Alterations in endogenous opioid neurotransmission associated with acute and long-term use of drugs of abuse

by

Emily Buitron Nuechterlein

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Doctoral Committee:

Professor Jon-Kar Zubieta, Chair
Professor J. Wayne Aldridge
Professor Daniel J. Clauw
Professor Robert A. Koeppe
Professor Stephan F. Taylor

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ABSTRACT

The endogenous opioid neurotransmitter system (EOS) has been implicated in a wide array of behavioral processes, including reinforcement, pain modulation, mood disorders, social interactions, and the placebo effect. These intertwining factors make an understanding of the EOS's role crucial for developing effective therapies for a range of disorders. We used positron emission tomography to investigate whether acute and long-term administration of two drugs of abuse, nicotine and opioid analgesics, are associated with alterations in endogenous μ-opioid neurotransmission. We found that compared to healthy controls, overnight-abstinent smokers showed significant decreases in μ-opioid receptor (MOR) availability in the thalamus and bilateral basal ganglia, regions previously implicated in drug reinforcement and addiction. Moreover, these alterations in neurotransmission were associated with measures of both nicotine dependence and craving. When overnight-abstinent smokers were subsequently given a denicotinized (DN) cigarette to smoke, they showed decreases in MOR availability in the right nucleus accumbens and thalamus, likely related to endogenous opioid release in response to the expectation of receiving nicotine. This placebo effect associated with the DN cigarette appeared to mask the effects of the regular nicotine cigarette smoked afterwards.

We also examined how opioid analgesics affect MOR neurotransmission in patients with chronic back pain. Our results indicated that decreases in the integrity of the endogenous opioid system, as indicated by a reduced ability to release endogenous opioids in response to pain, were associated with both higher clinical back pain ratings and with greater hedonic responses to the

administration of an exogenous opioid drug. Patients using opioid analgesics for at least one year showed decreased experimental pain-induced MOR activation and a lower number of available free MORs at baseline in regions of the brain implicated in pain modulation and drug abuse, such as the nucleus accumbens and amygdala. With the high prevalence of nicotine smoking, as well as the increasing use of opioid analgesics, it is crucial to understand how endogenous opioid mechanisms are implicated in the reinforcing and long-term effects of these drugs. This knowledge will help suggest avenues to explore while determining what treatments may be most successful in individual patients.

CHAPTER I

Introduction

THE ENDOGENOUS OPIOID SYSTEM (EOS)

"Of all the remedies it has pleased almighty God to give man to relieve his suffering, none is so universal and so efficacious as opium."—Thomas Sydenham (1624-1689)

Opium, a drug made from the opium poppy, has been known for thousands of years for its analgesic and euphoric properties. We now know that opium causes these effects by activating opioid receptors in the brain. Morphine, the active ingredient in opium, was isolated in 1806 by Friedrich Sertürner, but not until 1973 was it demonstrated that opioids such as morphine were binding to receptors located in the brain (Pert and Snyder, 1973; Simon *et al*, 1973; Terenius, 1973). This suggested that there were also endogenous neurotransmitters being produced within the body that could bind to these same receptors. These neurotransmitters and their opioid receptors make up what is today known as the endogenous opioid system (EOS), a system that has been implicated in a number of roles including reward, analgesia, sexual activity, learning and memory, and stress (Bodnar, 2013).

There are four main types of receptors that make up the EOS. These include the classical μ, δ, and κ receptors as well as the more recently-discovered nociception/orphanin FQ (N/OFQ) receptor. All are G protein-coupled receptors consisting of 7 transmembrane regions (Trigo *et al*, 2010). They couple to inhibitory G proteins, initiating a cascade leading to decreased

production of cyclic adenosine monophosphate (cAMP) and cellular hyperpolarization, which reduces cellular excitability. It is now believed that multiple subtypes of some of these receptors also exist, through mechanisms such as alternative splicing and receptor heteromerization (Feng *et al*, 2012; Gretton and Droney, 2014).

Opioid receptors are located widely throughout the central and peripheral nervous system. The endogenous ligands that bind to the three classical receptor types are derived from three major precursor proteins: proopiomelanocortin (POMC), proenkephalin (PENK), and prodynorphin (PDYN). The opioid peptides that are produced from these precursors include ß-endorphins, enkephalins, dynorphins, and neo-endorphins. Some of these peptides bind to more than one of the classical receptor types with varying levels of affinity, while others are more selective. Unlike ligands for the classical receptor types, ligands for the N/OFQ receptor have not been found to activate any of the other receptor types (McDonald and Lambert, 2005).

The μ-opioid receptor (MOR) system is one of the most frequently studied parts of the EOS, and will be the focus of my research discussed in the following chapters. The highest densities of MORs are located in the basal ganglia and thalamus, as well as the periaqueductal gray and to a lesser extent the frontal and parietal cortices (Frost *et al*, 1985). The endogenous opioid peptides ß-endorphin and endomorphin show a particularly strong affinity for these receptors, and these are also the receptors through which morphine exerts its analgesic and euphoric effects.

CONNECTION BETWEEN THE EOS AND REINFORCEMENT/SUBSTANCE ABUSE

Neurocircuitry involved in reinforcement

The ability of organisms to repeat actions that result in positive outcomes is key to their survival and reproduction. In order to assist with this, animals have evolved a system of "reinforcement", where certain actions produce feelings of reward and pleasure that increase the likelihood that the animal will repeat those actions. Actions such as sexual reproduction, social bonding, eating, and drinking all commonly result in this type of positive reinforcement. The network of brain regions involved in reinforcement includes the ventral tegmental area (VTA), nucleus accumbens (NAC), amygdala (AMY), ventral pallidum (VP), thalamus (THA), and periaqueductal grey (PAG), among other regions (Le Merrer *et al*, 2009).

Mesolimbic dopaminergic projections from the VTA to the NAC are generally considered the main players in reinforcement, and animal and human studies have long shown dopamine's importance in this reward network (Di Chiara *et al*, 2004; Wise, 2008). However, opioid receptors are also located throughout the regions involved in reinforcement, and their role in modulating this circuitry is becoming increasingly well defined. Animal studies have provided a significant amount of evidence showing the influence of opioid receptors in reinforcement. These studies demonstrate that stimulation of MORs in certain regions of the NAC of rats causes an increase in both the "liking" and the "wanting" of a food reward (Pecina and Berridge, 2005). The hypothalamus, VTA, amygdala, and VP all also contain regions that increase food intake in rodents when their MORs are stimulated (Le Merrer *et al*, 2009). MOR antagonists introduced to the NAC, on the other hand, decrease a rat's preference for food generally considered especially palatable by rodents, but does not alter intake of a less palatable food. MOR knockout mice showed decreased food-anticipatory activity (Kas *et al*, 2004) as well

as decreased motivation to consume food (Papaleo *et al*, 2007). The involvement of the EOS in social interactions can be seen through the release of endogenous opioids in response to social play in rats (Vanderschuren *et al*, 1995), as well as the fact that young mice that lack MORs show decreased maternal attachment (Moles *et al*, 2004). In humans, the administration of the opioid antagonist naloxone modulates responses to reward and losses during a gambling task (Petrovic *et al*, 2008), increasing unpleasantness ratings for losses and decreasing the pleasure of rewards.

Substance abuse

While reinforcement is crucial in guiding animals towards beneficial behaviors, such as reproducing and locating sustenance, drugs of abuse are able to hijack this reinforcement circuitry, resulting in detrimental outcomes such as addiction. Drug addiction is generally characterized as a chronically relapsing "compulsion to use one or more drugs of abuse, the inability to control drug intake and continued drug use despite negative consequences" (Pierce and Kumaresan, 2006, p216). Substance abuse and addiction are major health problems worldwide, resulting in costs of over $700 billion annually in the United States alone due to decreased productivity as well as health and crime-related costs (NIH, 2015). Along with the financial cost, addiction impacts the social aspects of society, often harming interpersonal relationships and causing increases in crime as well as problems with health and employment.

There have been several theories put forward on the mechanisms through which addiction occurs. Positive-reinforcement models of addiction focus on the euphoric and other mood-elevating effects felt when individuals consume a substance of abuse, and consider them the main driver in addiction. The positive feedback received after initiation of drug use spurs the individual to keep utilizing the drug. However, this theory is insufficient to explain why

individuals can persist in taking their preferred drug of abuse even in the face of severe personal or societal consequences, since it is unlikely the short-term positive effects induced by the drug would be able to outcompete the negative consequences associated with addiction.

Another theory of addiction, the opponent-process theory, focuses more on the role of negative reinforcement (Solomon, 1980). In this theory, while drug use begins due to positive reinforcement, such as the euphoria induced by the drug of abuse, as drug use continues the motivation gradually shifts and negative reinforcement becomes the primary player. After the initial euphoric effect of using a drug such as heroin has ceased, individuals start feeling aversive withdrawal effects. As the drug use is repeated more frequently, the euphoric effect may start to decrease as habituation occurs, but the withdrawal effects become more severe. This drives individuals to continue taking the drug simply to stave off the aversive effects, even when no longer feeling the positive effects from the drug use. However, although there is evidence in support of many parts of this theory, it is unable to explain other observations, including why addicted individuals feel persistent craving and relapse years after discontinuing drug use, long after withdrawal effects are no longer being felt (Robinson and Berridge, 1993).

More recently, the incentive sensitization theory of addiction has posited that addiction is a result of a hypersensitization of mesolimbic reward circuitry in the brain that occurs in response to drugs of abuse (Robinson and Berridge, 1993). These increases in the attractiveness of drugs of abuse and their associated stimuli lead to compulsive drug craving. Furthermore, this theory suggests that drug "wanting" and drug "liking" can be disassociated, and addiction may be caused by excessive drug "wanting" even without changes in the circuitry controlling the pleasurable effects of the drug. These theories are not all mutually exclusive, and it is quite

likely that the process of becoming addicted to a drug involves aspects of the various theories at different time points.

Neurotransmitter systems involved in substance abuse

Most substances of abuse, including psychostimulants, alcohol, and nicotine, have been shown to either directly or indirectly activate the mesolimbic dopamine system discussed earlier, resulting in an increased release of dopamine (Pierce and Kumaresan, 2006). However, although dopamine is the most well-known neurotransmitter affecting drug reward and reinforcement, the EOS has increasingly been shown to mediate responses to many drugs of abuse (Contet *et al*, 2004; Gerrits *et al*, 2003). Some drugs of abuse, such as heroin and prescription opioids, act directly on opioid receptors to exert their effects. Important evidence of the role of MORs in morphine reward has come from MOR knockout mice. Unlike wild-type mice, mice lacking MORs did not show morphine-induced conditioned place preference (CPP), suggesting MORs are necessary for the reinforcing effects of morphine (Matthes *et al*, 1996).

There is also evidence that even in drugs of abuse that do not directly bind to opioid receptors, such as cocaine, nicotine, and alcohol, the EOS plays some sort of modulatory role. In one example, opioid antagonists were shown to reduce cocaine reinforcement in rodents (Kuzmin *et al*, 1997), while MOR agonists can increase cocaine self-administration (Corrigall *et al*, 1999). MOR knockout mice also find cocaine, along with nicotine and alcohol, less reinforcing than wild-type mice (reviewed in Le Merrer *et al*, 2009). Similar to rodent studies, human studies have also shown that naltrexone, an opioid receptor antagonist, is able to reduce the rewarding effects of cocaine in cocaine-dependent individuals (Kosten *et al*, 1992) as well as

decrease ethanol self-administration (Volpicelli *et al*, 1992). Naltrexone is currently approved for use in treating both alcohol-dependent and opioid-dependent patients.

Along with the manipulations available in animal studies, neuroimaging studies have shown promise for identifying the regions involved in drug abuse in humans (Fowler *et al*, 2007; Parvaz *et al*, 2011). Techniques available range from electroencephalography studies, which have been able to associate nicotine administration with changes in brain wave frequencies (Domino, 2003), to functional magnetic resonance imaging (fMRI) studies, such as one study that showed increased activation in the anterior cingulate and dorsolateral prefrontal cortex when cocaine-dependent subjects were shown drug-related stimuli (Maas *et al*, 1998). Recently, new techniques such as positron emission tomography (PET) have allowed us a greater understanding of how drugs of abuse alter neurochemistry *in vivo*. These PET studies and their conclusions will be discussed further in Chapter 2.

Variations in substance abuse susceptibility

There are large interindividual differences in how susceptible or resilient people are to becoming addicted to drugs, and research has suggested that both environmental and genetic components play a role in these differences. Environmental factors that increase the initiation of drug use in animal models include exposure to psychological stress, early social isolation, and prenatal drug exposure (reviewed in Gerrits *et al*, 2003). Twin studies have also suggested that genetic factors play a role in drug abuse susceptibility (Kendler *et al*, 2003; Tsuang *et al*, 1998; van den Bree *et al*, 1998).

There are a number of pathways involved in the development of drug addiction that could be altered by genetic polymorphisms, including variations in dopaminergic and serotonergic

pathways along with opioidergic pathways. One genetic polymorphism of the opioid pathway that has been scrutinized for its possible association with drug abuse is a single nucleotide polymorphism (SNP) affecting the μ-opioid receptor (*OPRM1*) gene. This polymorphism, referred to as the A118G polymorphism, results in the substitution of aspartic acid for asparagine at amino acid 40. The G allele in this polymorphism results in MORs that bind ß-endorphin more tightly and increase its potency by a factor of three (Bond *et al*, 1998). Although there is some evidence that this gene polymorphism can indeed be associated with different aspects of drug abuse (Ray *et al*, 2006; Verhagen *et al*, 2012), there are conflicting reports of which variant leads to greater vulnerability, as well as cases where no relationships were found (Arias *et al*, 2006; Coller *et al*, 2009).

CONNECTION BETWEEN THE EOS AND PAIN

Neurocircuitry involved in pain

Endogenous opioids play a major role in modulating both physical and emotional pain. Physical pain results from stimulation of nociceptors in response to actual or imminent tissue damage. When these nociceptors are activated by noxious stimuli, they send signals via the spinal cord to the brain, resulting in the perception of pain as a type of negative feedback. Along with this ascending pain system, there is also a descending pain pathway. This "top-down" regulation has a significant impact on how an individual experiences pain. These are the circuits that placebo-induced pain relief, a mechanism that recruits the endogenous opioid system, is believed to act through. The brain regions that are involved in pain modulation significantly overlap with those involved in reinforcement, with the amygdala, PAG, and thalamus all

implicated, along with regions such as the anterior cingulate cortex and insula (Bingel and Tracey, 2008; Ossipov *et al*, 2010).

Many neurotransmitters are involved in pain regulation, including substance P, glutamate, and serotonin. For the research discussed in this dissertation, the involvement of opioid peptides in pain control is most relevant. As mentioned earlier, exogenous opioid drugs such as opium and morphine have been used for centuries to relieve pain. However, in 1972 evidence was found that endogenous opioids were also involved in pain control. It had been shown a year earlier that electrical stimulation of the PAG caused reduced sensitivity to painful stimuli in rodents (Mayer *et al*, 1971). Akil and colleagues found that when rats were given the opioid antagonist naloxone before PAG stimulation, the analgesic effect of the stimulation was greatly reduced (Akil *et al*, 1972). This suggested that opioid receptors were crucial for mediating the analgesic effect seen by Mayer *et al*. (1971). In 2001, Zubieta *et al*. used positron emission tomography to show reductions in *in vivo* MOR availability in humans during a sustained pain challenge, consistent with pain-induced release of endogenous opioids (Zubieta *et al*, 2001). These reductions occurred in regions such as the thalamus, hypothalamus, PAG, NAC, AMY, insular cortex, and dorsal anterior cingulate cortex, regions previously associated with regulating affective and sensory responses to pain, and were negatively correlated with subjects' pain ratings. Recent studies have also shown that individuals with chronic pain conditions have altered μ-opioid system function, both at baseline and in response to a pain challenge (Harris *et al*, 2007; Martikainen *et al*, 2013).

Opioid analgesics

Opioid analgesics are widely considered the most effective drugs for treating acute pain. However, there is currently controversy on whether they are also suitable for treating patients with various types of chronic pain. There is evidence of side effects such as opioid-induced hyperalgesia, and taking opioids long-term leads to greater risks of tolerance, dependence, and opioid misuse and abuse, with some studies suggesting that more than 10% of chronic pain patients who take opioid analgesics end up misusing them (Garland *et al*, 2013). Patients who also suffer from disorders such as anxiety or depression or have a family or past history of drug abuse are at a particular risk of becoming dependent on these medications (Martel *et al*, 2014). The rise in opioid prescriptions over the past couple of decades has also coincided with an increased number of deaths due to prescription opioid abuse, demonstrating the risks involved in prescribing these drugs (Dunn *et al*, 2010). Along with the many disadvantages to opioid use, we still lack well-controlled studies that suggest these analgesics are particularly effective for treating chronic pain conditions. There is already evidence associating prescription opioid use in humans with volumetric changes in grey matter, changes in functional connectivity, and decreases in white matter anisotropy (Upadhyay *et al*, 2010; Younger *et al*, 2011). Whether these changes correlate with important functional effects is still under investigation. It also remains to be seen if long-term opioid analgesic use affects the endogenous μ-opioid system, one of the brain's principal pain regulatory systems that also is involved in many other crucial functions.

Opioid analgesics might still be the best treatment in some cases of pain, but it is important that researchers and physicians are aware of the risks associated with prescribing these drugs. As our understanding of opioid mechanisms and the interindividual differences involved

increases, the hope is that we will be able to predict which individuals would be most at risk or benefit the most if given opioids, and physicians could alter their recommendations accordingly.

PET IMAGING

Positron emission tomography (PET) is an imaging technique that began its development in the 1950s, with the use of positrons as a method of localizing brain tumors. By the 1970s, the first PET scanners similar to the ones in use today were being constructed and tested with human subjects (Nutt, 2002). In PET, biological molecules of interest are tagged with radioactive isotopes, such as ^{11}C, ^{18}F, ^{15}O, and ^{13}N, to form radiotracers. As the radioactive isotopes decay, the radiotracers release positrons, or "antielectrons". When these positrons collide with nearby electrons, an annihilation event takes place and gamma rays traveling in opposite directions are emitted. The PET scanner is able to detect these gamma rays, and through the process called coincidence detection in conjunction with image reconstruction from projections, it is able to determine where in the body the annihilation event took place. This allows researchers to track the position of the radiotracers. One of the earliest radiotracers developed that became commonly used was deoxyglucose tagged with ^{18}F (^{18}F-FDG) (Nutt, 2002), and this glucose analog is still widely used today to investigate tissue metabolism.

PET studies often discuss the binding potential (BP) associated with their radiotracer of interest. Binding potential is a measure of the number of available receptors to which the radiotracer can bind. It is derived from dynamic measures of the PET scan and is equivalent to the ratio of the receptor density, B_{max}, to the radiotracer equilibrium dissociation constant, K_D. Differences in BP between groups must be interpreted carefully. If one group has fewer free receptors (a lower BP) at baseline, it could be due to a down-regulation in the total number of

receptors, or an up-regulation of endogenous ligands competing for those same receptors. There are also other alterations, such as changes in binding affinity, that can affect BP values. Generally if an individual shows a decrease in BP after an intervention, it is considered an "activation" of the respective system, most likely due to increased release of endogenous ligands. An increase in BP, on the other hand, represents a "deactivation" of the system.

