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Exploring Potential Pharmacologic Treatments for Alcoholism: Can the Use of Drugs Selective for the μ-, δ-, and κ- Opioid Receptors Differentially Modulate Alcohol Drinking?

For the degree of Doctor of Philosophy

Is approved by the final examining committee:

Cristine Czachowski
Chair

Nicholas Grahame

Rob Stewart

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EXPLORING POTENTIAL PHARMACOLOGIC TREATMENTS FOR ALCOHOLISM: CAN THE USE OF DRUGS SELECTIVE FOR THE μ-, δ-, AND κ-OPIOID RECEPTORS DIFFERENTIALLY MODULATE ALCOHOL DRINKING?

A Dissertation

Submitted to the Faculty

of

Purdue University

by

Angela Nicole Henderson

In Partial Fulfillment of the

Requirements for the Degree

of

Doctor of Philosophy

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For Mom, Dad, and Chip: Thank you for your unconditional love, continued financial support, unwavering belief in my abilities, and for instilling in me the knowledge that I can achieve anything in life that I truly desire.

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To all the P and Long Evans Rats that sacrificed their lives to enable the completion of my dissertation as well as any one that had any involvement whatsoever, be it weigh a rat or bottle, order a drug, prepping alcohol or sucrose solutions, keeping my subjects well fed, hydrated, and in good health, allowing me to utilize their drug scales, or aiding in the editing, submission, and/or presentation of this document.

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ABSTRACT

Henderson, Angela Nicole Ph.D., Purdue University, May 2012. Exploring Potential Pharmacologic Treatments for Alcoholism: Can the Use of Drugs Selective for the μ-, δ-, and κ- Opioid Receptors Differentially Modulate Alcohol Drinking? Major Professor: Cristine L. Czachowski.

Naltrexone (NTX) is clinically efficacious at attenuating alcohol intake in non-abstinent alcoholics and, to a lesser extent, craving, independent of intake. While generally regarded as a non-selective opioid antagonist, NTX has been shown to have concentration dependent selectivity with lower doses (< 1.0 mg/kg) selective for the mu receptor and doses exceeding 1.0 mg/kg capable of binding to delta and kappa receptors. Like the mu system, the delta receptor system has also been implicated in mediating the rewarding effects of EtOH. In contrast, the role of the kappa system is less clear though recent evidence suggests that kappa activation may mediate EtOH aversion. Thus, the present study sought to evaluate the effects of both mu-selective and non-selective doses of naltrexone, the selective delta antagonist naltrindole (NTI), and the selective kappa agonist U50,488H (U50) in a paradigm that procedurally separates the motivation to seek versus consume a reinforcer to assess whether these receptor-selective drugs differentially affects these behaviors in both selected (alcohol-preferring P rats) and non-selected (Long Evans) rats, and whether these effects are specific to EtOH. Rats were trained to complete a single response requirement that resulted in access to either 2% sucrose or 10% EtOH for a 20-min drinking session. In three separate experiments, rats were injected (using a balanced design) with either vehicle or 1 of 3 doses of drug: U50 (IP; 2.5, 5.0, or 10.0mg/kg), NTI (IP; 2.5, 5.0, or 10.0 mg/kg), low NTX (SC; 0.1, 0.3, or 1.0 mg/kg) or high NTX (SC; 1.0, 3.0, or 10.0 mg/kg) on both consummatory and appetitive treatment days. Following either a 20 (U50), 15 (NTI), or 30 minute (NTX)

pretreatment, rats were placed into an operant chamber and intake (consummatory) or lever responses (appetitive) and response latencies were recorded. The results showed that overall: U50, NTI, and NTX attenuated intake and responding for sucrose and EtOH. Independent of reinforcer, LE rats were more sensitive to U50's effects on intake while P rats were more sensitive to the effects on seeking. P rats reinforced with EtOH were more sensitive to NTI's effects on intake and seeking than all other rat groups. P rats were more sensitive overall to lower doses of NTX than LE rats and lower doses of NTX were more selective in attenuating EtOH responding vs. sucrose. Higher doses of NTX suppressed intake and responding across both lines and reinforcers. These results demonstrate that craving and intake may be differentially regulated by the kappa, delta, and mu opioid receptor systems as a function of "family history" and suggest that different mechanisms of the same (opioid) system may differentially affect craving and intake.

INTRODUCTION

Currently, approximately 10 million Americans are suffering from alcohol use disorders (Grant et al., 2006), exacting an economic toll in excess of $184.6 billion dollars annually (Harwood et al., 1998). Beyond this financial incapacitation, the consequences of alcohol abuse and dependence are further reflected both by the sizeable numbers of health-related problems and vehicular-associated fatalities and the societal challenges of disrupted productivity and deterioration of familial and personal relationships (Harwood et al., 1998; Brennan et al., 1994). Attempts to combat these encumbrances through the extensive allocation of both federal and commercial resources have been directed towards the development of several possible pharmacotherapeutic interventions. To date, the culmination of this ever-advancing and enduring research has yielded three FDA approved medications for the management of alcohol dependence, each of which targets a distinct biochemical system.

Unlike other drugs of abuse, the biochemical properties of ethanol are such that they enable rapid diffusion and distribution throughout the body and central nervous system (CNS), as well as facilitate interactions with a host of neuromodulatory systems that ultimately yield ethanol's diverse assortment of behavioral and physiological effects. Though the exact mechanisms underlying these effects remain unclear, a multitude of systems have been implicated, including dopamine (DA), serotonin (5-HT), gamma aminobutyric acid (GABA), glutamate, opioid, adenosine, neuropeptide Y (NPY), and coticotropin releasing hormone (CRH; Durand & Barlow, 2000; Meyer & Quenzer, 2005; Dohrman et al., 1997; Lovinger, 1997; Froehlich, 1997; Mihic & Harris, 1997; Valenzuela, 1997; Gonzales & Jaworski, 1997; Di Chiara, 1997). As such, potential medications have been developed for the treatment/management of alcohol dependence that target each of these systems. One system that has gained extensive attention in its relationship to alcohol dependence is that of the endogenous opioid system.

The Endogenous Opioid System

An Overview

The endogenous opioid system, which has been implicated as having a role in regulating a diverse array of neurobiological functions, is comprised of three major groups of endogenous opioid peptides. These neuromodulating peptides include the endorphins, enkephalins, and dynorphins/neoendorphins which are derived from proopiomelanocortin (POMC; Nakanishi, 1979), proenkephalin (Noda et al., 1982), and prodynorphin (Kakidani et al., 1982), respectively. Studies have identified the arcuate nucleus of the medial basal hypothalamus, with projections to the limbic system and brain stem, as containing the greatest concentration of beta-endorphin cell bodies. In contrast, proenkephalin and prodynorphin cell bodies are widely distributed throughout the brain. Proenkephalin cell bodies are extensively found along the neuroaxis while prodynorphin cell bodies are prominent in several hypothalamic cell groups, especially those containing vasopressin producing cells of the neurosecretory nuclei and in cell groups along the brain stem (Akil et al., 1984).

Martin and colleagues (1976) were the first to speculate that there were multiple receptor subtypes based on their examination of morphine-like drugs in both nondependent and in morphine-dependent spinal dogs that experienced precipitated abstinence syndrome. Their findings resulted in the initial identification of the morphine (μ), ketocyclazocine (κ), and *N*-allylnormetazocine (SKF-10047; σ) receptor subtypes. The later discovery of yet another receptor subtype (delta; δ) named for the vas deferens where it was first identified (Lord et al., 1977; Kosterlitz et al., 1980), and the eventual revelation that the σ receptor was non-opioidergic (Mannalack et al., 1986), culminated in the recognition of the current mu (μ), delta (δ), and kappa (κ) opioid receptor subtypes. Of these G-protein coupled receptors, binding studies determined that the enkephalins bind selectively to the delta receptors and the dynorphins/neoendorphins selectively to the kappa receptors, while the beta-endorphins tend to demonstrate equal affinity for both the mu and delta receptor subtypes (Lord et al., 1977; Chavkin et al., 1982).

