which included seminal work in identifying opioid binding sites (Goldstein et al., 1971; Lord et al., 1977; Pert and Snyder, 1973), and characterizing the *in vivo* effects of various opioid agonists and antagonists (Kosterlitz and Robinson, 1958; Martin and Gorodetzky, 1965; McClane and Martin, 1976), it was determined that the therapeutic and unwanted effects of these ligands are mediated through the  $\mu$ -opioid receptor. Today,  $\mu$ -opioid receptor agonists, such as morphine, oxycodone, codeine, and fentanyl are routinely used in medical practice primarily for analgesia, but also to control cough, treat diarrhea, and ameliorate “air hunger” (Banzett et al., 2011; Dworkin et al., 2003; Zollner and Stein, 2007). Although  $\mu$ -agonists are unparalleled in their ability to treat pain of any modality, their therapeutic use is limited

by unwanted effects such as constipation, sedation, itch, physical dependence and their potential for abuse (Inturrisi, 2002; McNicol, 2008).

Pain has been referred to as the fifth vital sign, a designation that recognizes the salience of this symptom in human health (Lorenz et al., 2009). Adequate analgesia is the primary goal of pain management, and is regarded as “*a moral imperative, a professional responsibility, and the duty of people in the healing professions*” (Institute of Medicine, 2011). In 2010, the Patient Protection and Affordable Care Act directed the Institute of Medicine (IOM) to conduct research on ways to increase “the recognition of pain as a significant public health problem in the United States.” A subsequent report published by the IOM estimated that 100 million people in the United States live with chronic pain, and the economic costs associated with pain conditions are in excess of \$500 billion (Institute of Medicine, 2011).

At the same time, opioid abuse in the United States has reached epidemic proportions (Han et al., 2015; Maxwell, 2011). The prevalence of prescription opioid abuse has reached an all time high, and the estimated number of fatalities from overdose is almost 45 per day (Davis et al., 2013; Han et al., 2015). This has coincided with the resurgence in heroin abuse, which in part, has been attributed to the perception among opioid abusers that heroin is a cheaper alternative to prescription opioids (Jones et al., 2015). Taken together, the challenges faced by the medical community are clear: the abuse, misuse, and diversion of prescription opioid drugs is a serious public health problem that must be controlled in a manner that is sensitive to the legitimate and essential use of these medicines.

In recognition of the vital role that opioid medicines have in patient care, and the seriousness of the opioid epidemic, this thesis evaluates two potential strategies to improve the therapeutic use of opioids, with a particular emphasis on pain control. The first strategy explores the discriminative stimulus and analgesic effects of the nociceptin/orphanin FQ receptor (NOP) agonist, Ro 64-6198, in rhesus monkeys. As a target, the nociceptin (NOP) receptor has demonstrated several therapeutic advantages over the  $\mu$ -opioid receptor in non-human primates (NHPs). The second strategy develops an experimental framework to investigate how the intranasal (IN) route of drug administration may be employed to improve the therapeutic use of opioid agonists and antagonists. A comparison of the general pharmacodynamic properties of IN and other parenteral routes of administration is described.

### **A brief history of opioid receptor pharmacology**

The actions of opioid drugs are mediated through the binding of opioid receptors, and the constellation of effects that are produced vary depending on the selectivity of the drug or ligand for one or more of these receptors (Martin, 1983). In the mid-1960s, Martin and colleagues postulated, and provided some of the earliest evidence, that different opioid agonists had distinct pharmacological properties mediated through more than one receptor (Martin et al., 1965; Martin and Gorodetzky, 1965; McClane and Martin, 1967). The approach to establishing the existence of such receptors was two pronged, and rooted in the foundational principles of pharmacology. First, the effects produced by different opioid drugs of varying efficacy were thoroughly characterized across different functional preparations (*in vivo and in vitro*), followed by an evaluation

of the saturable and stereospecific binding of these drugs in different tissue fractions (Martin, 1983). Influential work in the field by Chang and Gaddum demonstrated that agonists acting at one or more receptor populations could be identified by comparing their rank order of potency across multiple assay systems (Chang and Gaddum, 1933). It was later shown that different receptor populations could be more accurately differentiated with selective antagonists according to the methods of Arunlakshana and Schild. This held that the potency of a given selective antagonist to shift the agonist dose-effect curve two log units to the right would be identical for agonists acting at the same receptor (Arunlakshana and Schild, 1959; Schild, 1947). Finally, it had been established that opioids bound to brain homogenates in a stereoselective and saturable manner (Simon et al., 1973). The application of these principles across multiple systems would eventually lead to the discovery of three distinct opioid receptors,  $\mu$ ,  $\kappa$ , and  $\delta$ .

*In vivo* evaluation supported the findings of distinct opioid systems; it was discovered that opioids could be broadly classified based on their relative efficacy to produce specific behavioral effects. Two general types of behavior were most commonly described, one corresponding to the effects of acute drug administration, and the other, the syndrome that was produced following discontinuation of the drug after a period of chronic administration (the “drug abstinence syndrome”) (Martin, 1979). In humans, following acute administration, it was noted that morphine-like drugs produced signs and symptoms such as itchy skin, euphoria, and talkativeness, while cyclazocine-like analgesic compounds were associated with somnolence, drunkenness, and at higher doses, dysphoria (Jasinski et al., 1968; Martin and Gorodetzky, 1965; McClane and Martin, 1976). Parallel work performed in animals (monkeys, chronic spinal dog)

also supported the division of opioid drugs into at least two classes based on acute effects and drug abstinence (Gilbert and Martin, 1976). In the case of drug abstinence, agonists within the same drug class would substitute, or suppress the abstinence syndrome, to an extent that was reflective of their efficacy. For example, in cyclazocine-dependent animals, morphine substitution did not suppress the abstinence syndrome produced following the discontinuation of cyclazocine, but these effects were completely reversed with ketazocine, while only partially suppressed with nalorphine (Gilbert and Martin, 1976). Drugs with no efficacy, such as naloxone, would precipitate the abstinence syndrome in subjects made physiologically dependent on either cyclazocine or morphine (Jasinski et al., 1967; Jasinski et al., 1968).

More evidence that opioids acted at a heterogeneous population of receptors was generated with *in vitro* experiments measuring smooth muscle contractions in the mouse vas deferens and/or guinea pig ileum (Hutchinson et al., 1975; Kosterlitz and Robinson, 1958; Lord et al., 1977). In 1977, Lord et al. published an elegant series of experiments combining the previously discussed pharmacological principles across several assays, which established definitively the existence of more than one opioid receptor type. Consistent with these findings, the relative potency of different opioid agonists was found to be assay-dependent, and therefore likely mediated through a heterogeneous population of receptors. It was determined that  $\mu$ -receptors (and to a lesser extent  $\kappa$ -receptors) predominated in the guinea pig ileum, while  $\delta$  receptors (and to a lesser extent,  $\mu$ -receptors) predominated in mouse vas deferens. Moreover the potency of naloxone to antagonize the effects of agonists in different preparations varied (e.g. the potency of naloxone to antagonize the effects of the endogenous opioid

peptide leu-enkephalin was almost 9x greater in the guinea pig ileum than the mouse vas deferens). Importantly, the results from these experiments *in vitro* were strongly correlated with *in vivo* preparations assaying analgesia, the suppression of drug abstinence syndrome, and the like. For example, the rank order potency of  $\mu$ -opioid agonists to inhibit smooth muscle contraction in the guinea pig ileum (etorphine> fentanyl> levorphanol> heroin>normorphine>codeine) was retained in preparations measuring human analgesia and in preclinical preparations using dogs and monkeys (Lord et al., 1977). Thus, the pharmacology of an opioid ligand could be reliably confirmed across multiple assay systems.

More than 30 years later, the existence of three opioid receptor types  $\mu$ ,  $\kappa$ , and  $\delta$  was further verified through molecular cloning (Waldhoer et al., 2004). Following that, a fourth member of the opioid family was identified entirely through amino acid sequence homology, and was eventually named the nociceptin/orphanin FQ peptide receptor (NOPr) (Mollereau et al., 1994). Although the receptor shared 60-80% sequence homology with the classic opioid receptors, NOPr had negligible affinity for the endogenous opioid peptides, and agonist stimulation of this receptor was not blocked by naloxone (Mogil and Pasternak, 2001). One year after its identification, the endogenous ligand for NOP was discovered and named nociceptin/orphanin FQ (Meunier et al., 1995). This peptide did not bind the canonical opioid receptors, but it shared high sequence homology with the kappa selective peptide dynorphin A. Although classified as an opioid receptor, considerable differences exist between  $\mu$ ,  $\kappa$ , or  $\delta$ , and NOPr.



## **The *Holy Grail* and less *Holy* alternatives**

The quest to discover new analgesics that are as powerful as morphine without the unwanted effects has been dubbed the Holy Grail of opioid research (Corbett et al., 2006). In part, the establishment of three distinct opioid receptors led to speculation that this may be possible, and helped to encourage a massive drug discovery effort between industry, government, and academia (Campbell and Lovell, 2012). The problems associated with opioid agonist therapies are evidenced by research estimating that 50% of patients report side effects that limit their effectiveness, and between 10-30% of patients discontinue their medication despite an established need for pain control (Labianca et al., 2012; McNicol, 2008). One meta-analysis concluded that the most common side effects of opioid analgesics were constipation, sedation, nausea, vertigo, vomiting, and itch (Furlan et al., 2006). While opioid abuse and dependence are not considered “common” side effects *per se*, the risk of iatrogenic drug addiction remains a concern among practitioners (Inturrisi, 2002; Kouyanou et al., 1997). The efforts to improve upon  $\mu$ -agonist therapy is complicated by the knowledge that most of the unwanted effects are on-target (i.e. mediated through the  $\mu$ -opioid receptor), meaning that the therapeutic and unwanted effects are difficult to disassociate.

The efforts undertaken to improve the medical use of opioids at the  $\mu$ -receptor, has been an equally important goal of opioid pharmacology. In general, these innovations have been quite successful in their own right, and may be considered in two categories. The first is the development of  $\mu$ -opioid receptor ligands with pharmacodynamic and pharmacokinetic profiles that make them well suited for a specific medical purpose. Among these buprenorphine, remifentanyl, loperamide,

naloxone and naltrexone are notable. Buprenorphine is a  $\mu$ -opioid receptor partial agonist that has become a mainstay in the treatment of opioid abuse and dependence (Comer et al., 2005). Among buprenorphine's numerous positive pharmacological attributes, it has an unparalleled safety margin compared to other  $\mu$ -agonists, and when used in the treatment of opioid abuse can help prevent relapse and overdose (Li et al., 2014; Walker et al., 1995). Remifentanyl is a short acting anesthetic routinely used in surgery (Scott and Perry, 2005). It is a full  $\mu$ -agonist that is rapidly degraded by esterases in the blood following administration, and thus its therapeutic effects can be initiated and terminated with unprecedented alacrity (Stroumpos et al., 2010). Naltrexone is a  $\mu$ -opioid antagonist that is used to treat opioid and alcohol dependence (Comer et al., 2006). Recently, methylated derivatives of naltrexone have been FDA approved to treat opioid induced constipation (Camilleri, 2011). Naloxone, another  $\mu$ -opioid antagonist, is used routinely to reverse opioid toxicity (Kim and Nelson, 2015). Finally, loperamide, an over-the-counter medication used to treat diarrhea is also a  $\mu$ -opioid agonist (the slowing of gastrointestinal transit was one of the earliest recognized medical benefits of opium derivatives). Loperamide remains unscheduled, and is not considered to have an abuse liability because it has poor solubility when administered systemically, and low absorption through the GI tract (Baker, 2007).

The second set of innovations generally concern formulation strategies and routes of administration, which complement a particular opioid drug for a specific indication. Long-acting formulations reduce the need for repeat drug administration, and provide lasting pain relief (Argoff and Silvershein, 2009). Abuse deterrent formulations prevent the extraction of active principles for use in an illicit manner (Vosburg et al.,

2012; Vosburg et al., 2013). The spinal administration of opioids (either intrathecal or epidural), is preferred over systemic administration in some clinical situations. Implanted intrathecal pumps with  $\mu$ -agonists (typically morphine) are given to patients with chronic intractable pain and/or end of life pain, and provide rapid and robust relief from suffering (Grass, 1992). Epidural or intrathecal administration of morphine is also the preferred opioid intervention for obstetric pain during childbirth (Gogarten, 2003). While this has not solved the problem of unwanted effects, these examples illustrate how innovations in the medicinal chemistry, formulation and/or route of administration can change the properties of opioid drugs that result in positive therapeutic benefits.

Drugs that bind other opioid targets, such as agonists at  $\delta$  and  $\kappa$ -receptors, have also been shown to produce analgesia in humans and animals (of the two,  $\kappa$ -receptor agonists are generally considered to have greater analgesic efficacy) (Negus et al., 1998; Walker and Young, 1993). However, selective ligands for these receptors produce unwanted effects that are equally or more medically complicated than  $\mu$ -agonist ( $\kappa$ -agonists have been shown to produce dysphoria, and  $\delta$ -agonists are convulsive) (Comer et al., 1993; Kumor et al., 1986). The strategy of designing non-selective opioid agonists (ligands that bind to more than one opioid receptor) has yielded mixed results. At least one such compound, butorphanol (a  $\mu/\kappa$ -agonist), has been FDA approved for use in man. Although the discovery of a “Holy Grail” remains the hope, it has not produced a compound (opioid or non-opioid) that has supplanted the clinical use of  $\mu$ -opioid agonists.

## **Once more into the breach in the quest for the *Holy Grail*:**

### **NOPr agonists**

The development of NOPr agonists as novel analgesic agents had an inauspicious beginning. When the endogenous NOP peptide was first identified and given to rodents, it was found to induce nociception (unlike its canonical opioid counterparts) and was given the name “nociceptin” (Meunier et al., 1995). Nevertheless, interest in this target as a novel opioid analgesic continued. The initial series experiments conducted in rodents with peptidic and small molecule NOPr agonists revealed complex effects on pain that varied as a function of route of administration and the pain modality (e.g., Mogil and Pasternak, 2001; Schroder et al., 2014). It was subsequently determined that NOPr agonists were not pronociceptive in rodents, but the broadest spectrum of analgesic efficacy was found only following intrathecal administration. While systemic administration of NOPr agonists was generally shown not to be effective against acute thermal nociceptive stimuli, positive results with this route have been achieved in neuropathic and inflammatory pain models (Khroyan et al., 2011). Interestingly, systemic administration, or supraspinal activation of NOPr receptors was found to produce a functional antagonism of  $\mu$ -agonist effects (Khroyan et al., 2009a; Khroyan et al., 2009b). These findings have led to commercial interest in developing mixed acting  $\mu$ /NOPr agonists for pain control, at least one of which cebranopadol, is in Phase III (Linz et al., 2014).

Based on the results in rodents, it was surprising to learn that the effects of NOPr agonists in rhesus monkeys were considerably more straightforward. Studies in non-human primates demonstrated that the small molecule NOPr agonists Ro 64-6198 and

SCH 221510 given systemically produced strong analgesia (comparable with full agonists at  $\mu$ -receptors, such as alfentanil) (Cremeans et al., 2012; Kangas and Bergman, 2014; Ko and Naughton, 2009). Identical results were obtained after intrathecal administration of nociceptin and other peptidic agonists (Ko and Naughton, 2009; Ko et al., 2006) Moreover, these effects were found in assays of acute thermal nociception as well as capsaicin-induced allodynia. Finally, these drugs did not produce reinforcing effects, nor did they cause pruritus or respiratory depression (Ko et al., 2009). These results supported the continuing interest in the therapeutic profile of NOPr agonists, and their potential advantages over  $\mu$ -agonists. However, other than analgesia, the behavioral effects of NOPr agonists in rhesus monkeys have remained uncharacterized (most of the studies have noted their relative lack of effects on a target behavior).

Drug discrimination studies have been used extensively to characterize the interoceptive effects of psychoactive drugs (e.g., Colpaert, 1999). Previous work in the laboratory demonstrated that the discriminative stimulus effects of  $\mu$ ,  $\kappa$ , and  $\delta$  agonists are behaviorally and pharmacologically selective (Woods et al., 1988). In addition to providing information about the similarities and differences in the central effects of psychoactive drugs, drug discrimination techniques can also measure the potency of drugs to alter rates of responding. This provides an opportunity to measure the behavioral disrupting effects produced by drug administration. Through the use of selective antagonists, the pharmacological specificity of the discriminative stimulus and behavioral disrupting effects may be established (Woods et al., 1988).

In contrast to  $\mu$  and  $\kappa$  agonists, previous studies had suggested that the analgesic effects of NOPr agonists were observed with doses that did not produce sedation or behavioral disruption. Based on these findings, it was plausible that NOPr agonists would produce discriminative stimulus effects with doses that were smaller than those required to produce analgesia. If true, this would be a significant departure from the commonly observed order of potency for most opioids to produce these behavioral effects (i.e. discriminative stimulus effects > rate suppression > analgesia), and demonstrate one way in which NOPr agonists may have a superior therapeutic profile to MOP agonists. The primary purpose of the first set of experiments in this thesis was to characterize the discriminative stimulus effects of NOPr agonists in non-human primates and to compare the order of potency of NOPr agonists to produce stimulus, rate suppressing, and analgesic effects with that of the  $\mu$ -receptor agonist, fentanyl.

**And another less *Holy* alternative:**

**Intranasal opioid administration**

Even though the unwanted effects from  $\mu$ -agonists are salient, the medically indispensable nature of opioids requires innovation in formulation and administration. Furthermore, the application of a particular opioid therapy should be aligned with the therapeutic goals, and used in consideration of the risks, benefits and alternatives. For example, in the context of pain control, the onset and duration of action, as well as the magnitude of effect should be congruent with the frequency, intensity, and duration of the pain modality (Inturrisi, 2002). The general recognition of these principles follows the

use of many different opioid agonists, which are employed in a variety of formulations and through several routes of administration (fentanyl patches, intrathecal morphine pumps, oral loperamide, IV remifentanyl).

Although the bioavailability of some opioids following IN administration has been documented, other than butorphanol, the practice of administering opioids intranasally has only recently become more common following the approval of IN fentanyl for breakthrough cancer pain (Grassin-Delyle et al., 2012; Prommer and Thompson, 2011). In general, the nose is an attractive site for drug delivery due to the relatively large surface area for absorption, and the ability to avoid hepatic and GI first-pass metabolism. From a clinical perspective, this route of administration provides a non-invasive method of parenteral systemic drug delivery, and produces a prompt onset of action that is comparable to IV injection (Foster et al., 2008; Gourlay and Benowitz, 1997). There have been reports of direct absorption to the CNS following IN administration, and while there is considerable interest in exploring this possibility, evidence for this pattern of absorption in primates is limited (Dhuria et al., 2010; Scheibe et al., 2008)

The physiochemical properties of ligands that make them suitable for IN absorption are not radically different than those for other parenteral routes of administration (good solubility, low molecular weight, high lipophilicity, if targeting CNS) (Arora et al., 2002). However, there are some notable differences that have important implications for drug development and therapeutics (for review, see Arora et al., 2002). The size of the intranasal cavity limits the volume that can be delivered into the nose without jeopardizing the reliability of dosing, and this amount varies from species to species. Normal mechanisms of mucosal clearance and drainage may limit drug contact

with anatomical regions critical for absorption. Lastly, there are enzymes present in the nose, including cytochrome P450s and P-glycoproteins, which may break down peptides and small molecules or prevent their absorption (Wioland et al., 2000; Zhang et al., 2005). There is a considerable body of research on formulations designed to enhance IN absorption that may circumvent some of these issues, but they are beyond the scope of the work presented here.