There are several radiotracers currently being used to study the EOS. ^{11}C-Carfentanil (^{11}C-CFN), the first tracer to be introduced in 1985 to study the EOS in humans, is a selective MOR agonist (Frost *et al*, 1985). Shortly after ^{11}C-CFN, a non-selective opioid antagonist, ^{11}C-diprenorphine, came into use (Jones *et al*, 1988). It binds with similar affinities to μ, δ, and κ receptors. Other tracers commonly used today include ^{18}F-cyclofoxy, which marks both μ and κ receptors, and ^{11}C-methylnatrindole, which is selective for δ receptors (Henriksen and Willoch, 2008). The radiotracer ^{11}C-GR103545, a κ agonist, has just recently been tested in humans (Naganawa *et al*, 2014), as has the N/OFQ receptor antagonist ^{11}C-NOP-1A (Lohith *et al*, 2012). Still more radiotracers targeting various opioid receptors are currently in development.

The development of PET has provided an *in vivo*, noninvasive method of following the actions of biologically significant molecules. Limitations of PET include the expense, as well as the difficulty of interpreting any alterations seen in BP, given the multiple factors that could cause such a change. However, the benefits of incorporating PET studies to fill in the gaps left by animal and other human studies are substantial.

CURRENT DIRECTIONS

The high cost of drug dependence, both to the individual and to society as a whole, makes increasing our understanding of the mechanisms involved in the development of this condition a

crucial undertaking. It is becoming increasingly clear that neurotransmitter systems such as the EOS play a vital role in drug addiction. Because the endogenous opioid system is involved in both the analgesic and rewarding effects of opioids, as well as having a role in functions such as stress, social attachment, and mood disorders, understanding the alterations in this neurotransmitter system that are associated with both acute and long-term drug use is essential. If interindividual variations in EOS can be linked to an individual's response to drugs of abuse, it would further increase our ability to personalize our treatment of patients taking potentially addictive drugs.

In the following chapters I will first review the studies that have been conducted using positron emission tomography to examine the EOS in individuals at various stages of the drug abuse cycle. I will then present data on the results of a study using PET to examine the alterations seen in the μ-opioid systems of smokers compared to healthy controls, as well as the acute effects of smoking denicotinized and nicotinized cigarettes on these individuals. Finally, I will show how the acute subjective effects of opioid agonists, another class of drugs with high abuse potential, can be correlated with interindividual differences in pain-induced endogenous opioid function in a group of chronic pain patients. The alterations in MOR neurotransmission resulting from long-term use of opioid analgesics in these patients will then be discussed.

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CHAPTER II

Use of Positron Emission Tomography to Examine Opioid System Functioning in Addiction[1]

ABSTRACT

Animal and human studies have long suggested a critical role for the endogenous opioid system (EOS) in reward and addiction. The development of positron emission tomography (PET) has allowed researchers to investigate the functioning of the opioid system *in vivo*. The resulting studies have confirmed that alterations in the EOS are involved in both acute and long-term responses to drugs of abuse. As drug abusers maintain abstinence, there are also indications that some of these dysregulations will begin to normalize. The full potential of this methodology needs to be further explored in future studies.

INTRODUCTION

Addiction is an incredibly difficult psychiatric condition to treat. Nearly 60% of all patients seeking treatment for substance abuse or dependence relapse, and there are currently few pharmacological treatments available to counteract the addiction process (McLellan *et al*, 2000). However, one set of pharmacological agents that act to either fully or partially interfere with activity at the opioid receptor have proven to help stem cravings and deter relapse. In the early

[1] This work reflects collaboration with Dr. Tiffany Love and Dr. Jon-Kar Zubieta at the University of Michigan

1980s, work pioneered by scientists including Charles O'Brien and colleagues indicated that use of the opioid receptor antagonist naltrexone could reduce the rate of relapse among alcoholics (Pettinati *et al*, 2006). Since that time, more evidence has emerged which indicates that naltrexone may also be useful for treating methamphetamine dependence (Karila *et al*, 2010), and certain full or partial opioid agonists, such as methadone or buprenorphine, may significantly aid in the treatment of populations abusing opioid drugs (e.g. Bart, 2012; Gerra *et al*, 2006).

Mounting evidence suggests the endogenous opioid system (EOS) plays a key role in the addiction process. While it is certainly no surprise the EOS is responsive to opioid agonists that are frequently abused (e.g. heroin, morphine, oxycodone), it is now understood that this system is also engaged in response to many other drugs of abuse including cocaine, methamphetamine and alcohol (Trigo *et al*, 2010). While a vast amount of what is known about the opioid system in addiction has been borne from animal and post-mortem human studies, relatively recent breakthroughs in neuroimaging technologies have enabled the study of opioid system functioning in living human beings. This review will discuss the use of positron emission tomography (PET) to enquire into the state of opioid system functioning in substance dependent populations and summarize current progress.

OPIOID SYSTEM OVERVIEW

There are several major receptor subtypes that respond to endogenous and exogenous opioids – μ, κ, δ, and N/OFQ. Opioid receptors are widely distributed in the brain and each belongs to the G-protein-coupled receptor (GPCR) superfamily. Activation of these receptors results in the inhibition of adenlyl cyclase and voltage-gated Ca2+ channels, and the stimulation of inwardly-rectifying K+ channels. The net result is inhibition of presynaptic release of a range

of neurotransmitters. Endogenous ligands for these receptors include endorphins, endomorphins, and enkephalins, which display preferences for μ- and δ- receptors, and dynorphin and nociception, which principally bind to κ- and N/OFQ receptors, respectively (Table 2.1).

While the relative receptor distribution varies depending on the type of opioid receptor, there are widespread concentrations of each of the receptor types throughout the central nervous system. Relevant to the discussion of addiction and the role of the opioid system, however, are the heavy concentrations of opioid receptors within the motivational circuitry. These include various areas within the limbic system: the hippocampus and amygdala, prefrontal cortex, the dorsal and ventral striatum as well as within the ventral tegmental area (VTA) (see Frost *et al*, 1985; German *et al*, 1993; Peckys and Landwehrmeyer, 1999). Prior to the 1970s, the anatomical distributions of each opioid receptor type in humans was determined via post-mortem studies; however, following the development of the first positron emission tomography (PET) opioid radiotracers for humans, it was possible to conduct this research in living humans (Frost *et al*, 1985).

POSITRON EMISSION TOMOGRAPHY

In use in clinical populations since the late 1970s, positron emission tomography (PET) has granted researchers the opportunity to observe specific neurochemical processes *in vivo.* PET is a nuclear medicine imaging technique that utilizes compounds tagged with radioisotopes (e.g. ^{11}C, ^{18}F, ^{68}Ga,^{15}O, ^{13}N) to track biochemical processes of interest (Phelps, 2004). These compounds, commonly referred to as radioligands, are typically administered intravenously, subsequently taken up by the body, and, utilizing specialized equipment, can be detected and localized.

The radioisotopes used in PET imaging are atoms with unstable nuclei that will decay to a more stable form by emitting a positron (i.e. the electron's antiparticle). Radioisotopes can be generated through the use of a cyclotron, which can alter the number of protons or neutrons in the nucleus of a particular target via particle acceleration. Having the same mass as an electron but opposite in charge, when a positron collides with an electron the result is called annihilation. During this process, the mass of the electron and positron are converted into energy and a pair of 511 keV gamma rays will be emitted in opposite directions from the site of the annihilation. These gamma rays are detected by PET scanner equipment using a method dubbed coincidence detection (Phelps, 2004) and the anatomical site of positron annihilation can be determined through reconstruction from projections.

By attaching these positron-emitting radioisotopes to molecules of interest, a variety of neurochemical processes can be tracked. In the case of opioid system research, investigators often utilize radioligands designed to monitor opioid receptor availability. The primary measure used to describe receptor availability is binding potential (B_{max}/K_D), which incorporates both the density of available receptors (B_{max}) and the affinity to which the radioligand binds to the given receptor (K_D) (Phelps, 2004).

The first opioid receptor radioligand used in human PET imaging was ^{11}C-carfentanil (^{11}C-CFN). ^{11}C-CFN is an opioid agonist derived from fentanyl that is highly selective for the μ subtype of opioid receptor (OR) (Frost *et al*, 1985). This was followed by development of ^{11}C-diprenorphine (Jones *et al*, 1988), ^{11}C-methylnatrindole (Madar *et al*, 1996), and ^{18}F-cyclofoxy (Theodore *et al*, 1992) (Table 2.2). There are also several opioid radiotracers currently in development including 11C-GR103545 (κ-OR agonist) and ^{11}C-PEO/^{18}F-PEO (μ-agonist, κ-OR agonist) (Marton *et al*, 2009; Naganawa *et al*, 2014).

OVERVIEW OF OPIOID PET STUDIES IN HUMANS

There has been a myriad of opioid PET studies conducted in an attempt to understand the interaction between drugs of abuse and the EOS. These studies have encompassed the entire cycle of drug abuse, from the acute effects of the drug, both in naïve and frequent users, to the chronic effects seen in drug users undergoing recovery. In the following sections, we discuss some of the important findings from opioid PET studies, how they fit in to the rest of the drug abuse literature, and gaps that still need to be filled.

Acute effects of substance use

While numerous preclinical studies indicate endogenous opioids are released in response to consumption of drugs of abuse, relatively few investigations have examined this phenomenon in humans. Recent opioid PET imaging studies indicate acute recreational drug use can interfere with μ-opioid receptor (MOR) activity. For example, acute alcohol consumption is associated with a decrease in MOR binding potential (BP) in the bilateral nucleus accumbens (NAC) as measured by ^{11}C-CFN, which indicates alcohol consumption can promote endogenous opioid release. Such decreases in MOR BP were noted in both non-alcohol dependent subjects as well as in heavy drinkers, suggesting that this effect was not purely related to alcohol dependence or craving (Mitchell *et al*, 2012). However, greater decreases in MOR BP were correlated with greater subject ratings of feeling the "best ever" after alcohol consumption, implicating endogenous opioid release as a factor in the positive reinforcement that occurs when drinking alcohol.

Studies on the acute effects of nicotine smoking on the endogenous opioid system have been inconsistent. In a pilot study of 6 male smokers, MOR BP decreased in the anterior cingulate cortex (ACC) after subjects smoked a regular nicotine cigarette compared to after

smoking a denicotinized cigarette, suggesting endogenous opioid release in that region. However, MOR BP also increased in the left amygdala (AMY), left ventral basal ganglia, and right thalamus, which was interpreted as a possible sign that the denicotinized cigarette itself might have induced endogenous opioid release due to expectations and non-nicotine elements of smoking, which overwhelmed some of the effects of the nicotine cigarette. A later study similarly showed both increases (L putamen, L AMY, and L NAC) and decreases (medial prefrontal cortex, R ventral striatum (vStr), L insula (INS), and R hippocampus) in MOR BP after smoking the nicotine cigarette (Domino *et al*, 2015). In contrast, though, are two other studies that did not report any changes in BP (Kuwabara *et al*, 2014; Ray *et al*, 2011). The gender ratio of the subjects differed among these studies, as well as the timing and manner of smoking the two types of cigarettes. These differences may have contributed to the conflicting results.

Acute drug effects on opioid system activity have also been observed following amphetamine administration, although the results are again somewhat mixed. Colasanti and colleagues found that oral amphetamine administration caused a decrease in MOR BP in a number of brain regions, including the thalamus, cingulate, vStr, and frontal cortex (Colasanti *et al*, 2012). These results were recently replicated in a separate group of participants (Mick *et al*, 2014). A study by Guterstam and colleagues, however, failed to find any significant alterations in MOR BP in healthy men after intravenous amphetamine administration compared to placebo administration (Guterstam *et al*, 2012). Differences in route of administration and timing of the PET scan, among other aspects, may account for the differing results.

While there have only been a handful of studies examining acute drug administration effects on opioid system activity, initial evidence suggests that in at least some cases the changes

in receptor availability seen in humans mirror what is observed in animals, whose endorphin levels appear to increase during alcohol, nicotine, and psychostimulant administration (reviewed in Trigo *et al*, 2010). These endogenous opioids that are released then bind to receptors and initiate an intracellular signaling cascade that can lead to an increase in dopamine release, a neurotransmitter implicated in the rewarding effects of most drugs of abuse.

Chronic effects of substance use

Along with the observable changes during acute drug administration, alterations in opioid system function also generally occur with chronic drug use. These changes appear to be linked to both length of abstinence and craving. The majority of PET studies conducted on the opioid system in people with substance use disorder (SUD) have focused on individuals at various stages of abstinence. When Weerts and colleagues conducted research with alcohol-dependent (AD) subjects, they observed them after five days of abstinence. Both AD subjects and controls underwent two PET scans, one using CFN and one using ^{11}C-methylnaltrindole (MeNTL), a δ-opioid receptor (DOR)-selective ligand. AD subjects showed higher MOR BP than controls in the cingulate, amygdala, vStr, insula, thalamus, caudate, putamen, and globus pallidus. The amount of recent alcohol drinking was positively correlated with DOR BP in the caudate of AD subjects, indicating alcohol consumption may lead to decreased release of the endogenous opioids that bind to delta opioid receptors. However, in contrast to MOR BP, no differences were found in DOR BP between groups (Weerts *et al*, 2011). An examination of BP for the non-selective opioid ligand ^{11}C-diprenorphine (DPN) in AD patients after 2 weeks of abstinence found a negative correlation between lifetime alcohol consumption and ^{11}C-DPN BP in the brainstem. Although the results did not reach statistical significance, abstinent AD subjects showed a trend towards increased ^{11}C-DPN BP compared to controls, similar to what was seen in

the previous study (Williams *et al*, 2009). When CFN scans of AD subjects who had been abstinent between 1 and 3 weeks were compared to those of controls, MOR BP was again shown to be significantly elevated in the AD subjects in both the vStr and putamen (Heinz *et al*, 2005). Higher MOR BP in the frontal cortex and vStr was associated with increased craving in these individuals, implicating craving as either a possible cause or a result of the increased MOR availability seen. These changes in BP are consistent with rodent studies that showed that MOR density increased when animals receiving chronic ethanol treatment underwent abstinence (Djouma and Lawrence, 2002). However, not all the data on AD subjects match: Bencherif and colleagues reported lower MOR BP in the right dorsolateral prefrontal cortex (DLPFC), anterior frontal cortex, and parietal cortex of AD individuals on the 4th day of abstinence (Bencherif *et al*, 2004). The reasons for this inconsistency are unclear, but could simply be due to the small sample size (8 AD males). Subjects had also been abstinent for a shorter period than those in the study by Heinz and colleagues, and Weerts and colleagues included both male and female subjects in their study.

Opioid and cocaine use can also affect EOS functioning in chronic substance abusers. Opioid-dependent individuals examined using ^{11}C-DPN PET exhibited increases in average global opioid receptor BP compared to controls on their 10th day of abstinence (Williams *et al*, 2007). Williams and colleagues also observed region-specific heightened ^{11}C-DPN BP in the ACC, R AMY, putamen, and portions of the orbitofrontal cortex of opioid-dependent individuals as compared to controls. Similar increases were seen in the MOR BP of cocaine-dependent subjects undergoing early withdrawal (Zubieta *et al*, 1996). A longitudinal study also investigating alterations in cocaine-dependent subjects scanned subjects at day 1, week 1, and week 12 after entering a treatment facility, in order to examine how MOR BP changed (Gorelick

et al, 2005). Compared to controls, after a day of abstinence cocaine users showed an increase in MOR BP in the prefrontal, inferior frontal, and dorsolateral prefrontal cortices, as well as the lateral temporal cortex and anterior cingulate. A week later, MOR BP in the DLPFC and lateral temporal cortex had decreased to the levels seen in controls. By 12 weeks, only the anterior cingulate and anterior frontal regions still showed a significant increase in MOR BP. These results help elucidate the relationship between length of cocaine abstinence and changes in MOR BP, suggesting that as abstinence continues, the changes associated with cocaine use can be at least partially reversed.

Studies comparing smokers versus nonsmokers have also been conducted, again with inconsistent results. In the pilot study mentioned earlier, overnight-abstinent male smokers showed decreased MOR BP in the rostral ACC, thalamus, NAC, and AMY compared to controls (Scott *et al*, 2007). Two more recent studies did not show differences in MOR BP between smokers and controls, although one did find that within smokers there was a negative correlation between MOR BP in the bilateral superior temporal cortices and nicotine dependence as measured by the Fagerström Test for Nicotine Dependence (Kuwabara *et al*, 2014; Ray *et al*, 2011). The decreased MOR BP seen by Scott and colleagues in cigarette smokers after a few hours of abstinence contrasts with the effects seen after longer periods of abstinence in many other drugs of abuse. This may be partially explained by the fact that cigarette smokers were likely undergoing physical withdrawal symptoms, while most other studies on abstinent subjects waited until physical withdrawal symptoms had subsided.

There is evidence from several studies that EOS activity can also be used to predict how intensely a drug user will crave his or her drug of choice, as well as the related measure of how likely an abstinent subject is to relapse. In general, it appears that high MOR BP in areas of the

brain such as the ACC and AMY correlates with more intense craving during withdrawal. This has been shown for both cocaine and alcohol-dependent subjects, as well as in heroin-dependent subjects being maintained on buprenorphine (Gorelick *et al*, 2005; Greenwald *et al*, 2003; Williams *et al*, 2009; Zubieta *et al*, 1996). Since craving appears to be an important factor in determining whether an abstinent patient will relapse (Rohsenow *et al*, 2007), understanding and being able to modulate the factors that influence craving would be extremely helpful in preventing relapse.

As the link between craving and relapse might predict, when researchers directly investigated relapse rates in patients, similar correlations to those found in craving were uncovered. Cocaine-dependent subjects who stayed abstinent the longest after entering a program tended to have had lower MOR BP before treatment in the ACC, medial frontal, middle frontal, middle temporal, and sublobar insular gyri, compared to patients who relapsed more quickly (Ghitza *et al*, 2010). As mentioned earlier, many of the regions with significantly higher MOR BP in cocaine-users versus controls tended to normalize as abstinence continued (Gorelick *et al*, 2005). This suggests that opioid system functioning can begin to recover if subjects are able to remain abstinent for prolonged periods, although more research is needed to examine whether similar trends are seen in subjects dependent on other drugs of abuse. A subsequent study by Gorelick and colleagues correlated the decrease in MOR BP between week 1 and week 12 of abstinence with measures of treatment outcome (Gorelick *et al*, 2008). They found a positive correlation between the decrease in binding and time before relapse in the right inferior frontal cortex, bilateral temporal cortex, and left thalamus, and a negative correlation in the bilateral frontal cortex. When patients were split into individuals who relapsed within 10 days and those who took longer to relapse, those who relapsed earlier had more minimal decreases in

binding between 1 and 12 weeks in the right vStr, right orbitofrontal cortex, right frontal cortex, and left anterior temporal cortex. Baseline MOR BP at 1 week was also greater in the temporal cortex, left OFC, and right vStr of the early relapsers (Gorelick *et al*, 2008).

Not all studies on people with SUD are consistent in equating higher MOR BP with increased craving. Although results from the study on AD subjects by Williams and colleagues were similar to those seen in other drugs of abuse, there has also been research suggesting that AD subjects sometimes show a different pattern. Bencherif *et al.* (2004) and Weerts *et al*. (2011) both found that alcoholic dependent subjects showed an inverse correlation between MOR BP and alcohol craving. Additional research is needed to clarify these conflicting results.