While binding studies attempting to identify regional brain distributions of the various receptor subtypes differ as a function of species (Bonnet et al., 1981; Chang et

al., 1979, 1981; Ninkovic et al., 1981), there is some consistency as to the general dissemination of receptor subtypes. For example, the densest areas of mu receptors tend to be thoughout the caudate-putamen, neocortex, thalamus, nucleus accumbens, hippocampus, amygdala, dorsal horn of the spinal cord (on the presynaptic terminal of nociceptive primary afferent fibers), the periaquaductal gray, and the median raphe. In contrast, delta receptor densities seem to be the greatest in the olfactory bulb, neocortex, caudate-putamen, and nucleus accumbens while being virtually absent from the thalamus, hypothalamus, and brain stem (Goodman et al., 1980). Kappa receptors show the greatest concentrations in the nucleus accumbens, claustrum, dorsal endopiriform nucleus and the interpeduncular nucleus (Nock et al., 1988).

More recently, the opioid-receptor like 1 (OPL1; Meunier et al., 1995; Reinscheid et al., 1995) receptor (also referred to as the NOP receptor) was identified as was its endogenous ligand, nociceptin/orphanin FQ (N/OFQ). Despite having a similar structure to dynorphin A (Nothacker et al., 1996, Reinscheid et al., 1996; the endogenous ligand for the kappa opioid receptor), and expressing moderate to high densities in the amygdala, medial prefrontal cortex, ventral tegmental area, lateral hypothalamus, the bed nucleus of the stria terminalis, and the nucleus accumbens (Neal et al., 1999; all of which are implicated in the brain reward circuit), N/OFQ fails to bind to the mu, delta, and/or kappa opioid receptors (Reinscheid et al., 1998). Further, naloxone fails to antagonize (Mollereau et al., 1994) and opioid ligands fail to activate the NOP receptor (Reinscheid et al., 1998). In addition, the effects of N/OFQ are unaffected by naloxone (Darland et al., 1998; Henderson & McKnight, 1997). Therefore, while the activation of the OPL1 receptor may result in a similar intracellular cascade noted with the classic opioid receptors (Darland et al., 1998; Hawes et al., 2000; Henderson & McKnight, 1997; Meunier, 1997; Reinscheild et al., 1995), the pharmacological actions are not the same, which led the International Union of Basic and Clinical Pharmacology (IUPHAR) to classified NOP as a non-opioid member of the opioid receptor family (for a review, see Lambert, 2008).

Role in Mediating Reinforcement

Studies assessing the rewarding properties of the opiate agonists morphine and heroin found that following systemic administration rats displayed a significant preference for the environment where the drug had previously been administered. This preference was not observed following saline administration, demonstrating the ability of the opiate agonists to produce a conditioned place preference (CPP; van der Kooy, 1987) indicating that the rats found the drug effects to be rewarding (Bozarth & Wise, 1981a; Mackey & van der Kooy, 1985; Mucha & Herz, 1985; Spyraki et al., 1983). Pretreatment with the opiate antagonist naloxone was found to block CPP development (interpreted as blocking the rewarding effects of the drugs; Bozarth & Wise, 1981a; Mucha & Herz, 1985), while both pretreatment with the dopamine antagonist haloperidol and the lesioning of the mesolimbic dopaminergic system with 6-hydroxydopamine (6-OHDA) were able to attenuate CPP development to heroin (Spyraki et al., 1983). Taken together, these results suggest that rewarding effects of systemically administered opiate agonists are mediated in part by the dopamine reward pathway.

While the systemic administration of morphine and other opiate agonists have been repeatedly demonstrated to produce a strong CPP in rats (Bozarth & Wise, 1981a; Mackey & van der Kooy, 1985; Mucha & Herz, 1985; Rossi & Reid, 1976; Spyraki et al., 1983; van der Kooy et al., 1982), the observation that lesioning the dopaminergic pathway could attenuate a morphine-induced CPP pointed to the reinforcing effects of opiates as being centrally mediated. Van der Kooy and colleagues (1982) found that while systemic administration of 0.08 mg/kg of morphine was the least effective dose capable of producing a CPP, only 10 μg (or 0.03 mg/kg) was required centrally to elicit a CPP to morphine. Thus, this led to the initial theory that perhaps the reinforcing effects of opiates were mediated centrally and the aversive ones peripherally (Bechara & van der Kooy, 1985, 1987; Bechara et al., 1987). However, Bals-Kubik and colleagues (1989) found that central administration of naloxone, the kappa agonist U50,488H, and the mu antagonist CTOP produced significant conditioned place aversions, indicating that the aversive effects of opiates are also centrally mediated.

Neurochemically, the reinforcing effects of various drugs of abuse have been purported to be regulated by the mesocorticolimbic dopamine pathway with dopaminergic processes originating in the ventral tegmental area (VTA) giving rise to the mesolimbic fibers (Ungerstedt 1971; Simon et al., 1976) that project to structures closely associated with the limbic region, particularly the nucleus accumbens shell and the prefrontal cortex (PFC; Koob, 1992; Spanagel, 2009; for a review see Oswald & Wand, 2004). Initial findings that 6-OHDA-induced lesions attenuated the CPP for heroin (Spyraki et al., 1983) and that the reinforcing effects of opiates are centrally mediated suggest that this pathway could be regulating opiate reinforcement.

In support of this notion, a multitude of studies have shown that the administration of various mu and delta agonists, including morphine, beta-endorphin, and methionine- and leucine-enkephalin produce reinforcing effects either through the development of a CPP or by the willingness of the animals to work to self-administer them directly into the VTA (van der Kooy et al., 1982; Bozarth & Wise, 1981b; Phillips & LePaine 1980, 1982; Katz & Gormezano, 1979; Belluzzi & Stein, 1977; Belluzzi & Wise, 1977; Van Ree et al., 1979; Wei & Loh, 1976) and are blocked by central or systemic pretreatment with naloxone (Bozarth & Wise, 1981b). In fact, the ability of the endogenous opioid system to induce a conditioned place preference in Sprague-Dawley rats was determined to be contingent upon both brain region and receptor specificity such that infusion of the mu agonist DAMGO elicited a significant place preference when infused into the VTA but not the nucleus accumbens. Contrastingly, infusion of the kappa agonist U50,488H and the dynorphin derivative E-2078 produced significant place aversions when infused both into the VTA and the nucleus accumbens (Zangen et al., 2002; Bals-Kubik et al., 1993). These results suggests that the VTA is responsible for mediating the reinforcing effects of the opiates through activation of the mu and/or delta receptors while the kappa system, acting in opposition to the mu and delta system, is able to block the reinforcing effects of opiates via receptors located within or near the VTA and nucleus accumbens.

Microdialysis and electrophysiological studies soon began to investigate the role of the opiates in regulating dopamine release along the mesolimbic pathway.

Electrophysiological studies determined that systemic and local administration of morphine significantly increased the firing rate of dopamine neurons in the VTA and increased dopamine turnover (indicative of increased release) in the nucleus accumbens (Nowycky et al., 1978; Matthews & German, 1984). Using microdialysis techniques to measure extracellular synaptic dopamine concentrations as an index of dopamine transmission in freely moving rats, Spanagel and colleagues (1992, 1999) determined that administration of the mu agonist DAGO and antagonist CTOP into the VTA dose-dependently increased and blocked spontaneous dopamine release in the nucleus accumbens, respectively, while neither had any effect on dopamine when microinjected into the nucleus accumbens. In contrast, microinjection of the kappa agonist U69593 and antagonist norbinaltorphamine (nor-BNI) failed to affect dopamine release when microinjected into the VTA but respectively decreased and increased dopamine release when administered into the nucleus accumbens.

Similarly, both self-administration of heroin into the VTA and systemic administration of morphine facilitated dopamine release in the nucleus accumbens (Xi et al., 1998; Di Chiara & Imperato, 1988). Pretreatment with the mu antagonist beta-funaltrexamine (β-FNA) blocked the morphine-facilitated increase in dopamine release (Di Chiara & Imperato, 1988). The effects of heroin-induced dopamine release were able to be mediated by the kappa receptor in the nucleus accumbens such that pretreatment with the kappa agonist U50,488H or coadministration of prodynorphin A resulted in decreases in dopamine release while pretreatment with the kappa antagonist nor-BNI actually potentiated dopamine release in the nucleus accumbens (Xi et al., 1998). Beta-endorphin and the enkephalins have also been shown to be rewarding by increasing dopamine release in the nucleus accumbens (Koob, 1992), the effects of which can be blocked by the administration of the opiate antagonist naltrexone (Gonzales & Weiss, 1998).