The therapeutic potential for IN opioid administration is best illustrated with IN fentanyl and naloxone (NLX). The treatment of episodic breakthrough cancer pain, which is unpredictable and highly distressing periods of intense suffering, has been improved with the use of IN fentanyl (Kongsgaard et al., 2014). The ability of patients to control drug administration, and the prompt onset of relief that typically occurs only in the clinic with medication delivered by injection, has improved treatment satisfaction (Karlsen et al., 2013). IN NLX has gained acceptance as an alternative to intramuscular or intravenous administration in the treatment of opioid overdose. Again, the prompt onset of action, and the elimination of needles has made this appealing for use in emergency medicine, particularly when treating intravenous drug users who have a high incidence of blood borne diseases and poor intravenous access.

The majority of preclinical research conducted on IN drug administration, with the notable exception of studies investigating the effects of oxytocin, has been performed in rodents. Across all species, studies of IN drug administration typically focus on pharmacokinetics (PK), and attempts to extrapolate conclusions about pharmacodynamics based on these data may produce misleading conclusions. For example, in one published PK study in humans, it was reported that the bioavailability of



NLX following IN administration was only 4% relative to IV injection. As a result, the authors concluded that NLX would be least effective when administered by this route (Dowling et al., 2008). Like many PK studies of this kind, the authors relied on venous sampling to measure the concentration of naloxone in the blood. However, studies with IN nicotine and fentanyl show that, unlike when drugs are administered IV or IM, venous sampling may significantly underestimate the bioavailability of drug at the target, and is a poor predictor of pharmacodynamics (Gourlay and Benowitz, 1997; Guthrie et al., 1999; Moksnes et al., 2008). Subsequent evidence from studies comparing IV and IN NLX in the context of drug overdose, have suggested the therapeutic effect are actually quite similar (Kerr et al., 2009). The application of other methods that could more directly determine the extent of ligand-target engagement, such as receptor occupancy studies with PET, have not been used to address the suitability of IN administration to produce desired therapeutic effects.

The purpose of the last series of experiments in this thesis was to develop and validate a procedure to measure the behavioral effects of IN opioid delivery in rhesus monkeys with the hope of establishing its translational relevance in preclinical drug development. There is good evidence to suggest that NHP models of IN drug effects may be of greater translational value relative to experiments performed in rodents. Rats and mice have larger nasal cavities relative to their body size, and a larger nasal surface area overall for drug absorption (Gross et al., 1982). Moreover, the cellular composition of the rodent nasal cavity is split equally between olfactory epithelium, which are key mediators in direct nose-to-brain absorption, and other cell types (Dhuria et al., 2010; Harkema et al., 2006; Hoekman and Ho, 2011). Olfactory epithelium

composes only 3% of nasal cavity volume in humans and 7% in monkeys. Thus, the different patterns of drug deposition, absorption, and distribution, between rodents and primates, following IN drug administration may limit the reliability of rodent models to predict clinical relevant pharmacodynamic end-points.

The lack of such translational reliability has been noted as a limiting factor in at least one industry perspective published by Pfizer evaluating the potential of direct nose-to-brain absorption (although the same principles are applicable to any goal with intranasal delivery) (Landis et al., 2012). Perhaps, this is the reason many therapeutics that are administered IN are reformulated medicines that have already gained FDA approval through another route. This strategy, while commercially viable, ignores the advantage that IN delivery offers at the early stages of drug development. Thus, ligands with therapeutic potential that do not have “good drug properties” associated with oral administration may be unnecessarily abandoned.

The first set of studies established the procedure for measuring the analgesic effects of intranasally administered opioids using fentanyl, buprenorphine, and the opioid peptide, MMP 2200. Basic pharmacodynamic comparisons between IN and IM administration were characterized for fentanyl and buprenorphine in studies of acute thermal nociception. The generalizability of this procedure was then assessed in another pain modality, capsaicin-induced allodynia, where the ability of MMP 2200 to reverse allodynia after IN administration was evaluated. Previous studies conducted in rhesus monkeys showed that IM MMP 2200 reversed capsaicin-induced allodynia through a peripheral mechanism of action (Do Carmo et al., 2008). It was used in these

experiments to establish proof-of-concept that the analgesic effects of peptides could be detected following IN delivery in this preparation.

Finally, the IN administration of opioids was extended to include naloxone, the  $\mu$ -receptor antagonist. The importance of opioid antagonists in therapeutics has been reaffirmed as the rates of opioid abuse and overdose reach epidemic proportions in the United States (Dart et al., 2015; Wermeling, 2013). While field medical studies have provided evidence that IN NLX can reverse opioid toxicity, its effectiveness compared to other more commonly used parenteral routes of administration remain unclear (Kerr et al., 2008; Kerr et al., 2009; Zuckerman et al., 2014). For example, the potency of IN NLX to block  $\mu$ -agonist effects, or the reliability of IN dosing in terms of receptor engagement, have never been systemically evaluated. The procedures for evaluating IN opioids that were developed in this thesis provided a chance to investigate some of these issues, and illustrate how this model may be used to answer scientific questions that are relevant to public health. The last set of studies evaluated the potency of IN and IV NLX to block the antinociceptive effects of fentanyl. Since the same magnitude of behavioral effect may be produced with different levels of receptor occupancy, the degree of receptor availability following equipotent doses of NLX given IV and IN was measured using positron emission tomography (PET). This provided the first direct comparison of receptor occupancy across routes of administration.

## **Specific Aims**

*Specific Aim 1.* NOPr agonists have been reported to produce antinociceptive effects in rhesus monkeys with comparable efficacy to  $\mu$ -opioid receptor agonists, but without their limiting side effects. There are also known to be species differences between rodents and NHPs in the behavioral effects of NOPr agonists. The aims of this study were to: 1) determine if the NOPr Ro 64-6198 could be trained as a discriminative stimulus, 2) evaluate its pharmacological selectivity as a discriminative stimulus, and 3) establish the order of potency with which Ro 64-6198 produces discriminative stimulus effects compared with analgesic effects in NHP.

*Specific Aim 2.* The anatomical and physiological features of the nose that enable rapid and efficient drug absorption to the systemic circulation, and possibly to the CNS, make developing drugs for IN administration appealing. This study sought to establish the first procedure to measure the analgesic effects of IN opioids in rhesus monkeys. The initial experiments compared the ability of fentanyl and buprenorphine to increase tail-withdrawal latency from 50°C water across two routes of administration (IN versus IM). The second experiment aimed to validate these procedures using a different pain modality, capsaicin-induced allodynia, and to evaluate the opioid peptide MMP 2200 following IN administration.

*Specific Aim 3.* Treatment with NLX can reverse opioid toxicity if administered promptly following an overdose. The efforts to expand the use of NLX have included the IN route of administration, however, questions exist regarding the potency and effectiveness of IN NLX relative to more common parenteral routes of administration, such as intravenous injection (IV). The purpose of this study was to compare the potency of IN and IV NLX to block the antinociceptive effects of the  $\mu$ -opioid agonist fentanyl, and to measure the receptor occupancy produced with equipotent doses of NLX across routes of administration using PET imaging.

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## CHAPTER TWO

### Characterization of the Discriminative Stimulus

#### Effects of a NOP Receptor Agonist

#### Ro 64-6198 in Rhesus Monkeys<sup>1</sup>

#### Introduction

The nociceptin/orphanin FQ receptor (NOP) is a seven-transmembrane domain receptor that was first cloned in 1994, and was noted to share significant sequence and structural homology with the classic opioid receptors  $\mu$ ,  $\kappa$ , and  $\delta$  (Mollereau et al., 1994). Despite these similarities, the canonical endogenous opioid peptides have negligible affinity for NOPr, as does the opioid antagonist naloxone. One year later, two separate groups identified a 17-amino acid peptide that bound with high affinity to NOPr as the endogenous ligand (Meunier et al., 1995; Reinscheid et al., 1995). This peptide was given two names, nociceptin and orphanin FQ (N/OFQ), and was found to share considerable sequence homology with the  $\kappa$ -selective peptide dynorphin A. Functional experiments *in vitro* demonstrated that NOPr,  $\mu$ ,  $\kappa$ , and  $\delta$  all coupled predominantly to

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<sup>1</sup> This chapter was accepted for publication in the Journal of Pharmacology and Experimental Therapeutics

the  $G\alpha_{i/o}$  class of G-proteins, and produced analogous signal transduction following agonist stimulation (inhibition of adenylyl cyclase, and  $Ca^{2+}$  conductance; stimulation of  $K^+$  conductance).

Given its classification, the initial behavioral pharmacology focused on evaluating the role of NOP in modifying pain-related behaviors. The results from many of these experiments were nuanced relative to  $\mu$ -agonist effects, and there were considerable differences noted between rodents and non-human primates (NHPs). In rodents, the analgesic response to NOPr agonists varied depending on the route of administration, and the type of pain stimulus. There is now general agreement that NOPr agonists produce analgesia in rodents when given intrathecally (IT) across a variety of pain assays. Systemic or supraspinal administration in rodents does not produce antinociception against an acute thermal stimulus (tail-flick, hot-plate), but may have positive effects in inflammatory, neuropathic, and chronic pain states (for review, see Lambert, 2008; Mogil and Pasternak, 2001; Schroder et al., 2014).

In rhesus monkeys, however, NOPr agonists given systemically or spinally were shown to be antinociceptive, antiallodynic, and anti-inflammatory (Kangas and Bergman, 2014; Ko and Naughton, 2009; Ko et al., 2009). When administered IT, N/OFQ produced antinociception that was less potent, but similar in magnitude, to morphine. However, unlike morphine, or other  $\mu$ -opioid receptor agonists given i.t., N/OFQ did not produce respiratory depression or pruritus. The first small molecule agonist to be tested systemically in monkeys, Ro 64-6198, was reported to produce acute thermal antinociception and to be antiallodynic (Ko et al., 2009). When compared with alfentanil, Ro 64-6198 was equipotent in producing antinociception, but was not

self-administered, and did not produce respiratory depression or itch. The antinociceptive effects of Ro 64-6198 were selectively blocked by the NOPr antagonist J-113397 and not by the opioid antagonist naltrexone. Although not systematically evaluated, NOPr agonists were reported to produce analgesia in the absence of sedation, unlike agonists at  $\mu$  and  $\kappa$ -opioid receptors (Ko and Naughton, 2009; Podlesnik et al., 2011). Furthermore, whereas the preclinical literature in rodents showed a complex relationship between the NOP system and analgesia, the data in monkeys appeared to be more straightforward.

To our knowledge, other than analgesia, there have been no other reports identifying behaviors that are modified by NOPr agonists in NHPs (most of the studies have noted their relative lack of effects on a target behavior). Drug discrimination procedures are widely used to study the interoceptive properties of CNS drugs, and are well suited for establishing the *in vivo* selectivity of ligands from a broad array of drug classes. Previous research has established that agonist stimulation of  $\mu$ ,  $\kappa$ , and  $\delta$ -opioid receptors produced discriminative stimulus effects that are pharmacologically selective (Colpaert, 1999; Woods et al., 1988). Previous drug discrimination studies with Ro 64-6198 in rats showed that it possessed stimulus properties that were unlike opioid agonists at  $\mu$ ,  $\kappa$ , and  $\delta$ -opioid receptors (Recker and Higgins, 2004). These effects were selectively blocked with the NOPr antagonist, J-113397, but not with the opioid antagonist naltrexone. This study successfully established that, in rodents, the discriminative stimulus effects of Ro 64-6198 were selectively mediated through NOPr, and were pharmacologically and behaviorally distinct from the stimulus effects produced through traditional opioid receptors. However, these experiments did not examine



whether the stimulus effects of Ro 64-6198 were distinguishable from drugs in other pharmacological classes. Furthermore, the apparent species differences in terms of other NOPr agonist effects raised the possibility that these findings may not be generalizable to NHP.

The purpose of the present experiments was to determine if rhesus monkeys could be trained to discriminate Ro 64-6198, and to compare the relative potencies of Ro 64-6198 and the  $\mu$ -agonist fentanyl in measures of analgesia, discriminative stimulus effects, and the ability to suppress rates of responding. We also wanted to test whether the stimulus effects of Ro 64-6198 were similar to other opioids, and drugs from different classes.

## **Methods**

### **Subjects**

In the drug discrimination studies, six adult rhesus monkeys (one female, weight, 8 kg; five males, weights, 9-12.5 kg). were individually housed in steel cages (83.3 cm high x 76.2 cm wide x 91.4 cm deep) on a 12hr light/12 hr dark schedule. Their diet consisted of Lab Fiber Plus Monkey Diet (PMI Nutrition Intl. LLC.) that was supplemented with fresh fruit daily. Water and enrichment toys were continuously available in the home cage. All of the monkeys used in this experiment had served as subjects in other studies, and had prior drug histories. In the antinociception experiments, three separate adult male rhesus monkeys (weights, 10-11.4 kg) were employed and housed under the same conditions as the drug discrimination animals.

One female monkey that was trained to discriminate fentanyl (weight, 8kg) was also used in the antinociception studies. The antinociception experiments in this animal commenced 118 days after drug discrimination training was discontinued. All animals were maintained and experiments were performed, in accordance with the Institutional Animal Care and Use Committee, at the University of Michigan.

### Apparatus

Drug discrimination experiments were conducted in the home cage seven days a week beginning at 12p. A metal panel was mounted on either side of the cage (20 cm wide x 28 cm high) that housed three response levers with three stimulus lights located 5 cm above each lever. A food dispenser was located on the same side as the operant levers. In an adjacent room, computers with MED-PC software controlled all of the experimental procedures (Med-Associates, Georgia, Vermont, USA).

For warm-water tail-withdrawal studies, monkeys were trained to sit in Plexiglas primate chairs that were 1.5 m in height. A tea kettle with hot water was maintained at approximately 100°C. Cold tap water and hot water were mixed together in a Thermos and calibrated with a total immersion thermometer in order to obtain the desired temperatures. Tail-withdrawal latencies were measured using a digital stopwatch.

### Drug Discrimination Procedure

Monkeys were trained to discriminate either fentanyl 0.01 mg/kg from vehicle (n=3; monkeys BC, MI, BU) or Ro 64-6198 (0.18 mg/kg, FA; 0.1 mg/kg, IE and ST) from vehicle in a two-lever, single component drug discrimination procedure. FA had no history of operant conditioning, while IE had previous experience responding for

intravenous (IV) drug administration and food, and ST had a history of responding for food. MI and BU had no previous operant training before this experiment, and BC had prior experience responding both for food and IV drug administration.

The session began with the illumination of two green stimulus lights above the right and left lever. Monkeys were trained to respond on a fixed-ratio (FR) 30 schedule for a single 300 mg food pellet (Bio Serv, Dustless Precision Pellets® Primate, Purified: 300 mg, Banana Flavor). Drug and vehicle/sham- paired levers were randomly assigned to each monkey. Pre-treatment time varied by drug condition; fentanyl animals were given an injection 20 minutes before the beginning of the session and Ro 64-6198 animals were injected 30 minutes prior to the session. Following administration of the training drug or sham/vehicle injection, completion of a FR 30 on the condition-appropriate lever extinguished the green stimulus lights and illuminated the center red stimulus light that signaled the delivery of a food pellet. Completion of a FR 30 on the inappropriate lever extinguished the green stimulus lights and initiated a 10s time out (TO) during which responses had no scheduled consequence. Any responses emitted on the incorrect lever reset the response requirement on the condition-appropriate key. The session ended when the monkey received 75 food pellets or after 60 minutes elapsed.

In order to meet criteria for drug testing, subjects were required to emit no less than 85% of their total responses on the condition-appropriate lever, and to complete the first FR in 45 responses or less. Stimulus control was deemed adequate for testing when monkeys met criteria for six consecutive sessions out of seven days of training. Test conditions were identical to training sessions except that completion of a FR 30 on

either key produced a food pellet. Subsequent test sessions were carried out following two consecutive training sessions during which the monkey met the criteria. If the subject failed to meet criteria during one of these sessions, testing was suspended until the criteria were again satisfied for two consecutive sessions.

Drug substitution studies were performed using selective opioid agonists and other drugs that are known to produce stimulus effects from different pharmacological classes. The  $\mu$ -agonists fentanyl and buprenorphine, the  $\kappa$ -agonist ketocyclazocine, and the  $\delta$ -agonist SNC 80 were used to determine if Ro 64-6198 had interoceptive effects in common with drugs that act at these opioid sites. Ketocyclazocine's  $\kappa$ -mediated effects have been demonstrated in discrimination studies in rhesus monkeys (Hein et al., 1981); SNC 80's  $\delta$ -mediated effects have likewise been shown in rhesus monkeys (Brandt et al., 1999). The non-opioid drugs diazepam, phencyclidine (PCP), and chlorpromazine were used to further test the selectivity of Ro 64-6198's interoceptive effects. These drugs were tested up to doses that suppressed rates of responding or where the limits in drug solubility were reached. Drugs were considered to have partially substituted for the training stimulus if responses on the drug-appropriate lever were in excess of 50%, and full generalization was interpreted as greater than 85% responding on the drug-paired lever. The ability of antagonists to alter the stimulus effects of fentanyl and Ro 64-6198 was investigated by pretreating animals with antagonists 40 and 50 minutes, respectively, prior to the beginning of the session.

#### Acute thermal antinociception

For measurements of antinociception, monkeys were trained to sit in a primate chair and their tails were periodically immersed in water heated to 38, 42, 46, or 50° C.

Temperatures were tested randomly at each time point with approximately 30-60s between each measurement. The session began with baseline measurements recorded at each temperature; this was followed by a series of injections (drug or saline) in thirty-minute cycles. Two different experimenters, who were blind to the water temperature, tested each temperature once 20 minutes following the injection. Water heated to 50° C reliably produced nociception and the subject typically withdrew its tail from the water in 2-5s. Effective opioid analgesics raise the nociceptive threshold at this temperature and increase the tail-withdrawal latency as compared with saline treatment.

### Data Analysis

Drug discrimination data are presented as a percent of drug-appropriate lever responding and plotted as a function of dose. If a test compound was administered to a monkey more than once, an average was calculated for drug-appropriate responding in that condition. Mean data are presented as an average of all the monkeys in the group.

In the warm-water tail-withdrawal study, each point represents an average of two sessions with a single monkey. The averages for each monkey were then converted to percent maximum possible effect (%MPE) using the following calculation:  $\%MPE = [(test\ latency - control\ latency) / (20\ s\ cut-off\ latency - control\ latency)] \times 100$ .

### Drugs

All drugs were administered intramuscularly (IM) in volumes between 0.1- 2 ml. Fentanyl (Sigma Aldrich, St. Louis, MO), ketocyclazocine (Sterling-Winthrop Research Institute, Rensselaer, NY), phencyclidine (PCP) (Warner-Lambert/Parke-Davis, Ann Arbor, MI), naltrexone (NIDA, NIH), and chlorpromazine (Sigma Aldrich, St. Louis, MO)

were all dissolved in sterile water. Ro 64-6198 (Hoffman-La Roche, Basel, Switzerland) was dissolved in 10% DMSO, 10% Tween 80, and 80% sterile water for a final ratio of 1:1:8. J-113397 (K. Rice, NIDA, NIH) was dissolved in sterile water with 1.1 eq of 1M HCl. Diazepam and flumazenil (Hoffman-La Roche, Basel, Switzerland) were dissolved in 50% ethanol, 30% Alkamul, and 20% sterile water. SNC 80 (K. Rice, NIDA, NIH) was dissolved in 0.5% HCl.

## **Results**

Monkeys trained to discriminate Ro 64-6198 from vehicle required an average of 75 sessions (range, 56-95) to acquire stimulus control. FA, the first monkey to be trained, met criteria after 95 sessions and 3 different changes in dose (0.03, 0.32, and 0.18 mg/kg). The remaining monkeys, IE and ST were trained to discriminate 0.1 mg/kg of Ro 64-6198, which required 56 and 75 sessions, respectively. The three monkeys in the fentanyl group were all trained to discriminate 0.01 mg/kg of fentanyl from saline and required an average of 97 sessions (range, 91-105) to reach criteria. All monkeys in the fentanyl group started training at 0.0056 mg/kg before the dose was increased.