Treatment

As an alternative to complete abstinence from drugs, some opioid dependent individuals can be treated using full or partial opioid agonists such as methadone and buprenorphine (BUP). Methadone is a synthetic opioid and BUP is a μ-opioid partial agonist and κ/δ antagonist. Both drugs will decrease opioid withdrawal symptoms and craving without producing euphoria, and will block symptoms produced by other opioids. They provide a safer and more controlled alternative for opioid-dependent individuals, who can either maintain their methadone/BUP doses long-term or take advantage of the milder forms of withdrawal that those drugs produce to gradually stop opioid use altogether.

To examine the effects of methadone on the EOS, former heroin users on long-term methadone maintenance were compared to healthy controls using ^{18}F-cyclofoxy, a μ- and κ-opioid receptor antagonist. The group on methadone was shown to have significantly lower binding in areas such as the thalamus, caudate, ACC, middle temporal cortex, and middle frontal cortex (Kling *et al*, 2000). However, binding of ^{11}C-diprenorphine did not appear to differ

between heroin-dependent individuals maintained on methadone and controls (Melichar *et al*, 2005). To explain this, Melichar and colleagues posited that methadone may only be required to bind to a very small percentage of receptors to be clinically effective, which ^{11}C-diprenorphine PET may be unable to detect. They also pointed out that while ^{11}C-diprenorphine has been shown to label internalized receptors, studies have not yet been done to determine whether the same is true for ^{18}F-cyclofoxy. Therefore, if methadone treatment causes opioid receptor internalization, it might explain why binding differences were seen when using ^{18}F-cyclofoxy, but not when using ^{11}C-diprenorphine.

In a preliminary study examining the effects of buprenorphine on three heroin-dependent subjects and controls, it was found that BUP dose-dependently decreased MOR BP in many regions of the brain (Zubieta *et al*, 2000). After treatment with BUP was ceased and subjects were on placebo, the heroin-dependent subjects were compared to controls. Significant increases in MOR BP were shown in the anterior cingulate and inferofrontal cortex of the heroin-dependent subjects, while the PFC and ventral caudate showed a trend in that direction. A later study with a larger sample size also showed that BUP dose-dependently decreased MOR BP in all the regions of interest (ROIs) investigated (PFC, ACC, NAC, AMY, thalamus, and caudate) as well as in the whole brain analyses (Greenwald *et al*, 2003). This decrease in MOR BP was associated with decreased craving and fewer withdrawal symptoms. Greenwald and colleagues also examined how MOR BP changed after heroin-dependent subjects who had been taking BUP daily discontinued their dose (Greenwald *et al*, 2007). As time since the last BUP dose increased from 4 to 76 hours, whole-brain MOR availability compared to controls taking placebo increased from 30% to 82%. To examine the effectiveness of the BUP at blocking the actions of opioids, subjects were given hydromorphone, an opioid agonist, at various time points. At both 4 and 28

hours after BUP administration, subjects reported no significant opioid agonist symptoms due to hydromorphone administration. By 52 and 76 hours after the last BUP dose, however, hydromorphone was able to produce significant increases in agonist symptoms.

The effects of naltrexone, a non-selective opioid receptor antagonist, and naloxone, an inverse agonist, have also been studied by several researchers. Like methadone and BUP, naltrexone can be used to treat opioid dependence, although it is more commonly used to treat alcohol dependence. Since it is an antagonist, it blocks the effects of opioids but does not reduce craving. In AD subjects, naltrexone prevents the alcohol from releasing endogenous opioids, which prevents some of the pleasurable effects of alcohol (Latt *et al*, 2002). Naloxone, on the other hand, is most frequently used to treat opioid overdose. The effect of naltrexone on the μ- and δ-opioid systems was examined using CFN and ^{11}C-methyl naltrindole PET (Weerts *et al*, 2008). Alcohol-dependent subjects were admitted to a clinical research unit to undergo alcohol withdrawal. PET scans were obtained before naltrexone was administered and after withdrawal symptoms abated on the 5th day. On day 15, subjects began receiving oral naltrexone doses daily, and a second set of PET scans was conducted 3 days later. Results from the CFN scan showed an average 94.9% (±4.9% SD) decrease in MOR BP throughout the brain. DOR BP was also shown to decrease during naltrexone administration, although to a lesser extent and with larger interindividual variation: BP decreased by 21.1% (±14.49% SD) across brain ROIs compared to the earlier scan. The day after their first set of PET scans, these same subjects were given increasing doses of naloxone while their plasma ACTH and cortisol concentrations were recorded. This provided information on baseline opioid system function, since naloxone would be expected to inhibit the baseline endogenous opioid inhibitory tone that normally regulates ACTH secretion, resulting in increased ACTH and cortisol release. The relationship between

opioid receptor availability and cortisol release in response to naloxone was investigated in these AD subjects and compared to controls (Wand *et al*, 2012, 2013). In healthy controls, Wand and colleagues found negative correlations between DOR BP in the vStr, cingulate cortex, and fusiform gyrus, and the area under the cortisol response curve (Wand *et al*, 2013). These correlations were not seen in AD subjects. Similarly, healthy subjects showed a negative correlation between MOR BP and cortisol response in nine ROIs studied, including the vStr, thalamus, and AMY. Again, no correlations were found in the AD subjects, suggesting a disruption in these individuals of the normal relationship seen between the opioid system and the HPA axis (Wand *et al*, 2012).

At-risk populations

PET studies investigating individual differences in the EOS could also be useful in predicting who may be at higher risk for developing a drug addiction later in life. Generally, individuals with substance abuse problems have higher ratings of impulsivity than healthy controls, and there is some indication that impulsivity is negatively associated with treatment outcome (Bickel *et al*, 1999; Krishnan-Sarin *et al*, 2007; Madden *et al*, 1997). Evidence indicates a two-way connection between drug abuse and impulsivity: individuals with higher impulsivity are at greater risk of using drugs, and drug use is associated with increases in impulsivity (de Wit, 2009; Diergaarde *et al*, 2008; Jentsch, 2008; Perry *et al*, 2008). The association between impulsivity and MOR BP was examined by Love *et al.* (2009), who found that people with high impulsivity scores have greater MOR BP in the right ACC, medial frontal cortex, ventral basal ganglia, and AMY. This is interesting given that opioid, cocaine, and some alcohol-dependent individuals who are abstinent and craving also showed heightened MOR

availability within regions such as the cingulate, putamen, and AMY when compared to control populations.

Genetic variations are also believed to play a role in predisposing certain individuals to develop an addiction. One example is the *OPRM1* A118G polymorphism. The less common G variant of this polymorphism has been linked in previous studies to increased risk of addiction, although results are inconsistent (Haerian and Haerian, 2013; Ray *et al*, 2012; Verhagen *et al*, 2012). In a recent CFN PET study, Ray *et al*. (2011) observed that smokers with a G allele exhibit decreased MOR BP after smoking in the left ACC, AMY, right caudate, and thalamus. Individuals with a G allele also appear to have decreased global MOR BP, as shown in a study examining both 5-day abstinent AD individuals and healthy controls (Weerts *et al*, 2013). The effects of other genetic variations on the EOS of humans should also be examined using PET.

DISCUSSION

This review has focused on studies examining *in vivo* alterations of the human EOS in individuals at various stages of the drug abuse cycle. Earlier animal studies have shown that along with the well-known connections to the dopaminergic system, reward and addiction are also linked to changes in the endogenous opioid system (reviewed in Di Chiara *et al*, 2004; Trigo *et al*, 2010). The advent of PET has provided a crucial tool that allows us to study these processes in humans.

Much has already been learned about the EOS through studies on animals, such as its ability to modulate reinforcement properties of various drugs and to affect craving and relapse (see Gerrits *et al*, 2003; Trigo *et al*, 2010). MOR knock-out mice, as well as animals given MOR antagonists, seem to find drugs of abuse less reinforcing, suggesting that MORs are especially

crucial in mediating the rewarding effects of these drugs. One known mechanism through which the opioid system plays a role in drug abuse is by modulating the function of the dopaminergic system. Dopaminergic activation, particularly in the NAC, is believed to be the primary reinforcing effect in most drugs of abuse (Di Chiara *et al*, 2004). Injections of both μ- and δ-opioid receptor agonists lead to increased dopamine (DA) levels in the vStr in rats. Rats also learn to self-administer infusions of both of those agonists into their VTA, indicating that the infusions are reinforcing (Devine *et al*, 1993; Devine and Wise, 1994). The knowledge obtained from these types of animal studies can now be elaborated upon in humans using PET studies, which have the unique ability to focus on alterations in neurotransmitter systems of interest while subjects are awake and performing tasks.

As our ability to synthesize radiotracers targeting specific receptors of interest grows, PET studies will also be useful for differentiating between the activities of the various types of opioid receptors in humans. Although the μ-opioid system is generally thought of as the main player when discussing the role of the EOS in addiction, there is increasing evidence that the other receptors, especially the k-opioid receptors, play critical roles as well. While the δ-opioid system often acts similarly to the μ-opioid system, agonists for k-receptors have aversive effects in animals and may interfere with the rewarding effects of drugs of abuse (see Wee and Koob, 2010). There are a number of cases where μ and k agonists appear to have opposing effects, including how they alter drinking behavior, locomotion, and DA release, and it has been suggested that the aversive effects of k-opioid agonists may contribute to the drug withdrawal symptoms seen in people with SUD. Animal and post-mortem human studies both suggest that drug abusers have an upregulated κ-opioid system (reviewed in Wee and Koob, 2010). Animals studies have also found that during withdrawal from cocaine, ethanol, and opioids, levels of the κ

agonist dynorphin appear to increase in the NAC and AMY (reviewed in Koob, 2008). This increase in dynorphin levels is hypothesized to lead to a downregulation of the dopaminergic system, which may cause some of the aversive effects associated with withdrawal (Wee and Koob, 2010). As radiotracers focusing exclusively on the κ receptors become better developed, these studies should be replicated *in vivo* in humans.

Since the development of opioid receptor specific PET radiotracers, our options for investigating the dysregulation of the human EOS that occurs in response to drugs of abuse have expanded. We are able to examine opioid receptor functioning and determine how receptor availability changes in patients at different stages of drug abuse. The majority of the studies reviewed in this paper agree that acute drug administration decreases MOR BP, while in recently-abstinent drug users, whether they are dependent on opioids, cocaine, or alcohol, MOR BP is increased compared to controls. Animal studies suggest that this initial decrease in MOR BP after acute drug administration is likely due to the release of endogenous opioids, which then compete with the radiotracers for receptors. The increase in binding potential seen in human chronic drug users undergoing abstinence could be explained by an upregulation of MORs, a decrease in endogenous opioids, or some combination. However, two studies examining postmortem brains of opioid-dependent individuals did not find any significant difference in MOR density or affinity, which suggests that alterations in endogenous opioid levels may be primarily responsible for the differences in binding (Gabilondo *et al*, 1994; Schmidt *et al*, 2001). In rodents chronically exposed to cocaine, the MOR system appears to show activation in a variety of regions, and withdrawn animals have shown higher levels of MOR mRNA in the frontal and cingulate cortices (reviewed in Yoo *et al*, 2012). In the PET studies reviewed here, the degree to which BP is increased often appears to predict the strength of the craving that an

individual feels for a drug and the likelihood of relapse. As the length of abstinence increases, MOR BP may normalize back to the levels found in controls in many of the brain regions, as occurred in a sample of cocaine-dependent subjects followed for several months (Gorelick *et al*, 2008).

In the PET studies discussed above, the NAC was a region that often showed alterations in the EOS in response to drugs of abuse. The NAC is part of the mesolimbic pathway, which connects the VTA to the limbic system. This pathway is a major player in drug addiction, believed to mediate "acute reinforcing effects of drugs and various conditioned responses related to craving and relapse" (Feltenstein and See, 2008, p265). As Mitchell and colleagues show, after acute alcohol intake there is a decrease in MOR BP in the NAC, which is associated with subjects feeling the "best ever" (Mitchell *et al*, 2012). Alterations in NAC BP in people with SUD are also seen in a number of the other studies discussed. The NAC is a key structure in reinforcement and addiction, and as mentioned earlier, most drugs of abuse are believed to obtain their reinforcing properties due to the release of DA in the NAC (Volkow *et al*, 2012). Generally, GABA interneurons inhibit the activity of the dopaminergic neurons in this area. When μ- or δ-opioid receptors in the NAC or VTA are activated, they inhibit the inhibitory GABA interneurons. This disinhibits the dopaminergic neurons, leading to increased DA release in the NAC and presumably causing the rewarding effects of opioid drugs (Trigo *et al*, 2010). In the above study by Mitchell and colleagues, decreased MOR BP likely indicated an increase of endogenous opioids in the NAC following alcohol consumption. This could then act to disinhibit the dopaminergic neurons and increase DA release, producing the reported reinforcing effect.

The cingulate cortex and DLPFC are two other regions that were frequently implicated in the reviewed PET studies. Both of these regions are part of the mesocortical dopaminergic pathway, which connects the VTA to various cortical areas and is believed to be involved in the "conscious drug experience, drug craving and a loss of behavioural inhibition related to compulsive drug-seeking and drug-taking behaviours" (Feltenstein *et al*, 2008, p265). The relevance of these regions in reward and drug abuse has been examined in humans using functional magnetic resonance imaging (fMRI), which uses changes in blood-oxygen-level dependent (BOLD) signal intensity as a way of estimating changes in neural activity. An fMRI study by Maas *et al.* (1998) reported increased BOLD activation in the anterior cingulate and the left DLPFC in cocaine-dependent individuals compared to controls when shown drug cues, with stronger activation corresponding to greater craving. Activation of the DLPFC may also be important for self-regulation of craving, according to an fMRI study conducted by Kober *et al.* (2010). Subjects were scanned while considering how smoking or eating would make them feel at that moment, as well as while employing a cognitive strategy meant to decrease craving: considering the long-term consequences associated with the appetitive stimuli. Increases in BOLD activity in the DLPFC and decreases in vStr activity were both associated with how effectively subjects were able to decrease their craving using that strategy. This is consistent with the suggestion that individuals who have problems with substance abuse may show decreased prefrontal control over regions associated with reward, such as the vStr.

As this review shows, PET studies have affirmed the role of the EOS in the pathophysiology of various stages in the drug abuse cycle. However, despite the potential, there have been relatively few PET studies examining the opioid system in people suffering from drug abuse, and practically none on populations believed to be at increased risk of drug abuse. Of the

PET studies that do focus on the EOS, the vast majority uses either radiotracers specific to the μ-opioid system or tracers that bind to multiple receptor types. Since kappa receptors often show effects opposing those of the other receptors, non-specific tracers could mask the true effects of each of the receptor types. Future studies should increase our examination of changes in κ- and δ-opioid system function in relation to drug addiction, since there is growing evidence that these systems may also have important roles to play in addiction.

TABLES

Table 2.1: Endogenous opioid receptor preferences.

Endogenous Opioid	Receptor Preference
Endorphins	μ, δ
Enkephalins	μ, δ
Nociceptins	N/OFQ
Endomorphins	μ, δ
Dynorphins	κ, N/OFQ

Table 2.2: Common opioid radioligands and receptor targets.

Radioligand	Receptor Selectivity
^{11}C-carfentantil	μ-OR agonist
^{11}C-diprenorphine / ^{18}F-diprenorphine	μ-OR antagonist, δ-OR agonist, κ-OR agonist
^{11}C-methylnatrindole	δ-OR antagonist
^{18}F-cyclofoxy	μ-OR antagonist, κ-OR antagonist

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CHAPTER III

Nicotine-Specific and Non-Specific Effects of Cigarette Smoking on Endogenous Opioid Mechanisms[2]

ABSTRACT

This study investigates differences in μ-opioid receptor mediated neurotransmission in healthy controls and overnight-abstinent smokers, and whether these differences are affected by *OPRM1* A118G genotype. It also examines the effects of smoking denicotinized (DN) and average nicotine (N) cigarettes on the μ-opioid system. Positron emission tomography (PET) with ^{11}C-carfentanil was used to determine regional brain μ-opioid receptor (MOR) availability (non-displaceable binding potential, BP_{ND}) in a sample of 19 male smokers and 22 nonsmoking control subjects.

Nonsmokers showed greater MOR BP_{ND} than overnight abstinent smokers in the basal ganglia and thalamus bilaterally. BP_{ND} in the basal ganglia was negatively correlated with baseline craving levels and Fagerström scores. Interactions between group and genotype were seen in the nucleus accumbens bilaterally and the amygdala, with G-allele carriers demonstrating lower BP_{ND} in these regions, but only among smokers.

After smoking the DN cigarette, smokers showed evidence of MOR activation in the thalamus and nucleus accumbens. No additional activation was observed after the N cigarette, with a mean effect of increases in MOR BP_{ND} (i.e., deactivation) with respect to the DN cigarette

[2] This work reflects collaboration with Lisong Ni, Dr. Edward Domino, and Dr. Jon-Kar Zubieta at the University of Michigan

effects in the thalamus and left amygdala. Changes in MOR BP_{ND} were related to both Fagerström scores and changes in craving.

This study showed that overnight-abstinent smokers have lower concentrations of available MORs than controls, an effect that was related to both craving and the severity of addiction. It also suggests that nicotine non-specific elements of the smoking experience have an important role in regulating MOR-mediated neurotransmission, and in turn modulating withdrawal-induced craving ratings.

INTRODUCTION

With over one billion smokers worldwide, nicotine dependence is a major health concern. There are more than 5 million deaths associated with tobacco each year, and tobacco smoking is the most prevalent cause of "preventable disease and death" in the United States (Graul and Prous, 2005). There are substantial lines of evidence pointing to a strong link between nicotine use and endogenous μ-opioid mechanisms, which may mediate some of nicotine's addictive properties and distress during withdrawal (for review see Pomerleau, 1998).

Animal and cell culture studies suggest that acute nicotine induces endogenous opioid release (Boyadjieva and Sarkar, 1997; Davenport *et al*, 1990). However, attempts to translate these initial findings into human studies have led to inconsistent results. Studies examining changes in MOR availability (binding potential, BP) after smoking denicotinized (DN) versus average nicotine (N) cigarettes with positron emission tomography (PET), an indirect measure of changes in neurotransmitter release and μ-opioid receptor activation, have found both reductions in BP (suggesting activation of neurotransmission) and increases (deactivation) in different regions of the brain (Domino *et al*, 2015; Scott *et al*, 2006). Alternatively, some studies have not

found any significant differences in MOR binding after smoking N versus DN cigarettes (Kuwabara *et al*, 2014; Ray *et al*, 2011). Measures at baseline have also shown either lower MOR BP in smokers compared to nonsmoking controls (Scott *et al*, 2006), or no significant differences between groups (Kuwabara *et al*, 2014). The μ-opioid system is also known to respond to positive expectancies, including the so-called placebo effect (Pecina *et al*, 2015a; Scott *et al*, 2008; Zubieta *et al*, 2005), which may impact the effects of both DN and N. This effect could be particularly prominent in studies conducted after nicotine abstinence, when craving and positive expectancies are highest. This was initially suggested in a small pilot study (Scott et al., 2006), and could potentially contribute to inconsistencies in results across study designs.