Interestingly, increasing doses of opiates tend to decrease behavior, creating depressed-like states despite the observed dose-dependent increases in dopamine. Throughout the rest of the nervous system, the direct action of opiates has been demonstrated as inhibitory (North & Tonini, 1977; Mihara & North, 1986; North et al.,

1987; Johnson & North, 1992). This led to the theory originally put forth by Gysling and Wang (1983) that perhaps the excitatory effects of the opioids was due to their inhibition of secondary, non-dopaminergic neurons that were otherwise inhibiting dopaminergic cells. Further, it was noted that the destruction of dopamine-containing cells in the VTA failed to alter mu opioid binding (Dilts & Kalivas, 1989), and that the VTA content of GAD, a marker for GABA, in the VTA was unaltered following the destruction of GABAergic innervations to the VTA, suggesting the presence of GABA-containing cells within the VTA (Fonnum et al., 1977).

Taken together, these observations suggested that the opioids were exerting their effect by binding to GABA interneurons that were able to directly inhibit dopamine neurons. Subsequent examinations of this hypothesis revealed that the administration of the $GABA_A$ and $GABA_B$ agonists muscimol and baclofen dose-dependently decreased dopamine in the nucleus accumbens (Westerink et al., 1996; Xi & Stein, 1998) while $GABA_A$ and $GABA_B$ antagonists bicuculline and 6-OH saclofen (but not CGP52432) significantly increased dopamine release in the nucleus accumbens (Westerink et al., 1996; Xi & Stein, 1998). Further, the systemic administration of 6-OH saclofen was shown to block the baclofen-induced decrease in dopamine release in the nucleus accumbens, suggesting that GABA interneurons are tonically inhibiting dopaminergic neurons in the VTA (Xi & Stein, 1998). Johnson and North (1992) determined that mu receptor activation was responsible for the hyperpolarization of the secondary GABAergic cells and consequent increase in dopamine firing in the nucleus accumbens as DAMGO but not U50,488H was able to elicit this response. Therefore, the endogenous opioid system is capable of modulating dopamine release directly via receptors located in the nucleus accumbens and indirectly through the modulation of GABA interneurons in the VTA that otherwise tonically inhibit dopamine release (Johnson & North, 1992; Gianoulakis, 1996; Herz, 1997; Margolis et al., 2003; Wise, 1996; Fichna et al., 2007).

Interactions with Alcohol

Studies examining the rewarding effects of ethanol show strikingly similar patterns to those of the opiates. Acute administration of low doses of ethanol have been shown consistently to dose-dependently increase dopamine turnover, dopamine

transmission in the mesolimbic system, and dopamine release in the nucleus accumbens (Gessa et al., 1985; Imperato & Di Chiara, 1986; Di Chiara & Imperato, 1988; Weiss et al., 1993; Pontieri et al., 1995; Kohl et al., 1998; Philput & Kirstein, 1998), the effects of which are blocked by the systemic administration of the opioid antagonist naltrexone (Benjamin et al., 1993). Electrophysiology studies have found that ethanol increased firing rates of dopamine neurons in the VTA (Brodie et al., 1990, 1995, 1999; Bunney et al., 2001), while animal studies revealed that rats willingly self-administer ethanol into the VTA (Gatto et al., 1994; Rodd-Henricks et al., 2000; Rodd et al., 2004, 2005). Several studies have also determined that ethanol increases in vivo and in vitro release of beta-endorphin in the brain and pituitary gland (Keith et al., 1986; Gianoulakis et al., 1989; Gianoulakis, 1990; de Waele et al., 1992; Gianoulakis & Barcomb, 1987; Jarjour et al., 2009). Additionally, low doses of morphine have been shown to increase ethanol consumption whether administered systemically (Reid & Hunter, 1984) or centrally (Liseman, 1989). Thus, these findings suggest that ethanol reinforcement is at least in part dependent upon the activation of the endogenous opioid system such that its activity regulates the reinforcing effects of ethanol both directly and indirectly.

Conclusions

Taken together, the above information relays the idea that the reinforcing effects of opiates are modulated via the mesolimbic dopaminergic system. Additionally, drugs that facilitate the activation of the mu and delta opioid receptors are generally accepted as being rewarding while those that activate the kappa receptors are considered aversive. Further, the nonspecific opioid antagonists naloxone and naltrexone have repeatedly been demonstrated to block the reinforcing effects of these drugs, whether administered centrally or systemically. Ethanol, a drug that lacks a specific receptor of its own, has been shown to have activation patterns remarkably similar in the brain to those observed following the administration of various opiates, such as morphine. Specifically, both central and systemic administration of ethanol increase firing rates of dopaminergic neurons and subsequent dopamine release in the nucleus accumbens. Ethanol has also been shown to increase the release of the endogenous opioids beta-endorphin and enkephalin, and to be rewarding when administered directly into the VTA. Finally, as

with the opiates, the rewarding effects of ethanol can be blocked via naloxone and naltrexone administration. These findings suggest that the rewarding effects of ethanol are mediated, at least in part, both by direct and indirect activation of the endogenous opioid system.

Naltrexone

If the reinforcing effects of ethanol and the opiates are mediated through similar neural mechanisms, then opiate antagonists should modulate ethanol intake. As naltrexone has been shown to successfully reduce the intake of opiates in both animal and human studies, the next logical step was to test its efficacy in reducing ethanol intake. Indeed, studies conducted in rats, mice, and monkeys showed that the opiate antagonists naltrexone and naloxone reduced ethanol intake across a wide variety of behavioral paradigms. Naltrexone reduced homecage drinking (Lê et al., 1993; Gardell et al., 1997a; Goodwin et al., 2001; Koistinen et al., 2001; Stromberg et al., 1998, 2001, 2004; Kim et al., 2004; Mhatre et al., 2004; Ciccocioppo et al., 2007) and responding for ethanol in operant studies maintained on both fixed (Lê et al., 1999; Middaugh et al., 1999; Sabino et al., 2006; Ji et al., 2008; Kuzmin et al., 2007, 2008; Williams & Broadbridge, 2009) and progressive ratio (Rodefer et al., 1999; Carroll et al., 2000) schedules. Further, naltrexone was able to attenuate ethanol-associated behaviors following dependence induction using vapor chambers (Gilpin et al., 2008; Walker & Koob, 2007) as well as to block reinstatement (Lê et al., 1999; Bienkowski et al., 1999; Buraltini et al., 2006) and the alcohol deprivation effect (ADE; Hölter & Spanagel, 1999).

Clinically, O'Malley and colleagues (1992) conducted a 12 week double-blind, placebo-controlled clinical trial of naltrexone as a potential treatment for alcoholism. The results revealed that patients receiving naltrexone drank on half as many days and consumed 66% fewer standard drinks than those receiving placebo. Naltrexone-treated patients also had better treatment outcomes in terms of increased abstinence and decreased relapse rates. In a similarly designed study, Volpicelli and colleagues (1992) likewise concluded that naltrexone was efficacious in decreasing craving, drinking days, and relapse rates. This effect was especially pronounced among those patients that experienced a "slip" as evidenced by the fact that of those that sampled alcohol during

the course of treatment, only 50% of naltrexone treated participants relapsed, defined as consuming 5 or more drinks per occasion, compared to 95% of placebo-treated participants. Based on the results of these two simultaneously released reports, naltrexone was approved by the Food and Drug Administration in 1993 for the treatment of alcohol dependence.