In Ro 64-6198-trained animals, increasing doses of the training drug produced increases in responding on the drug-appropriate lever (Figure 1.1A). On average, fentanyl substitution in Ro 64-6198-trained animals did not produce significant increases in responding on the drug-appropriate key that satisfied the *a priori* criteria for stimulus generalization (Figure 1.1B). Doses of fentanyl between 0.0056- 0.01 mg/kg produced slight increases (range, 15-36%) in the number of responses on the drug lever in all

three Ro 64-6198-trained animals, while simultaneously producing large decreases in the rates of responding. Peculiarly, the lowest dose of fentanyl tested, 0.001 mg/kg, produced full generalization in monkey IE, but elicited responding only on the vehicle-appropriate lever in both ST and FA. In fentanyl-trained animals, fentanyl produced dose-dependent increases in drug-lever responding (Figure 1.2A). Tests of stimulus generalization with Ro 64-6198 did not engender responding on the drug-appropriate lever up to doses that suppressed rates of responding (Figure 1.2B).

When Ro 64-6198-trained animals were pretreated with 1 mg/kg of the NOPr selective antagonist J-113397, responding on the drug-paired lever was completely abolished. However, pretreatment with a  $\mu$ -selective dose of naltrexone (0.03 mg/kg) did not alter responding on the Ro 64-6198-paired lever (Figure 1.3A). In fentanyl-trained animals, responding on the drug-paired lever was blocked by pretreatment with 0.03 mg/kg of naltrexone but was not altered by pretreatment with J-113397 (Figure 1.3B). No significant effects were found on rates of responding in either experiment.

In order to further verify that Ro 64-6198 did not produce  $\mu$ -mediated stimulus effects *in vivo*, fentanyl-trained animals were pretreated with a dose of J-113397 (1 mg/kg) that completely abolished the stimulus effect of Ro 64-6198 in Ro 64-6198-trained animals at the highest dose tested (0.32 mg/kg). The rationale for this experiment was to test if eliminating the stimulus effects produced through NOP would unmask any  $\mu$ -receptor mediated stimulus effects of Ro 64-6198. Under these conditions, fentanyl-trained animals responded only on the vehicle-appropriate lever (data not shown).

The *in vivo* pharmacological selectivity of Ro 64-6198 was further tested with the  $\kappa$ -agonist, ketocyclazocine, the  $\delta$ -agonist, SNC 80, the  $\mu$ -agonist, buprenorphine, the NMDA antagonist, phencyclidine (PCP), the non-selective dopamine antagonist, chlorpromazine, and the GABA<sub>A</sub> allosteric modulator, diazepam. Up to doses that suppressed rates of responding, ketocyclazocine, SNC 80, PCP, and chlorpromazine did not produce drug-lever responding in animals trained to discriminate Ro 64-6198 (Figure 1.4A). Overall, buprenorphine did not produce significant increases in drug lever responding in Ro 64-6198-trained animals. However, IE, the monkey that generalized to fentanyl at the lowest dose tested, also exhibited complete stimulus generalization to the lowest dose of buprenorphine (0.01 mg/kg), and partial generalization at the middle and high doses.

In fentanyl-trained animals, ketocyclazocine, PCP, chlorpromazine, and diazepam did not produce increases in drug lever responding (Figure 1.4B). The  $\mu$ -agonist buprenorphine produced dose-dependent increases in drug lever responding, and fully generalized to a fentanyl cue. SNC 80 also produced dose-dependent increases in the drug lever responding in fentanyl-trained animals and, at the two highest doses tested, produced partial generalization to a fentanyl stimulus.

Drug substitution studies with diazepam produced dose-dependent increases in Ro 64-6198-lever responding that, on average, met criteria for partial generalization (Figure 1.5A). All three subjects partially or fully generalized to at least one dose of diazepam, and at every dose tested, two out of three animals made fifty percent or more of their responses on the drug-appropriate lever. Ro 64-6198-lever responding following



diazepam was unaltered by J-113397 1 mg/kg (Figure 1.5B), and the potency of the Ro 64-6198 training dose was similarly unaffected by flumazenil 1 mg/kg (data not shown).

In the warm-water tail-withdrawal procedure, fentanyl produced dose-dependent increases in tail-withdrawal latency in all four subjects, and in the same animals, doses of Ro 64-6198 up to 0.32 mg/kg did not increase tail-withdrawal latency in 3 of 4 animals. In one animal (WD), there was an increase in tail-withdrawal latency from 2.7s at baseline to 15.2s, or 72% of maximum percent effect, at the highest dose tested (0.32 mg/kg) (Figure 1.6).

## **Discussion**

This is the first study to demonstrate that a NOPr agonist, Ro 64-6198, can be trained as a discriminative stimulus in rhesus monkeys. The interoceptive effects of Ro 64-6198 were distinct from those mediated through other opioid receptors as evidenced by the lack of stimulus generalization to selective opioid agonists, and the ability of selective NOPr antagonists to block the Ro 64-6198-induced cue. Additional drug substitution studies with chlorpromazine and PCP, which have both been trained as discriminative stimuli (Goas and Boston, 1978; Solomon et al., 1982), also failed to generalize, indicating that these drugs and the receptors they bind do not contribute to a Ro- 64-6198 stimulus.

While Ro 64-6198 was shown to be 100-fold more selective for NOP *in vitro*, it possesses reasonable affinity for the  $\mu$ -opioid receptor ( $K_D$  approximately 50 nM) where it functions as a full agonist (Jenck et al., 2000; Wichmann et al., 2000). Generally,

studies evaluating the effects of Ro 64-6198 on analgesia, anxiety, and locomotion in rodents, and on analgesia in monkeys, have shown these effects are not sensitive to the opioid antagonists naloxone and naltrexone (Higgins et al., 2001; Ko et al., 2009; Varty et al., 2005). The absence of stimulus generalization to a fentanyl cue in animals trained to discriminate Ro 64-6198, and likewise, the absence of stimulus generalization to a Ro 64-6198 cue in animals trained to fentanyl, supports previous findings in rat and monkey that Ro 64-6198 has no appreciable efficacy at the  $\mu$ -opioid receptor *in vivo*. To test this further, we attempted to “unmask” the  $\mu$ -agonist effects of Ro 64-6198 by pretreating fentanyl-trained animals with J-113397 before administering a large dose of Ro 64-6198. Even under conditions where NOP activity was silent and the dose of Ro 64-6198 was high compared to doses that produce other behavioral effects, the animals responded only on the vehicle-appropriate lever, further confirming that Ro 64-6198 lacks  $\mu$ -agonist effects. While it is possible that even higher doses of Ro 64-6198 may display some  $\mu$ -opioid receptor like stimulus effects, the dose tested here was high enough to produce significant behavioral disruption. Thus, it is unlikely that Ro 64-6198 has any behavioral effects mediated through the  $\mu$ -opioid receptor.

Curiously, the Ro 64-6198-trained monkey IE reported that the lowest dose of fentanyl (0.001 mg/kg) fully generalized to the training drug and this effect decreased as the dose of fentanyl was increased. Consistent with this pattern of responding, IE completely generalized to the  $\mu$ -receptor partial agonist buprenorphine at low doses, while larger doses only produced partial generalization. IE had an extensive i.v. drug history that included working for cocaine, remifentanyl, and methylphenidate, as well as for food, under a variety of different schedules. While we cannot rule out prior

experimental history as a reason for this unusual pattern of stimulus generalization, it is not apparent what specific aspects are impacting these data. Individual differences in the pharmacodynamics and/or pharmacokinetics of drugs are common, and may be caused by genetic differences in neurobiological makeup and in how the drug is absorbed, distributed, and metabolized. If significant overlap between the behavioral effects of Ro 64-6198 and  $\mu$ -agonists continue to be reported in rhesus monkeys or any other species, then pharmacogenetic studies looking at genetic polymorphisms in the  $\mu$  and NOPr, as well as the enzymes known to metabolize Ro 64-6198 may help provide an explanation for these findings.

The current study found that Ro 64-6198-trained animals partially generalized to the benzodiazepine diazepam. Several studies in rats have reported that Ro 64-6198 produced anxiolytic-like effects in the elevated plus-maze, fear-potentiated startle, and the modified Geller-Seifter conflict test (Goeldner et al., 2012; Jenck et al., 2000; Varty et al., 2005). These anxiolytic-like effects were similar in magnitude to those produced with diazepam and alprazolam, but unlike the benzodiazepines, Ro 64-6198 was anxiolytic-like at doses that did not disrupt motor or cognitive performance. In rhesus monkeys, we found that diazepam produced partial generalization to Ro 64-6198, and that these effects were blocked with flumazenil but not with J-113397. Additionally, the potency of the Ro 64-6198 training dose was unaltered by pretreatment with flumazenil. This suggests that Ro 64-6198 and diazepam share components of their interoceptive effects, but that they are not mediated through a common receptor. *In vitro* binding data support that Ro 64-6198 has no significant affinity for the GABA<sub>A</sub> channel or the benzodiazepine-binding site on GABA<sub>A</sub> (Wichmann et al., 2000). To the extent that the

interoceptive effects produced with diazepam are related to its therapeutic efficacy, these data may support the use of Ro 64-6198 as a novel anxiolytic.

Consistent with the previous literature (France et al., 1992), monkeys readily learned to discriminate between fentanyl and vehicle. The effects of fentanyl were antagonized with  $\mu$ -selective doses of naltrexone but not with J-113397. Ro 64-6198, diazepam, chlorpromazine, ketocyclazocine, and PCP did not produce stimulus generalization in fentanyl-trained animals, while the  $\mu$ -agonist buprenorphine produced full generalization. Surprisingly, the  $\delta$ -selective agonist SNC 80 produced partial generalization to a fentanyl cue. To our knowledge this is the first time that a  $\delta$ -agonist has partially generalized to a  $\mu$ -receptor stimulus in monkeys, although rats and monkeys trained to discriminate SNC 80 and other  $\delta$ -agonists have shown stimulus generalization to drugs from other pharmacological classes such as ketamine, cocaine, and amphetamine (Brandt et al., 1999; Suzuki et al., 1997a; Suzuki et al., 1997b). These results taken in the context of the previous literature suggest that the stimulus effects of SNC 80 maybe more complex than previously thought. In general, however, these findings confirm that the stimulus effects of  $\mu$ -agonists are selective.

Overall, in contrast to the apparent species differences in the antinociceptive effects, there is good concordance between the stimulus effects of Ro 64-6198 in rat and monkey. In rats trained to discriminate Ro 64-6198 from saline, morphine 6 mg/kg and buprenorphine 0.1 mg/kg, produced 40 - 50% responding on the drug-appropriate lever, but these doses significantly suppressed rates of responding, and there was considerable variability among subjects (Recker and Higgins, 2004). This is consistent with the present study in that Ro 64-6198-trained monkeys responded on the drug-

appropriate lever when tested with doses of fentanyl and buprenorphine that also suppressed rates of responding, except that neither drug met criteria for generalization. Likewise, in both rat and monkey, stimulus effects of Ro 64-6198 were blocked only with J-113397 and not naloxone or naltrexone, and drug substitution studies with selective opioid agonists at  $\kappa$  and  $\delta$ -receptors did not produce stimulus generalization in these animals.

In the present study, Ro 64-6198 did not produce antinociception against an acute thermal nociceptive stimulus (50° C water) in 3/4 monkeys tested. One monkey showed increases in tail-withdrawal latency at the highest dose of Ro 64-6198 (0.32 mg/kg), which produced 72% of the maximum possible effect. In contrast, the  $\mu$ -agonist fentanyl produced maximum antinociception in 4/4 animals tested. The analgesic potency and efficacy of fentanyl in this experiment are consistent with previously published studies, and the order of potency with which fentanyl was discriminated, suppressed rates of responding, and produced analgesia is consistent with the pharmacodynamic profile of this drug across the literature (i.e., potency order of discrimination > rate suppression > analgesia) (Dykstra et al., 1988; France et al., 1992; Stevenson et al., 2003). If this same order of potency held true for NOPr agonists, analgesia should have been observed at doses of 0.32 mg/kg of Ro 64-6198. However, very limited analgesia was observed even at this large dose of Ro 64-6198. Interestingly, in previous studies with rhesus monkeys, a dose of 0.03 mg/kg Ro 64-6198 was reported to produce analgesia in the same thermal nociception assay as that used here (e.g. Sukhtankar et al., 2014; Ko and Naughton, 2009; Ko et al., 2009). This

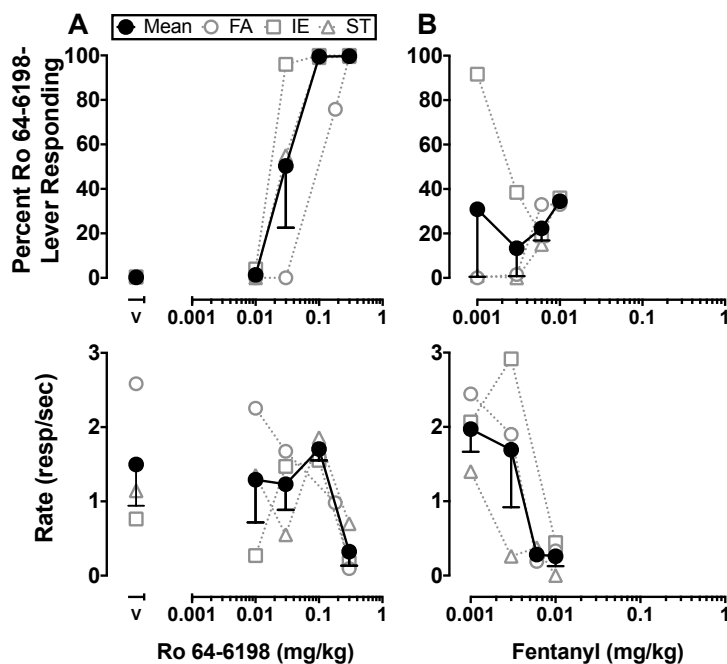
is an order of magnitude less than would be expected to produce analgesia if the commonly observed order of behavioral potency held true with this NOPr agonist.

The reasons for the differences in the antinociceptive efficacy of Ro 64-6198 between studies are not clear. Limits in drug solubility prevented increasing the dose of Ro 64-6198 above 0.32 mg/kg, but this dose was more than an order of magnitude greater than the reported ED<sub>50</sub> for antinociception published in previous studies using analogous procedures (Cremeans et al., 2012; Ko et al., 2009). When monkeys self-administering remifentanyl were pretreated with Ro 64-6198 0.32 mg/kg IV, there was a comparable decrease in rates of responding and the authors reported that this dose produced general sedation (Podlesnik et al., 2011). Even when accounting for differences in potency as a function of route of administration, there appears to be little difference in the potency of Ro 64-6198 to produce sedation in the present study and in studies where Ro 64-6198 was found to be antinociceptive. These findings suggest that the antinociceptive effects of Ro 64-6198 may be more variable than previously described.

Since additional NOPr agonists and antagonists were not characterized in these procedures, the results from this study may not be broadly generalizable to the whole class of compounds. This is a limitation of the present investigation. Other small molecule NOPr agonists, such as SCH 221510, have also been shown to produce antinociception in rhesus monkeys using the warm-water tail-withdrawal assay (Cremeans et al., 2012). SCH 221510 was subsequently found to produce analgesia in a novel food-reinforced antinociceptive assay where squirrel monkeys were trained to pull a lever heated at different temperatures for various periods of time (Kangas and

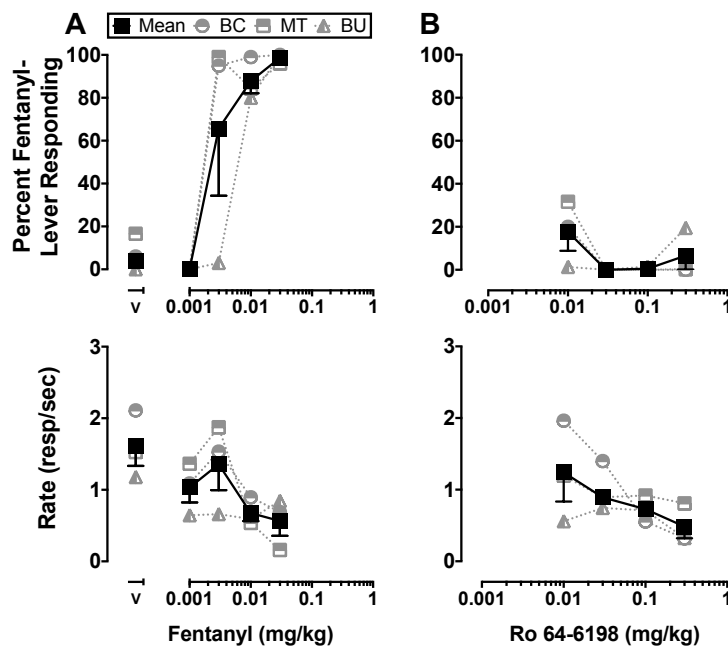
Bergman, 2014). This procedure appeared to be quite sensitive to different analgesics when compared to assays that use warm water as the thermal stimulus, and not all drugs that were found to be antinociceptive in warm-water tail-withdrawal were antinociceptive in this procedure. It will be interesting to learn whether Ro 64-6198 is antinociceptive in this assay. The results from these studies illustrate the importance of testing potential therapeutics in a variety of species, with different procedures, across different laboratories.

In sum, this study establishes that Ro 64-6198 can be trained as a discriminative stimulus in rhesus monkeys and that these effects are selectively mediated through the NOPr. The stimulus properties of Ro 64-6198 are not like other opioid agonists, but may share similarities with diazepam. Future studies should examine the extent to which drugs that act at the GABA<sub>A</sub> receptor generalize to Ro 64-6198, and whether the stimulus effects produced with Ro 64-6198 are characteristic of all NOPr selective ligands. Finally, Ro 64-6198 failed to produce antinociception in the present study suggesting that more research is needed to assess Ro 64-6198 and other NOPr agonists as novel agents for pain control.



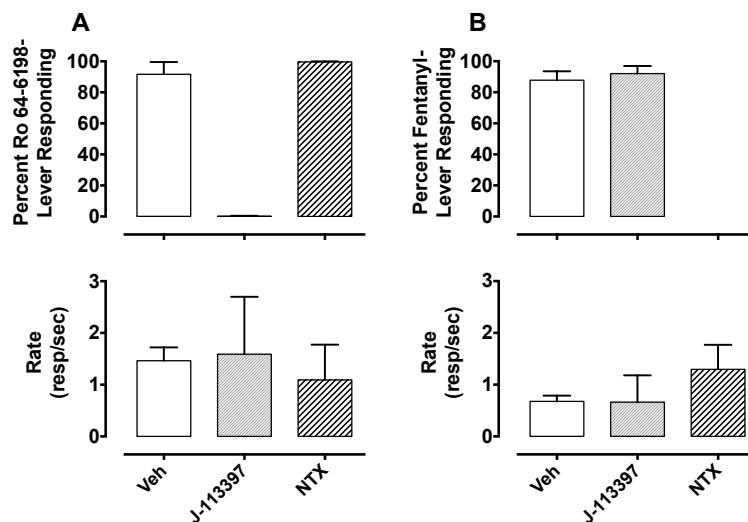
**Figure 1.1 Discriminative stimulus effects Ro 64-6198 in Ro 64-6198-trained monkeys.** Discriminative stimulus and response rate effects of Ro 64-6198 in Ro 64-6198-trained animals (A) and drug substitution studies with fentanyl (B). Data from individual monkeys (FA, IE, ST) and their mean (+/- SEM) are plotted (n=3). FA was trained to discriminate Ro 64-6198 0.18 mg/kg, and IE and ST were trained to 0.1 mg/kg. Abscissae: Dose in milligrams per kilogram, and vehicle responding (V). Ordinates: Percent Ro 64-6198-Lever Responding and Response Rate (responses/second).