To explore this possibility, the current study examined MOR non-displaceable BP (BP_{ND}) (Innis *et al*, 2007) at baseline, preceding and following DN and N cigarettes, and again during DN and N smoking using a single blind design. In this analysis, effects of DN and N cigarette smoking on subsequent BP_{ND} values were further controlled by their corresponding baseline values to provide a corrected measure of μ-opioid system activation (changes in BP_{ND}) after DN and N smoking. Smokers were studied after verified overnight abstinence when craving for cigarettes would be high. The effects of a common functional polymorphism of the MOR, known to influence both baseline MOR BP_{ND} (Pecina *et al*, 2015b; Ray *et al*, 2011; Weerts *et al*, 2013) and the activation of the μ-opioid system during positive expectations (Pecina *et al*, 2015b) were also evaluated in these analyses.

PATIENTS AND METHODS

Participants and study design

Twenty-four smokers and 22 healthy non-smokers between the ages of 20 and 35 were recruited by advertisement for this study. All participants were male and right-handed, not on any medication, and had no history or current signs of psychiatric or physical illnesses. Participants were excluded if they used any drugs of abuse besides tobacco smoking. Written informed consent was obtained from all subjects, and the study was approved by the Institutional Review Board for Human Subject Research and the Radioactive Drug Research Committee at the University of Michigan.

Five individuals from the experimental group had to be excluded from the analyses. Three had missing elements of MRI or PET data, and one subject was discovered to be a nonsmoker. Another participant tested positive for opioids at the time of his PET scan. The final sample was 19 male smokers between the ages of 20 and 35 (mean ± SD: 25.3±4.4 years) and 22 non-smokers (mean ± SD: 24.2±3.7 years). Participants smoked between 6 and 30 cigarettes a day (mean ± SD: 18.4±5.6), and had Fagerström Scale of Nicotine Dependence (FSND) scores ranging from 2 to 8.3 (mean ± SD: 5.5±1.9) with 10 being most dependent (Heatherton *et al*, 1991).

Smokers were instructed to cease smoking the night before the 8:30 AM scans, resulting in 8-12 hrs of abstinence. Compliance was tested using a carbon monoxide (CO) detector (Vitalograph Breath CO Model BC1349, Vitalograph Inc., Lenexa, KS) with a requirement of CO levels < 10 parts per million (p.p.m.) prior to scanning (Domino and Ni, 2002).

In the overall protocol, PET scanning was conducted using the radiotracers ^{11}C-carfentanil (^{11}C-CFN) and ^{11}C-raclopride (^{11}C-RCL) targeting μ-opioid and dopaminergic D2/D3

receptors, respectively. Only the ^{11}C-CFN data is reported here. Smokers participated in the trials on two separate days, with two consecutive 90 min PET scans each day (Figure 3.1). For the first half of each scan the participants were simply instructed to lie still, providing a baseline measure. Between 43 and 53 min after tracer administration, smokers were directed to smoke either two DN cigarettes (0.08 mg nicotine/cigarette, 9.1 mg tar/cigarette) or two N cigarettes (1.01 mg nicotine/cigarette, 9.5 mg tar/cigarette) through a one-way airflow system. Smokers received the N cigarettes second in order to prevent the effects of the nicotine from carrying over into the DN condition. Each day, participants received one scan using the tracer ^{11}C-CFN and one using the tracer ^{11}C-RCL. The order was counterbalanced the second time the participants came in. The data from the ^{11}C-RCL scans has been previously reported (Domino *et al*, 2012; Domino *et al*, 2013), as has a different analysis of the ^{11}C-CFN data (Domino *et al*, 2015).

Prior to scanning, smokers were asked to rate a 1-10 visual analog scale (VAS) for "craving", "relaxed", "sickness", "wakefulness", and "nervousness". They repeated this at 30 and 60 min into each of the scans (once before smoking and once after smoking). At the 30 and 60 min time points participants also completed the Positive and Negative Affectivity Schedule (PANAS; Watson and Clark, 1999), Profile of Mood States (POMS; McNair *et al*, 1971), and Spielberger State Anxiety Inventory (STAI; Spielberger *et al*, 1983).

Six of the healthy controls were asked to smoke a sham cigarette (unlit cardboard cylinder) in place of either the DN or N cigarette. The remaining 16 controls simply underwent one 90 min baseline ^{11}C-CFN scan in which they were asked to lie in the scanner with no intervention.

Scanning protocol and data acquisition

Participants were placed in a Siemens HR+ scanner and data were collected as previously described (Domino *et al*, 2012; Scott *et al*, 2006). Briefly, scans were acquired in three-dimensional mode (reconstructed FWHM resolution ~5.5 mm in-plane and 5.0 mm axially, with septa retracted and scatter correction). Images were reconstructed using iterative algorithms (brain mode; FORE/OSEM four iterations, 16 subsets; no smoothing) into a 128x128 pixel matrix in a 28.8 cm diameter field of view. Attenuation correction was done using a 6 min transmission scan (^{68}Ge source) obtained before the radiotracer was injected. Image data was transformed into two sets of parametric maps: a tracer transport measure (K1) and a receptor related measure (BP_{ND}) using a modified Logan graphical analysis (Logan *et al*, 1996) with the occipital cortex as a reference region.

A light forehead restraint was used on each participant to reduce movement, Two intravenous (antecubital) lines were placed. ^{11}C-CFN was synthesized at high specific activity through the reaction of ^{11}C-methyliodide and a non-methyl precursor (Jewett, 2001). The tracer was administered through one of the intravenous lines, beginning with a bolus containing half of the tracer. The other 50% was administered continuously during the scan. Throughout the scan, each participant's blood pressure and heart rate were recorded.

A high-resolution anatomical MRI image was obtained for each participant using a 3 Tesla scanner (Signa, General Electric, Milwaukee, WI). The acquisition sequence used was an axial SPGR IRpPrep MR (TE = 5.5, TR = 14, TI = 300, flip angle = 20°, NEX = 1, 124 contiguous images, 1.5mm thickness), followed by axial T2 and proton density images (TE = 20 and 100, respectively; TR = 4000, NEX = 1, 62 contiguous images, 3mm thickness).

Participants were also asked to complete several functional MRI tasks while in the scanner, the results of which will be published separately.

Bloods/Genotyping/Plasma nicotine

Before the first scan, 10 ml of venous blood was drawn from each participant. Blood was processed by the Michigan Center for Translational Pathology laboratory biorepository and Michigan Sequencing Core for analysis and subsequently reconfirmed (Domino et al., 2012). Each participant's genotype at the *OPRM1* A118G polymorphism was determined. Participants were divided into two groups based on whether they carried at least one rare G allele (G*) or whether they were homozygous for the A allele. Nicotine plasma levels were also determined by drawing blood samples just before smoking (min 43) and at five other time points after smoking initiation (49, 59, 65, 75, and 95 min after tracer administration). Blood samples were analyzed by MEDTOX Laboratories, Inc. (St. Paul, MN).

Data analysis

Image preprocessing

PET data consisted of two early BP_{ND} values acquired at baseline (10-40 min post-tracer administration, one of them after overnight abstinence and the second after an initial DN smoking session), and two late session BP_{ND} values (45-90 min post-tracer administration) acquired after DN and N smoking. Each participant's PET images were coregistered to their own MRI image using Matlab (MathWorks, Natick, Massachusetts) and SPM8 (Wellcome Trust Center for Neuroimaging, London, United Kingdom) software, and then were linear and non-linear warped using VBM8 Toolbox (Christian Gaser, University of Jena, Department of Psychiatry, Jena, Germany) within SPM8 to match the Montreal Neurological Institute (MNI) stereotactic atlas orientation. Coregistration and warping were checked visually for each

participant, and a 3-D Gaussian filter (FWHM 6 mm) was applied to each scan. Voxel-by-voxel whole-brain analyses were performed on the participants, and significance thresholds estimated using image smoothness, the Euler characteristic, and the number of voxels in the gray matter (Worsley *et al*, 1992). Further analyses were performed on extracted data using SPSS Statistics v. 20 (IBM Corp, Armonk, NY). Differences in BP_{ND} values were examined to investigate differences between baseline values, changes in BP_{ND} as a result of DN and N smoking, and the effect of the *OPRM1* A118G polymorphism on these measures.

Craving, Fagerström scores, and plasma nicotine levels in smokers:

We focused on craving ratings and their change within and between scans using SPSS. Paired t-tests were used to compare VAS craving ratings at 30 min and 60 min (before and after DN and N) and Pearson correlations were calculated between VAS craving and Fagerström scores. The effect of smoking DN and N cigarettes on plasma nicotine levels was determined using paired t-tests between levels acquired prior to smoking (min 43) and post-smoking samples at min 49, 59, 65, 75, and 95 after tracer administration. Plasma nicotine data was not collected on one subject during the DN scan, and on another subject during the N scan.

Effects of group and genotype on baseline MOR BP_{ND}:

A 2x2 whole-brain analysis of covariance (ANCOVA) in SPM8 was used to examine the effect of group (smokers vs. controls) and genotype (AA vs. *G at the A118G polymorphism) on baseline MOR BP_{ND} (10-40 min post-tracer administration), as well as any interactions between group and genotype. The genotype frequencies for smokers were 14AA, 4AG, and 1GG; for nonsmokers they were 15AA, 5AG and 2GG.

Effects of DN and N smoking on MOR BP_{ND}.

Differences between baseline BP_{ND} values preceding DN (after overnight abstinence) and after DN (prior to N), reflecting potential effects of DN smoking on endogenous opioid release, were examined in SPM8 using voxel-by-voxel paired t-tests with scan order as a covariate. Because of some technical difficulties with the image data, this sample included n=16 smokers. Similar analyses were conducted to examine differences between DN and N effects, controlling for their preceding baseline BP_{ND} values and scan order (n=19).

Regions that were significant after cluster-level FWE correction ($p<0.05$) or approached significance ($p<0.06$) were extracted for further analysis using MarsBaR (Brett *et al*, 2002). Only areas with BP_{ND} values greater than 0.1 were included in the analyses, to exclude regions with nonspecific binding. Correlations between BP_{ND} in these regions of interest (ROIs), baseline VAS craving, and participants' Fagerström scores were examined using SPSS and Pearson correlations at $p<0.05$.

RESULTS

Baseline MOR BP_{ND} in smokers compared to non-smokers

Two-way ANCOVA showed an effect of group on MOR BP_{ND} at baseline. Healthy controls showed higher BP_{ND} than smokers in the left and right basal ganglia (BG) and the thalamus (THA) (Figure 3.2a,b). BP_{ND} in the left and right BG of smokers were negatively correlated with baseline craving (L BG: $r(17)=-0.67$, $p=0.002$; R BG: $r(17)=-0.68$, $p=0.001$) (Figure 3.2c). Fagerström score was also negatively correlated with MOR BP_{ND} in the same regions (L BG: $r(17)=-0.55$, $p=0.014$; R BG: $r(17)=-0.54$, $p=0.017$) (Figure 3.2c).

Effects and interactions of 118G allele and smoking status on baseline MOR BP_{ND}

Two-way ANCOVA showed an overall significant effect of genotype, with G-allele carriers showing lower baseline BP_{ND} than AA homozygotes throughout brain areas with specific binding, most prominently in the orbitofrontal cortex (OFC), anterior cingulate cortex (ACC), insular cortex, nucleus accumbens (NAC), and THA (Figure 3.3a).

An interaction between group and genotype was also observed in the NAC bilaterally, as well as in the right AMY (Figure 3.3b,d). For controls, A118G genotype did not have a significant effect on baseline BP_{ND} in those regions (L NAC: $F(1,36)=0.135$, $p=0.719$; R NAC/AMY: $F(1,36)=0.355$, $p=0.555$). G-carrier smokers, however, showed lower BP_{ND} values compared to AA homozygotes (L NAC: $F(1,36)=10.921$, $p=0.002$; R NAC/AMY: $F(1,36)=12.531$, $p=0.001$) (Figure 3.3c).

Craving, plasma nicotine levels, and Fagerström scores in smokers

Craving scores were significantly reduced after both the DN cigarette ($t(18)=5.89$, $p<0.0001$) and the N cigarette ($t(18)=6.64$, $p<0.0001$) compared to ratings prior to smoking. Craving during N scans was significantly lower than during DN scans, both at 30 min (post-DN smoking, pre-N smoking) ($t(18)=3.34$, $p=0.004$) and at 60 min (post-N smoking) ($t(18)=3.71$, $p=0.002$) (Figure 3.4a). No significant differences were found in the degree of craving relief afforded by N compared to DN cigarettes (30-60 min) ($t(18)=-0.35$, $p=0.732$). There were also no significant effects of *OPRM1* A118G genotype on craving measures.

Smokers with higher Fagerström scores had higher baseline craving ($r(17)=0.69$, $p=0.001$) and a greater reduction in craving after both DN ($r(17)=0.65$, $p=0.003$) and N cigarettes ($r(17)=0.51$, $p=0.026$). Both the DN and the N cigarette significantly increased plasma nicotine levels from pre-smoking levels (DNpre vs. DNpost: $t(17)=-3.26$, $p=0.005$; Npre vs.

Npost: $t(17)=-3.953$, $p=0.001$), but with plasma nicotine levels at all time points after N being significantly higher than the corresponding plasma nicotine levels after DN ($p<0.001$) (Figure 3.4b). After DN smoking, plasma nicotine levels peaked at min 59 (mean ± SD: 3.2 ± 1.9 ng/ml), and after N smoking levels peaked at min 65 (mean ± SD: 16.4 ± 8.1 ng/ml).

Effect of DN smoking

Early baseline scan (10-40 min) MOR BP_{ND} (before DN) was significantly higher than that following DN (before N) in an area that included the THA and the right NAC (Figure 3.5), suggesting a substantial activation of endogenous opioid neurotransmission in these areas after DN that persisted into the following scanning period. No significant correlations were found between the changes in BP_{ND} and Fagerström scores, craving ratings, or plasma nicotine levels. There were also no significant effects of *OPRM1* A118G genotype.

Effect of N smoking

To isolate the effects of DN versus N smoking, we controlled for the above baseline differences in MOR BP_{ND} during the early scans preceding DN and N. For this purpose we first subtracted the late portion of each scan (DN or N) from the earlier baseline portion and then examined differences between DN and N smoking effects. In these analyses, we observed significantly greater activation of neurotransmission during DN smoking compared to N smoking, and net increases in BP_{ND} in the THA and left AMY during the N scan compared to the DN scan, suggesting an overall effect of deactivation of neurotransmission during N smoking relative to DN smoking in these regions (Figures 3.5b, 3.6). There were no significant increases in MOR BP_{ND} during DN compared to N scans. No significant effects of *OPRM1* A118G genotype were observed in these analyses.

Fagerström scores were negatively correlated with changes in BP_{ND} in the THA during the DN scan ($r(15)=0.52$, $p=0.031$) and the N scan ($r(15)=-0.65$, $p=0.005$), with greater relative activation of neurotransmission observed in those with higher nicotine dependence scores. The change in craving scores after DN smoking were also inversely associated with changes in MOR BP_{ND} in the THA ($r(15)=-0.64$, $p=0.006$). No significant associations were observed for changes after N smoking.

DISCUSSION

This manuscript examined changes in endogenous μ-opioid receptor mediated mechanisms in addicted smokers during DN and N cigarette smoking in relation to a control sample of non-smokers. We observed reductions in baseline receptor availability (BP_{ND}) in smokers after overnight abstinence compared to non-smoking controls. These group effects were observed in the BG bilaterally and in the THA, regions involved in reward responsiveness and habitual behavior, and sensory integration, respectively (Graybiel, 2008; Haber and Knutson, 2010; Tyll *et al*, 2011). Reductions in the BG were further associated with Fagerström and craving scores, suggesting an association between the effects of chronic tobacco smoking on MORs and the severity of nicotine addiction. These data are also consistent with that acquired in an initial pilot study from our group, where reductions in MOR BP_{ND} were observed in a small sample of smokers after DN cigarette smoking in the rostral ACC, THA, NAC, and AMY, in comparison with non-smoking controls (Scott *et al*, 2006). Previous work by Ray *et al.* (2011) and Kuwabara *et al.* (2014), however, did not find significant differences in receptor availability between controls and smokers, a discrepancy that may be secondary to differences in the samples studied or in the study designs. Both Ray *et al.* (2011) and Kuwabara *et al.* (2014) studied males

and females, who present substantial differences in MOR BP_{ND} under resting conditions (Zubieta *et al*, 1999), potentially increasing the variance of the measures. In contrast, the present study and Scott *et al.* (2006) included only males. Participants in this study also had average peak nicotine levels above 16 ng/mL, while in the study by Kuwabara *et al*. (2014) nicotine levels appear to have peaked around 7 ng/mL. This may have been too low a level to show significant group differences in binding, as previous EEG studies have suggested that increases of plasma nicotine levels greater than 10 ng/mL are needed before brain waves are significantly altered by smoking (Kadoya *et al*, 1994). There is also evidence that smokers self-regulate smoking to obtain nicotine boosts of 10 ng/mL per cigarette, a level that may be necessary to obtain many of the positive subjective effects of nicotine (Russell *et al*, 1995). In addition, the present study acquired baseline measures after overnight abstinence without any intervention, while others (Kuwabara *et al*, 2014; Ray *et al*, 2011) used scans acquired after DN smoking as surrogate baselines, which may introduce an additional confound, as noted below. Similarly to the present report, Kuwabara *et al*. (2014) observed negative correlations between MOR BP_{ND} and Fagerström score during the DN condition, albeit in different regions.

The observed reductions in baseline MOR BP_{ND} would be consistent with a chronic activation of this neurotransmitter system as a result of smoking and subsequent receptor downregulation, effects similar to those observed for dopamine and dopamine D2 receptors (Brody *et al*, 2004). It is also possible that overnight nicotine abstinence induced acute increases in endogenous opioid production, as suggested by data in animal models (Houdi *et al*, 1998; Isola *et al*, 2002), further reducing the receptor availability measures acquired in vivo.

In animal models, chronic exposure to nicotine has been shown to reduce MOR concentrations, an effect that was linked to the development of tolerance to nicotine (Galeote *et*

al, 2006). However, the opposite effect has also been observed, with increases in MOR receptor concentrations after chronic administration that were associated with nicotine-induced reward conditioning (Walters *et al*, 2005). These upregulatory effects have also been shown to be sex dependent, more prominent in female than in male rodents, and associated with reductions in intracellular content of met-enkephalin (Wewers *et al*, 1999).

Smoking status also interacted with *OPRM1* A118G genotype, whereby reductions in MOR BP_{ND} were observed in G allele carriers but only in smokers, not in non-smoking controls, similar to data presented in Ray et al. (2011). Presence of the G allele has been associated with lower levels of MOR mRNA expression in human post-mortem tissue (Zhang *et al*, 2005), in cultured cells (Kroslak *et al*, 2007), and in animal models (Mague *et al*, 2009; Wang *et al*, 2012). In larger healthy control samples, G allele carriers have also shown reductions in MOR BP_{ND} in multiple cortical and subcortical brain regions, when compared to AA homozygotes (Pecina *et al*, 2015b). The significant interaction between smoking status and genotype found in this study are therefore likely to represent an additive effect of chronic smoking and G-allele reducing MOR availability, which would not be present in the control group and may not have been detected with the small control sample sizes employed in this report and in Ray et al. (2011). Peciña et al. (2015b) studied a much larger healthy control sample, but did not include smokers. In smokers, lower levels of receptor availability were associated with higher Fagerström nicotine dependence scores and higher craving ratings in both the present report and in Kuwabara et al. (2014). In non-smokers, lower regional MOR BP_{ND} values in G-allele carriers have been linked to higher trait NEO Neuroticism scores, and specifically to the two subscales Vulnerability to Stress and Depression (Pecina *et al*, 2015b).