Following these preliminary results, numerous studies have since been dedicated to examining the role of naltrexone on ethanol consumption in the clinical population. In contrast to earlier findings that showed naltrexone to have overwhelmingly positive effects on several drinking outcomes (O'Malley et al., 1992; Volpicelli et al., 1992), subsequent studies revealed somewhat mixed results regarding the efficacy of naltrexone relative to placebo. Overall, however, trends garnered from meta-analyses suggest that the majority of clinical trials result in a modest positive effect of naltrexone over placebo, the strongest effects involving the reduction of heavy or excessive drinking (Kranzler & Van Kirk, 2001; Srisurapanont & Jarusuraisin, 2005; Pettinati et al., 2006). In contrast, naltrexone has been shown to fail in enhancing ethanol abstinence (Garbutt et al., 1999). Of further interest, as with other pharmacotherapies aimed at treating alcoholism, one of the most important predictors in treatment outcome with naltrexone is compliance (Anton et al., 1999; Chick et al., 2000a; 2000b; Volpicelli et al., 1997; Monti et al., 2001) such that there is a direct correlation between percent treatment compliance and positive outcomes. The greatest reason cited for noncompliance with naltrexone is adverse side effects; the principal complaint of which is nausea (cited by approximately 10 percent of naltrexone users) followed by fatigue and headache (Croop et al., 1997). Additionally, while it has been reported that patients need to comply with their medication approximately 70-90% of the time for naltrexone to be efficacious (Volpicelli et al., 1997; Chick et al., 2000a), noncompliance rates remain somewhere between 20-60% for daily naltrexone users (Bouza et al., 2004).

Findings From Opioid Receptor Selective Drugs

Despite problems with noncompliance, naltrexone is the most efficacious pharmacotherapy currently available for the management of alcohol dependence and provides evidence that the endogenous opioid system is a fruitful target in regulating

alcohol reinforcement. Studies utilizing drugs that are selective for individual receptor subtypes within the endogenous opioid system have been employed to help elucidate the potential mechanisms by which naltrexone may be mediating its reinforcing effects. These results suggest potential treatment options that may have fewer side-effects, thereby increasing adherence to medication and ultimately resulting in better treatment outcomes.

Drugs Targeting the Mu Opioid Receptor

While several studies have implicated the mu opioid receptor as the featured opioid receptor subtype mediating ethanol reinforcement, there is a surprising dearth of literature utilizing mu-selective antagonists evincing this point. A quick survey of those that have been examined, revealed beta-funaltrexamine to effectively reduce ethanol intake in both Wistar (Stromberg et al., 1998) and High Alcohol Drinking (HAD; Krishnan-Sarin et al., 1998) rats but not in C57BL/6 mice (Lê et al., 1993) in limited access paradigms. CTOP, a peptidic antagonist that can only be administered centrally due to an inability to cross the blood brain barrier (Gulya et al., 1988), has been shown to attenuate ethanol intake in both Alko-Alcohol (AA) preferring rats (Hyytiä, 1993) and C57BL/6 mice (Kim et al., 2000). Additionally, naloxonazine has been shown to decrease ethanol intake (Honkanen et al., 1996). Interestingly, naloxonazine has been shown to be a selective μ_1 antagonist; however, while the μ_1 receptor has been implicated as regulating behaviors related to supraspinal analgesia, feeding, and prolactin release, it is not involved in mediating either respiratory depression or physical dependence of morphine (Zhang & Pasternak, 1981a, 1981b; Spiegel et al., 1982; Ling & Pasternak, 1983; Ling et al., 1984, 1985, 1986).

Part of the reason for such a deficit in research specifically examining the role of the mu receptor in mediating ethanol reinforcement could be due to the fact that previous findings have consistently indicated that across a range of doses, naltrexone and naloxone demonstrate selectivity for the mu receptor compared to both the delta and kappa opioid receptors. In fact, given the wealth of studies that have shown naltrexone (which is already FDA approved for the treatment of alcohol dependence) to be efficacious in decreasing ethanol self-administration across a variety of paradigms (Mhatre et al., 2004;

Gardell et al., 1996; 1997a; 1997b; Goodwin et al., 2001; Stromberg et al., 1998, 2001, 2004; Liseman, 1989; Ji et al., 2008; Parkes & Sinclair, 2000; Koistinen et al., 2001; Perfumi et al., 2003; Ciccocioppo et al., 2007; Lê et al., 1993; Kim et al., 2004; Kamdar et al., 2007; Kuzmin et al., 2007, 2008; Bienkowski et al., 1999; Williams & Broadbridge, 2009; Walker & Koob, 2007; Czachowski & DeLory, 2009; June et al., 1998; Gilpin et al., 2008; Sabino et al., 2006; Middaugh et al., 1999, 2000; Heidbreder et al., 2007; Escher & Mittleman, 2006), there seems little reason to further explore other antagonists. However, many of the studies that examine the role of naltrexone on ethanol reinforcement do so across a wide dose-response range which fails to effectively tease apart doses that are supposedly selective for the mu receptor from those that are more non-selective.

Specifically, doses of naltrexone and naloxone that were less than 30nM or 1.0 mg/kg have been shown to selectively bind to mu receptors with an affinity of 0.86 compared to delta 0.06 and kappa 0.08. (Paterson et al., 1984). Further studies have demonstrated that low doses of naloxone and naltrexone bind preferentially to mu receptors, (< 1.0 mg/kg) while increasing concentrations of the antagonist are less selective and show a greater capacity to bind to delta and kappa receptors, rendering higher doses of naltrexone and naloxone as nonspecific opioid antagonists (Childers et al., 1979; Paterson et al., 1984; Takemori & Portoghese, 1984; Emmerson et al., 1994; Mhatre & Holloway, 2003; Wang et al., 2001; 2007). Although the use of a more selective mu antagonist, such as beta-funaltrexamine (β-FNA), would provide more insight into the role of the mu receptor in mediating various aspects of ethanol reinforcement, β-FNA is an irreversible antagonist that also has short term affinity for the kappa receptor, which could potentially confound any results aiming to specifically assess the role of the mu receptor (Corbett et al., 1993; Rothman et al., 1988; Ward et al., 1982).

Drugs Targeting the Delta Opioid Receptor

The role of the delta opioid receptor has long been associated with mediating the positive reinforcing effects of ethanol, in part due to the ability of beta-endorphin to bind with equal affinity to both the delta and mu opioid receptors (Lord et al., 1977; Bals-

Kubik et al., 1990). While further research using more selective agonist and antagonist implicate the mu opioid receptor in mediating the reinforcing effects of morphine and ethanol, other research has suggested that there is a far more complex relationship between these two receptor subtypes (Traynor & Elliott, 1993). For example, the mu and delta receptor subtypes have been found to exist on the same neuron (Rogers & Henderson, 1990). In addition, ligands for the mu receptor have been shown to inhibit delta ligand binding in both a competitive and noncompetitive nature (Rothman et al., 1988). This led to the proposal of a mu/delta receptor complex, activated by beta-endorphin and capable of being blocked by both selective delta and mu ligands (Schoffelmeer et al., 1990; Bals-Kubik et al., 1990; Traynor & Elliott, 2003). Given that ethanol has been shown to induce the release of beta-endorphin in the brain (Keith et al., 1986; Gianoulakis et al., 1989; Gianoulakis, 1990; de Waele et al., 1992; Gianoulakis & Barcomb, 1987; Jarjour et al., 2009), and that both mu and delta selective ligands can effectively antagonize this receptor complex, it is of interest to know whether delta-selective antagonists may offer a benefit over mu-selective or non-selective antagonists as potential pharmacotherapies for alcoholism.

The limited research that has examined the role of various delta receptor antagonists on mediating ethanol reinforcement has been somewhat mixed. For example, use of the delta antagonist ICI 174,864 was able to attenuate intake in a limited access paradigm in both High Alcohol Drinking (HAD; Froehlich et al., 1991) and Sprague-Dawley rats (Franck et al., 1998), but unable to reduce ethanol at 1 and 4 hours following administration in Alko-Alcohol (AA) preferring rats (Honkanen et al., 1996). Similarly, SoRI-9409, but not naltrindole, was able to attenuate intake of intermittent access to 20% ethanol in Long Evans rats (Nielsen et al., 2008). Likewise, naltrindole was ineffective at reducing ethanol consumption in Wistar rats (Stromberg et al., 1998). However, naltrindole did decrease ethanol intake in non-selected Sprague-Dawley rats (Franck et al., 1998) and in rats and mice that have a preference for ethanol (HAD, P, and C57BL/6) in limited access paradigms (Lê et al., 1993; Krishnan-Sarin et al., 1995a; Kim et al., 2000). Naltrindole administration has also been demonstrated to attenuate ethanol self-administration and intake in operant paradigms in Alko-Alcohol (AA) preferring and in

outbred Wistar rats (Hyytiä & Kiianmaa, 2001) as well as to block cue-induced reinstatement of ethanol seeking in Wistar rats (Ciccocioppo et al., 2002; Marinelli et al., 2009). Given that naltrindole seems to more consistently attenuate ethanol reinforcement and is a non-peptidic, potent delta receptor antagonists (Takemori & Portoghese, 1992), devoid of the neurotoxic properties found with other delta antagonist (ICI 174,964; Long et al., 1988), it is the most promising target for a delta-selective pharmacotherapy for alcohol use disorders.