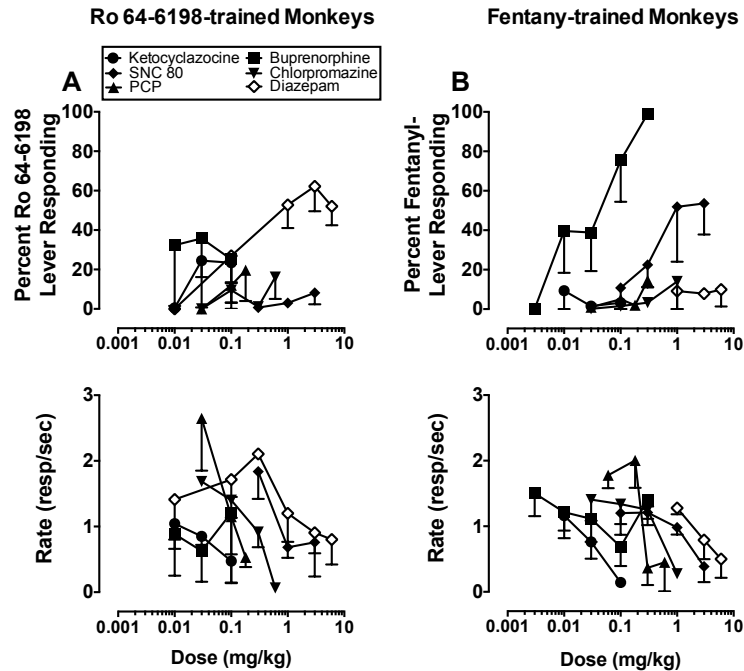


**Figure 1.2 Discriminative stimulus effects of Ro 64-6198 in fentanyl-trained**

**Monkeys.** Discriminative stimulus and response rate effects of fentanyl in fentanyl-trained animals (A) and drug substitution studies with Ro-64-6198 (B). Data from individual monkeys (BC, MT, BU) and their mean (+/- SEM) are plotted (n=3). All animals were trained to discriminate fentanyl 0.01 mg/kg. Abscissae: Dose in milligrams per kilogram, and vehicle responding (V). Ordinates: Percent Fentanyl-Lever Responding and Response Rate (responses/second).

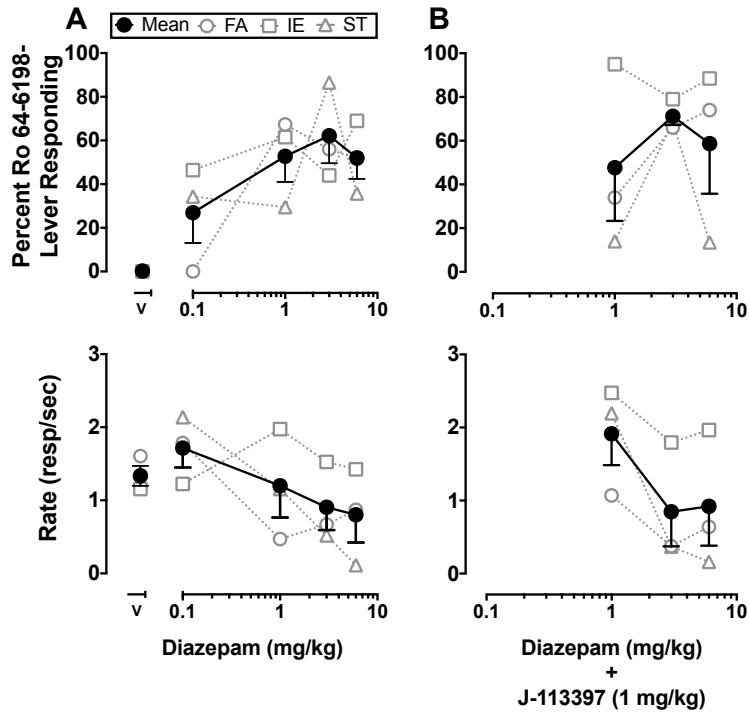


**Figure 1.3 The effects of selective antagonists in Ro 64-6198 and fentanyl-trained monkeys.** The effect of J-113397 (1 mg/kg) and naltrexone (0.03 mg/kg) on the discriminative stimulus and response rate effects of the training dose in Ro 64-6198-trained animals (A) and fentanyl-trained animals (B). Data are presented as the mean and (+/- SEM) for each group of monkeys (n=3). Abscissae: Vehicle (Veh), J-113397, and naltrexone (NTX) in the presence of the training drug. Ordinates: Percent Ro 64-6198-Lever Responding (A), Percent Fentanyl-Lever Responding (B), and Response Rate (responses/second).



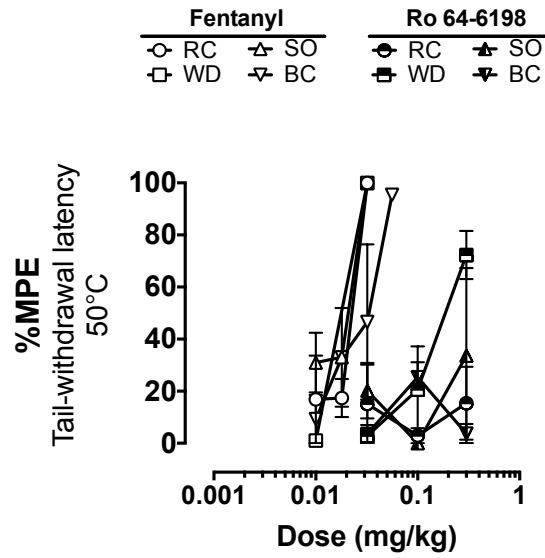
**Figure 1.4 Drug substitution studies in Ro 64-6198 and fentanyl-trained**

**Monkeys.** Discriminative stimulus and response rate effects of drugs that did not substitute for a Ro 64-6198 stimulus (A) and discriminative stimulus and response rate effects of drugs that did not substitute for a fentanyl stimulus (B). Data are presented as the mean of three animals (+/- SEM). Abscissae: Dose in milligrams per kilogram. Ordinates: Percent Ro 64-6198-Lever Responding (A), Percent Fentanyl-Lever Responding (B), and Response Rate (responses/second).



**Figure 1.5 Diazepam substitution studies in Ro 64-6198-trained animals.**

Discriminative stimulus and response rate effects of diazepam in Ro 64-6198-trained monkeys. Diazepam alone (A) and following pretreatment with J-113397 (1 mg/kg) (B). Data from individual monkeys and the mean (+/- SEM) are presented. Abscissae: Dose in milligrams per kilogram of diazepam and vehicle responding (V). Ordinates: Percent Ro 64-6198-Lever Responding and Response Rate (responses/second).



**Figure 1.6 Antinociception studies with Ro 64-6198 and fentanyl.** Effects of fentanyl and Ro 64-6198 in the warm-water tail-withdrawal assay at 50°C. Data are presented from individual monkeys as the mean of 2 observations (+/- SEM) and were converted to maximum percent effect (MPE).

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**CHAPTER THREE**

**A Model to Measure the Analgesic Effects of  
Intranasally Administered Opioids  
in Rhesus Monkeys**

**Introduction**

Intranasal (IN) drug administration is a non-invasive, rapid, and efficient route of drug delivery (Pires et al., 2009). Currently, IN administration is used for a variety of therapeutics including, vaccines, chemotherapies, and analgesics. The therapeutic possibilities for IN drug administration are far-reaching and include: the administration, outside a clinical setting, of biologics and peptides as therapeutics; the ability to target drugs directly to the brain and CNS while reducing peripheral side effects; and the ability to achieve therapeutic effects at lower absolute doses with the potential to reduce toxicity (Born et al., 2002; Campbell et al., 2012; Dhuria et al., 2010; Fortuna et al., 2014; Miyake and Bleier, 2015).

Studies performed in rodents and primates have demonstrated that IN drug administration circumvents hepatic first pass metabolism, avoids GI decomposition, and provides a rapid onset of action (e.g. Fortuna et al. 2014; Dhuria et al. 2010).

In humans and NHP drug absorption in the nasal cavity occurs primarily in the respiratory zone located between the inferior and middle turbinate (Grassin-Delyle et al., 2012). This region has the highest surface area in the primate nose and contains a dense vascular network composed of the sphenopalatine, facial, and ophthalmic veins, which drain into the jugular, and back to the superior vena cava (Gourlay and Benowitz, 1997; Guthrie et al., 1999).

In general, therapeutics delivered IN are primarily absorbed into the systemic circulation via the respiratory zone. However, several lines of research have suggested that drug administration into the nasal cavity may permit direct absorption to the brain that circumvents the blood brain barrier (BBB) (Dhuria et al., 2010; Johnson et al., 2010; Miyake and Bleier, 2015). Although the exact path that drugs travel to directly access the brain remains an active area of investigation, studies have shown that olfactory receptor neurons (ORN) located in the superior turbinate are competent to enable this pattern of absorption (Thorne et al., 1995). It has also been proposed that trigeminal nerves, which innervate the respiratory epithelium, may also play a role (Johnson et al., 2010). While this remains a promising area of research, evidence for direct drug absorption from the nose into the brain is limited (Scheibe et al., 2008).

To date, preclinical research on IN drug administration has primarily been conducted in rats. Although rodents have been invaluable in establishing what is known about intranasal drug absorption and nasal pathology, there are anatomical differences between rodents and primates that may be translationally important (Harkema et al., 2006). For example, the ratio of the nasal cavity surface area to volume was found to be largest in rats (51.5 cm<sup>3</sup>), compared to 7.75 cm<sup>3</sup> in rhesus monkeys and 6.4 cm<sup>3</sup> in

humans (Gross et al., 1982; Smith et al., 2004). The larger ratio indicates rodents may have a greater potential for drug absorption compared to primates. Additionally, the respiratory epithelium composes 80-90% of the nasal passage in primates, while the rat nasal passage is equally divided between respiratory and olfactory epithelium. Since drug deposition into each of these regions may represent a different route of absorption (CNS vs. systemic), preclinical research on intranasal delivery would benefit from more studies conducted across species.

Recently, the intranasal administration of opioids (agonists and antagonists) has demonstrated great clinical value. Despite their well-known side effects, opioid agonists remain the most widely used agent to control moderate to severe pain. Relief from acute, unpredictable, and highly distressing pain events, such as break-through cancer pain, has been improved with IN formulations of fentanyl (Karlsen et al., 2013; Kongsgaard et al., 2014). IN naloxone, an opioid antagonist, is being used routinely to reverse opioid toxicity (Kerr et al., 2008; Wermeling, 2013). Furthermore, the ease of administration, the rapid onset of action, and the elimination of needles have paved the way to increase the distribution of naloxone as one means to reduce the rising number of fatalities from opioid overdose (Rando et al., 2015).

Although IN administration has been shown to have clinical benefits, there remains no procedure to reliably evaluate the analgesic effects of intranasally administered opioids in NHP even though preclinical studies in this species have contributed to opioid pharmacology and the development of therapeutics. The primary purpose of this study was to validate a procedure to measure the analgesic effects of intranasal opioids, and to conduct an exploratory analysis of the pharmacodynamic

differences between IN and IM administration. For these experiments, two small molecule  $\mu$ -opioid receptor agonists with different efficacies, fentanyl and buprenorphine, were evaluated in the warm water tail-withdrawal procedure. As a secondary goal, we explored the possibility of extending this procedure to other pain modalities, such as capsaicin-induced allodynia, and evaluated the potential of this model to detect the activity of peptidic opioid ligands, such as MMP 2200.

## **Methods**

### *Subjects*

Adult male rhesus monkeys (n=3) served as subjects in these studies (weights, 10.2 - 11.4 kg). Monkeys were individually housed in custom-built steel cages (83.3 cm high x 76.2 cm wide x 91.4 cm deep) and kept on a 12hr light/12 hr dark schedule. Monkeys were fed Lab Fiber Plus Monkey Diet (PMI Nutrition Intl. LLC.) that was supplemented with fresh fruit daily, and water and enrichment toys were continuously available in the home cage. The animals were maintained, and all experiments were performed in accordance with the University of Michigan University Committee on Use and Care of Animals.

### *Surgery*

Monkeys were surgically implanted with indwelling venous catheters that were attached to ports located under the skin for the administration of anesthetic propofol. For surgery, all monkeys were anesthetized with ketamine (10 mg/kg) and xylazine (2 mg/kg). Intravenous catheters were placed in an accessible vein and passed subcutaneously to the animal's back where it was attached to the port (Access

Technologies, Intisil™ catheter, Attachable 5 French [0.30"/0.7mm ID x 0.065"/1.7mm OD x 24"/60 cm], Skokie, Illinois).

### *Drug administration*

Intranasal administration was performed with a modified nasal atomizer (MAD) (LMA® MAD Nasal™ Intranasal Mucosal Atomization Device, Research Triangle Park, NC) that was attached to a 1 ml syringe. The Styrofoam stop, and the butterfly handles were removed from the MAD to ensure a good fit into the monkey's nostril. Immediately prior to drug administration, the monkey was anesthetized with propofol (3.3 ml of 10 mg/ml), which was administered through the IV port. Following the onset of anesthesia, the monkey was reclined to an angle of approximately 45° degrees, the atomizer was inserted into the right or left nostril (approximately 3 cm), and the plunger was depressed while holding the opposing nostril closed. The monkey was held in the reclined position for three minutes following drug administration. Fentanyl and buprenorphine were delivered in volumes between 0.05 ml and 0.4 ml. Buprenorphine was not administered at a volume of more than 0.2 ml in a single nostril due to the viscous nature of the vehicle. MMP 2200 was administered in volumes between 0.3-1.0 ml—no more than 0.5 ml per nostril. Intramuscular drug administration was performed in the standard manner except that propofol anesthesia was administered prior to injection.

### *Antinociception studies*

Monkeys were trained to sit in a Plexiglas primate chair that measured 1.5 m in height. The bottom portion of the tail was shaved and periodically dipped into water heated to 38, 42, 46, or 50° C. The desired temperature was achieved by mixing hot

water from a kettle (maintained at approximately 100°C) with cold tap water in a Thermos, which was calibrated using a total immersion thermometer. Tail-withdrawal latency was measured using a digital stopwatch.

The session began with baseline determinations performed at each temperature. Two experimenters measured tail-withdrawal latency at every temperature. The experimenter who was measuring tail-withdrawal latency was blind to the temperature being prepared by the other. Monkeys were anesthetized with propofol as described above. Following drug or vehicle administration IM or IN, the monkey was gently stimulated (rubbing its head and chest) to encourage rapid recovery from anesthesia. A monkey was determined suitable for testing when it could successfully track the experimenter's finger with its eyes, and when it was capable of withdrawing its leg following stimulation from the experimenter. Tail-withdrawal latency was measured subsequently at 10, 20, 40, 60, 80, 100 and 120 minutes. No more than one session was performed per week.

#### *Capsaicin-induced allodynia*

Allodynia experiments were conducted based on methods that have been reported elsewhere (Ko et al., 1998). Pilot experiments were performed using two doses of capsaicin (0.1 mg and 0.32 mg), while measuring tail-withdrawal latency at different time points relative to drug administration following capsaicin (5, 10, 15, 30, 45, 60 min). The final parameters were chosen based on their ability to produce reliable and reversible allodynia across all subjects.

Following baseline tail-withdrawal determinations, animals were anesthetized with propofol, as described above, and were given a dose of drug or saline IN. Fifteen

minutes later, capsaicin 0.32 mg was administered subcutaneously (SC) 5-8 cm from the bottom of the tail, and 15 minutes following capsaicin administration, tail-withdrawal latencies were measured at 38, 42, and 46° C. Water heated to 42° C was chosen as the thermal stimulus for these experiments because it was the highest temperature that reliably produced the maximum tail-withdrawal latency in the absence of capsaicin, while still producing rapid tail removal following capsaicin administration.

### *Data analysis*

For measures of antinociception, the average tail-withdrawal latency was calculated for each individual monkey, and converted to percent maximum possible effect (%MPE) using the following calculation:  $\%MPE = [(test\ latency - control\ latency) / (20\ s\ cut-off\ latency - control\ latency)] \times 100$ . If a condition was run more than once, average tail-withdrawal latency was calculated for that condition within subject and then averaged across subjects. MPE data is presented as an average of three monkeys  $\pm$  SEM. The data were analyzed by repeated measures ANOVA, and post-hoc analyses were conducted using the Holm-Sidak correction for multiple comparisons.

Tail-withdrawal latencies for capsaicin- induced allodynia were reported in seconds (s) and were not converted to MPE. Due to the variability across subjects, data are presented from individual monkeys.

### *Drugs*

Fentanyl (Sigma Aldrich, St. Louis, MO), and MMP 2200 (generously provided by Dr. Robin Polt) were dissolved in sterile saline. Buprenorphine (NIDA, NIH) was dissolved in 40% beta-cyclodextrin. Capsaicin (Sigma Aldrich, St. Louis, MO) was

dissolved in 50% ethanol. Propofol (10 mg/ml) was manufactured by Sagent Pharmaceuticals (Schaumburg, IL).

## **Results**

### *Acute thermal antinociception*

The time to recovery from propofol anesthesia did not differ between routes of administration for either fentanyl or buprenorphine experiments [Fentanyl mean: [IN] 332.5 s ( $\pm$  9.39); [IM] 343.2 s ( $\pm$  12.18 SEM). Buprenorphine mean: [IN] 342.5 s ( $\pm$  9.96); [IM] 358.8 s ( $\pm$  18.54)]. In the time course data presented in Figures 2.1 and 2.3, the measurement of tail-withdrawal latency at time-point 0 corresponds to the first measurement taken after the subject had recovered from anesthesia.

Figure 2.1 illustrates the tail-withdrawal latency, over a period of 120 min, from water heated to 50° C following the IN (2.1A) or IM (2.1B) fentanyl administration (0.01-0.032 mg/kg). The largest dose of IN fentanyl (0.032 mg/kg) increased tail-withdrawal latency to 70% MPE at time-point 0, and tail-withdrawal latency remained at 75% MPE or higher for 120 min (range, 70-100%). Smaller doses of IN Fentanyl (0.01 and 0.018 mg/kg) increased tail-withdrawal latency beginning at time-point 0 to 35 and 45% MPE, respectively. For the remainder of the session, IN fentanyl 0.01 mg/kg produced changes in tail-withdrawal latency between 16-59% MPE, and IN fentanyl 0.018 mg/kg produced a range of tail-withdrawal latencies between and 15-62% MPE.

IM fentanyl administration also increased tail-withdrawal latency from 50°C water. IM fentanyl (0.032 mg/kg) increased tail-withdrawal latency to 61% MPE at time point 0, and produced a range of effects between 49-100% MPE over the course of 120



minutes. A dose of 0.018 mg/kg IM increased tail-withdrawal latency to 9% MPE at the 0 time point, and produced a range of tail-withdrawal latencies between 8-47% thereafter. IM fentanyl (0.01 mg/kg) produced tail-withdrawal latencies similar to those measured following saline injection throughout the 120-minute session.

Figure 2.1 presents the average peak tail-withdrawal latency produced with fentanyl across time. There was a main effect of dose on tail-withdrawal latency, but no effect of route [Dose:  $F(3,6) = 19.82, p \leq 0.01$ ]. IN fentanyl produced dose-dependent increases in tail-withdrawal when compared to saline administration [Fentanyl dose (mg/kg) vs. saline: 0.01,  $p \leq 0.01$ ; 0.018,  $p \leq 0.01$ ; 0.032,  $p \leq 0.001$ ]. Following IM administration, the two largest doses of fentanyl (0.018 and 0.032 mg/kg) produced a significant increase in tail-withdrawal latency [Fentanyl dose (mg/kg) vs. saline: 0.018,  $p \leq 0.05$ ; 0.032,  $p \leq 0.001$ ]. An analysis of dose x route indicated a significant difference between IN and IM fentanyl only at a dose of 0.01 mg/kg ( $p \leq 0.05$ ).