During the smoking phases of the study, both DN and N cigarettes significantly reduced craving after overnight abstinence, an observation that parallels the results of prior studies examining the effect of DN and N smoking on subjective reports of tobacco withdrawal symptoms, including craving (Butschky *et al*, 1995; Pickworth *et al*, 1999). The non-nicotine effects of smoking were highlighted in a recent study showing that smokers would preferentially self-administer DN cigarette puffs over intravenous nicotine (Rose *et al*, 2010), suggesting the importance of secondary, nicotine non-specific, reinforcing effects of smoking, which include sensory aspects such as the taste, smell, and feel of the cigarette smoke. In our study we observed lower craving levels immediately prior to and after N smoking compared to the same periods in the preceding DN scans, further suggesting that smoking the DN cigarette after overnight abstinence had substantial effects that carried over into the subsequent N smoking scanning period. The effects of both DN and N smoking were also dependent on the severity of nicotine addiction, with higher Fagerström scores associated with greater craving after overnight abstinence and more profound reductions in craving ratings after both DN and N cigarettes.

These non-specific effects of smoking, observed at the behavioral level, were paralleled by the results of the molecular measures acquired. In the experimental design employed, baselines were acquired prior to DN and N smoking acquisitions, which were not randomized in order. The rationale for this design was that the baseline periods, acquired early in the scan, would allow for the assessment of a "true" baseline prior to the introduction of any challenge. Comparisons between the effects of DN and N smoking can then be carried out across early periods as well as across late periods with similar signal to noise ratios, as tracer decay may differentially affect BP_{ND} estimates acquired early and late during scanning. Potential carry-over

effects of the DN challenge would then be reflected in the following baseline, while N smoking, expected to have substantial carry over effects, would not affect DN BP_{ND} estimates.

We observed significant reductions in MOR BP_{ND} from the period preceding compared to that following DN smoking in the THA and AMY, suggesting that DN smoking alone elicited a substantial release of endogenous opioid peptides. In parallel, and accounting for differences in baseline values across scans, relatively greater activation of endogenous opioid neurotransmission was observed for DN, in relation to N smoking during the late scanning periods. In the THA, Fagerström scores were correlated with activation of neurotransmission after smoking both the DN and the N cigarettes. The change in craving ratings was likewise associated with differential levels of activation in neurotransmission after DN smoking in the THA.

These results then suggest that after overnight abstinence, when craving is highest, nicotine non-specific effects are prominent, inducing the release of endogenous opioids and μ-opioid receptor activation, which in the present study largely obscured additional effects of nicotine during N smoking, and are consistent with prior experimental observations (Butschky *et al*, 1995; Pickworth *et al*, 1999; Rose *et al*, 2010).

MORs in the THA and AMY, regions implicated in the regulation of sensory and emotionally relevant information (Gallagher and Chiba, 1996; Tyll *et al*, 2011), are known to be implicated in homeostatic responses of the organism to salient environmental cues. In the context of pain, but also in Major Depression, activation of MOR-mediated neurotransmission by expectations of symptom relief during placebo administration has been observed in these and other brain regions (Pecina *et al*, 2015a; Scott *et al*, 2008; Zubieta *et al*, 2005). It is therefore likely that DN smoking, particularly after overnight abstinence, when nicotine withdrawal

distress and craving are high, would represent a strong environmental cue that induced a potent activation of MOR-mediated neurotransmission independent of nicotine content in the cigarettes.

These observations are relevant for the treatment of nicotine addiction. They would imply that under similar conditions of taste and appearance, reductions in nicotine content in cigarettes, particularly those consumed during early abstinence, would be possible and still afford craving relief in nicotine-addicted individuals. This would allow for progressive reductions in nicotine consumed shortly after awakening, one of the hallmarks of nicotine dependence.

Further exploration of these processes appears warranted, given the high retention of addiction among heavy smokers. The results acquired in this study highlight the importance of non-specific elements of cigarette smoking. Future studies examining the effects of nicotine and smoking on brain function should take these processes into account by using sham comparison controls and employing full randomization of DN and N smoking.

FIGURE LEGENDS

Figure 3.1: Experimental design. Participants were scanned on two separate days, receiving one RCL and one CFN scan each day. Scanning was counterbalanced between participants who either went through Protocol A their first day and Protocol B their second day or vice versa. Participants began smoking the DN cigarette on minute 43 of the first scan and the N cigarette on minute 43 of the second scan.

Figure 3.2: Relationship between MOR BP and smoking status. a) Regions where MOR BP_{ND} is greater in controls than in overnight abstinent smokers. b) Cluster size, cluster-level FWE-corrected p-value, Z-value, and coordinates of regions. c) Plot showing Pearson correlations between MOR BP_{ND} and craving or Fagerström score in overnight abstinent smokers. * Regions where FWE corrected cluster significance is <0.05.

Figure 3.3: Interaction between smoking status and A118G genotype. a) Regions where AA individuals have higher baseline MOR BP_{ND} than *G individuals. b) Regions where there is an interaction between group and genotype. c) Bar graph of the mean baseline MOR BP_{ND} ±1 SEM for regions shown in 3.3b. In the left nucleus accumbens (L NAC) and right nucleus accumbens/amygdala (R NAC/AMY), the presence of the 118G allele was associated with decreased BP_{ND} in smokers, but not in controls. d) Table showing cluster size, cluster-level FWE-corrected p-value, Z-value, and coordinates of regions showing an interaction between group and genotype. * Regions where FWE corrected cluster significance is <0.05.

Figure 3.4: Change in craving and plasma nicotine levels in response to DN and N smoking. a) Mean visual analog scale ratings for craving (from 1-10, with 10 being highest craving). Ratings were taken before each DN scan (Pre-scan), at 30 min into the DN and N scans (before receiving the cigarettes: Pre-DN, Pre-N), and at 60 minutes into the DN and N scans, (after receiving the cigarettes: DN, N). b) Mean plasma nicotine levels (ng/mL) after smoking either the DN or the N cigarettes. Error bars show ±1 SEM. *p<0.01. **p≤0.001.

Figure 3.5: Effects of DN smoking. a) Brain regions showing greater MOR BP_{ND} before the DN cigarette than after the DN cigarette. b) Cluster size, cluster-level FWE-corrected p-value, Z-value, and coordinates of the regions of interest shown in Figure 3.5a, as well as regions where the change in BP from the early to the late portion of the scan differs between the DN and N scans ((early-late) D > (early-late) N) (Figure 3.6). * Regions where FWE corrected cluster significance is <0.05.

Figure 3.6: Effects of N smoking compared to DN smoking. a) Regions where the increase in MOR BP_{ND} from the early (min 10-40) to the late (min 45-90) portion of the scan is greater in N scans than in DN scans. b) MOR BP_{ND} in the left amygdala and thalamus during the early and late portions of both the DN and the N scans. c) Change in MOR BP_{ND} from the early to the late portion of the DN and N scans, and for the baseline scans of the healthy controls (HC). Error bars show ±1 SEM.

FIGURES

Figure 3.1: Experimental design

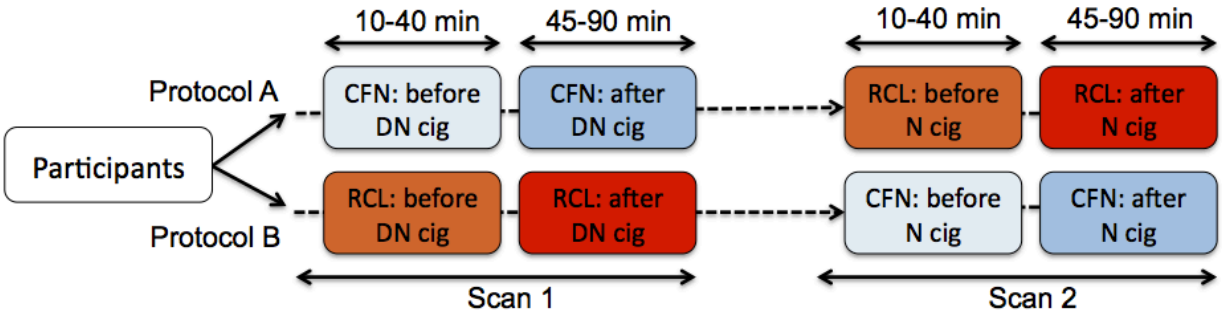
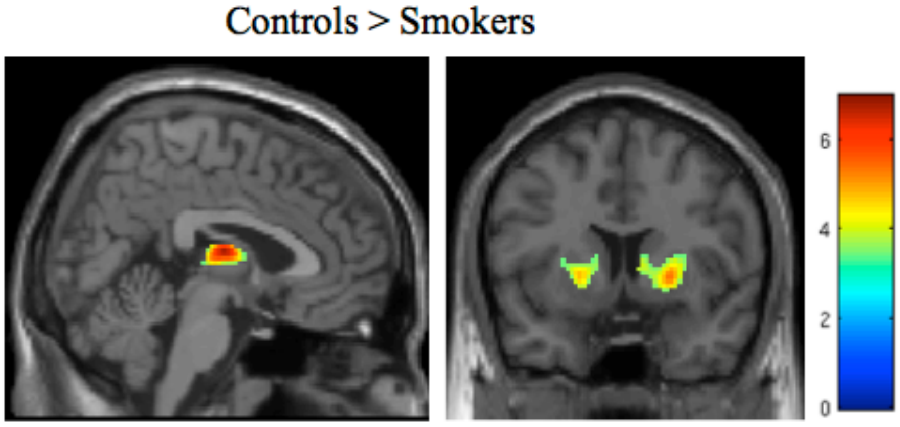


Figure 3.2: Relationship between MOR BP and smoking status

a)



b)

Regions	Cluster size (mm^3)	P-value (cluster level, FWE corrected)	Z-value	x y z (mm)
Controls > Smokers				
L BG	2216	0.055	4.14	-20 6 0
R BG	3976	*0.007	4.62	24 6 0
THA	3496	*0.012	5.5	2 -12 10

c)

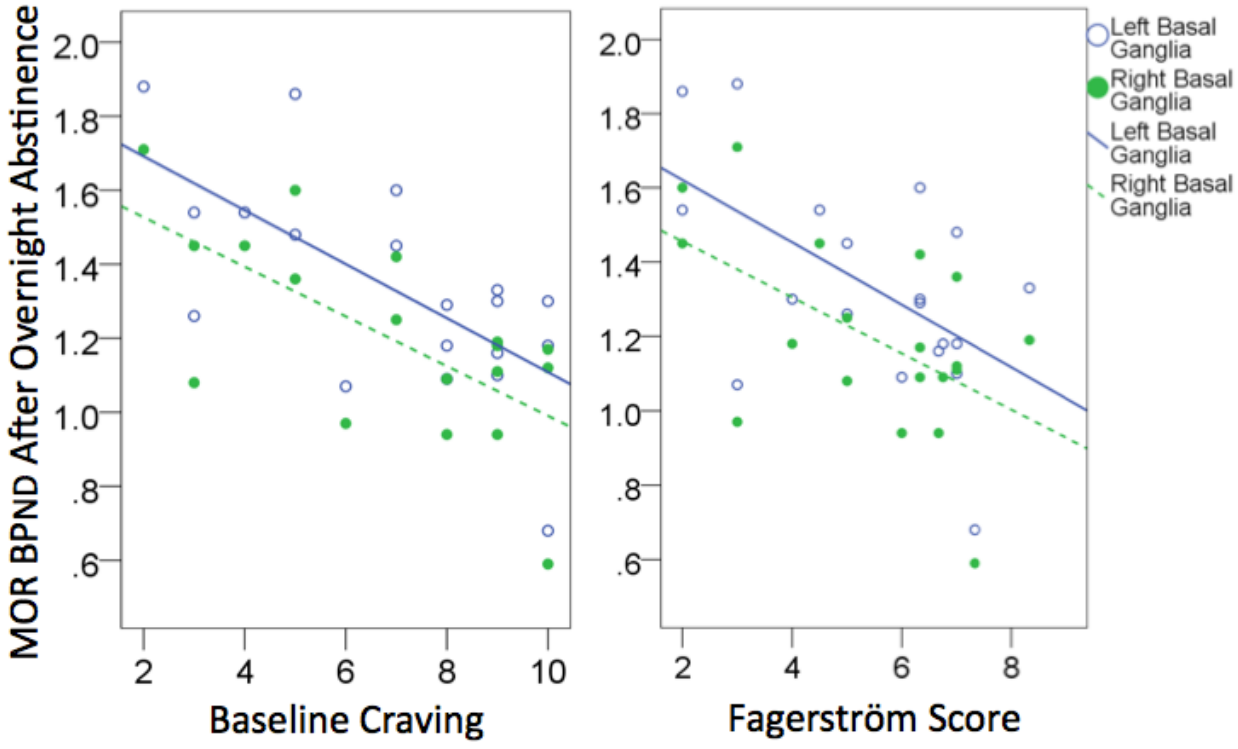
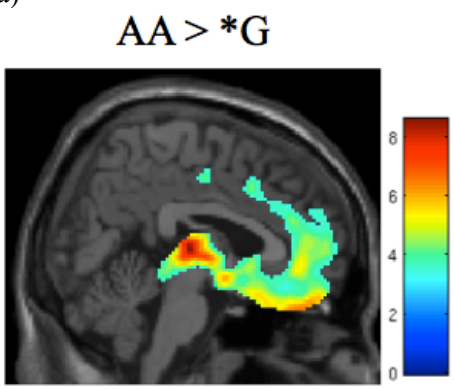
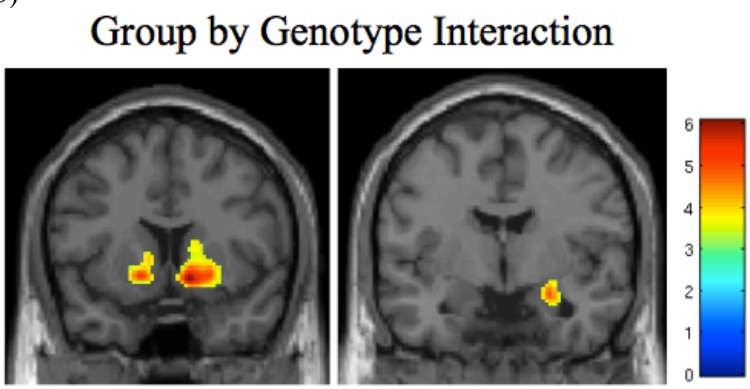

MOR BPND After Overnight Abstinence
2.0
1.8
1.6
1.4
1.2
1.0
.8
.6
2
4
6
8
10
Baseline Craving
2
4
6
8
Fagerström Score
Left Basal Ganglia
Right Basal Ganglia
Left Basal Ganglia
Right Basal Ganglia

Figure 3.3: Interaction between smoking status and A118G genotype

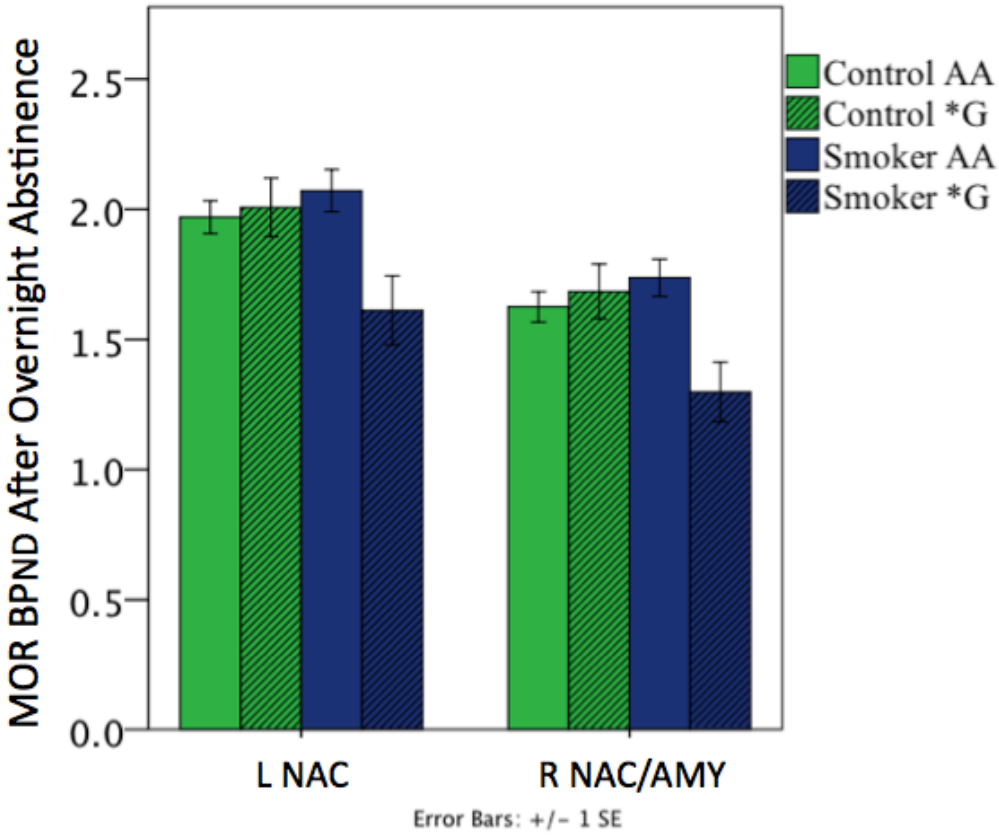
a)



b)



c)

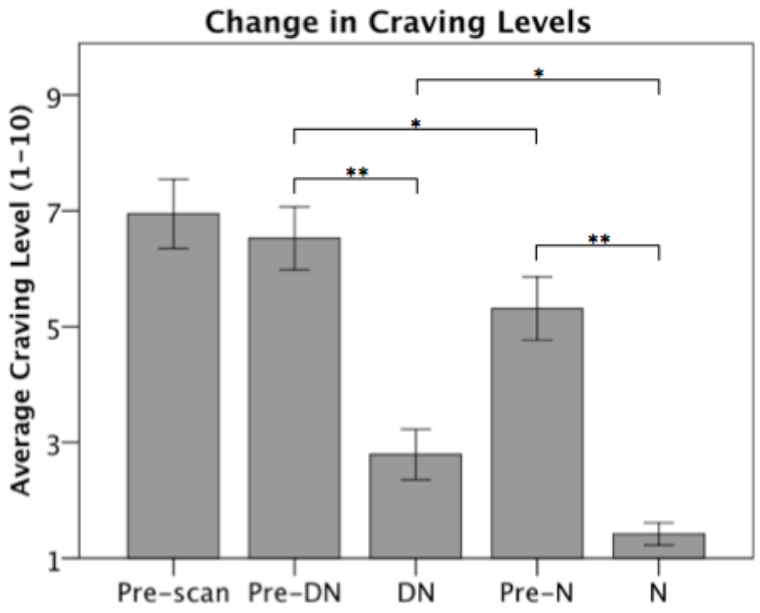


d)

Regions	**Cluster size (mm^3)**	**P-value (cluster level, FWE corrected)**	**Z-value**	**x y z (mm)**
Group by Genotype Interaction				
L NAC	2152	0.059	4.54	-14 6 -8
R NAC merging into R AMY	7064	* <0.001	5.01	12 10 -8

Figure 3.4: Change in craving and plasma nicotine levels in response to DN and N smoking

a)



b)