Drugs Targeting the Kappa-Opioid Receptor

Unlike the mu and delta opioid receptor systems, the role of the kappa opioid receptor in influencing the reinforcing effects of alcohol and other drugs of abuse is less clear. It appears that the activation of the kappa system acts in an opposite manner to that of the mu (and delta systems) across a wide range of parameters, including drinking, locomotion, place conditioning, body temperature, and dopamine and serotonin release (Hartig & Opitz, 1983; Locke et al., 1982; Iwamoto, 1981; Mucha & Herz, 1985; Chen et al., 2005; Handler et al., 1992; Di Chiara & Imperato, 1988; Tao & Auerbach, 2002, 2005; see Wee & Koob, 2010 for a review). While antagonists at the mu and delta receptors tend to decrease ethanol reinforcement, kappa antagonists have varying effects. For example, in nondependent animals, nor-BNI (a kappa receptor antagonist) has been shown to decrease ethanol intake in mice (Logrip et al., 2008), to increase ethanol intake in rats (Mitchell et al., 2005), or to have no effect on operant self-administration of ethanol in rats (Doyon et al., 2006) or monkeys (Williams & Woods, 1998). In contrast, studies conducted in animals that have been made dependent (either through the use of vapor inhalation chambers or a “chronic” drinking history exceeding 16 months) have found nor-BNI capable of attenuating ethanol reinforcement (Hölter et al., 2000; Walker & Koob, 2008; Walker et al., 2010). As such, the effects of the kappa system on ethanol reinforcement have recently been shown to be more consistent in studies using dependence induction models.

In contrast to kappa antagonists, kappa agonists seem to more consistently decrease ethanol reinforcement. Studies using the kappa agonists U50,488H, endolin (CI-977), and bremazocine have found decreases in ethanol drinking in rats and mice

(Lindholm et al., 2001; Nestby et al., 1999; Logrip et al., 2008; Cosgrove & Carroll, 2002). U50,488H has also been shown to block the rewarding effects of ethanol by blocking conditioned place preference to ethanol in DBA/2J mice (Logrip et al., 2009). Therefore, while studies that have used kappa receptor antagonists found somewhat inconsistent results, those using kappa receptor agonists consistently found reduced ethanol reinforcement. As such, the kappa agonist, *trans* –(+)-3,4, Dichloro-N-methyl-N [2-(1-pyrrolidinyl)cyclohexyl] benzeneacetamide methanesulfonate salt (U50,488H), which is a potent and selective kappa receptor agonist with little effect at either the delta or mu receptors and can be administered systemically (Lahti et al., 1982; Vonvoightlander et al., 1983; Tang & Collins, 1985), should be considered as a potential pharmacotherapy to mediate the reinforcing effects of ethanol.

Use of Animals Models is Assessing Drug Efficacy

Animal Models of Alcoholism

When examining various subpopulation of alcoholics, clinically, naltrexone has been found to be more efficacious in reducing drinking in samples of the population that have been identified as having an early age of onset (defined as before the age of 25; Rubio et al., 2005; Rohsenow et al., 2007; Tidey et al., 2008) and a greater family history of alcohol problems (FH^+ with greater than 20% of family members having alcohol problems; King et al., 1997; Monterosso et al., 2001; Rohsenow et al., 2007; Tidey et al., 2008). Similarly, results examining the specific opioid receptor genes have found that functional variants in genes associated with both dynorphin (PDYN, the endogenous ligand for the kappa receptor; Williams et al., 2007), and the kappa opioid receptor gene (OPRK1) are significantly correlated with alcoholism (Xuei et al., 2006; Zhang et al., 2008; Edenberg et al., 2008). While the results differ as a function of ethnicity, gender, and genotype concerning the ability of naltrexone to be more efficacious in those with the Asn40Asp variant of the mu opioid receptor gene (OPRMI, see Ray et al., 2011 for a review), studies examining the effects of a functionally similar polymorphism (OPRM1 C77G) in rhesus monkeys found that monkeys with the G77G genotypes have an increased preference for alcohol consumption over their G77C counterparts, and that

naltrexone is more efficacious in reducing ethanol preference and self-administration than in C77C or C77G genotyped monkeys (Barr et al., 2007; Barr et al., 2010; Vallender et al., 2010).

In addition to looking at individuals that have allelic variations that confer a differential response to naltrexone, other studies have extended this to examine the role of different alcohol typologies. For example, Kiefer and colleagues (2007) found that when identifying a subset of individuals as either presenting with Cloninger's Type I (late onset) or Type II (early onset) alcoholism, that those individuals classified as having Type II alcoholism were significantly more responsive to the effects of naltrexone compared to those diagnosed with Type I. Specifically, Type II classified alcoholics showed significant benefits from the use of naltrexone treatment (versus placebo) over their Type I counterparts, including increased time to lapse/first drink and relapse and better outcomes in terms of final abstinence. In addition to being characterized as having an earlier onset, Type II alcoholism has traditionally been thought of as familial alcoholism (see Cloninger, 1981; 1987), with designants possessing genetic/hereditary components that appear to be correlated with a more favorable response to naltrexone treatment. Taken together, this information would suggest that those individuals that have a genetic predisposition towards alcoholism may be differentially affected by naltrexone than those that do not.

Given that naltrexone has been shown to be more efficacious in reducing ethanol in non-abstinent patients with alcoholism (i.e., among those patients that experienced a "slip" during treatment; Volpicelli et al, 1992; Heinälä et al., 2001), it is important to identify an animal model that displays a similar phenotype and propensity for alcohol intake. Drinking behavior in the alcohol-preferring P rat has been well characterized as this line satisfies the criteria of an animal model of the propensity to drink alcohol (Lester & Freed, 1973; Cicero, 1979; McMillen, 1997). Specifically, the P rat, which has been selectively bred for both increased ethanol preference and increased ethanol intake (Li et al., 1993) voluntarily consumes at least 5 grams of ethanol per kilogram of body weight (g/kg) per day. The P rat also consumes ethanol for its pharmacological effects as opposed to solely for caloric or taste values (Bice & Kiefer, 1990; Gatto et al., 1994;

Lankford et al., 1994), attains pharmacologically relevant blood alcohol concentrations (BACs; Li et al., 1979; Lumeng & Li, 1986), develops tolerance and experiences withdrawal, and will self-administer ethanol via operant procedures (Murphy et al., 1989; Rodd-Henricks et al., 2002; for a review, see McBride & Li, 1998).

Further, P rats have demonstrated a willingness to work for ethanol access and to maintain high ethanol intakes in operant paradigms where the response requirement was continually increased in order to gain access to the reinforcer in both limited and 24-hour access paradigms (Ritz et al., 1994; Files et al., 1998) P rats were also willing to work much harder than either replicate of the high alcohol drinking (HAD) rats in 20 minute operant sessions to access either an ethanol or sucrose reinforcer, with breakpoints 2-3 times higher than HAD1 and HAD2 rats (Czachowski & Samson, 2002). Taken together, the P rat would be a valid model of a FH^{+} drinking phenotype in which to examine the effects of receptor-selective drugs on ethanol reinforcement.

Assessing Different Components of Reinforcement

Although previous studies have found naltrexone to be most effective in non-abstinent patients with alcoholism by reducing relapse to heavy drinking (effectively decreasing alcohol consumption; Volpicelli et al, 1992; Heinälä et al., 2001), other studies have found naltrexone to be effective during periods of abstinence independent of ethanol consumption –or, in other words, while the subject is not under the pharmacological effects of alcohol. These observations led to the hypothesis that the efficacy of naltrexone is a function of its ability to extinguish the reinforcing properties associated with alcohol-related stimuli (cues associated with drinking) that otherwise elicit appetitive motivational response through conditioned endogenous opioid release which can be blocked with naltrexone treatment (Swift, 1995; Monti et al., 1999; Katner et al., 1999; Katner & Weiss, 1999; Ciccocioppo et al., 2003). Further, studies on craving have revealed that in abstinent alcoholics, exposure to alcohol cues increased alcohol craving and associated physiological responses (Cooney et al., 1997; Rohsenow et al., 1994). However, as noted by Flannery and colleagues (2001), craving for alcohol is not necessarily directly associated with alcohol drinking, particularly in studies involving the use of medications targeting decreased ethanol consumption.