Figures 2.3A and 2.3B present the tail-withdrawal latency, over a period of 120 minutes, from water heated to 50° C following IN or IM buprenorphine administration (0.1-1 mg/kg). Doses of IN buprenorphine (0.1 - 1 mg/kg), produced 50% MPE or more beginning at the 20-minute time point that continued within the range of 49-80% for 120 minutes.

IM buprenorphine also produced increases in tail-withdrawal latency. Following IM administration, doses of 0.32 and 1 mg/kg increased tail-withdrawal latency to 47 and 52% MPE at 20 minutes, which continued with a similar magnitude for 120 minutes. The smallest dose of IM buprenorphine (0.1 mg/kg) produced an increase in tail-

withdrawal latency of 48% at 60 minutes, which continued within the range of 32-52% until the 120-minute time point.

Figure 2.4 presents the average peak tail-withdrawal latency produced with buprenorphine across time. There was a main effect of buprenorphine dose on tail-withdrawal latency, but no effect of route [Dose:  $F(3,6) = 11.41, p \leq 0.01$ ]. IN and IM buprenorphine produced significant increases in tail-withdrawal latency at all doses (see figure caption), but there were no differences between dose, or between routes of administration.

### *Allodynia*

Pilot studies to optimize the experimental parameters (dose of capsaicin, water temperature, time-intervals for measuring tail-withdrawal) took one month. Each animal participated in 2-4 experiments during that time. Over a subsequent period between 12-15 weeks, two subjects (ST and RD) became desensitized to the effects of capsaicin. This manifested in the failure of capsaicin to produce decreases in tail-withdrawal latency from 42°C over time (a temperature that in absence of capsaicin is non-noxious and produces the maximum tail-withdrawal latency). For the purposes of evaluating the antiallodynic effects of IN MMP 2200, data are presented only for these two subjects prior to the last control experiment with IN saline where the allodynic effects of capsaicin were verified.

As shown in Figure 2.5, the administration of SC capsaicin 0.32 mg into the tail produced a decrease in tail-withdrawal latency from water heated to 42°C in all animals [mean: 4.89 s (SEM  $\pm$  0.588)]. IN MMP 2200 (1 and 3.2 mg/kg) produced a complete reversal of capsaicin-induced allodynia in monkey ST, while 3.2 mg/kg increased the

average tail-withdrawal latency for monkey WD from 4.9 to 15 s. In subject RD, neither 3.2 mg/kg nor 10 mg/kg produced a notable increase in the average tail-withdrawal latency. Overall, IN MMP 2200 reversed allodynia to varying degrees in 2 of 3 animals tested.

## **Discussion**

The present study was the first to successfully establish a procedure for evaluating the antinociceptive and antiallodynic effects of intranasally delivered opioids in rhesus monkeys. In the initial experiments, the pharmacodynamics of two well-studied opioid agonists, fentanyl and buprenorphine, were compared across two routes of administration (IN and IM) in the warm water tail-withdrawal assay. The results from this study showed that fentanyl and buprenorphine produced dose-dependent increases in tail-withdrawal latency across both routes of administration. Overall, the potency of fentanyl and buprenorphine did not significantly differ between routes, although there was a trend for IN administration to produce increases in tail-withdrawal latency at doses that were either inactive, or not significantly different from saline when administered IM. Relative to IM administration, there was also a trend for buprenorphine and fentanyl to produce a faster onset of action when given IN. Lastly, the opioid peptide MMP 2200 was found to reverse allodynia in 2 out of 3 monkeys following IN delivery. The ability to study this compound more thoroughly was limited by the finding that 2 out of 3 monkeys became desensitized to the effects of capsaicin.

In measures of acute thermal nociception, the largest dose of fentanyl (0.032 mg/kg) given IN and IM produced a comparable onset of action, with a similar

magnitude and duration of effect. At smaller doses of fentanyl (0.01 and 0.018 mg/kg), IN administration produced sizable increases in tail-withdrawal latency while the same doses administered IM were not significantly different from those following saline. This difference was most clearly illustrated with IN fentanyl (0.01 mg/kg), which produced a mean increase in tail-withdrawal latency across the entire session of 40% MPE, while IM administration of the same dose produced an 11% change. In general, fentanyl, which has been studied extensively in this procedure given IM/SC, does not produce significant increases in tail-withdrawal latency at this low dose. The overall potency for fentanyl to produce antinociception was similar to previously published studies (Banks et al., 2010; Ko et al., 1998; Stevenson et al., 2003).

Since IN and IM fentanyl (0.018 mg/kg) showed a very different time course, the data were reanalyzed to look at the effect of route averaged across time. In contrast to peak effects, when MPE was averaged across the session, IM fentanyl (0.018 mg/kg) did not produce significant increases in tail-withdrawal latency compared to saline whereas IN fentanyl (0.018 mg/kg) produced effects that were significantly greater than those produced with saline. These data demonstrated that IM fentanyl 0.018 mg/kg was capable of producing the same amplitude of effect, but that IN administration was associated with greater increases in tail-withdrawal latency per unit of time. The same analysis performed with fentanyl (0.01 and 0.032 mg/kg) corroborated the lack of effect found with IM fentanyl 0.01 mg/kg (relative to IN administration), and the comparable effects on tail-withdrawal produced with IN and IM fentanyl at 0.032 mg/kg.

The ability of IN and IM buprenorphine to produce antinociception was assessed in the same procedure. Doses of buprenorphine (0.1- 1 mg/kg) administered IN and IM

produced increases in tail-withdrawal latency that plateaued between 66- 80% MPE. Across routes of administration, there were no significant differences noted among peak effects or when averaged across time (data not shown). This “flattening” of the dose effect curve was consistent with buprenorphine’s partial agonist action, and a similar dose response function can be seen for other effects such as respiratory depression (Cowan et al., 1977a; Cowan et al., 1977b; Kishioka et al., 2000; Walker et al., 1995). This was in sharp contrast to fentanyl, which over a smaller range of doses (0.01-0.032 mg/kg) was able to produce the maximum effect in this procedure, and the peak effects measured at the highest dose of fentanyl (0.032 mg/kg) reached 100% MPE in all three monkeys.

There was a trend for fentanyl and buprenorphine to have a faster onset of action when given IN. For fentanyl, this was most apparent when comparing the time course of tail-withdrawal latency produced with 0.018 mg/kg, which was active given by both routes of administration. The dose of IN fentanyl produced a 50% increase in tail-withdrawal latency at the 0 time point, while when given IM 0.018 mg/kg fentanyl did not produce an increase of this magnitude until 40 minutes. The tendency for IN buprenorphine to produce a faster onset of action was likewise more apparent with smaller doses. At the lowest dose of buprenorphine (0.1 mg/kg), IN administration produced a 53% increase in MPE at the 20-minute time point, while IM buprenorphine produced only a 22% change that did not reach 50% until the 100-minute time point. The rapid onset of effects that may be achieved with IN administration, and which do not require any form of injection, is one of the most appealing characteristics of this route of administration. Some clinical studies have suggested that the

pharmacodynamics and pharmacokinetics of IN fentanyl, as well as other drugs such as nicotine, are similar those produced by IV injection (Foster et al., 2008; Gourlay and Benowitz, 1997; Veldhorst-Janssen et al., 2010). Thus, IN administration may provide a spectrum of effects that are not routinely available outside of a clinical setting.

In general, ligands with high lipophilicity are more soluble in membranes and more easily traverse the BBB regardless of route of administration. This is particularly important when the target of therapeutic action is located within the CNS.

Buprenorphine (Log P = 4.98) and fentanyl (Log P = 4.05) are two highly lipophilic agonists that readily cross the BBB, and the use of such compounds may have made the opportunity to find differences in the onset of action, and the potency of these drugs as a function of route more likely (Cheng et al., 2007). It would be interesting to learn if opioid agonists with relatively lower lipophilicity such as morphine (Log P = 0.76) and oxycodone (Log P = 0.82) (Cheng et al., 2007; Tetko et al., 2005), would produce similar differences given IN and IM. Future studies may want to investigate how relative lipophilicity, and other physiochemical properties, impacts the antinociceptive effects of opioid agonists administered intranasally.

We were also interested in adapting this procedure to measure other pain modalities such as allodynia. Previous studies have shown that SC injection of capsaicin into the tail of rhesus monkeys produced a transient allodynia that was sensitive to the effects of compounds that stimulate peripheral opioid receptors in the absence of central opioid receptor activation (Caterina et al., 1997; Do Carmo et al., 2008; Ko et al., 1998). Thus, the effects of peptides and small molecule opioid agonists that do not pass through the BBB may be detected in this preparation. MMP 2200 was

derived from the endogenous opioid leu-enkephalin, and is a mixed-acting  $\mu/\delta$ -agonist that was shown to reverse allodynia in rhesus monkeys through a peripheral mode of action (Do Carmo et al., 2008). There are certain medical conditions where effective pain control, with opioids or other drugs, can be achieved in the absence of CNS effects, and there are circumstances where this may be preferred (Stein and Lang, 2009; Vadivelu et al., 2011). Lastly, since one of the potential benefits of IN administration is the ability to administer molecules that are generally not suitable for oral administration (e.g. peptides and proteins), we wanted to test if this model could be used to evaluate such compounds.

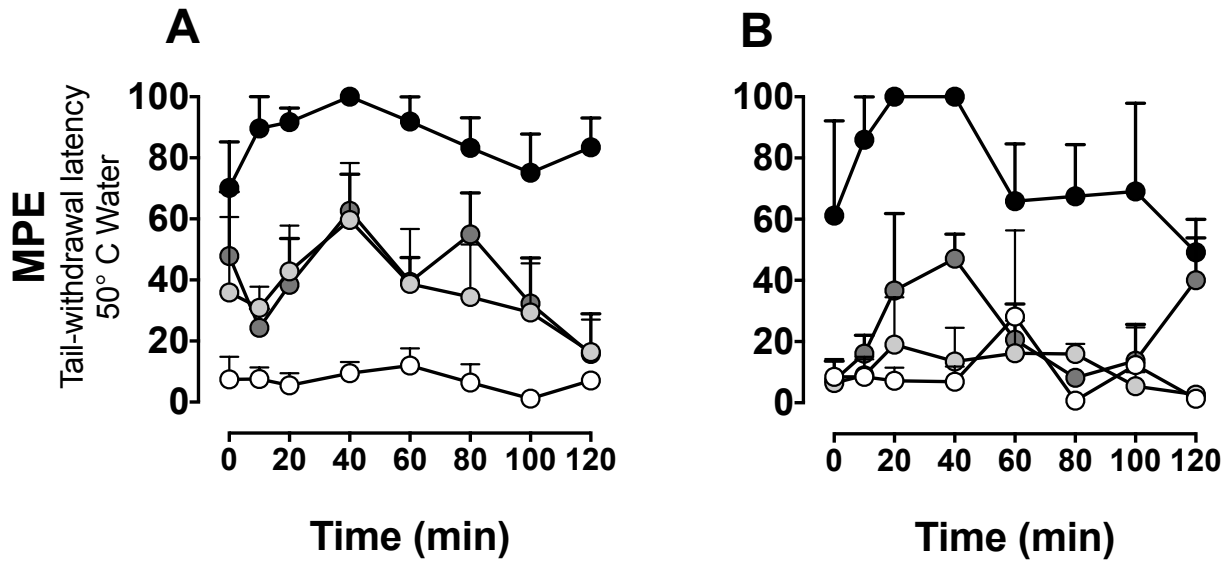
In two out of three animals, IN MMP 2200 (1.0 and 3.2 mg/kg) produced complete, and near-complete reversal of capsaicin-induced allodynia, which was generally consistent with previously published studies (Do Carmo et al., 2008). In one animal, doses up to 10 mg/kg did not significantly increase tail-withdrawal latency, however higher doses could not be evaluated because this subject had developed desensitization to the allodynia produced with capsaicin. Although the present study did not verify that the effects of MMP 2200 were opioid-mediated, previous work in rhesus monkeys demonstrated these effects were blocked with  $\mu$ -selective doses of naltrexone, the  $\delta$ -opioid antagonist naltrindole, and quaternary naltrexone (Do Carmo et al., 2008). However, it was also determined that doses of MMP 2200 (32 and 56 mg/kg) that suppressed rates of responding were not blocked with opioid antagonists suggesting that non-opioid receptors may be partially responsible for the effects of MMP 2200 at higher doses. These data provide preliminary suggestive evidence that MMP 2200 was bioavailable, and antiallodynic following IN delivery. Future studies assessing the

analgesic effects of MMP 2200 should consider examining this route of administration more thoroughly.

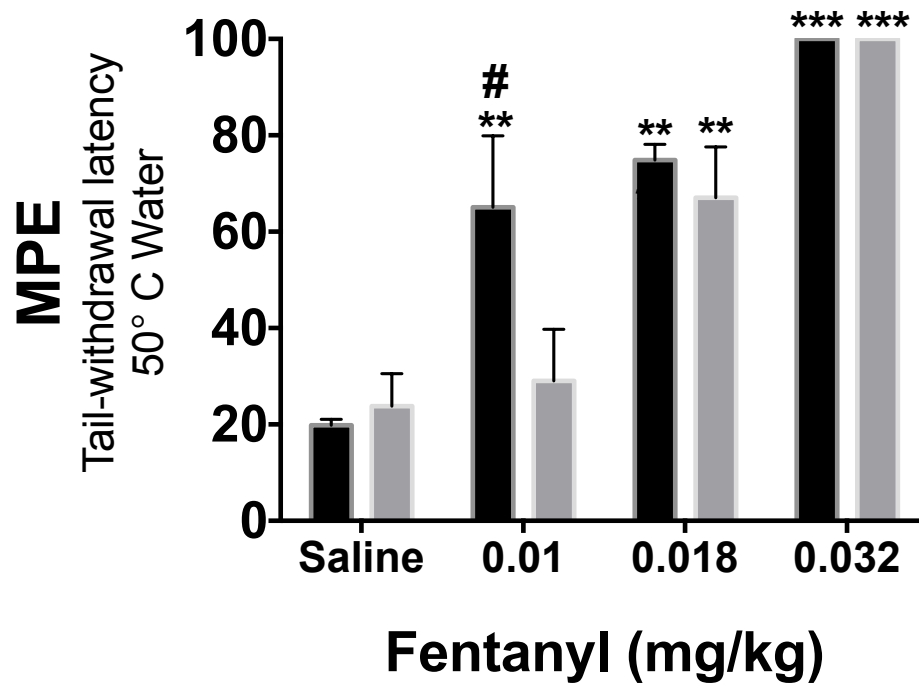
The finding that desensitization occurred to capsaicin-induced allodynia was surprising. Although this has not been reported elsewhere in the NHP literature, there are some notable differences between the current procedure, and those used previously. For example, this experiment used larger dose of capsaicin than had been employed in the past (0.32 mg vs. 0.1 mg). It is also possible that repeated administration of this dose over time produced receptor desensitization. Additionally, there are lines of evidence suggesting drug interactions between propofol, capsaicin, and the receptors they stimulate (Fischer et al., 2010; Ji et al., 2013; Nishimoto et al., 2015; Wickley et al., 2010). Like capsaicin, propofol is an agonist of TRPV1, and additionally, TRPA1, another transient receptor potential-receptor that is co-localized with TRPV1 on nociceptive afferents, including sensory neurons (Mickle et al., 2015). Both receptors are thought to play a role in mediating the effects of neuropathic and inflammatory pain. The co-activation of these receptors with selective agonists has been reported to produce complex effects including sensitization, as well as desensitization at the receptor level. These effects of cross-receptor sensitization and desensitization have also been extended to the actions of propofol and capsaicin, specifically. While, the exact basis for capsaicin desensitization in this preparation remain unclear, it would be interesting to learn if these results are generalizable to different chemical irritants and anesthetics. At the least, it suggests that a dose of capsaicin (0.32 mg) given by injection into the tail of rhesus monkeys may not be appropriate for repeated use in these experiments.



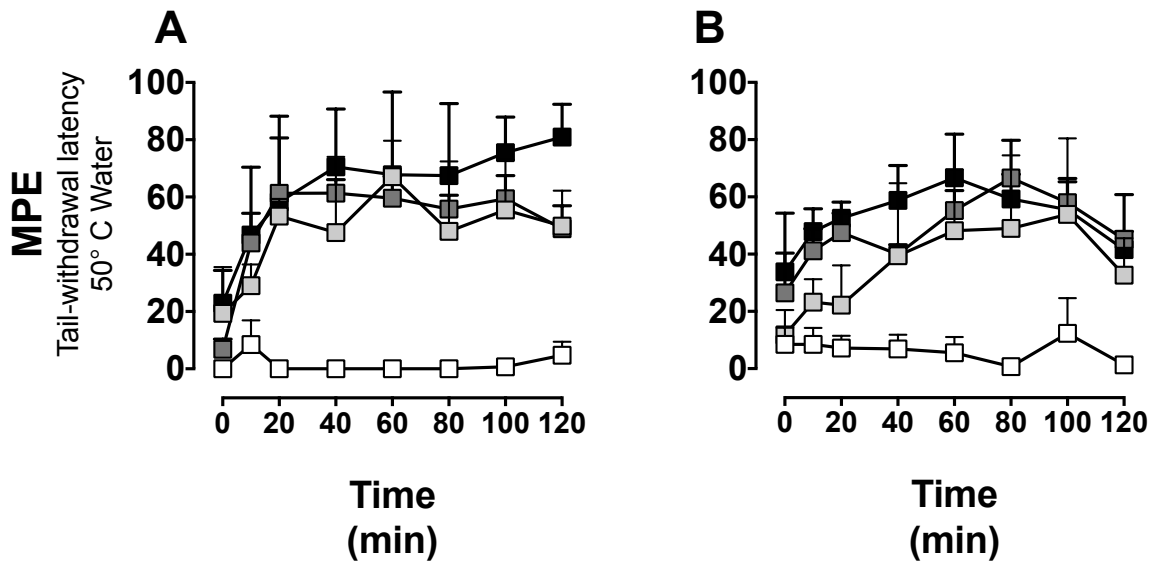
Intranasal drug administration has considerable potential for a variety of therapeutics. We have successfully demonstrated a procedure that can be reliably used to detect the antinociceptive effects, and to a lesser extent, the antiallodynic effects, of intranasal opioids. The study confirms that IN administration with these drugs has a prompt onset of action, and may allow therapeutic effects to be achieved with lower doses relative to other routes of administration. We also presented preliminary evidence that the opioid peptide MMP 2200 produces antiallodynia when given IN. These procedures are suitable to investigate novel or clinically used agents that may be used to improve opioid therapy.