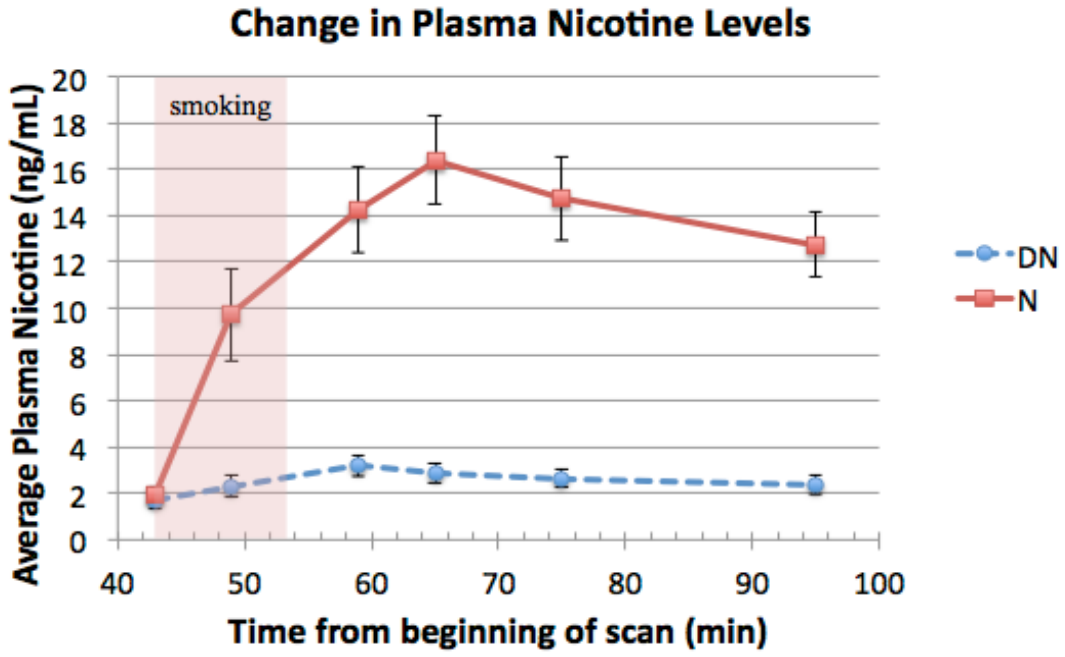
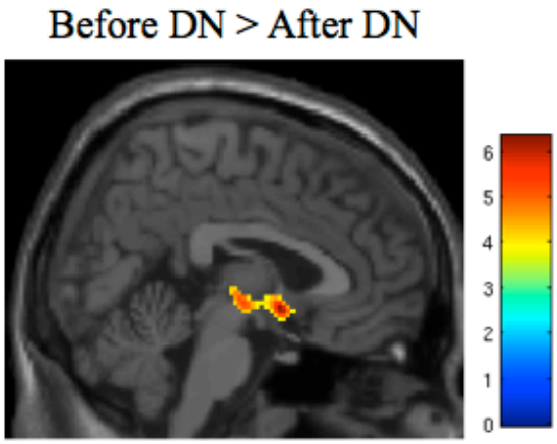


Figure 3.5: Effects of DN smoking

a)

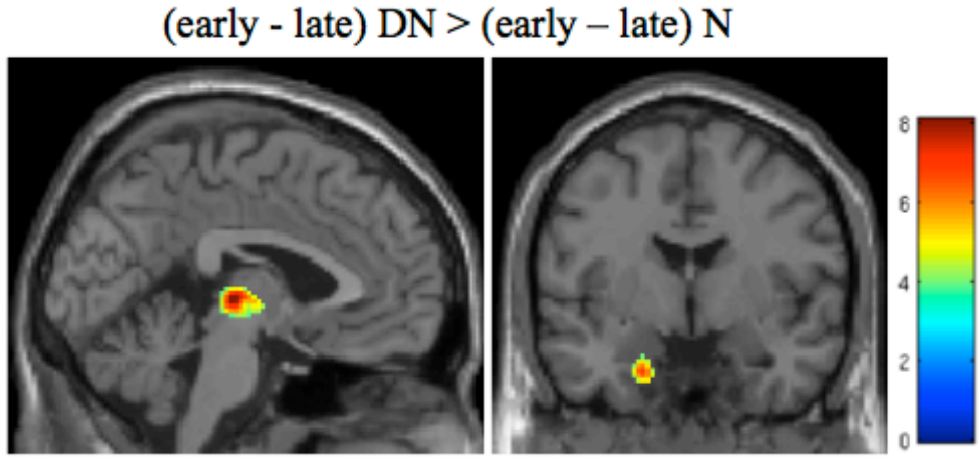


b)

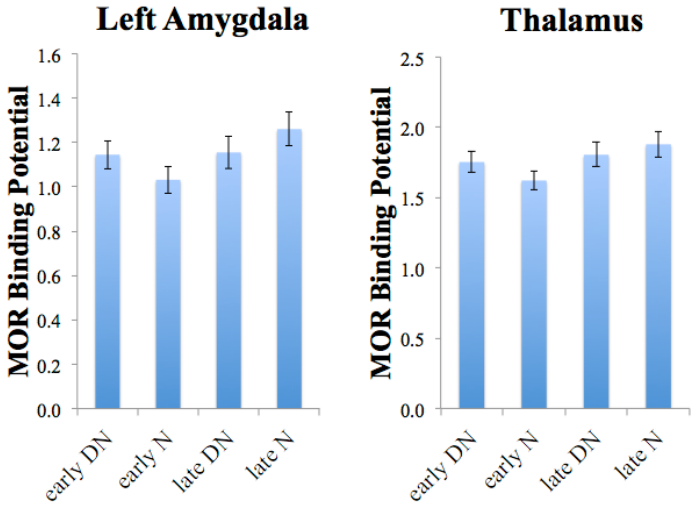
Regions	Cluster size (mm^3)	P-value (cluster level, FWE corrected)	Z-value	x y z (mm)
Before DN > After DN				
R NAC merging into THA	2288	*0.002	4.28	6 4 -6
(early-late) D > (early-late) N				
L AMY	528	0.054	4.48	-20 -2 -30
THA	1624	* <0.001	4.95	-2 -20 2

Figure 3.6: Effects of N smoking compared to DN smoking

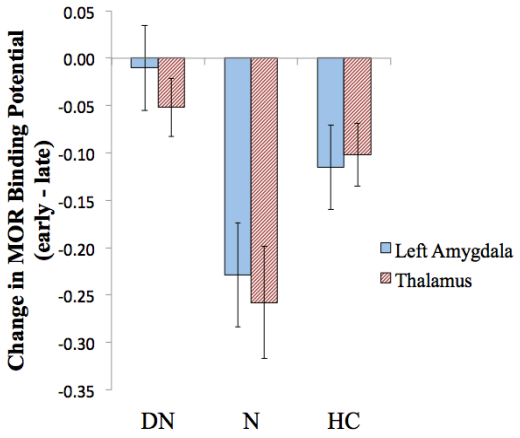
a)



b)



c)



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CHAPTER IV

Interactions of Acute and Chronic Opioid Administration with Endogenous Opioid System Function in Chronic Back Pain Patients[3]

ABSTRACT

One-third of American adults suffer from chronic pain, and many are treated with opioid analgesics. Opioid drugs have proven useful for controlling acute pain, but chronic use may lead to pain chronicity as well as tolerance and dependence, and few studies have examined the effects of long-term opioid use on the brain. Determining the neurobiological consequences of long-term use and identifying characteristics that predict individual responsiveness to opioid medication would inform clinicians treating chronic pain.

We hypothesized that individual differences in MOR function would relate to a patient's response to the opioid drug fentanyl, and that long-term opioid use would be associated with decreased μ-opioid receptor (MOR) function. We used positron emission tomography with the radiotracer ^{11}C-carfentanil to compare the μ-opioid systems of chronic non-neuropathic back pain patients taking opioids long-term (CNBP+O) with those not taking opioids (CNBP-O). We also related CNBP-O patients' subjective responses to fentanyl with measures of their endogenous opioid system function.

Our results demonstrated that individuals that showed decreased pain-induced activation of MOR neurotransmission in the thalamus, left nucleus accumbens (NAC), and left amygdala

[3] This work reflects collaboration with Dr. Martikainen, Chelsea Cummiford, Dr. Love, Dr. Green, and Dr. Zubieta at the University of Michigan, Dr. Stohler, at Columbia University, and Dr. Greenwald at Wayne State University

(AMY) reported stronger "good drug effects" when given fentanyl. Compared to CNBP+O subjects, CNBP-O subjects had higher baseline MOR binding in the left nucleus accumbens (NAC). During a sustained pain challenge, CNBP-O subjects showed greater MOR activation in the left NAC and right amygdala. Further information on the relationships between opioid use and μ-opioid system function is valuable for determining the benefits and risks of opioid analgesics.

INTRODUCTION

Chronic pain is one of the most prevalent medical issues affecting us today, with recent estimates suggesting that approximately 37% of adults suffer from some form of persistent pain state (Tsang *et al*, 2008). Despite the large number of people affected, available treatments remain largely unsatisfactory. Opioid analgesics are one of the most common treatments and are a well-proven therapy during short-term use. However, despite their increasing use (Boudreau *et al*, 2009), they are of questionable or unproven efficacy during chronic administration (Martell *et al*, 2007) and can be associated with tolerance over time, leading to higher dosing and the potential development of opioid misuse and abuse (Compton and Volkow, 2006). In addition, opioid analgesics have in some preclinical and clinical studies been associated with the development of hyperalgesia (Chang *et al*, 2007; Tompkins and Campbell, 2011).

Opioid medications currently in use are agonists of the μ-opioid receptor (MOR), which mediates both their analgesic and tolerance effects. This receptor system forms part of the endogenous opioid mechanisms implicated in the internal regulation of stressors, including the experience of pain in both its sensory and affective-emotional domains (Akil *et al*, 1972; Zubieta *et al*, 2001). The acute administration of opioid drugs effectively activates these receptors,

however the effects of their chronic administration have not been well studied in humans (Fields, 2011; Sullivan and Howe, 2013). Chronic opioid treatment induces the downregulation of MORs and their transduction mechanisms in animal models, an effect that has been associated with tolerance and reduced analgesic efficacy (Williams *et al*, 2013). There is also some suggestion that chronic opioid administration impairs the production of endogenous opioid peptides in rodents (Gudehithlu *et al*, 1991; Van Bockstaele *et al*, 2000), which in turn may impair endogenous pain and emotion regulatory mechanisms.

In addition to their role in pain regulation, MORs are critically implicated in the reinforcement and addiction potential of opioid medications (reviewed in Trigo *et al*, 2010). The euphoric effect elicited by opioids, high addiction potential, and their wide availability has contributed to prescription opioids being the second most commonly abused drugs in the US (Wilson, 2007). Potential misuse and abuse of opioids is of particular concern and difficult to address in chronic pain patients, in whom both positive (analgesia) and negative effects (tolerance and dependence) may manifest (see Fields, 2011; Garland *et al*, 2013; Sullivan *et al*, 2013). The dysregulation of this neurotransmitter system may also have implications for domains beyond pain, as it has been centrally implicated in social behavior (Hsu *et al*, 2013; Panksepp *et al*, 1980), as well as in the pathophysiology of mood and anxiety disorders (Hsu *et al*, 2015; Kennedy *et al*, 2006; Liberzon *et al*, 2007; Pecina *et al*, 2015a), which are themselves highly comorbid with persistent pain syndromes.

There are also alterations in opioid receptor availability and endogenous opioid system function in persistent pain syndromes, with decreased baseline opioid receptor availability reported in neuropathic pain syndromes (Jones *et al*, 2004; Maarrawi *et al*, 2007; Willoch *et al*, 2004) and fibromyalgia (Harris *et al*, 2007), but also both regional upregulations of MOR

availability and reductions in endogenous opioid system function in non-neuropathic back pain (CNBP) (Martikainen *et al*, 2013). These variations in MOR availability would be expected to induce variability in the acute effects of opioid drugs. However, this has not been studied directly in humans.

This study examines the subjective, acute effects of cumulative doses of fentanyl, a short-acting MOR agonist, as a function of receptor availability (non-displaceable binding potential, BP_{ND} (Innis *et al*, 2007)) in a sample of patients diagnosed with CNBP. This syndrome has been previously associated with increases in MOR availability in the thalamus and amygdala, but with substantial interindividual variability (Martikainen *et al*, 2013). In addition, we examined the effects of chronic opioid administration on MOR BP_{ND} and the responses of the endogenous opioid system to an experimental sustained pain challenge in CNBP patients treated chronically with those medications.

We hypothesized that the subjective effects of fentanyl would be positively associated with receptor availability in CNBP patients. In addition, it was believed that the chronic administration of opioid drugs would induce reductions in MOR BP_{ND}, potentially through a combination of occupancy and downregulation. Chronic opioid use was also hypothesized to lead to a dysregulation of endogenous opioid system function, resulting in reduced opioid release during an experimental pain challenge.

PATIENTS AND METHODS

Participants

Participants were 50 right-handed individuals between 20 and 50 years old (mean (±SD) age=38.5±9.4 years) diagnosed with CNBP and recruited through a specialized pain clinic at a

university hospital. Eligible patients reported back pain for at least one year with a pain rating between 3 and 8 on a visual analog scale (VAS) of 1 to 10. Average baseline back/neck pain intensity was 57.3±23.3 on a VAS of 0 to 100. Participants were excluded if they had a current or past history of neurological or psychiatric disorders other than depression or anxiety. Other exclusionary criteria included the current use of recreational drugs, exercising more than an hour a day, and drinking more than 10 units of alcohol per week. Participants were grouped into those who had not taken regular opioid medication for at least the past year (CNBP-O) and those who were currently taking opioid analgesics and had been taking them for a minimum of one year (CNBP+O) at stable doses. In this group current daily doses, in morphine equivalents, were 36.1±28.4 mg/day (range 6.8-100 mg/day). CNBP+O volunteers were instructed to take their morning dose of medications as usual. All studies were conducted in the afternoon, with the first scanning period starting at 1:30 PM.

Written informed consent was obtained from all patients, and the study was approved by the Institutional Review Board and the Radioactive Drug Research Committee. The protocol was in accordance with the Declaration of Helsinki.

Study design

Subjects completed two 90-minute positron emission tomography (PET) scans using the MOR selective radiotracer ^{11}C-carfentanil. The two scans, a baseline and a scan that included an experimental pain challenge, were conducted in a randomized and counterbalanced order (Figure 4.1). Scanning protocols were identical to those reported previously (Martikainen *et al*, 2013). A subset of CNBP-O patients also underwent fentanyl administrations on a separate day.

Pain challenge

During the pain challenge scan, subjects received two 20-minute IM saline injections in the masseter muscle via a computer-controlled closed-loop system (Stohler and Kowalski, 1999; Zhang *et al*, 1993): a nonpainful 0.9% isotonic saline injection, and a painful 5% hypertonic saline injection. Subjects were blind to the order of administration or the lateralization of the challenge. However, to simplify data analyses they always received the isotonic saline in the right masseter during the early portion of the pain scan (minutes 5-25) (a control, pain expectation condition). Hypertonic saline was injected into the left masseter muscle during minutes 45-65 of the scan. This method of sustained painful stimulation during PET allows the determination of acute changes in MOR BP_{ND} associated with the activation of endogenous opioid release, measures which are related to individual assessments of the pain experience (Scott *et al*, 2006; Zubieta *et al*, 2001). VAS ratings of pain (0 no pain, 100 most pain imaginable) were acquired via an electronic VAS placed in front of the scanner gantry and the infusion rate adjusted to maintain a sustained pain at moderate intensity (43.7±18.1). This feedback mechanism ensured that the pain intensity was standardized across individual subjects and subject groups (Stohler *et al*, 1999).

Subjective assessments

Measures of clinical pain included the McGill Pain Questionnaire (MPQ; Melzack and Torgerson, 1971), the Pain Catastrophizing Scale (PCS; Sullivan *et al*, 1995), the Beliefs in Pain Control Questionnaire (Internal Locus of Control, I-LOC BPCQ; Skevington, 1990), the Center for Epidemiological Studies Depression scale (CESD; McCallum *et al*, 1995), the Positive and Negative Affect Schedule (PANAS; Watson and Clark, 1999), and 0-100 VAS ratings of back/neck pain intensity and unpleasantness, acquired prior to scanning. Ratings of experimental

pain included MPQ and 0-100 VAS ratings of intensity and unpleasantness acquired after completion of the masseter pain challenge, and PANAS ratings, collected before and after (min 35, min 75) the experimental pain challenge.

Scanning protocol and data acquisition

PET scans were acquired with a Siemens HR+ scanner (Knoxville, TN) in 3D mode with septa retracted and scatter correction (reconstructed full width at half-maximum (FWHM) resolution 5.5 mm in-plane and 5.0 mm axially). ^{11}C-carfentanil was synthesized at high specific activity (>2000 Ci/mmol) by the reaction of ^{11}C-methyl triflate with desmethyl carfentanil as previously described (Dannals *et al*, 1985; Jewett, 2001). Half of the ^{11}C-carfentanil dose was administered as a bolus, and half as a continuous infusion, to more rapidly achieve steady state levels.

Participants lay supine in the scanner, with a light forehead restraint to reduce head movement. Intravenous (antecubital) lines were placed in both arms, one to administer the ^{11}C-carfentanil, and one for blood sampling. Prior to scanning, 23-gauge needles were placed bilaterally in the masseter muscles of each participant and connected to either the isotonic or hypertonic saline.

A high-resolution anatomical T1-weighted magnetic resonance (MR) image was also obtained for each participant for the purposes of spatial normalization. Images were collected on a Sigma LX 3T scanner (Sigma LX; General Electric, Milwaukee, WI), with 3D inversion recovery-prepared fast spoiled gradient recalled (SPGR) acquisition (echo time=1.9 ms; repetition time=9.2 ms; inversion time=500 ms; flip angle=15º; bandwidth=16 kHz; number of excitations=1; 256x256 matrix; field of view=25/26 cm; number of contiguous images=154; isotropic voxel size=1 mm).

Fentanyl procedure

This procedure was designed to assess the subjective effects of fentanyl in CNBP-O patients, and test whether these were associated with PET MOR functional measures. Subjects received a total of four 10 mL IV infusions 20 minutes apart, with each infusion lasting two minutes. A placebo infusion of 0.9% NaCl was always given first, followed by three cumulative infusions of fentanyl in 0.9% NaCl. For each fentanyl infusion, participants were given a dose of 0.1 mg/70 kg (1.43 mcg/kg). Participants were told that any of the 4 infusions could be either a placebo or fentanyl. Participants were asked to rate on a scale of 0 ("not at all") to 4 ("extremely") how much they felt symptoms such as "coasting", "backache", and "relaxed", and completed a series of visual analog scales asking them to rate their moods/feelings ("any drug effect", "good drug effect", "bad drug effect", "high", "like the drug effect", "stimulated", and "sedated") on a 0-100 VAS. Scales were completed before the infusions began and 5 minutes after each infusion. After each infusion participants also chose whether they would prefer the drug they just received versus payments ranging from $0.25-$15.