Therefore, in order to best assess the value of potential future pharmacotherapies aimed at treating alcohol dependence, it is important to use a model that is capable of independently assessing both the appetitive motivational responses and the actual consumption of a drug. One such paradigm is the sipper-tube model (Samson et al., 1998, 1999, 2000) which procedurally separates ethanol seeking and drinking by requiring rats to complete an operant response requirement (RR) in order to gain access to a reinforcer (i.e., ethanol) for 20 minutes. Further, by eliminating the need for a contingent operant response that regularly precedes access to a reinforcer at frequent intervals, the sipper-tube model avoids the confound of the pharmacological effects of ethanol interfering with an animal's physical ability to emit a response. Relevant to the present investigation, the sipper tube model allows for drugs to be assessed on their ability to differentially attenuate consumption and the motivation to seek a drug.

Selectivity for Ethanol Versus Non-Drug Reinforcement

Clinically, while naltrexone has been found to reduce sucrose intake (Fantino et al., 1986), the majority of clinical trials do not directly assess the selectivity of a drug for alcohol compared to other reinforcers. However, naltrexone has been shown to attenuate the reinforcement of internet pornography, exercise, cocaine addiction, bulimia, pathological gambling, compulsive sexual addiction, kleptomania, and self-injurious behaviors including trichotillomania (Bostwick & Bucci, 2008; Daniel et al., 1992; Kosten et al., 1992; Marrazzi et al., 1995; Kim et al., 2001; Raymond et al., 2002; Grant & Kim, 2005; Symons et al., 2004; Carrion, 1995). In the animal literature, the ability of naltrexone to selectively attenuate ethanol occurs only at relative low doses, providing an extremely narrow therapeutic range of selectivity before which saccharin and sucrose are also significantly reduced (Czachowski & DeLory, 2009; Kuzmin et al., 2007; Goodwin et al., 2001; Ji et al., 2008). The ability of naltrexone to blunt the reinforcing effects of such a multitude of activities and other reinforcers could partially contribute to the decreased compliance observed in the clinical population. Therefore, and pursuant to the present investigation, it is important that any subsequent treatments developed for modulating alcoholism be selective in their modulation of ethanol reinforcement. Through the use of animal models, testing various drugs on their ability to not only

modulate the seeking and consuming of ethanol but also other palatable reinforcers, such as saccharin and/or sucrose, gives a better indication of the mechanism by which a drug may be mediating ethanol-reinforced behaviors. That is, whether any effects observed on ethanol are mediated by activation of a pathway that is specific to ethanol reinforcement or rather by interfering with pathways implicated in mediating other phenomena, such as feeding or general reinforcement.

Study Rationale and Purpose

Studies that have been conducted examining the role of drugs selective for individual receptor subtypes within the endogenous opioid system suggest their potential as future pharmacotherapies in the management of alcohol dependence. However, little is known about the relative efficacy of these drugs owing to the different conditions under which they have been assessed. Therefore, the general purpose of the present investigation was to compare the effects of three receptor-selective drugs using identical paradigms and samples to ultimately determine whether these drugs selectively mediate certain aspects of ethanol reinforcement, and whether any of these drugs may confer treatment advantages over naltrexone.

In pursuit of these goals, several factors were considered. First, the use of drugs selective for the mu, delta, and kappa receptor subtypes may help elucidate the mechanisms by which these component of the opiate system may be contributing to the maintenance of ethanol reinforcement. Specifically, the goal was to establish if the drugs were capable of attenuating both ethanol consumption and seeking or if they were specific for one aspect of ethanol reinforcement relative to the other. This was accomplished through the use of the sipper-tube model which procedurally separates the two components of reinforcement as previously described. Additionally, it was necessary to determine whether the effects of these receptor selective drugs were specific to ethanol or may generalize to other palatable reinforcers, such as sucrose. This was best accomplished by testing a second, non-drug reinforcer (i.e., sucrose), under identical conditions to those assessing ethanol reinforcement thus enabling direct comparisons. Finally, it was of interest to determine whether the effects of these drugs differ as a function of drinking phenotype as has been suggested in the literature. This was

examined through the utilization of two distinct samples of rats displaying different alcohol-related phenotypes. Specifically, drug outcomes were compared among rats that have been selected for a propensity to drink high amounts of alcohol (P) and outbred Long Evans (LE) rats that have not experienced any selection but have been shown to work for and consume ethanol (Czachowski & Samson, 1999).

Investigation Aims

The specific aims of the present investigation were:

(1) To examine the effects of systemically administered antagonists/agonist selective for the mu (naltrexone < 1.0 mg/kg), delta (naltrindole), and kappa (U50,488H) opioid receptors that have been shown to modulate ethanol reinforcement in a paradigm that separately assesses seeking and consumption of a reinforcer. The efficacy of these antagonists/agonist were compared to those of non-selective doses of naltrexone using the same paradigm.

(2) To evaluate the selectivity of these drugs in modulating ethanol reinforcement by comparing the observed outcomes with those of a second, palatable reinforcer (sucrose); and, when applicable, to assess which dose(s) confers the greatest selectivity in attenuating ethanol versus sucrose reinforcement.

(3) To determine whether selective breeding for high ethanol intake (P rat) confers a greater sensitivity to the effects of these opiate drugs on altering ethanol and/or sucrose seeking and/or consumption when compared to unselected rats that display moderate ethanol intake (Long Evans rat).

Hypothesized Results

Given that the mu and delta opioid receptor systems have been heavily implicated in regulating the reinforcing effects of ethanol, and that they have been demonstrated to attenuate both home cage drinking and cue-induced reinstatement for ethanol, it was expected that both low dose naltrexone (mu-selective) and naltrindole would dose-dependently decrease both the consumption of and appetitive responding for ethanol.

Additionally, given that the clinical literature suggests a role of family history of alcoholism in conferring a greater sensitivity to the effectiveness of naltrexone, it was predicted that the P rats, which represent the FH^+ phenotype, would show a leftward shift in the dose-response relative to Long Evans rats (FH^-) following both low dose naltrexone and naltrindole treatment. Further, as naltrexone has been shown to be most efficacious following a "slip" such that it prevents a full relapse, it was hypothesized that lower doses of naltrexone would be more efficacious in attenuating ethanol intake relative to seeking. Finally, because lower doses of naltrexone have been shown to be selective for ethanol as opposed to other reinforcers such as water, sucrose, and/or saccharin, it was hypothesized that at doses < 1.0 mg/kg, naltrexone would selectively attenuate ethanol drinking.

Contrastingly, very little is known about the role of the kappa system in mediating ethanol reinforcement, other than the observation that the use of kappa agonists more consistently decrease the intake of ethanol than do selective kappa receptor antagonists. Given how few studies there are available, it is difficult to make any hypotheses concerning either the selectivity of U50,488H for ethanol (versus sucrose) reinforcement or any populations differences among rat phenotypes. However, it was predicted that U50,488H would attenuate ethanol consumption based on studies that found U50,488H to decrease ethanol intake. Additionally, because U50,488H was found to both block an ethanol-induced CPP in mice and induce a strong conditioned place aversion when administered alone, indicating that the environment had been used as a cue in gauging the rewarding/aversive effects of a drug, it was hypothesized that U50,488H would also be effective at attenuating appetitive responding for a reinforcer.