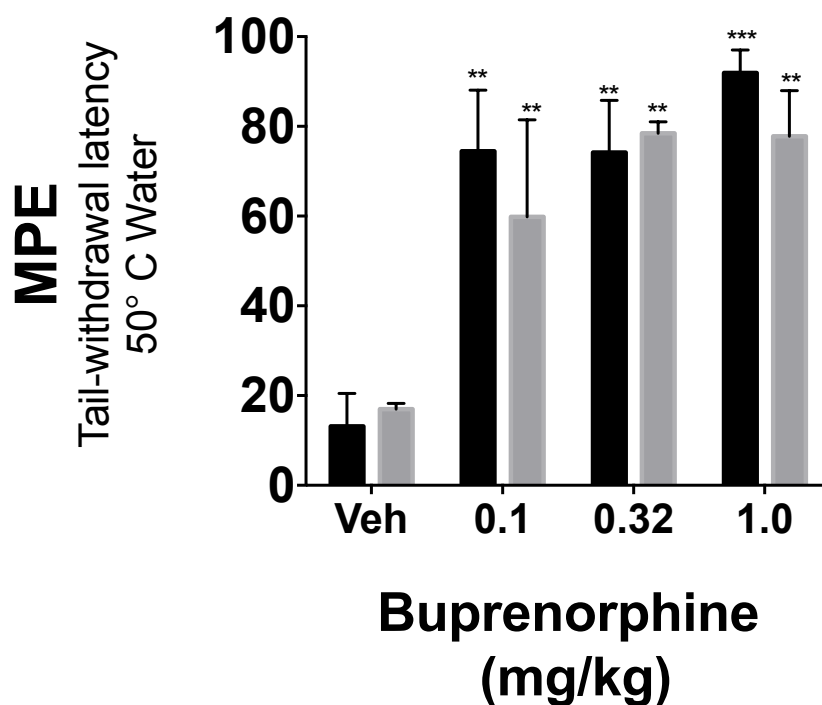
**Figure 2.1 Time course of intranasal and intramuscular fentanyl in the warm water tail-withdrawal assay.** Mean ( $\pm$ SEM) tail-withdrawal latency from 50°C water expressed as maximum percent effect (MPE) and plotted as a function of dose and time for IN (A) and IM (B) fentanyl administration in rhesus monkeys (n=3). Symbols: (*open circles*) saline; (*light gray circles*) Fentanyl 0.01 mg/kg; (*dark gray circles*) Fentanyl 0.018 mg/kg; (*black circles*) Fentanyl 0.032 mg/kg. Abscissae: Time in minutes (min). Ordinates: Tail-withdrawal latency presented as maximum percent effect (MPE).



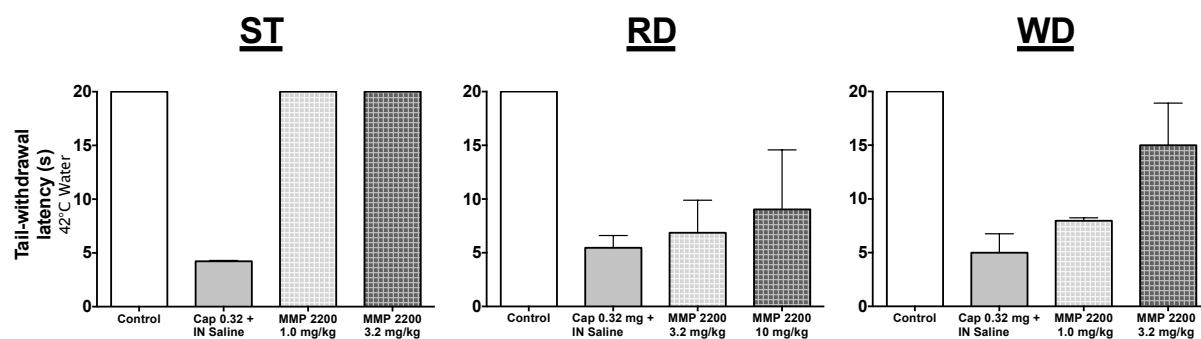
**Figure 2.2 Peak effects of intranasal and intramuscular fentanyl in the warm water tail-withdrawal assay.** Averaged ( $\pm$  SEM) peak measurement of tail-withdrawal latency taken from the time course data for fentanyl administered IN (*black bars*) and IM (*gray bars*). Abscissae: Dose of fentanyl saline, 0.01, 0.018 and 0.032 mg/kg. Tail-withdrawal latency presented as maximum percent effect (MPE). Significant differences between buprenorphine and saline are indicated by. \*  $p \leq 0.05$ , \*\*  $p \leq 0.01$ , \*\*\*  $p \leq 0.001$ . Significant differences between routes of administration are indicated by #  $p \leq 0.05$ .



**Figure 2.3 Time course of intranasal and intramuscular buprenorphine in the warm water tail-withdrawal assay.** Mean ( $\pm$ SEM) tail-withdrawal latency from 50° C water expressed as maximum percent effect (MPE) and plotted as a function of dose and time for IN (A) and IM (B) buprenorphine administration in rhesus monkeys (n=3). Symbols: (*open circles*) saline; (*light gray circles*) buprenorphine 0.1 mg/kg; (*dark gray circles*) buprenorphine 0.32 mg/kg; (*black circles*) buprenorphine 1.0 mg/kg. Abscissae: Time in minutes (min). Ordinates: Tail-withdrawal latency presented as maximum percent effect (MPE).



**Figure 2.4 Peak effects of intranasal and intramuscular buprenorphine in the warm water tail-withdrawal assay.** Averaged ( $\pm$  SEM) peak measurement of tail-withdrawal latency taken from the time course data for buprenorphine administered IN (*black bars*) and IM (*gray bars*). Saline and buprenorphine doses 0.1, 0.32 and 1.0 mg/kg. Tail-withdrawal latency presented as maximum percent effect (MPE). Significant differences between buprenorphine and saline are indicated by. \*  $p \leq 0.05$ , \*\*  $p \leq 0.01$ , \*\*\*  $p \leq 0.001$ .



**Figure 2.5 Effects of IN MMP 2200 on capsaicin-induced allodynia.** Mean tail-withdrawal latency from 42°C water before and after 0.32 mg capsaicin in individual monkeys administered IN saline or different doses of MMP 2200. Abscissae: Latency to withdrawal tail from 42°C water in seconds (s). Ordinates: Control measurements taken before capsaicin administration, IN saline followed an injection of 0.32 mg capsaicin into the tail, and different doses on IN MMP 2200.

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## CHAPTER FOUR

### **Intranasal versus Intravenous Naloxone: Behavioral Effects and Receptor Occupancy Studies Using PET Imaging**

#### **Introduction**

Opioid antagonists, such as naloxone (NLX) and naltrexone (NTX), are used therapeutically to treat opioid dependence and for the reversal of opioid-induced toxicity (Comer et al., 2006; Kampman and Jarvis, 2015; McAuley et al., 2015). Over the last several years, there has been a dramatic increase in the number of opioid overdoses in the United States, which cause almost 45 deaths per day (Jones, 2012; Levy et al., 2015). The reasons for this have been attributed to many factors including, the increase in prescription opioid treatment, an increase in prescription opioid abuse, and a resurgence in heroin use (Cicero et al., 2014; Dart et al., 2015).

Naloxone is a competitive, neutral antagonist that binds with high affinity to the  $\mu$ -opioid receptor, which is responsible for mediating both the therapeutic and toxic effects of most clinically used opioid agonists. Once only available to paramedics and other clinical personnel, there has been a concerted effort to widen access to NLX as a response to the US opioid epidemic (Fortuna et al., 2014; Wermeling, 2013). Naloxone “kits” are now being distributed to a broader range of first responders, including police

officers, as well as to opioid users themselves. Thus, the availability of NLX, particularly for intranasal (IN) use, is increasing (Doe-Simkins et al., 2014; Rando et al., 2015; Wheeler et al., 2015).

IN drug delivery is an emerging route of administration that is currently used for a variety of therapeutics including vaccines, chemotherapies, analgesics, and emergency rescue medications (Pires et al., 2009). It is attractive from a clinical and medications development perspective because it has several advantages over oral, and more common parenteral routes of delivery, such as the avoidance of GI decomposition and hepatic first-pass metabolism (Fortuna et al., 2014). In general, IN administration is an efficient, non-invasive, and rapid delivery method to the systemic circulation, although some have argued that IN drug delivery has the potential to result in direct absorption to the CNS that circumvents the blood brain barrier (BBB) (Dhuria et al., 2010). However, evidence for this type of absorption is limited (Scheibe et al., 2008).

Traditionally, naloxone was administered either intramuscularly (IM) or intravenously (IV) in a suspected case of opioid overdose. Both routes of administration are sufficient to reverse respiratory depression, which is the basis of opioid toxicity, and there are generally no adverse effects associated with its use (Boyer, 2012; Kerr et al., 2008). For the past several years, IN administration of NLX has been evaluated as an effective and rapid alternative to IM and IV administration. This route has the advantage of reducing needle stick injuries when working with a population that is at increased risk for carrying infectious diseases, such as HIV and Hepatitis C (Akselrod et al., 2014; Broz et al., 2014; Kerr et al., 2008). In addition, many intravenous drug users (IDUs)

have poor intravenous access, which is a limitation to administering NLX intravenously (Coffin et al., 2012; Maliphant and Scott, 2005).

Although IN NLX is gaining acceptance, there have been no laboratory-controlled studies evaluating the potency of NLX to reverse opioid agonist effects as a function of route of administration, and some practitioners still question the effectiveness of IN NLX compared to IV or IM delivery (Zuckerman et al., 2014). In general, field research suggests that IN NLX reliably reverses opioid toxicity in humans, however inconsistent study designs, primary endpoints, and preparations of NLX (dose, concentration, volume etc.) make it difficult to assess how the pharmacodynamics of NLX change with different routes of administration. For example, in an unblinded, randomized trial of IM versus IN naloxone (2 mg) for opioid overdose in humans, it was found that IM naloxone restored respiration more quickly than IN naloxone (6 and 8 min, respectively), although both routes effectively rescued patients from overdose (Kelly et al., 2005). A follow-up study found that using a more concentrated form of naloxone at the same dose resulted in no difference in the latency of IN and IM naloxone to restore respiration (Kerr et al., 2009). Barton et al. compared naloxone IV (1-2 mg) or IN (2 mg) for opioid overdose in humans, and found that both produced clinically significant recovery within 3 minutes (Barton et al., 2005). However, the paramedic subjectively determined the choice of which route to use, the initial dose of drug IV, and the presence or absence of clinically significant effects. Lastly, Sabzghabae and colleagues (2014), in an unblinded, randomized study of IN versus IV naloxone (0.4 mg) administered in a poison control center for suspected opioid overdose, reported that IN naloxone produced significantly higher levels of consciousness. Both routes of administration were found to restore

respiration within 3 minutes, and the authors attributed the rapid recovery with IN administration to drug being directly absorbed into the CNS. These studies, while essential, were performed in emergency situations where rigorous experimental controls are difficult to establish, and the patient's drug history, or the time elapsed since agonist administration, cannot be known. Thus, it is impossible to make any concrete pharmacological characterizations about IN naloxone based on this research alone.

Despite the widespread use of IN NLX, there are no laboratory-controlled (clinical or preclinical) studies systematically evaluating the potency of NLX to antagonize opioid effects as a function of route of administration. We have developed a novel procedure to measure the behavioral effects of opioid drugs following IN administration in rhesus monkeys. The goals of this study were to evaluate the potency of IN and IV NLX to antagonize the antinociceptive effects of the  $\mu$ -agonist fentanyl. Using a parallel design, we also evaluated the ability of IN and IV naloxone to displace [ $^{11}\text{C}$ ]carfentanil ([ $^{11}\text{C}$ ]CFN) using positron emission tomography (PET) imaging.

## **Methods**

### *Subjects*

Three male rhesus monkeys (weights, 10-11.4 kg) were subjects in the antinociception studies and two females (weights, 7.4-8.5 kg) were used in the PET study. All monkeys were individually housed in steel cages (83.3 cm high x 76.2 cm wide x 91.4 cm deep) on a 12hr light/12 hr dark schedule. Monkeys were fed Lab Fiber Plus Monkey Diet (PMI Nutrition Intl. LLC.) that was supplemented with fresh fruit daily. Water and enrichment toys were available continuously in the home cage. Each

monkey had served in previous studies and had an extensive drug history. All animal studies were conducted in accordance with the standards set by the University of Michigan University Committee on Use and Care of Animals.

### *Surgery*

Monkeys were surgically implanted with ports to allow for convenient IV administration of fentanyl and propofol. Surgeries were conducted under sterile conditions with the use of anesthesia (ketamine, 10 mg/kg; xylazine, 2 mg/kg). IV catheters were implanted in an accessible vein, and then passed subcutaneously to the animal's back where it was attached to a port that was maintained subcutaneously (Access Technologies, Intisil™ catheter, Attachable 5 French [0.30"/0.7mm ID x 0.065"/1.7mm OD x 24"/60 cm], Skokie, Illinois).

### *Drug Administration*

Intranasal administration was performed with a modified nasal atomizer (MAD) (LMA® MAD Nasal™ Intranasal Mucosal Atomization Device, Research Triangle Park, NC) that was attached to a 1 ml syringe. The Styrofoam stop, and the butterfly handles were removed from the MAD to ensure a good fit into the monkey's nostril. Prior to drug administration, the monkey was anesthetized with propofol (3.3 ml of 10 mg/ml), which was administered through the IV port, and flushed with 3 ml of sterile saline. Following the onset of anesthesia, the monkey was reclined to an angle of approximately 45° degrees, the atomizer was inserted into the right or left nostril (approximately 3 cm), and the plunger was depressed while holding the opposing nostril closed. The monkey was held in the reclined position for three minutes following drug administration. NLX was delivered in a volume between 0.05 and 0.2 ml. The monkey remained in the reclined

position for three minutes. IV NLX and fentanyl administration also occurred through the IV port, and were flushed through with 3 ml of sterile saline.

### *Antinociception*

Antinociception was measured using the warm-water tail-withdrawal procedure (Dykstra and Woods, 1986). Monkeys were trained to sit in Plexiglas primate chairs that measured 1.5 m in height. A tea kettle with hot water was maintained at approximately 100°C. Cold tap water and hot water were mixed together in a Thermos and calibrated with a total immersion thermometer in order to obtain the desired temperatures. Tail-withdrawal latencies were measured using a digital stopwatch.

The session began with baseline determinations of tail-withdrawal latency in water heated to 38, 42, 46, or 50° C. Two experimenters alternated in measuring tail-withdrawal latency and preparing the water bath; the experimenter measuring tail-withdrawal latency was blind to the water temperature. Two determinations were made at each temperature. Monkeys were anesthetized with propofol prior to IN or IV NLX administration. Ten minutes after NLX (or saline) delivery, IV fentanyl (0.018 mg/kg) was administered and measurements of tail-withdrawal latency began ten minutes later. Subsequent measurements were recorded at 20, 30, 40, 50, 60, and 90 minutes following fentanyl injection.

### *Primate MicroPet Imaging*

Imaging studies were performed in two mature female rhesus monkeys. The animals were anesthetized in the home cage with ketamine and transported to the PET facility. Subjects were intubated for mechanical ventilation, and anesthesia was continued with isoflurane. Anesthesia was maintained throughout the duration of the



PET scan. A venous catheter was inserted into one hind limb and the monkey was placed on the PET gantry with their head secured to prevent motion artifacts. The nasal atomizer was placed into the nasal passage and secured with surgical tape to prevent movement. The atomizer was attached to extension tubing so the dose could be administered without interfering with the data collection. Following a transmission scan, IV or IN NLX (or saline) was administered. Ten minutes later, 3.5 mCi - 5.3 mCi of CFN was administered in a bolus dose over 1 minute. Emission data were collected beginning with the injection and continued for 60 min (12x5 min frames). Data were corrected for attenuation and scatter, and reconstructed using the 3D maximum a priori method (3D MAP algorithm).

[<sup>11</sup>C]CFN was prepared as previously described (Shao et al., 2011). Briefly, [<sup>11</sup>C]methyl triflate was bubbled through a solution of desmethyl carfentanil - tetrabutyl ammonium salt (0.4 mg in dimethyl sulfoxide (100 µL)) at 15 mL/min for 3 min at room temperature. After production, 1% ammonium hydroxide (1 mL) was added to the reaction vessel. The crude reaction mixture was diluted with 5 mL of 1% ammonium hydroxide, and the resulting mixture was passed through a 3M Empore C2 extraction disk where the [<sup>11</sup>C]CFN was trapped. The disk was then washed with 3 mL of 10% n-propanol followed by 7mL Milli-Q water to remove impurities. The disk was then dried (He gas) for 1 min, and the [<sup>11</sup>C]CFN was eluted off with ethanol (0.5 mL) and diluted with Sterile Water for Injection, USP (9.5 mL). The formulated product was then passed through a 0.22-µm filter into a sterile dose vial and analyzed for pH, radiochemical purity and cold mass.

#### *Data analysis*

## Antinociception

In the studies of antinociception, the mean tail-withdrawal latency was calculated for each individual monkey, and then averaged across subjects. These values were converted to percent maximum effect (%MPE) using the following calculation:

$\%MPE = [(test\ latency - control\ latency) / (20\ s\ cut-off\ latency - control\ latency)] \times 100$ . Data were analyzed by repeated measures analysis of variance using the Holm-Sidak correction for multiple comparisons.

## PET Analysis

The dynamic sequence of PET images were summed and volumes-of-interest (VOIs) were drawn manually on multiple planes for the thalamus, striatum, pons, temporal cortex, frontal cortex and parietal cortex of the control scan for each monkey. Images from all subsequent [ $^{11}C$ ]CFN scans following NLX blocking studies were registered to the that monkey's baseline scan using the NeuroStat package available on the web, in order to allow image data to be extracted using the same set of VOIs. These data were used to construct brain tissue-radio activity curves that were then analyzed with the method of Logan using the occipital cortex as a reference region (Logan et al., 1996). Changes in receptor occupancy were estimated from the distribution volume ratio (DVR) calculated from each VOI in the baseline scan compared with the DVRs obtained from the NLX blocking studies, using the formula:

$$\text{occupancy (\%)} = 100 * [1 - (DVR_{\text{block}} - 1) / (1 - DVR_{\text{base}} - 1)].$$

## *Drugs*

Fentanyl (Sigma Aldrich, St. Louis, MO), and naloxone (NIDA, NIH) was dissolved in sterile saline, and buprenorphine (NIDA, NIH) was dissolved in 40% beta-

cyclodextrin. Propofol (10 mg/ml) was manufactured by Sagent Pharmaceuticals (Schaumburg, IL).

[<sup>11</sup>C]CFN was prepared as previously described (Shao et al., 2011). Briefly, [<sup>11</sup>C]methyl triflate was bubbled through a solution of desmethyl carfentanil - tetrabutyl ammonium salt (0.4 mg in dimethyl sulfoxide (100 µL)) at 15 mL/min for 3 min at room temperature. After production, 1% ammonium hydroxide (1 mL) was added to the reaction vessel. The crude reaction mixture was diluted with 5 mL of 1% ammonium hydroxide, and the resulting mixture was passed through a 3M Empore C2 extraction disk where the [<sup>11</sup>C]CFN was trapped. The disk was then washed with 3 mL of 10% n-propanol followed by 7mL Milli-Q water to remove impurities. The disk was then dried (He gas) for 1 min, and the [<sup>11</sup>C]CFN was eluted off with ethanol (0.5 mL) and diluted with Sterile Water for Injection, USP (9.5 mL). The formulated product was then passed through a 0.22-µm filter into a sterile dose vial and analyzed for pH, radiochemical purity and cold mass.

## **Results**

Figure 3.1 presents the time course of effects for a fixed dose of IV fentanyl (0.018 mg/kg) alone, and in the presence of IN (A) and IV (B) NLX (0.0032-0.032 mg/kg) on tail-withdrawal latency. The 0 time point on the graph corresponds with IV fentanyl injection, which occurred 10 minutes after IV or IN NLX. IV Fentanyl (0.018 mg/kg) following saline administration produced peak antinociceptive effects of 97% MPE 10 minutes following injection, which decreased to 50% by the 50-minute time point, and then to 37% at the end of the 90-minute session. Since there were no

statistical differences between saline conditions, these data were averaged across routes for the purpose of analysis.