Data analysis

Image preprocessing

PET images were reconstructed using iterative algorithms (brain mode; Fourier rebinning with ordered subsets-expectation maximization, four iterations, 16 subsets; no smoothing) into a 128x128 pixel matrix in a 28.8 cm diameter field of view. A 6-minute transmission scan (^{68}Ge source) run before the PET study was used for attenuation correction. Automated computer algorithms were used to correct for minor head motions during the scans, and images were then coregistered (Minoshima *et al*, 1993). Time points were decay-corrected during PET data reconstruction. Image data were transformed on a voxel-by-voxel basis into two sets of

parametric maps: (1) a tracer transport measure (K_1 ratio) and (2) a receptor-related measure during pain expectation and pain (non-displaceable binding potential, BP_{ND}).

A modified Logan graphical analysis (Logan *et al*, 1996) using the occipital cortex as a reference region was used to calculate tracer transport and BP_{ND}. A 6 mm FWHM Gaussian filter was applied to each scan, and scans were divided into early (minutes 5-40) and late (minutes 45-90) sessions and coregistered to the participant's anatomical image using Matlab (MathWorks, Natick, Massachusetts) and SPM8 (Wellcome Trust Center for Neuroimaging, London, United Kingdom) software. Images were then warped using VBM8 Toolbox (Christian Gaser, University of Jena, Germany) within SPM8 to match Montreal Neurological Institute (MNI) stereotactic atlas orientation with individual quality checks.

Comparison of PET data: CNBP-O vs CNBP+O

Fifty individuals participated in the PET portion of this study. This included 29 CNBP-O patients (14 males) and 21 CNBP+O patients (6 males). One CNBP-O subjects and three CNBP+O subjects had technical problems during the pain scans, and were not included in those analyses. Voxel by voxel analyses were performed using SPM8, with independent-sample t-tests used to compare MOR function between CNBP-O and CNBP+O participants. Fagerström scores, a measure of nicotine dependence (Heatherton *et al*, 1991), were used as a covariate to control for smoking status. Age and scan order were also included as covariates. Baseline BP_{ND} was determined using data from min 45-90 of each participant's baseline scan, while reductions in MOR BP_{ND} during the sustained pain challenge compared to the baseline scan were used as a measure of MOR activation. Voxel by voxel regression analyses were also conducted in a

combined group of CNBP subjects to determine associations between selected psychophysical measures and MOR measures.

For these and subsequent PET analyses, statistical significance was determined using Monte Carlo simulations with p=0.0001 and an alpha of 0.05. Individual MOR BP_{ND} values were extracted for each region of interest using MarsBaR and further analyses were conducted with SPSS.

Fentanyl procedure

Seventeen CNBP-O subjects completed the fentanyl procedure (6 males, 11 females; age=34.4±10.1 years). The acute effect of fentanyl was shown by comparing drug effects felt after the placebo vs the first dose of fentanyl via paired t-tests. The Benjamini-Hochberg procedure was used to control for a false discovery rate of 0.05. To examine whether endogenous opioid function could be related to fentanyl response, we tested whether the change in the 7 VAS scales after receiving fentanyl vs placebo, as well as the change in "backache", were correlated with subjects' MOR function at baseline and during pain. One of the subjects that received fentanyl did not complete the PET scans and was not included in these analyses.

RESULTS

Acute opioid effects in CNBP-O patients: Fentanyl procedure

Significant increases in "high", "coasting", "drunken", "rush", "soapbox (talkative)", and "friendly" ratings were observed after fentanyl compared to placebo, while a reduction in "backache" ratings was observed (Figure 4.2a). The subjective monetary value of the drug (amount of money in $) also increased significantly after fentanyl administration, as did

participant VAS ratings of "any drug effect", "good drug effect", "like the drug effect", "high", "stimulated", and "sedated" (Figure 4.2b).

No statistically significant correlations were found between baseline MOR BP_{ND} and VAS ratings of drug effects or backache. However, significant negative associations were observed between the change in MOR BP_{ND} during the sustained pain challenge, a measure of endogenous opioid system activation, and participants' ratings of "good drug effect". These associations were localized in the left nucleus accumbens (NAC), thalamus, and left amygdala (AMY) (Figure 4.2c,d; Table 4.1). Similar regions showed correlations with VAS "liked the drug effect", but only the thalamus reached statistical significance (Table 4.1). VAS ratings of "good drug effect" and "liked the drug effect" were highly correlated ($r(14)=0.91$, $p<0.001$), but these were not associated with ratings of back pain intensity. Of the significant regions above, the change in BP_{ND} in the left amygdala was the only one also associated with baseline ratings of back pain intensity ($r(14)=-0.65$, $p=0.007$).

Chronic effects of opioid administration: CNBP-O vs CNBP+O

There were no significant group differences in baseline back pain ratings (VAS pain intensity, VAS unpleasantness, MPQ (total, sensory and pain affect subscales), PCS total, I-LOC BPCQ, CESD, or PANAS scores. CNBP+O patients were older on average than CNBP-O patients (42.7±7.6 years vs. 35.4±9.5 years; $t(48)=-2.87$, $p=0.006$), and age was included as a covariate in subsequent analyses.

Baseline MOR BP_{ND}

CNBP+O subjects showed localized reductions in baseline MOR BP_{ND} in the left nucleus accumbens/ventral pallidum (NAC/VP), compared to CNBP-O subjects (Figure 4.3a; Table 4.2).

There were no regions where CNBP+O patients showed higher MOR BP_{ND} values than CNBP-O patients.

Across groups, MOR BP_{ND} in a region that incorporated the left NAC/VP and hypothalamus (HTH) was positively associated to subjective reports of masseter muscle pain intensity and unpleasantness during the experimental challenge (Figure 4.3b,d; Table 4.2). Positive associations were also observed between baseline BP_{ND} and the I-LOC of the BPCQ in the dorsal cingulate and the medial thalamus bilaterally (Figure 4.3c,e) and between baseline PANAS positive affect scores and MOR BP_{ND} in the right basal ganglia (Table 4.2). There were no associations between current opioid dose in morphine equivalents and baseline MOR $BP_{ND.}$

Activation of endogenous opioid neurotransmission during experimental pain

During the experimental pain challenge, CNPB+O subjects reported higher back pain unpleasantness scores than CNBP-O subjects ($t(44)=-2.48$, $p=0.013$). Other pain measures examined did not show statistically significant differences, although back pain intensity ($t(44)=-1.96$, $p=0.056$) and MPQ masseter pain sensory scores ($t(44)=-1.94$, $p=0.059$) approached significance, with CNPB+O subjects showing higher pain ratings. There were no group differences in changes in PANAS positive or negative affect during experimental pain.

CNBP-O subjects showed greater experimental pain-induced activation of endogenous opioid neurotransmission (reductions in BP_{ND} from baseline to pain challenge scans) in the left NAC and the right AMY (Figure 4.4a,b; Table 4.3). There were no regions where CNBP+O patients showed greater MOR activation than the CNBP-O sample during the experimental pain. Across subjects, baseline ratings of back pain intensity and unpleasantness were negatively associated with experimental pain-induced MOR system activation in the left amygdala (Figure

4.4c,d). Experimental pain-induced MOR system activation was also negatively correlated with back pain MPQ pain affect subscores in the left NAC (Table 4.3).

DISCUSSION

This paper examines interindividual variations in the effects of opioids administered acutely or chronically in patients diagnosed with CNBP. We related the responses of patients to two functional measures of the MOR system: MOR BP_{ND} at baseline, and endogenous opioid release when given a standardized sustained pain experimental challenge. When CNBP-O volunteers were administered fentanyl during the acute studies, their behavioral and back pain ratings were not associated with global or regional MOR concentrations. However, greater hedonic "good drug" and "liked the drug" effects were negatively associated with the capacity to activate the endogenous opioid system in response to an experimental sustained pain challenge in the thalamus, NAC, and AMY. Individuals who released the most endogenous opioids when undergoing a pain stressor reported fewer positive effects of the opioid fentanyl, while those showing a decreased ability to release endogenous opioids reported greater positive effects.

In previous work, reductions in experimental pain-induced activation of endogenous opioid neurotransmission were detected in the AMY of CNBP-O patients in comparison to healthy controls, though with substantial interindividual variation (Martikainen *et al*, 2015; Martikainen *et al*, 2013). The capacity to release endogenous opioid during experimental pain in the AMY in CNBP-O patients was related to better clinical pain ratings and the maintenance of positive affect in these patients.

Consistent with those results, here we also show that the baseline ratings of back pain intensity acquired prior to the PET scans were associated with the capacity to activate

endogenous opioid neurotransmission during the experimental challenge. These data then confirm that decreased endogenous opioid system integrity in CNBP patients not treated with opioids is related to both higher clinical pain ratings, as previously shown, as well as to increased hedonic responses to exogenous opiate administration. Notably, these associations were observed in a circuit where endogenous opioids are involved not only in pain regulation (THA, NAC, AMY) (Zubieta *et al*, 2001, 2002), but also in hedonic responses to MOR agonists and the incentive value of rewards (Mahler and Berridge, 2012; Pecina and Berridge, 2005; Smith and Berridge, 2007; Trigo *et al*, 2010). These results therefore suggest that in addition to more effectively regulating chronic pain, the integrity of the endogenous opioid system (but not necessarily MOR concentrations in these regions) would be protective against an exaggerated reinforcement by exogenous opiates in CNBP patients.

Chronic treatment with opioids in CNBP+O volunteers, compared to data from CNBP-O participants, was associated with reductions in baseline BP_{ND} in the NAC, a critical part of the mesolimbic reward system (Trigo *et al*, 2010), but also implicated in the internal regulation of pain (Scott *et al*, 2008; Zubieta *et al*, 2001). Perhaps surprisingly, these effects were not generalized, but were exclusively localized in this region. Chronic opioid administration is known to induce tolerance to its effects over time. This may occur through downregulatory effects of this neurotransmitter system (e.g., desensitization of transduction mechanisms), but not necessarily through reductions in receptor concentrations, which are internalized and recycled by some (e.g., etorphine, methadone) but not other (e.g., morphine) opioids (Christie, 2008; Gabilondo *et al*, 1994; Keith *et al*, 1998; Whistler *et al*, 1999).

Higher MOR BP_{ND} values in the NAC of CNBP patients were positively associated with experimental pain intensity and unpleasantness ratings, but not with clinical pain scores. This

may suggest that lower endogenous opioid system function, reflected by a lesser capacity to engage this neurotransmitter system during the experimental pain challenge, was associated with some degree of compensatory receptor upregulation in this region. In other brain areas (thalamus, dorsal anterior cingulate) receptor BP_{ND} was, in contrast, associated with ratings of a measure of "internal locus of control" (I-LOC), reflecting the subjectively assessed capacity of the individual to modify their pain by their own actions. Higher I-LOC scores have previously been associated with lesser disability and better treatment outcomes (Cheng and Leung, 2000; Harkapaa, 1991; Harkapaa *et al*, 1991; Williams and Gracely, 2006). Higher MOR BP_{ND} in the ventral basal ganglia was also associated with the maintenance of positive affect.

Chronic opioid treatment in the CNBP+O sample was additionally associated with a lesser capacity to activate endogenous opioid neurotransmission during the sustained pain challenge, compared to the CNBP-O volunteers, in both the NAC and AMY. The capacity to activate endogenous opioid neurotransmission during experimental pain was associated with clinical pain intensity and unpleasantness ratings in the AMY and with back pain MPQ pain affect ratings in the NAC, replicating and extending previous studies (Martikainen *et al*, 2015; Martikainen *et al*, 2013). These results appear consistent with those obtained in animal models, whereby the chronic administration of opioids has been shown to reduce the production of endogenous opioid peptides, potentially through the suppression of endogenous opioid gene expression (Borsook *et al*, 1994; Gonzalez-Nunez *et al*, 2013).

In this manuscript we report that the capacity to experimentally activate endogenous opioid neurotransmission, a measure of the integrity of this neurotransmitter system, is associated with both decreased hedonic responses to a MOR agonist in patients not treated with opioids, and well as with less severe back pain ratings in the combined group of CNBP patients.

Further, we report that the chronic administration of opioids in CNBP patients is linked to both reductions in receptor availability and in the capacity to activate endogenous opioid neurotransmission in response to changes in pain signal. These effects were related to the clinical presentation of the volunteers and took place in regions including the NAC and AMY, which are critically involved in motivated behavior as well as in pain control mechanisms (Baliki *et al*, 2010; Baliki *et al*, 2013; Martikainen *et al*, 2015; Martikainen *et al*, 2013).

As prescription opioid abuse becomes an increasingly severe public health problem, a better understanding of the mechanisms that are implicated in opioid reinforcing behavior in humans is critical. We show that the function of the endogenous opioid system is of importance in these processes, as well as in pain control, and that chronic opioid usage interferes with these systems. The information presented here supports the examination of novel therapeutic agents that, instead of directly acting on MORs, would enhance endogenous opioid peptide function, activating these receptor sites in a more physiological manner. In addition, these data suggest that genetic variations which impact the function of this neurotransmitter (e.g. Pecina *et al*, 2015b; Zubieta *et al*, 2003) may also contribute to pain chronicity and the potential for opioid abuse among patients afflicted with persistent pain that are treated chronically with opioid medications.

TABLES

Table 4.1: Regions showing a significant negative correlation between experimental pain-induced MOR activation and the subjective effects of a dose of fentanyl.

Region	**Cluster size (mm^3)**	**T**	**Z**	**x y z (mm)**
Good drug effect: Negative correlation with MOR activation				
L NAC	544	6.13	4.21	-14 14 -2
L AMY	320	6.87	4.47	-22 -8 -18
THAL	632	7.1	4.55	2 -14 0
Liked the drug effect: Negative correlation with MOR activation				
THAL	248	5.94	4.13	2 -14 2

Table 4.2: Regions showing significant differences in baseline MOR BP_{ND} between CNBP-O and CNBP+O patients, as well as regions where correlations were found between psychophysical measures and MOR BP_{ND} at baseline.

Region	Cluster size (mm^3)	T	Z	x y z (mm)
Baseline: CNBP-O>CNBP+O				
L NAC/VP	360	4.94	4.4	-14 2 -4
Baseline: Positive correlation with I-LOC (BPCQ)				
dACC	368	5.27	4.64	-10 -4 46
L THAL	664	6.18	5.25	-6 -14 0
R THAL	416	5.96	5.1	8 -14 2
Baseline: Positive correlation with masseter muscle pain intensity				
NAC/VP/HTH	1248	6.33	5.31	-2 6 -6
Baseline: Positive correlation with masseter muscle pain unpleasantness				
NAC/VP/HTH	2744	7.17	5.81	-2 6 -8
Baseline: Positive correlation with baseline positive affect (PANAS)				
R BG	368	4.65	4.17	18 20 0

Table 4.3: Regions showing significant differences in experimental pain-induced MOR activation between CNBP-O and CNBP+O patients, as well as regions where correlations were found between psychophysical measures and MOR activation during pain.

Region	Cluster size (mm^3)	T	Z	x y z (mm)
Pain: CNBP-O>CNBP+O				
L NAC	272	5.35	4.63	-16 0 -8
R AMY	264	5.56	4.77	24 -2 -16
Pain: Negative correlation with baseline back pain intensity				
L AMY	448	6.51	5.42	-22 -4 -24
Pain: Negative correlation with baseline back pain unpleasantness				
L AMY	376	5.7	4.91	-22 -2 -24
Pain: Negative correlation with baseline affective back pain (MPQ)				
L NAC	512	6.03	5.1	-8 6 -8
Pain: Positive correlation with masseter muscle pain intensity				
R AMY	440	6.79	5.59	18 -2 -20

FIGURE LEGENDS

Figure 4.1: Experimental design. Each participant received both a baseline scan and a pain scan. The scan order was counterbalanced. Participants were injected with isotonic saline as a non-painful control during the first half of the pain scan; during the second half participants were injected with hypertonic saline to produce moderate pain.

Figure 4.2: Subjective responses to administration of the opioid agonist fentanyl, and correlations with MOR activation. Significant differences were seen when comparing subject ratings of a) symptoms (High: $t(15)=-6.98$, $p<0.001$; Coasting: $t(15)=-5.17$, $p<0.001$; Drunken: $t(15)=-4.04$, $p=0.001$; Rush: $t(15)=-3.60$, $p=0.003$; Soapbox (talkative): $t(15)=-3.03$, $p=0.008$; Friendly: $t(15)=-2.71$, $p=0.016$; Backache: $t(15)=2.71$, $p=0.016$) and b) VAS and dollar value ($) questionnaire responses (Any drug effect: $t(15)=-8.34$, $p<0.001$; Good drug effect: $t(15)=-8.74$, $p<0.001$; Like the drug effect: $t(15)=-7.81$, $p<0.001$; High: $t(15)=-7.14$, $p<0.001$; Stimulated: $t(15)=-4.00$, $p=0.001$; Sedated: $t(15)=-3.83$, $p=0.002$); Value ($): $t(15)=-6.02$, $p<0.001$) after subjects received their first dose of fentanyl compared to after the placebo dose. c) Regions where the change in MOR BP_{ND} (MOR activation) is negatively correlated with subjective ratings of fentanyl's "good drug effect" in the left nucleus accumbens, left amygdala, and thalamus. d) Representative scatter plot showing the negative correlation found in the left nucleus accumbens between the "good drug effect" of fentanyl and the change in MOR BP_{ND} from the baseline to the hypertonic saline scan ($r(14)=-0.74$, $p=0.001$).

Figure 4.3: Group differences and correlations relating to baseline MOR BP_{ND} in CNBP patients. a) Significant reductions in baseline MOR BP_{ND} in the left nucleus accumbens/ventral pallidum are seen in CNBP+O subjects, when compared to CNBP-O subjects. b) Positive correlations were seen between masseter muscle pain unpleasantness ratings and baseline MOR BP_{ND} in the nucleus accumbens/ventral pallidum/hypothalamus. c) Positive correlations were seen between the strength of an individual's internal locus of pain control (I-LOC) and baseline MOR BP_{ND} in the right and left thalamus and dorsal anterior cingulate cortex. d) Example scatter plot of each individual's baseline MOR BP_{ND} in the nucleus accumbens/ventral pallidum/hypothalamus, versus their masseter muscle pain unpleasantness rating ($r(46)=0.530$, $p=0.0001$). e) Example scatter plot of each individual's baseline MOR BP_{ND} in the dorsal anterior cingulate cortex, versus their I-LOC rating ($r(46)=0.60$, $p<0.0001$).

Figure 4.4: Group differences and correlations relating to MOR activation in CNBP patients. a) MOR activation during pain anticipation is greater in CNBP-O subjects than in CNBP+O subjects in the left nucleus accumbens and right amygdala. b) Example graph showing group differences in the change in MOR BP_{ND} during the pain challenge in the right amygdala. c) Negative correlations were seen between VAS ratings of back pain intensity and MOR activation in the left amygdala. d) Scatter plot showing baseline back pain intensity versus MOR activation in the left amygdala ($r(44)=-0.45$, $p=0.002$).