METHODS

Subjects

Subjects were 136 age-matched experimentally-naïve adult male rats (n~ 34/study). A total of 64 P (69th-70th generation of selective breeding; obtained from the Indiana University School of Medicine, Indianapolis, IN) and 72 Long Evans rats [obtained from Harlan (Indianapolis, IN)] were used across four drug treatment studies. Each study used approximately 16 P and 18 Long Evans rats that were randomly assigned to one of two groups separated by reinforcer type: sucrose or ethanol (n = 8 P or 9 LE/group). Animals were housed individually in plastic tub-like cages and maintained in a vivarium on a 12:12 hour light/dark cycle (lights on at 700 am) with *ad libitum* access to both food (Lab Diet, 5001, PMI Nutritional International Inc., Brentwood, MO) and water except where noted. All animal care procedures were conducted in accordance with the NIH Guidelines for the Care and Use of Laboratory Animals (1996) and all protocols were approved by the Institutional Animal Care and Use Committee (IACUC).

Drugs

Ethanol was prepared volume/volume in water from 95% (v/v) and diluted to a final concentration of 10% (v/v). Sucrose was prepared weight/volume (w/v) and used as a solute at a final concentration of 2% (w/v). All drug solutions were prepared fresh each day just prior to the start of injections. *Trans –(+)*-3,4, Dichloro-N-methyl-N [2-(1-pyrrolidinyl)cyclohexyl] benzeneacetamidemethanesulfonate salt (U50,488H –a kappa agonist; Tocris Bioscience, Ellisville, MO) and (5α)-17-(Cyclopropylmethyl)-4,5-epoxy-3,14-dihydromorphinan-6-one hydrochloride (naltrexone hydrochloride; Tocris Bioscience, Ellisville, MO) were prepared by dissolving various concentrations of drug in saline. U50,488H (2.5, 5.0, and 10.0 mg/kg) was injected intraperitoneally (IP) in a volume of 1.0 ml/kg 20 minutes prior to the start of testing; saline was used as the

control. 17-(Cyclopropylmethyl)-6,7-dehydro-4,5α-epoxy-3,14-dihydroxy-6,7-2',3'-indolomorphinan hydrochloride (Naltrindole hydrochloride- a delta antagonist; Tocris Bioscience, Ellisville, MO) was prepared by dissolving various concentrations of drug in to sterile water and injected intraperitoneally (IP) in a volume of 1.0 ml/kg 15 minutes prior to testing in doses of 2.5, 5.0, and 10 mg/kg. Sterile water was used as the vehicle control for naltrindole. All drugs were prepared from their salt form.

As naltrexone has been shown to selectively bind to mu receptors at a dose of < 1.0 mg/kg (Childers et al., 1979; Paterson et al., 1984; Takemori & Portoghese, 1984; Emmerson et al., 1994; Mhatre & Holloway, 2003; Wang et al., 2001; 2007) and the mu-selective antagonists CTOP and β-FNA were not employed in the current design, naltrexone was utilized in doses of 0.1, 0.3, and 1.0 mg/kg as the mu-selective receptor antagonist. In addition, a second study assessed the effectiveness of naltrexone as a non-selective opioid antagonist at doses of 1.0, 3.0 and 10.0 mg/kg. Therefore, naltrexone (0.1, 0.3, 1.0, 3.0, and 10.0 mg/kg) was administered subcutaneously (SC) 30 minutes prior to testing; saline was use as the control.

Apparatus

Daily sessions were conducted in sound-attenuated standard operant chambers (Med-Associates; St. Albans, VT; USA; 30 x 30 x 24.5 cm). Each chamber was equipped with a house light, two retractable levers, and a retractable plastic bottle with rubber stopper and stainless steel spout with ball bearings to prevent leakage. The levers were located on either side of the house light and on the wall opposite the retractable sipper-tube. Electrical inputs and outputs from each chamber were controlled using Med-Associates software (Med-Associates).

Training and Ethanol Initiation

Upon arrival, all subjects were weighed and handled for a minimum of 3 days to allow for acclimation to their new environment. Daily sessions were then conducted 5 days/week at the same time each day during the light portion of the light cycle. During the first week of training only, rats were water restricted to facilitate lever pressing acquisition. After the initial week of training, all rats had *ad libitum* access to food and

water in the homecage throughout the duration of the experiment. Subjects were initially trained to press the active lever on a fixed-ratio (FR) schedule that allowed 15-30 seconds of access to the sipper-tube which contained 10% sucrose. During the initial three weeks of training, the subjects underwent a sucrose-fading procedure (Samson, 1986) whereby sucrose concentrations were decreased from 10% to either 0 or 2% and ethanol was gradually increased up to a concentration of 10% (final solutions: sucrose group: 2S; ethanol group 10E). At the same time, the FR was slowly increased from 1 to 4. The procedural separation of seeking (lever pressing) and consumption (fluid drinking) was subsequently implemented in such a way that the animals were required to make a response requirement (RR) of 4 lever presses on the active lever with in a 20 minute time limit. Following the completion of this response requirement, rats were given 20 minutes of uninterrupted access to the sipper-tube. The response requirement was gradually increased from 4 to 10, during which time a second, inactive lever was introduced. Responses on the inactive lever were recorded but had no programmed consequences. Rats were maintained on a RR10 for approximately 3 weeks before the consummatory phase of testing began (described in detail below). Subsequent to consummatory testing, the response requirement increased from 10 to 20 and rats were maintained on a RR20 for approximately two weeks prior to the initiation of the appetitive testing phase (described in detail below).

Consummatory Testing Sessions

During the consummatory testing phase (see Figure 1), rats were run 5 days/week over the course of 4 consecutive weeks. Mondays and Tuesdays of each week served as regular training days during which rats were required to make a response requirement of 10 lever presses in order to gain access to the reinforcer. Rats were divided into the following four drug testing experiments as follows:

Drug Study	Drug	Receptor Selectivity	Drug Doses
Experiment 1:	U50,488H	κ	0, 2.5, 5.0, & 10.0 mg/kg
Experiment 2:	Naltrindole	δ	0, 2.5, 5.0, & 10.0 mg/kg
Experiment 3a:	Naltrexone	μ	0, 0.1, 0.3, & 1.0 mg/kg
Experiment 3b:	Naltrexone	ns	0, 1.0, 3.0, & 10.0 mg/kg

On each Wednesday during the consummatory testing phase of an experiment, each rat was injected with one of four doses of drug (vehicle, low, medium, or high as determined from the literature; see above) in a within-subjects modified Latin Square design a specified time (15-30 minutes) prior to testing initiation. On testing days, the response requirement was lowered to 1 so that the rat was required only to make a single response on the appropriate lever (RR1) to gain access to the reinforcer to account for potential drug-related reductions in locomotor activity. On Thursdays, the response requirement was raised to 5, and on Fridays returned to 10. On testing days, intake (mls were recorded, g/kg were calculated), licks, and latencies to press and lick were recorded.

Appetitive Testing Sessions

Following the consummatory phase of testing, animals resumed regular sessions (no injections) for three weeks with the response requirement gradually raised from 10 to 20 (RR20) lever presses. On the Thursday immediately preceding the first week of appetitive testing, all animals underwent a non-injection extinction day to expose subjects to the extinction procedure (see Figure 1). This session consists of a 20-minute session during which responses on both levers were recorded but had no programmed consequences. During the four weeks of appetitive testing that followed, rats were injected twice/week (Tuesdays and Thursdays). The first weekly injection, on Tuesdays, served as a reinforced control session during which the animals were pretreated with the experiment's vehicle (saline or sterile water) in a volume equivalent to that on a drug testing day, and after the elapsed time (the same pretreatment time following a drug injection), placed into the operant chambers where the appropriate number of responses (RR20) resulted in reinforcer access. These sessions were conducted to prevent the

animals from learning to associate an injection with an extinction session. Thus, the sessions were run just like a drug injection day with the exception that (1) all animals were given vehicle and (2) the animals were reinforced after making the same number of responses (20) that were required on any non-injection training day. Similarly, on appetitive testing days (Thursdays) rats were injected with of one of four doses of drug (vehicle, low, medium, or high) in a within-subjects modified Latin Square design. On these days, following the drug injection and appropriate pretreatment time, the rats were placed in the operant chambers and experience a 20 minute extinction session (i.e., regardless of the number of responses made on the appropriate (active) lever, the reinforcer was never delivered). All other days (Mondays, Wednesdays, and Fridays) were run as regular reinforced training sessions. On appetitive testing days, active (reinforcer-associated) and inactive lever presses and latency to first lever press were recorded.