In the presence of IN NLX (0.032 mg/kg), the ability of fentanyl to produce increases in tail-withdrawal latency was greatly reduced. Whereas fentanyl alone increased tail-withdrawal between 80-97% MPE within the first 30 minutes, pretreatment with IN NLX (0.032 mg/kg) reduced this range of effects between 12-24% MPE. Likewise, IN NLX (0.01 mg/kg) reduced fentanyl-induced antinociceptive effects between 34-39% MPE within the first 30 minutes. Pretreatment with the lowest dose of IN NLX (0.0032 mg/kg) was less effective at altering the antinociceptive effects of fentanyl, producing tail-withdrawal latencies between 58-91% over the same time period. IV NLX (0.032 mg/kg) also blocked the antinociceptive effects of fentanyl, and decreased tail-withdrawal latencies between 0-3% MPE within the first 30 minutes. Similarly, the intermediate and smallest dose of IV NLX (0.01 and 0.0032 mg/kg) reduced the antinociceptive effects of IV fentanyl between 7-21% and 37-71% MPE, respectively.

Dose-response curves for IN and IV NLX were constructed by taking the average tail-withdrawal latency across the 90-minute session for each dose (Figure 3.2). Pretreatment with NLX given IV and IN produced dose-dependent antagonism of fentanyl-induced antinociception [Dose:  $F(3,6) = 19.49$ ,  $p \leq 0.01$ ]. For IN NLX, doses of 0.01 and 0.032 mg/kg were significantly different from fentanyl alone [IN NLX dose (mg/kg) vs. saline: 0.01,  $p \leq 0.01$ ; 0.032,  $p \leq 0.01$ ]. Likewise, IV NLX produced significant decreases in MPE relative to the effects with fentanyl alone [IV NLX dose (mg/kg) vs. saline: 0.0032,  $p \leq 0.05$ ; 0.01,  $p \leq 0.01$ ; 0.032,  $p \leq 0.001$ ]. No significant

differences were found between routes of administration in their ability to reverse fentanyl-induced antinociception.

Figure 3.3 presents the brain tissue time-radioactivity curves for [ $^{11}\text{C}$ ]CFN following IN saline, and IN and IV NLX (0.032 mg/kg) for selected brain regions. Under baseline conditions (IN saline) [ $^{11}\text{C}$ ]CFN showed rapid uptake into the brain that peaked between 5-10 minutes. Regions of high radiotracer uptake included the striatum, pons, and thalamus, and regions of lower uptake were found in cortical regions (anterior-temporal cortex and frontal cortex). Pretreatment with IN or IV NLX (0.032 mg/kg) resulted in a more rapid clearance of [ $^{11}\text{C}$ ]CFN from the brain. The change in DVR relative to [ $^{11}\text{C}$ ]CFN alone following IN NLX (0.032 mg/kg) showed a 56-66% decrease in available RO across the sampled brain regions (Figure 3.4). Blocking of [ $^{11}\text{C}$ ]CFN was highest in the parietal and frontal cortex and lowest in the temporal cortex following IN administration. IV NLX showed 67-83% change in RO, with the highest region of blocking in the frontal cortex and the lowest in parietal. Numerically, there was a trend for IV NLX to produce a greater decrease in RO than IN naloxone. Figure 3.5 presents representative microPET images, summed across the scan, for [ $^{11}\text{C}$ ]CFN alone (top row), and following blocking studies with IN (middle row) and IV NLX (0.032 mg/kg) (bottom row). These images confirm the reduction in tracer uptake in the presence of NLX.

## **Discussion**

The primary findings of this study are as follows. IN and IV NLX both produced dose-dependent decreases in fentanyl-induced antinociception. In general, the route of administration did not significantly alter the potency of NLX to block the antinociceptive

effects of IV fentanyl. The largest dose of NLX (0.032 mg/kg) given IN and IV completely antagonized increases in tail-withdrawal latency that were produced with fentanyl. At the intermediate dose (0.01 mg/kg), IN and IV NLX both produced significant decreases in antinociception, although the effects produced with IV NLX were numerically greater than those produced by IN route. A dose of 0.0032 mg/kg NLX given IV significantly decreased fentanyl-induced antinociception relative to fentanyl alone, while the same dose delivered IN produced no change. RO studies using PET were conducted with equipotent dose of NLX (0.032 mg/kg) given IN and IV. These studies showed that IV administration resulted in greater blocking of [ $^{11}\text{C}$ ]CFN binding, however the differences between routes were relatively small, and did not result in significant behavioral changes under these conditions.

Drug potency and bioavailability varies as function of route of administration. The extent to which pharmacodynamics are altered by changing the route of delivery depends on the drugs physiochemical properties, and other variables that govern absorption, distribution, and metabolism. For example, the peptide DAMGO, a highly potent and efficacious  $\mu$ -opioid receptor agonist, produces no measurable behavioral effects when given systemically, but following intrathecal (IT) administration, it produces antinociception, itch, and respiratory depression (Ko et al., 1998; Ko et al., 2006). Morphine produces antinociception when administered IV, IN, SC, and PO (orally), but the dose that produces a given level of effect varies considerably among routes (Grassin-Delyle et al., 2012). In rhesus monkeys, IT morphine produced maximum antinociception at a dose of 0.03 mg, however when given IM, doses between 3.2 and 5.6 mg/kg are required for the same level of effect (Lee et al., 2007; Maguire and

France, 2014). The onset and duration of action, as well as the spectrum of on-target and off-target side effects, may also vary with the route of administration. For example, compared to systemic administration, IT morphine has a longer duration of action, and was reported to produce significantly more pruritus (Ko et al., 2004).

Naloxone, which is normally administered IM or IV, is frequently given off-label through the IN route to reverse opioid toxicity. Results from studies conducted in humans have shown that IN NLX can be effectively used to reverse opioid toxicity. However, questions persist regarding the onset of action, duration of effect, and the potency of IN naloxone, as well as its overall effectiveness relative to other routes. In the present study, IN and IV NLX were found to be equipotent in reversing the antinociceptive effects produced with IV fentanyl. Doses of NLX that antagonized fentanyl were similar to those used in published studies to block different  $\mu$ -agonist effects in monkeys. For example, the antinociceptive effects of IT morphine (1 mg) were blocked with IM NLX 0.03-0.1 mg/kg in a shock titration paradigm (Yaksh, 1983). In morphine-dependent rhesus monkeys trained to discriminate 0.032 mg/kg of naltrexone, NLX 0.01-0.032 mg/kg SC completely substituted for a naltrexone stimulus, and likewise, NLX 0.01 mg/kg SC was found to precipitate withdrawal in monkeys that were made physically dependent on morphine (Gauthier and France, 1999; Valentino et al., 1983).

It has been suggested that IN administration results in direct absorption to the CNS (Dhuria et al., 2010), and some studies have attributed the rapid onset of action seen with IN NLX to this pattern of absorption (Sabzghabae et al., 2014). Typically, drugs that produce their effects in the CNS, are active at very small doses when

administered centrally. Given that the potency of IN and IV NLX were equivalent, and that a similar range of doses administered systemically have been shown to block various  $\mu$ -agonist effects, it is unlikely that IN administration in this study resulted in the direct absorption to the CNS.

The level of a receptor-mediated response to a drug stimulus is proportional to the percentage of receptors occupied. At the whole organism level, dose is one of the key variables that controls RO, and the effect produced with a given dose can vary depending on the route of administration. There are no published studies evaluating the percentage of receptors that need to be occupied by naloxone in order to block a specific  $\mu$ -agonist effect. In fact, few studies have investigated the pharmacokinetics of naloxone given by different of routes of administration in any context, or have correlated these results with behavioral end points.

In humans, the bioavailability of IN NLX was found to be 4% of IV administration (Dowling et al., 2008). This led to the supposition that the clinical response to IN naloxone might be less than that produced through other routes of administration. However, studies with IN nicotine and fentanyl have demonstrated that venous blood sampling, the method often used for PK evaluation, may underestimate the bioavailability of intranasally administered drugs (Gourlay and Benowitz, 1997; Guthrie et al., 1999; Moksnes et al., 2008). In a direct comparison of IV and IN nicotine, it was found that both routes of administration produced comparable bioavailability in the arterial circulation, and that drug concentration in this compartment more accurately predicted pharmacodynamic endpoints (Gourlay and Benowitz, 1997).



Since it is possible that the same level of behavioral effect can be produced with different receptor occupancies, particularly for a response where there is a receptor reserve, it was important to determine if equipotent doses of NLX given by different routes of administration were equivalent on a receptor basis. This would be particularly relevant in the context of evaluating IN and IV NLX under conditions where the percentage of agonist occupancy is relatively large (i.e. opioid overdose). IN and IV NLX were found in PET analysis to produce similar receptor occupancies although this varied slightly depending on the brain region. For example, in the pons, which controls aspects of the respiratory drive, NLX occupied 65% of the receptors available for binding following IN administration, whereas it occupied 75% of the available receptors following IV administration. However, in the parietal cortex, there was no difference in receptor occupancy between IV and IN administration. Overall, receptor occupancy with IV NLX was consistently larger than the occupancy produce with IN administration, but these differences were not sufficient to produce changes in behavior. These results demonstrate that IN and IV naloxone are nearly equivalent in two important pharmacodynamic dimensions that have clinical relevance.

This study had several limitations. Because the monkeys needed to receive anesthesia for IN administration it was not feasible to study whether the onset of action for NLX differed as a function of route. Since time is a critical factor in an opioid overdose this would have been valuable information. Clinically NLX is not administered to block the analgesic effects of opioid agonists, but to reverse opioid-induced respiratory depression. Although the dose of IV fentanyl used in this experiment was not toxic, IV fentanyl (0.018 mg/kg) has been shown to reduce the amount of respired air by

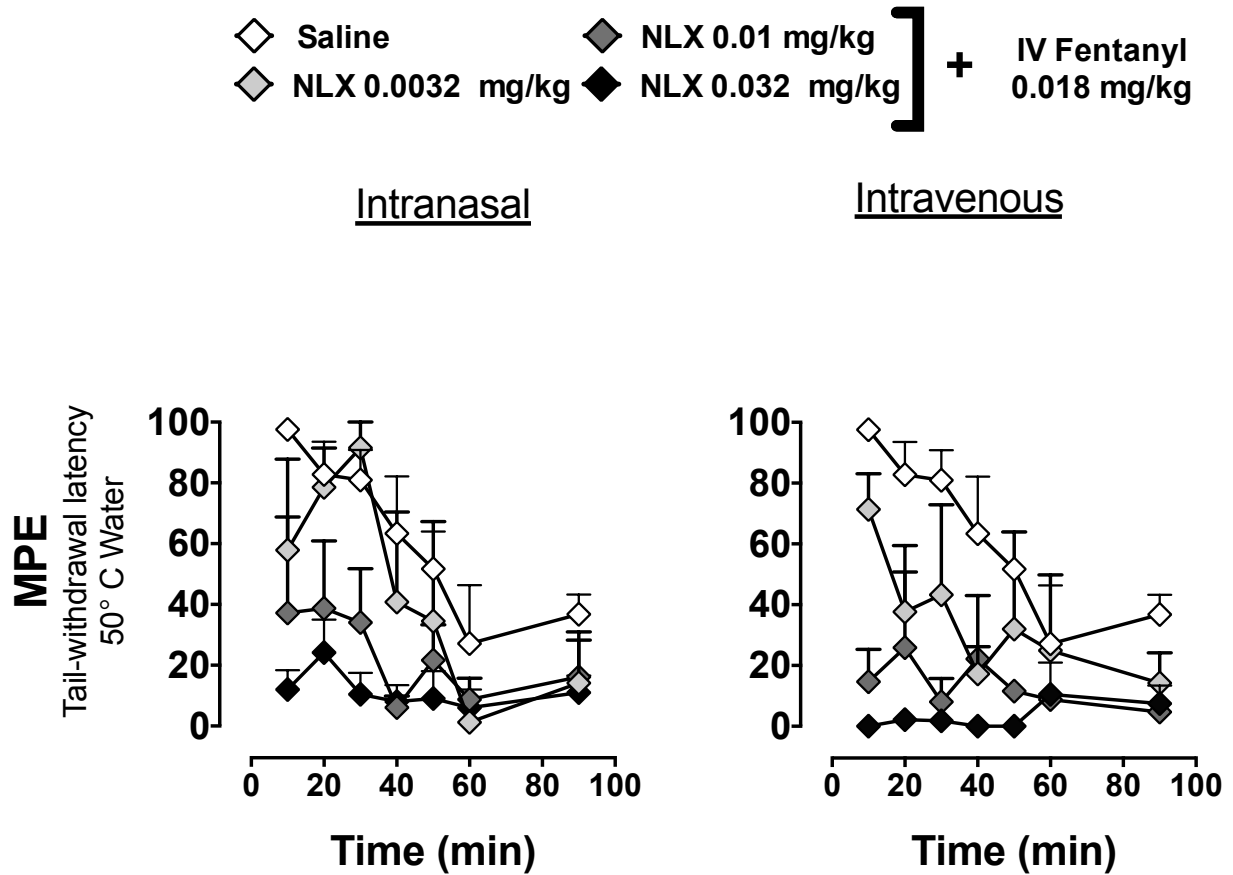
40% in rhesus monkeys. Increasing the dose of fentanyl by as little as a quarter log unit from this maximally effective dose has been reported to produce respiratory arrest in some animals (Ko et al., 2002; Saccone et al., unpublished observations). Although not a perfect proxy for measures of toxicity, the ability of NLX to reverse opioid action in this study was challenged by assessing behavior that is associated with relatively large-dose effects.

The modified nasal atomizer, which was adapted to fit into the monkey's nostril, was likely inserted further into the nose than would be possible had the Styrofoam stopper been in place. Some have argued that more posterior drug deposition, in proximity to the superior turbinate and cribriform plate, may enhance direct CNS absorption. However, it was found that the potency of IN NLX to block the effects of fentanyl was consistent with other routes of administration that result in systemic drug absorption. Lastly, in an opioid overdose situation the agonist would be on board prior to the administration of naloxone, while in the present study NLX was administered prior to fentanyl. Pharmacologically, however, this should not make a difference in terms of measuring antagonist potency because almost all clinically used opioids are competitive with the receptor.

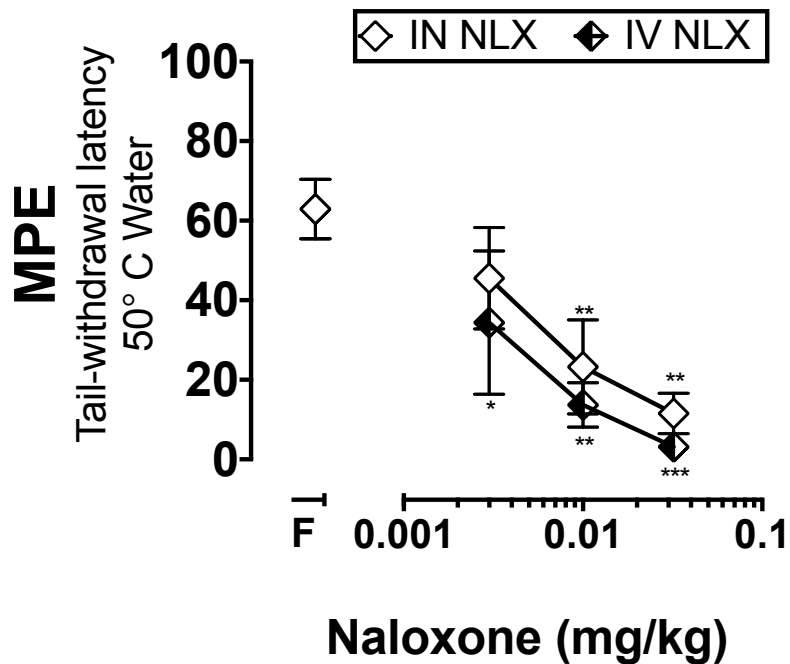
For IN administration, we elected to use parameters that would optimize IN drug delivery based on previous literature (Dhuria et al., 2010; Fortuna et al., 2014; Grassin-Delyle et al., 2012; Landis et al., 2012). The volume of administration was kept between 50-200  $\mu$ l in a single nostril, and the concentration of NLX varied between 1 - 2 mg/ml. Large solution volumes and low drug concentration have been reported to reduce the bioavailability and effectiveness of drugs given IN. To date, IN NLX has been given in

larger volumes, typically 1 ml of 1 mg/ml solution. Thus, the extent to which these findings would be generalizable to the current clinical standards is unclear. More research is needed to understand how the effects of drugs administered IN are altered by solution volume and dose.

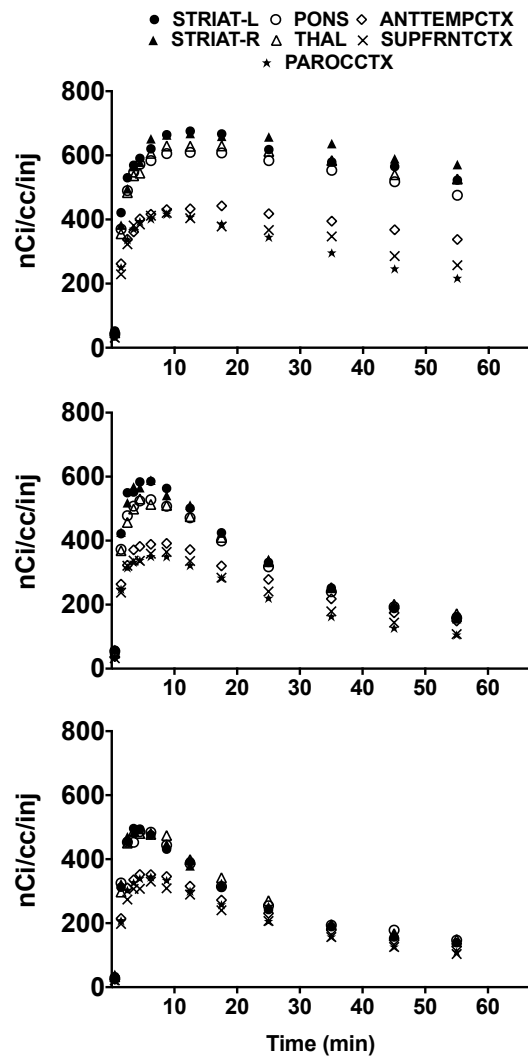
Overall, this study successfully established the first model to measure the behavioral effects of IN delivered opioid antagonists, and to evaluate receptor occupancy with PET following IN delivery. This route of administration remains promising for medication development and for improving treatment with currently used drugs.



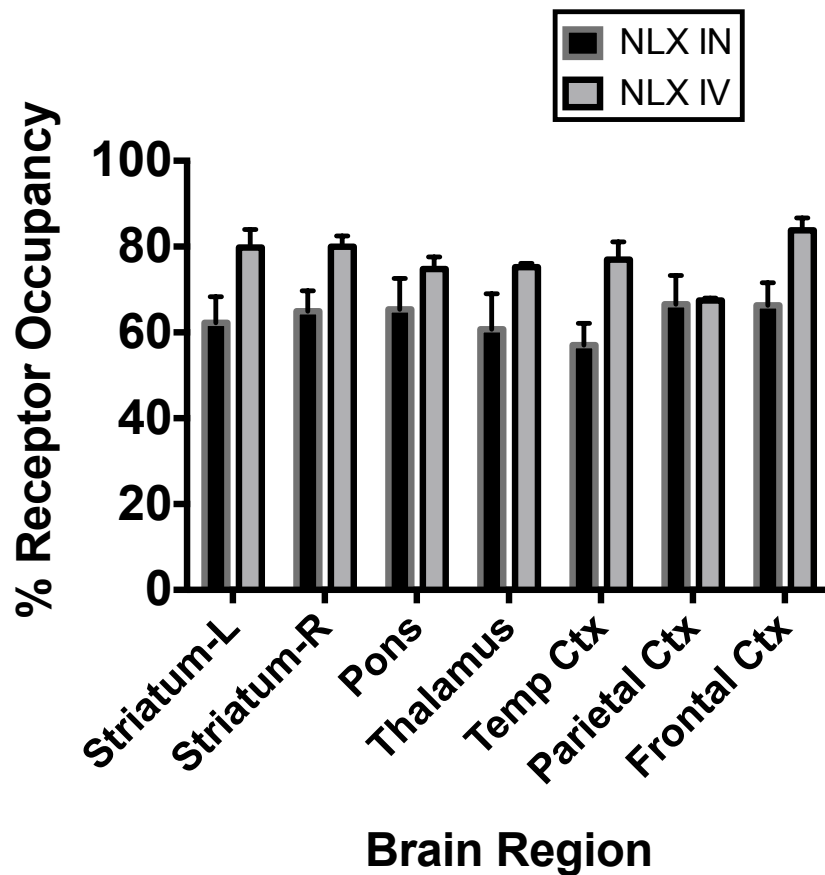
**Figure 3.1 Time course of intranasal and intravenous naloxone antagonism of fentanyl-induced antinociception.** Time course data for the effects of NLX (0.0032-0.032 mg/kg) administered IN (A) and IV (B) to block fentanyl-induced increases in tail-withdrawal latency in rhesus monkeys (n=3). Data are presented as the mean ( $\pm$  SEM) maximum percent effect and plotted as a function of time. Abscissae: Time in minutes (min). Ordinate: Tail-withdrawal latency presented as maximum percent effect (MPE).