FIGURES

Figure 4.1: Experimental design

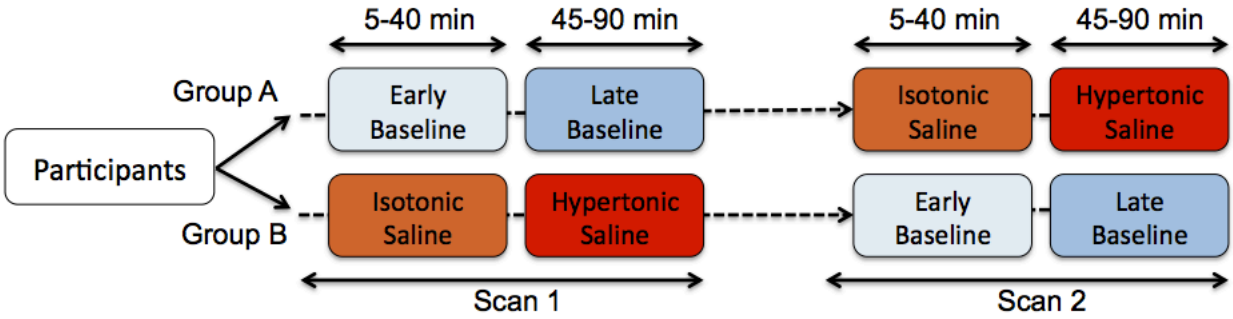


Figure 4.2: Subjective responses to administration of the opioid agonist fentanyl, and correlations with MOR activation

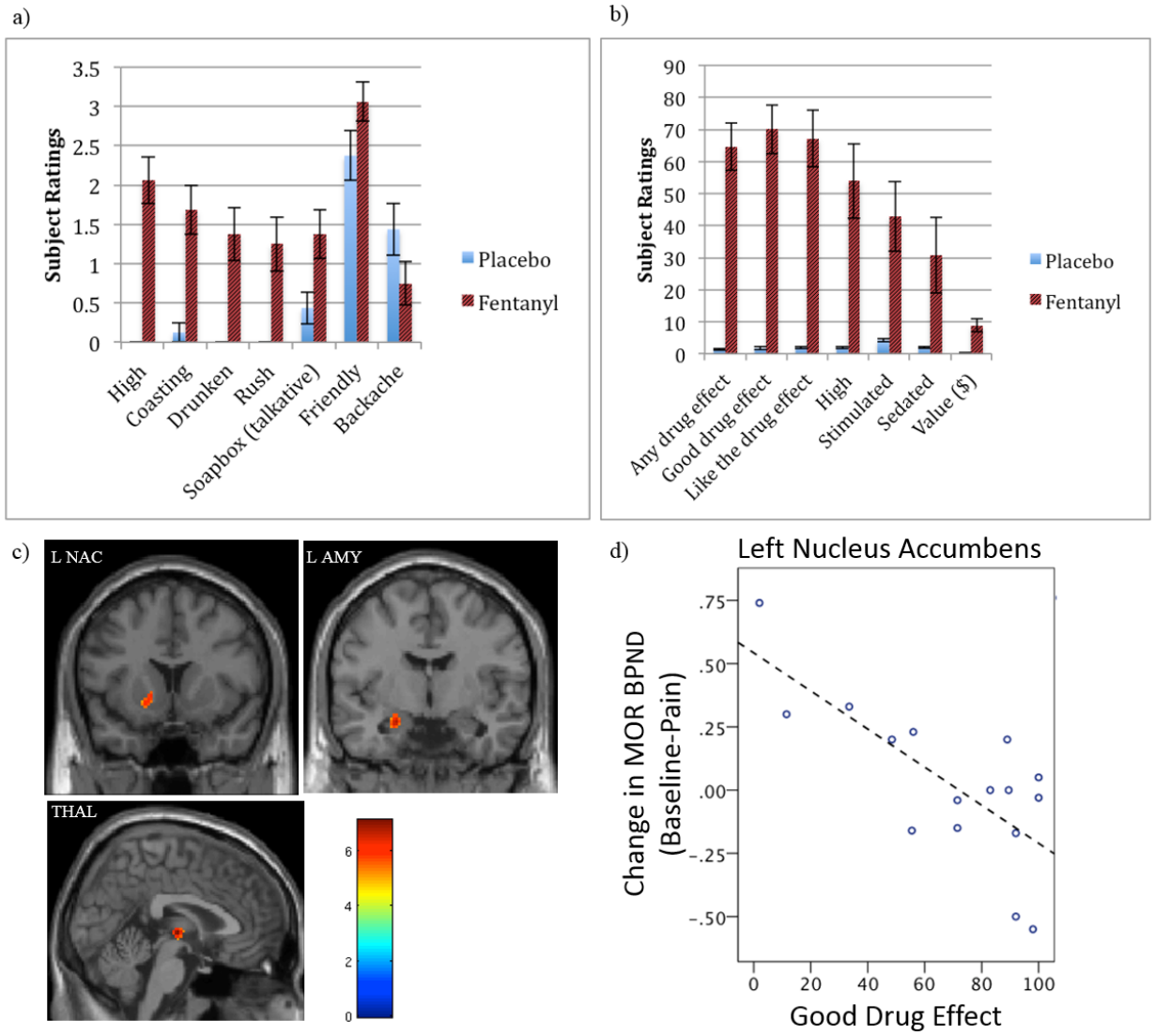


Figure 4.3: Group differences and correlations relating to baseline MOR BP_{ND} in CNBP patients

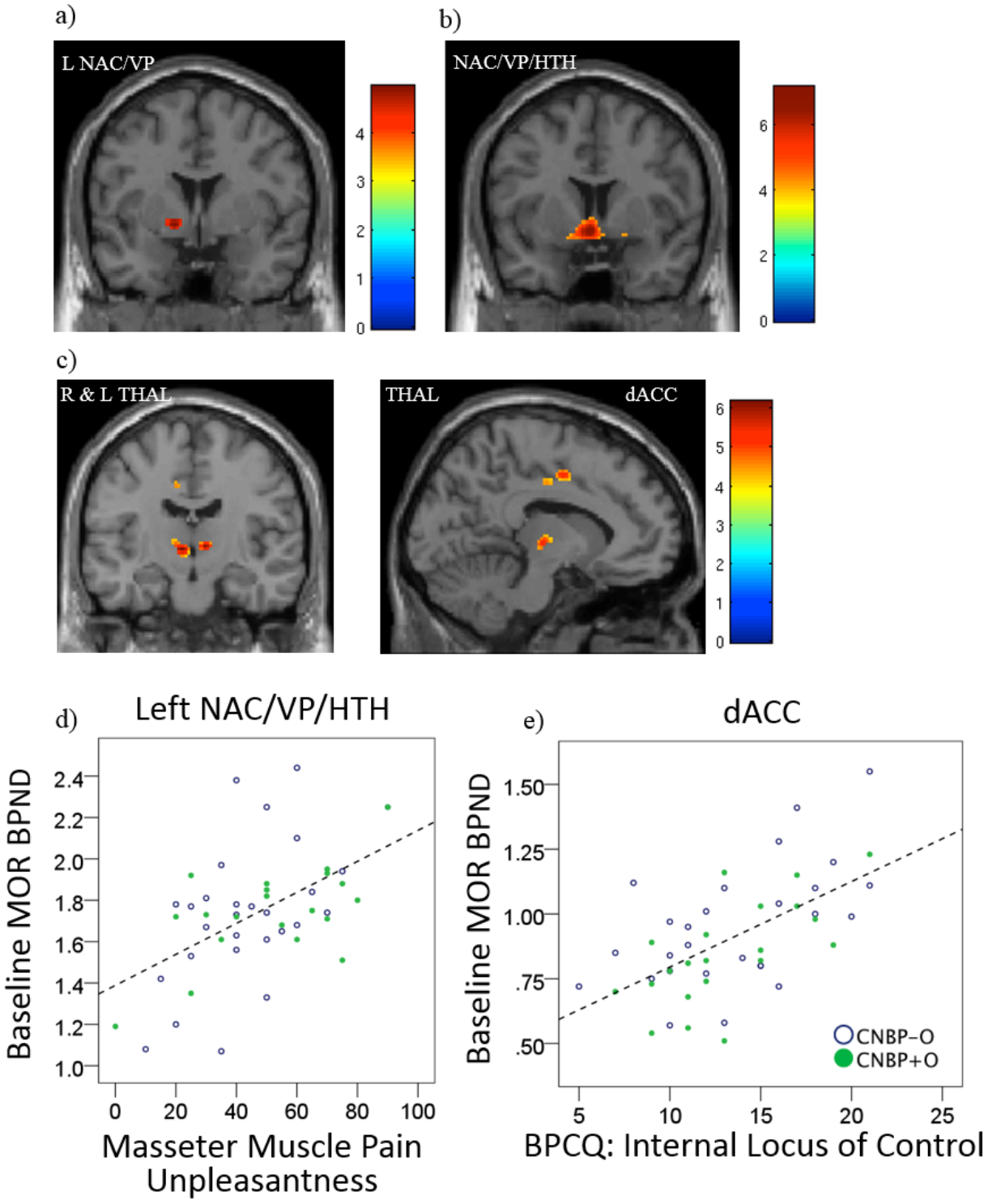
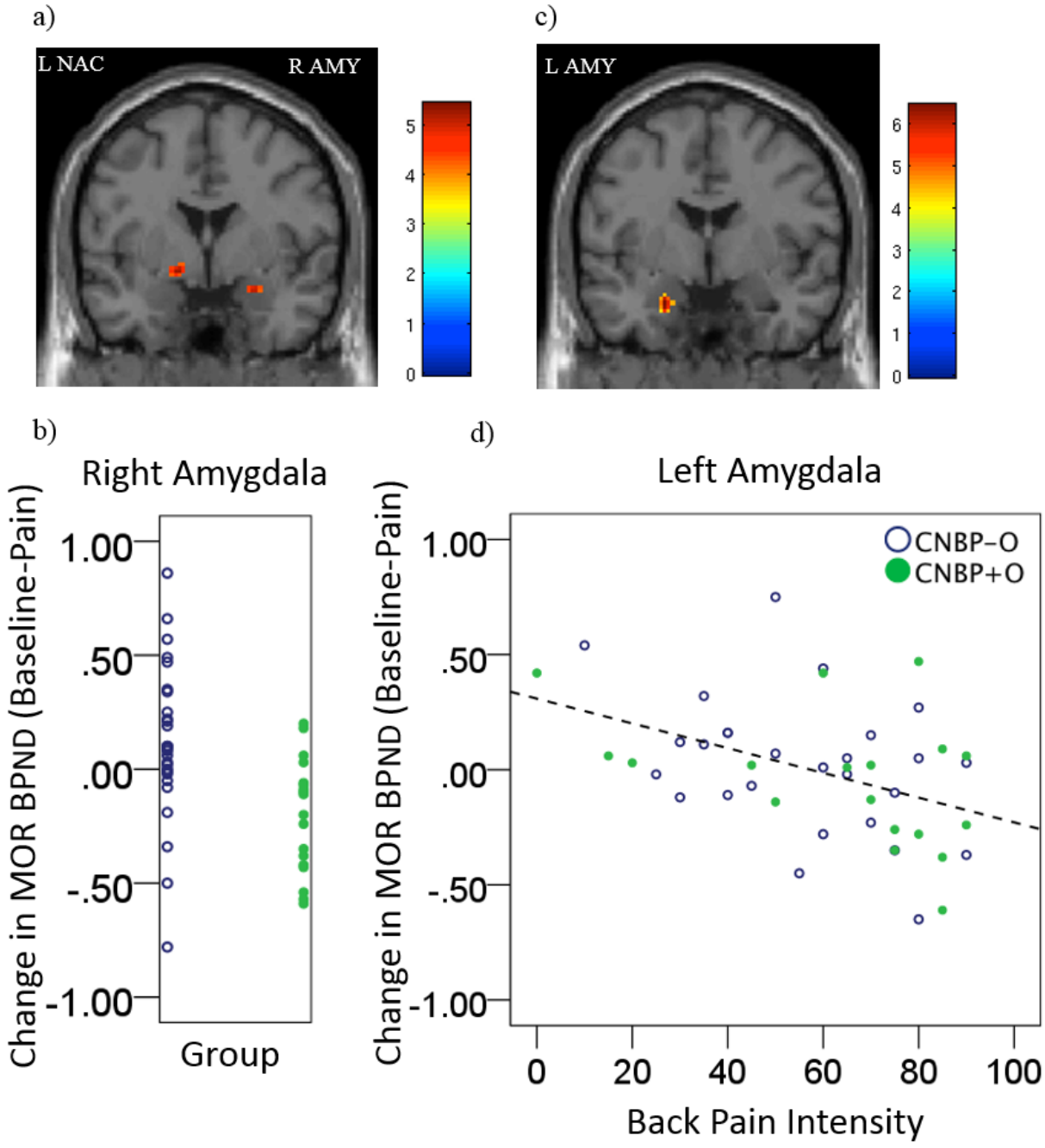


Figure 4.4: Group differences and correlations relating to MOR activation in CNBP patients



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CHAPTER V

Conclusion

DISCUSSION

The studies discussed above provide evidence that μ-opioid neurotransmission is involved in both acute and long-term effects of potential drugs of abuse such as nicotine and opioids. These data show group differences in EOS function between smokers and nonsmokers, as well as between opioid users and nonusers. However, our data also indicates that there is high interindividual variability throughout the groups. Decreased baseline MOR availability after overnight abstinence was associated with greater nicotine dependence in our smokers. Whether these differences in endogenous opioid neurotransmission existed before subjects began smoking, and potentially played a role in smoking initiation and continuation, or whether differences in nicotine dependence levels (or the associated craving) was what led to these variations needs to be further examined.

In our chronic pain patients, increased baseline MOR availability was correlated with higher positive affect and greater beliefs that subjects were in control of their own pain, aspects that have been associated with better coping strategies and more positive outcomes. Differences in how a patient's μ-opioid system responds to a sustained pain challenge could also be used to predict that individual's subjective response to an opioid agonist, a valuable ability for deciding whether opioid analgesics would be beneficial in treating a patient's pain.

This high variability in EOS function can be partially attributed to genetic factors that influence opioid neurotransmission, as indicated by the interaction between A118G genotype and smoking status that was associated with baseline MOR availability. A more complete understanding of the interindividual differences that affect opioid system functioning would significantly help in developing more personalized therapies for treating a number of disorders. Individuals naturally differ in their basal opioidergic tone, which has been shown to impact both stress responses as well as the ability to modulate pain (Buchsbaum *et al*, 1983; Chong *et al*, 2006; Koppert *et al*, 2005). Differences in the EOS have also been linked to personality traits, such as impulsivity, which are further known to correlate with vulnerability to addiction (Love *et al*, 2009). Studies using naloxone blockade to measure endogenous opioid activity have also found evidence of decreased activity in the hypothalamus of individuals with a family history of alcohol use (Wand *et al*, 1998).

As discussed earlier, G allele carriers at the A118G polymorphism appear to have decreased baseline MOR availability. The possible functional implications of these genetic alterations with regard to the potential to abuse drugs or the ability to modulate pain are currently being investigated (Pecina *et al*, 2015; Ray and Hutchison, 2004). Preliminary studies have suggested that *G individuals require higher doses of opioid analgesics to control their pain (Chou *et al*, 2006; Sia *et al*, 2008) and show an increased cortisol response to naloxone blockade (Chong *et al*, 2006). However, studies have so far not provided a clear answer on whether the A or G allele at this polymorphism is a risk factor for drug abuse (Chong *et al*, 2006). Other genetic polymorphisms that are known to alter endogenous opioid neurotransmission, such as the catechol-O-methyltransferase (COMT) polymorphism (Zubieta *et al*, 2003), would likewise be

good targets for future research as possible contributing factors for the high variation observed in how individuals respond to drugs of abuse, pain, and opioid analgesics.

Because of the wide variety of roles the EOS plays, it can be difficult to disentangle which changes are directly versus indirectly associated with alterations in EOS function. The EOS shows alterations in both mood disorders such as depression, which are often comorbid with chronic pain and drug abuse, as well as during the placebo effect, which can be involved in treating both depression and pain. Our data on smokers suggest that smokers' expectations of receiving nicotine could be just as relevant to the release of endogenous opioids as the nicotine itself, if not more so. The EOS's role in the placebo effect complicates studies that are attempting to examine this system in relation to specific drug effects, and necessitates that future study designs take into account the role that expectations play in an individual's response to a drug. It is essential to understand these interactions, as they may account for some of the differences seen between human studies, where expectations can play a major role, and animal studies.

Since both nicotine use and chronic back pain appear to be associated with alterations in EOS function, this neurotransmitter system could be at least partially responsible for the link seen between tobacco smoking and pain. Compared to the general population, patients suffering from chronic pain are about twice as likely to smoke (Ditre *et al*, 2011). Although most studies have not investigated the direction of causality between chronic pain and smoking, there has recently been evidence that individuals who are smokers are more likely to develop pain later in life (Goldberg *et al*, 2000). Whether smoking directly leads to an increased chance of developing pain, or whether both the likelihood of becoming a smoker and the likelihood of developing pain are linked to a third factor, is something researchers need to examine. If a third

factor, such as genetic differences in endogenous opioid tone, is associated with the development of both nicotine use and chronic pain, it could provide a potential method for physicians to determine who is at the greatest risk for developing these disorders and improve treatment options.

The EOS also provides a common link between opioid analgesics and nicotine use. Mice pretreated with nicotine appear to show decreased morphine-induced analgesia, suggesting that cross-tolerance can occur between nicotine and opioids (Zarrindast *et al*, 1999). There is also evidence that nicotine-dependent individuals are more likely to use prescribed opioids repeatedly (Skurtveit *et al*, 2010). Our research found that individuals with chronic pain, and particularly individuals on long-term opioids for their pain, show significant dysfunctions in MOR activation in response to experimental pain. Smoking might be an alternative way for these individuals to boost endogenous opioid activity in their bodies and at least temporarily decrease pain levels. The possibility that the opioid activation due to smoking might be mostly due to expectancy of receiving nicotine rather than the nicotine itself suggests interesting possibilities for future research.

LIMITATIONS AND FUTURE DIRECTIONS

The PET studies presented here are cross-sectional studies, which limits our ability to tell whether the differences seen in MOR function between smokers and nonsmokers, as well as between opioid users and nonusers, were a cause or an effect of the long-term drug use. In one of the few studies that has scanned chronic pain patients before and after initiating opioid use, Younger *et al*. (2011) found significant differences in grey matter morphology after only one month of analgesic use, indicating that opioid drugs are able to rapidly cause alterations in the

brain. Future research on the associations between opioid use and EOS function should use a similar protocol in order to elucidate whether the observed decreases in baseline MOR availability and opioid system activation associated with long-term opioid use are a direct effect of the opioid analgesics, or whether perhaps these alterations resulted in individuals being more likely to take opioids. Our fentanyl data gives rise to the intriguing possibility that a preexisting difference in EOS function may have been partially responsible for the differences seen between our opioid users and nonusers. Among our subjects that did not use opioids, individuals who reported the highest positive acute effects of fentanyl also had endogenous opioid systems that acted the most like those of our opioid users, with a decreased ability to release endogenous opioids in response to pain. This relationship bears further investigation.

Since we have shown that the *OPRM1* A118G genotype affected baseline MOR binding in our nicotine study, it might be predicted that this polymorphism will also alter how patients with chronic pain respond to opioid analgesics, as well as how they respond to an experimentally-induced increase in pain levels. Future analyses should investigate these effects in our sample of chronic pain patients, as well as the effects of other polymorphisms known to be involved in EOS function, such as COMT. Examining links between EOS function and subject responses to other tasks influenced by reinforcement networks, such as monetary incentive delay tasks, could also be productive.

Although our chronic pain patients included smokers, we did not have a large enough sample to compare the interactions between smoking, chronic pain, and opioid use in these subjects. Future work should attempt to increase recruitment of smokers in order to examine whether nicotine use, and the alterations in baseline MOR availability that we have found to be

associated with both craving and nicotine dependence, alters the associations currently shown between EOS function, opioid use, and both clinical and experimental pain.

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