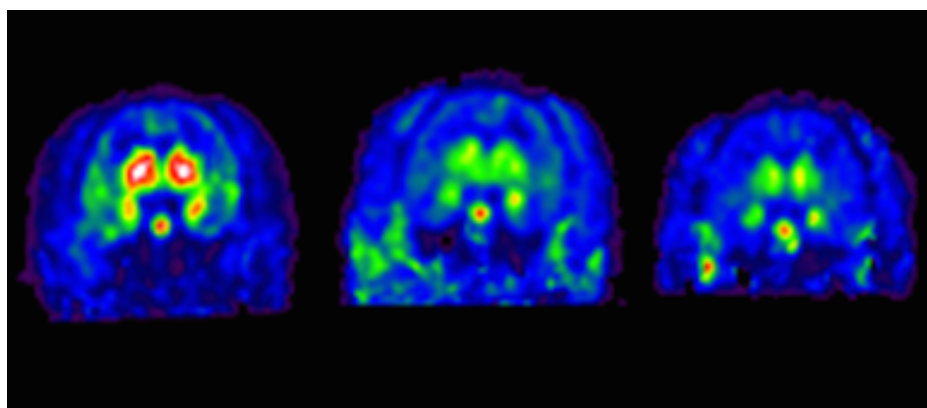
**Figure 3.2** The effects of intranasal and intravenous naloxone on tail-withdrawal latency averaged across the 90-minute session. Mean ( $\pm$ SEM) tail-withdrawal latency from 50° C water averaged across the 90-minute session produced with IV fentanyl alone and in the presence of IN and IV NLX (0.0032-0.032 mg/kg). Abscissae: IV fentanyl (0.018 mg/kg) alone (F) and doses of naloxone in milligrams per kilogram. Ordinates: Tail-withdrawal latency presented as maximum percent effect (MPE). Significant differences between fentanyl alone and different doses of naloxone are indicated by, \*  $p \leq 0.05$ , \*\*  $p \leq 0.01$ , \*\*\*  $p \leq 0.001$



**Figure 3.3 Brain tissue time-radioactivity curves for [<sup>11</sup>C]carfentanil alone, and in the presence of intranasal and intravenous naloxone.** Brain tissue time-radioactivity curves (TACs) for selected brain regions. TACs from [<sup>11</sup>C]CFN alone (*top panel*) and in the presence of NLX 0.032 mg/kg delivered IN (*middle panel*) and IV (*bottom panel*). Middle panel presents the TACs following IN NLX (0.032 mg/kg) administration. Abscissae: Time in minutes (min). Ordinates: Measures of radioactivity in nano-Curi's per cubic centimeter per milli-Curi's injected.



**Figure 3.4 Receptor occupancy estimates for intranasal and intravenous Naloxone.** Percent receptor availability in selected brain regions following IN and IV NLX (0.032 mg/kg). RO was estimated as the ratio of DVR values obtained from PET images with [<sup>11</sup>C]CFN alone relative to the DVRs calculated after NLX blocking experiments.



**Figure 3.5 microPET Images.** Representative coronal microPET images of [ $^{11}\text{C}$ ]CFN alone (*left*) and following blocking studies with IN (*middle*) and IV (*right*) NLX (0.032 mg/kg). All images are summed images 0–60 min following IV injection of the radiotracer.



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## **CHAPTER FIVE**

### **GENERAL DISCUSSION**

The introduction to this thesis argued that the primary concern regarding the use of opioids was balancing the legitimate therapeutic purpose of these medicines with their unwanted effects. Optimizing the medical use of opioids is to align the desired effect (onset and duration of action; magnitude of effect; mode of action) with the goals of therapy (Inturrisi, 2002). The unwanted effects of opioids that are related to drug abuse and addiction, and those that are therapeutically complicating, such as constipation, respiratory depression, and itch, all serve to interfere with either: a practitioners willingness to prescribe these medicines, the patients willingness to initiate or continue therapy, and/or for the drugs themselves to be effective (Jamison et al., 2014; Labianca et al., 2012; McNicol, 2008). As a result, continuing research efforts aim to identify new targets for pain control as well as methods to optimize the way opioids are clinically used.

The work presented here addressed two distinct strategies that have in common the goal of improving opioid therapy. In the first chapter, the behavioral pharmacology of

a new opioid target for pain control, NOPr, was investigated. The next two chapters focused on developing a procedure to measure the behavioral effects of intranasally administered opioids in rhesus monkeys. The general conclusions of this thesis were as follows.

The NOPr agonist Ro 64-6198 produced discriminative stimulus effects that are distinct from agonists at other opioid receptors, and may be similar to diazepam, in rhesus monkeys. In contrast to previous studies, Ro 64-6198 did not produce antinociception in the warm water tail-withdrawal assay indicating that the analgesic effects of this drug may be more variable than previously described.

Additionally, this thesis established a procedure to measure the ability of IN opioids to modify the effects of warm water on tail-withdrawal behavior in rhesus monkeys. The opioid agonists fentanyl and buprenorphine were shown to produce dose-dependent increases in tail-withdrawal latency following IN delivery. When compared to IM administration, there was a trend for fentanyl and buprenorphine to produce a faster onset of action, and larger increases in tail-withdrawal latency with smaller doses. This study also established preliminary evidence for the analgesic effects of an opioid peptide, MMP 2200, given intranasally in measures of capsaicin-induced allodynia.

Opioid antagonists also play a vital role in medicine, and this procedure was modified to study IN NLX in a manner that has become of public health relevance. A study was conducted comparing the potency and duration of effect of IV and IN NLX to block the antinociceptive effects of IV fentanyl. Additionally, brain  $\mu$ -receptor occupancy was measured using [ $^{11}\text{C}$ ]CFN and PET imaging following NLX given through both

routes of administration. These studies showed that IV and IN NLX were equipotent in antagonizing fentanyl-induced antinociception, and that these effects were produced with comparable receptor occupancy.

### **Characterizing the discriminative stimulus effects of Ro 64-6198**

This was the first study to train a NOPr agonist as a discriminative stimulus in rhesus monkeys. Previous work demonstrated that Ro 64-6198 produced discriminative stimulus effects in rats (Recker and Higgins, 2004). In general, there was agreement between the results from the discrimination studies in rats and rhesus monkeys with regard to the non-overlapping stimulus effects of selective opioid agonists. However, the question of whether Ro 64-6198 produced interoceptive effects that were similar to drugs from other pharmacological classes was left unaddressed in the rat study. Substitution experiments were performed with the non-opioid drugs PCP, chlorpromazine, and diazepam in monkeys trained to discriminate Ro 64-6198. Interestingly, diazepam was found to produce partial generalization to a Ro 64-6198 stimulus. Follow-up experiments revealed that diazepam and Ro 64-6198 produced common interoceptive effects that were likely mediated by two distinct receptors.

The generalizability of this finding to other benzodiazepines, or drugs acting at different sites on GABA<sub>A</sub>, was not evaluated in the current study, and is a noted weakness. Substitution studies with drugs such as muscimol (a direct acting GABA<sub>A</sub> agonist), midazolam (positive allosteric modulator at the BZD binding site), and pregnanolone (positive allosteric modulator at the neuroactive steroid site) would extend the current findings to drugs that stimulate the receptor through different mechanisms. It

would also be interesting to learn whether monkeys trained to discriminate diazepam generalized to Ro 64-6198. Nevertheless, this was an interesting finding given that Ro 64-6198 and other NOPr agonists produce anxiolytic-like behavior in rodents. Data from this study support the notion that NOPr agonists may be good anxiolytics to the extent that the therapeutic actions of benzodiazepines are related to their interoceptive effects.

The most surprising result from studies with Ro 64-6198 was the lack of antinociceptive effects particularly given the data from previous work showing that Ro 64-6198 produced analgesia that was comparable to alfentanil (Ko et al., 2009). This study evaluated doses ten times larger than those used in studies where Ro 64-6198 was antinociceptive, and a positive effect was found in only one animal at the largest dose tested (0.32 mg/kg). A range of doses (0.0032- 0.32 mg/kg) was tested in eight different animals throughout the course of these studies. Experiments began in three subjects using doses between 0.003 - 0.032 mg/kg. When those doses failed to produce an increase in tail-withdrawal latency, the dose range was increased by a  $\frac{1}{2}$  log unit, and then another  $\frac{1}{2}$  log unit after no positive effects were again found on tail-withdrawal latency. Experiments continued in this fashion with different animals for the next several months until we reached the solubility limit of Ro 64-6198. For the final series of experiments, doses between 0.032-0.32 mg/kg were administered to four animals.

Given that we tested a dose range over two log units, it is unlikely that the doses used in the final series of experiments were on the descending limb of a biphasic dose-effect curve. It should also be noted that the potency of other drugs to produce antinociception used throughout this thesis are in agreement with the published



literature. The reasons for the discrepancy remain unclear, and reemphasize the need to replicate research across laboratories.

Although these data question the strategy of developing selective NOPr agonists for pain control, they may have positive implications for the development of bifunctional or mixed acting agonists (ligands designed to stimulate more than one receptor) at NOPr and  $\mu$ -receptor. There are instances where opioids may be given in combination with benzodiazepines for the treatment of comorbid conditions associated with acute and chronic pain (Hawkins et al., 2015; Nielsen et al., 2015). At the clinical level, patients with chronic pain display a complex set of psychological and physical sequelae that often require multiple treatment modalities (Dale and Stacey, 2016; Kayhan et al., 2015). The co-administration of opioids and benzodiazepines has been shown to have additive or synergistic effects on respiratory depression, sedation, and measures of abuse liability, which results in increased morbidity for those who use these drugs in combination (Giummarra et al., 2015). Benzodiazepines alone have a risk of abuse, and they are also capable of producing physical dependence and an abstinence syndrome that may be life threatening (Woods et al., 1992). Bifunctional NOPr/ $\mu$ -agonists have been evaluated in preclinical models of chronic pain, and were generally shown to be effective (Khroyan et al., 2009; Schroder et al., 2014; Sobczak et al., 2014). Should a benzodiazepine-like component be identified in other NOPr agonists, it is possible that bifunctional ligands at NOPr and the  $\mu$ -receptor may reduce the need for combination therapy with these drugs, as well as address some of the psychological dimensions that are associated with chronic pain.

## **Intranasal drug administration: opioid agonists**

The benefits of IN administration include a rapid onset of action, the ability to administer drugs parenterally without the use of a needle, and the avoidance of GI and hepatic first-pass metabolism (Fortuna et al., 2014). Given these attributes, it is also possible that IN administration may result in the more routine development and use of peptides and proteins as therapeutics (these chemical entities are not easily adaptable to oral administration). The purpose of the first set of experiments was to develop a procedure for measuring the antinociceptive effects of IN opioid agonists in rhesus monkeys. Most of the preclinical work on IN drug administration has taken place in rats even though there are anatomical and physiological differences between rodents and primates that suggest the latter may have more translational relevance. The lack of good animal models that predict the effects of intranasally administered drugs in man has been cited as a reason why more therapeutics are not developed for this route of administration (Landis et al., 2012). Lastly, regardless of the models particular translational relevance, the importance of measuring the effects of drugs in different preparations is well recognized.

Initial experiments to characterize the pharmacodynamics of IN administration compared to IM delivery, and to validate the procedure across different pain modalities, were performed with a diverse set of opioid agonists: fentanyl, buprenorphine and the opioid peptide, MMP 2200. Experiments with IN fentanyl administration produced dose-dependent increases in tail-withdrawal latency, and consistent with the published literature, was found to be a full agonist under these conditions. At smaller doses, there was a trend for fentanyl delivered IN to produce larger increases in tail-withdrawal

latency, and for these effects to have a quicker onset of action and a longer duration of effect compared to IM administration. For example, IN fentanyl 0.01 and 0.018 mg/kg produced significant increases in tail-withdrawal latency across the 120-minute session, while the same doses of IM fentanyl did not (although IM fentanyl 0.018 mg/kg produced similar peak effects). The effects of IN fentanyl 0.018 mg/kg on tail-withdrawal latency were apparent immediately following recovery from propofol anesthesia, and remained above saline levels for 80 minutes. IM fentanyl 0.018 mg/kg increased in tail-withdrawal latency beginning at 20 minutes, and these effects had largely dissipated before the 60-minute time point. Fentanyl 0.01 mg/kg delivered IM was not behaviorally active.

Buprenorphine's effect on antinociception did not significantly differ between active doses or routes of administration. The observation that a relatively wide range of buprenorphine doses (0.1 -1.0 mg/kg) produced increases in tail-withdrawal latency that plateaued between 66-80% MPE strongly suggested that buprenorphine was a partial agonist in this procedure. Confirmation of this partial agonist effect could have been obtained by pretreating monkeys with buprenorphine prior to measuring the antinociceptive effects of fentanyl, however buprenorphine's identification as a partial agonist has already been well characterized in the literature (Cowan et al., 1977a; Cowan et al., 1977b; Walker et al., 1995). At smaller doses, there was a trend for IN buprenorphine to have a faster onset of action. IN buprenorphine (0.1 mg/kg) increased tail-withdrawal latency to 50% MPE within the first 20 min following recovery from anesthesia, while doses of IM buprenorphine did not produce increases of this magnitude until the 60-minute time point.

The conclusions regarding IN and IM administration were limited to opioids with high lipophilicity, which in general have an easier time crossing membranes and entering the CNS. Future studies may wish to investigate how the pharmacodynamics of IN opioids vary as a function of different physiochemical properties, such as lipophilicity, molecular weight, or degree of ionization. Additionally, changes in potency between routes of administration may have been more clearly differentiated using thermal stimuli of different intensities. In the warm water tail-withdrawal procedure, agonist potency and efficacy has been shown to depend on the water temperature (Walker et al., 1998). For fentanyl, increasing the temperature above 50° C may have more clearly separated the effect of route on tail-withdrawal latency, while lowering the temperature for buprenorphine below 50° C may have allowed more sensitivity to detect an effect between large and small doses by either route of administration.

The aim of the last experiment in this chapter was to apply this procedure to a different pain modality (capsaicin-induced allodynia), and to highlight one of the potential advantages of IN administration: the use of peptides as therapeutics. MMP 2200 is a mixed  $\mu/\delta$  agonist that was previously shown not to be active in measures of acute thermal nociception, but was able to reverse allodynia in rhesus monkeys (Do Carmo et al., 2008). The data from this experiment was limited because it was determined that over time the monkeys became desensitized to the effects of capsaicin. However, using data gathered only from experiments where the ability of capsaicin to produce allodynia was verified, MMP 2200 1 and 3 mg/kg were capable of reversing allodynia in 2/3 animals. The reasons for the development of desensitization to capsaicin are unclear, however there are a few noteworthy differences between the

current study and previous work. The present experiment used doses of capsaicin that were larger than those used in other studies, and propofol was given in order to administer MMP 2200 intranasally. There are reported drug interaction between the propofol and capsaicin, which makes an idiosyncratic drug interaction plausible. It would be interesting to learn if desensitization to experimental allodynia can be seen with different anesthetics and chemical stimuli.

### **Comparison of intranasal and intravenous naloxone in behavioral measures and receptor occupancy**

The investigation of intranasal opioid administration was extended to opioid antagonists as well. There were two reasons for its inclusion. First, opioid antagonists have a vital role in therapeutics. They are FDA-approved to treat opioid dependence, and are used to reverse opioid toxicity (Comer et al., 2006; Wermeling, 2013). Secondly, the use of IN NLX has been a critical factor in the decision to increase the distribution/availability of naloxone as a means to combat opioid overdose (Rando et al., 2015). These programs have extended naloxone access to more first responders (such as police officers) as well as opioid abusers, and the people who are close to them (Ray et al., 2015). There have even been documented instances of IN self-administration of naloxone (Green et al., 2014).

Although IN administration is gaining acceptance, and studies conducted in the field have indicated that it is effective in reversing opioid overdose in humans, there are still unresolved pharmacological questions regarding IN administration (i.e. potency, duration of effect, generally efficacy compared to other routes of administration). There

have been no laboratory controlled studies evaluating the potency of NLX to antagonize the effects of opioid agonists as a function of route of administration, nor has there been an assessment of how this translates into receptor occupancy at the  $\mu$ -opioid receptor.

Overall, IN and IV NLX were equipotent in antagonizing the effects of fentanyl in this procedure, however there were some notable differences between the routes of administration. At the largest dose tested (NLX 0.032 mg/kg), there was no apparent difference between routes of administration. There was a trend for IV NLX to produce a greater decrease in fentanyl-induced antinociception, particularly at lower doses (0.0032 mg/kg and 0.01 mg/kg). IN NLX 0.0032 mg/kg produced only very slight decreases in tail-withdrawal latency following fentanyl, and these effects were not significantly different relative to fentanyl alone. In contrast, IV NLX (0.0032 mg/kg) consistently produced effects that were numerically smaller at almost every time point both compared to fentanyl alone, and to IN NLX. These effects were found to be significantly different from fentanyl alone, but not different from the same dose of NLX given IN.

The PET studies revealed that IN and IV NLX administration resulted in similar receptor occupancies. Brain tissue time-radioactivity curves demonstrated that blocking studies with NLX given by either route produced more rapid clearance of [ $^{11}\text{C}$ ]CFN. This was more pronounced for IV NLX where the peak uptake of [ $^{11}\text{C}$ ]CFN in all brain regions was suppressed compared to the radiotracer alone, and the IN blocking studies. A Logan analysis showed a trend for IV NLX 0.032 mg/kg to produce slightly greater RO when compared to IN NLX, but this varied by brain region. The largest differences were noted in the striatum, and temporal cortex, while there appeared to be no difference in parietal cortex between routes of administration. The pons, a brain region that is critical

for controlling respiratory drive, showed only a modest difference between routes of administration (IN: 66% vs IV: 75%). At least with high doses of NLX, there appeared to be good concordance between the PET and studies of antinociception. The findings of this study offer support to the clinical research that has argued for IN NLX as an alternative first line treatment for opioid overdose.

Taken together, the conclusions from this thesis support the need for further research on the potential for NOPr agonists to be the illusive Holy Grail. The analgesic effects of this compound were not as reliable as previously reported, and additional work should be done to learn to more about the qualitative aspects of the NOPr-mediated stimulus, such as whether or not it may be aversive. On the other hand, the establishment of a procedure to measure the effects of IN opioid administration opens the possibility that new opioid drugs can be developed utilizing this route of administration which may have a positive implication for pain control, and the other therapeutic uses of opioids.

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