Blood Ethanol Levels (BELs)

All subjects reinforced with ethanol underwent one operant session either immediately after all testing was concluded or in between the consummatory and appetitive testing phases (see Figure 1) immediately after which they were gently restrained and blood samples were collected to assess blood ethanol levels (BELs). Immediately following the operant session, blood samples (100 µl) were collected from the cut tip of the tail in heparinized capillary tubes and placed on ice during the collection procedure. Immediately after blood collection, bloods were centrifuged and plasma samples (5 µl) were assessed using an Analox AM 1 analyzer (Analox Instruments LTD, Lunenberg, MA) to determine BELs (mg%). Ethanol concentration was determined with an amperometric oxygen electrode that measures oxygen consumption during the enzymatic oxidation of alcohol to acetaldehyde.

Data Analyses

The main dependent variables included in the analyses for each drug include intake (g/kg and ml/kg) for the consummatory phases and active and inactive lever presses for the appetitive phases. In order to determine intakes, prior to each session 25-

35 mL of ethanol or sucrose were measured to the nearest 0.1 mL using an electronic pipette and placed into a drinking tube with a double ball bearing to prevent leakage. At the conclusion of each session, the amount of liquid remaining in each drinking tube was again measured to the nearest 0.1 mL and the amount consumed recorded. To best assess the effectiveness of the various drug doses, all ethanol intakes were reported in g/kg and all sucrose intakes converted to ml/kg to account for any differences in body weights (which were measured daily). Results were analyzed using Mixed Factorial Analyses of Variance (ANOVA)s to assess the effects of drug (dose; vehicle and three doses of drug) as the within-subjects factor and Rat Population (Line; P, Long Evans) and Reinforcer Type (solution; sucrose, ethanol) as the between-subject factors. Post hoc tests were performed where appropriate, and in all cases, significance was determined at $p < 0.05$.

Separate one-way Repeated Measures (RM)-ANOVAs (with dose as the within subjects factor) were run for each group across experiments on intake (g/kg; ml/kg), and active and inactive lever presses to determine which doses were the most effective across each population and reinforcer subgrouping at attenuating intake and responding. Also, to assess whether any of the observed behavioral changes may be due to secondary motor effects of the drug (i.e., sedation), separate RM-ANOVAs were performed on the latency to lever press and latency to first lick (s). As indicated above, all post hoc analyses were performed where appropriate, and in all cases, significance was determined at $p < 0.05$.

Further, as a result of the experimental design, the dependent measure (intake) in the consummatory phase of each experiment is contingent upon a single appetitive response (RR1) being made in order to gain access to the reinforcer and subsequently initiate the 20-minute test trial. Consequently, those rats that failed to make the RR1 and gain access to the reinforcer were given an intake value of '0.' Because the variable of interest for consummatory testing is intake, and there is a separate procedure by which to measure the effects of each drug on the motivation to seek a reinforcer (the appetitive phase), two series of analyses were performed on the consummatory data. First, analyses were run with the inclusion of all subjects (all rats), regardless of whether or not the RR1 was met (a score of '0' was used for intake). Secondly, all analyses were run with the exclusion of any rats that did not meet the RR1 to prevent an artificial decrease of

reinforcer intake (those that gained access to the sipper) by the drug, resulting in a more conservative estimate of the drug effects. Both analyses are included for each drug across each experimental group where applicable. However, for more complicated analyses (anything more than a one-way RM ANOVA within a group) and in all written summaries/discussion, in order to better tease apart any drug effects on actual intake versus the seeking/appetitive process, the more conservatives intake values (those including only rats that gained access to the sipper/reinforcer) were used and are those that are referenced.

Finally, to overcome the challenges in assessing latency, in all instances where a lever response was made (during the consummatory phase), the time in seconds to first lever press (be it active or inactive) was used. If no lever response was made, there was no inclusion of those data in the final analyses as access to the sipper-tube was never acquired. Similarly, when assessing latency to first lick, if the RR1 was never met, these data too were excluded from the analyses. However, if the RR1 was met, but no licks were recorded, the maximum value of 1200s was included in the latency to first lick data. To account for a lack of lever responses, during the appetitive phase, a failure to initiate a single lever response resulted in a maximum session time of 1200s to be used as the latency to first lever press. Thus, during the appetitive phase, any effects of the drug (due to either impaired motivation to respond or inability to respond possibly due to the sedative effects of the drugs) were accounted for by measuring the latency to lever press. A failure to initiate any lever responses was recorded and analyzed as an appetitive response of ‘0.’

GENERAL RESULTS

Data from a total of 136 rats were included in the final analyses, with 134 and 131 included in the consummatory and appetitive phases respectively. Across experimental groups, rats were distributed as follows: kappa agonist U50,488H (experiment 1), n = 34; the delta antagonist naltrindole (experiment 2), n = 34; naltrexone as a selective mu receptor antagonist (experiment 3a), n = 34; naltrexone as a non-selective opioid antagonist (experiment 3b), n = 34. Table 1 shows the distribution of LE and P rats used in the final consummatory and appetitive analyses across each reinforcer for each experiment. P rats weighed an average of 258.0 ($\pm$ 4.9) and LE rats 265.0 ($\pm$ 3.2) g upon arrival (Tables 2-5 show the breakdown of weights across each experiment by group across three different time points: arrival, and just prior to the start of both the consummatory and appetitive phases of testing). Therefore, in order to account for any potential differences between rat populations in body weight across reinforcer conditions, all intakes were recorded and analyzed as a function of weight: ethanol (g/kg); sucrose (ml/kg).

Blood ethanol levels were collected either prior to the start of the appetitive phase (U50,488H and both naltrexone experiments) or subsequent to it (naltrindole only). These data (see Figure 2) reveal that, on average, across experiments, P rats consumed slightly more ethanol (0.927 $\pm$ 0.055 g/kg) than LE rats (0.735 $\pm$ 0.048 g/kg). However, average blood ethanol levels did not vary as a function of ethanol intake (P rats: 57.416 $\pm$ 3.942 mg%; LE rats: 57.811 $\pm$ 3.988 mg%). Further, in both P and LE rats, the average blood ethanol levels were significantly lower in the naltrindole-treated rats as opposed to those getting either U50,488H or naltrexone (see Table 6). While there was no drug on board during blood collections, the naltrindole-treated rats had 4-6 weeks more experience with ethanol and had twice as many drug injections prior to blood collection than did the U50,488H- and naltrexone-treated rats.

U50,488H

Experiment 1: Effects of the Selective Kappa Agonist U50,488H

Following training, rats in experiment 1 were given IP injections of saline, 2.5, 5.0, and 10.0 mg/kg of the kappa agonist U50,488H 20 minutes prior to testing. Rats were then assessed for ethanol (10% v/v) or sucrose (2% w/v) consumption during the consummatory phase and responding on the reinforcer-associated (active) and inactive levers during the appetitive phase. No rats were excluded from either the initial consummatory or appetitive analyses (see Table 2). The exclusion of all 'zero' values that resulted from failure to initiate the intake session (i.e., only subjects that gained access to the reinforcer) resulted in inclusion of the following number of subjects for each U50,488H dose (saline, 2.5, 5.0, and 10.0 mg/kg, respectively) across the following groups: P ethanol (8,7,7,4); LE ethanol (9,9,9,8); P sucrose (8,8,7,6); LE sucrose (9,9,8,8) rats.

Consummatory Phase

A series of one-way RM-ANOVAs were performed to assess the effects of dose of U50,488H on ethanol (g/kg) and sucrose (ml/kg) intakes in P and LE rats. Regardless of whether these subjects were removed from the analyses or whether a zero was assigned for total intake, a main effect of dose of U50,488H was found on ethanol intake (g/kg) in P rats: in all subjects [$F(3,21) = 15.592$, $p < 0.001$] and in only those that gained access to the sipper tube [$F(3,15) = 9.873$, $p < 0.001$]. Post hoc analyses revealed that when all subjects were included, all doses of U50,488H decreased ethanol consumption versus saline, and that administration of 10 mg/kg significantly lowered intake relative to 2.5 mg/kg of U50,488H (see Figure 3a). In contrast, among P rats that gained access to

the sipper tube, only 5.0 and 10.0 mg/kg effectively reduced ethanol consumption relative to saline (see Figure 3b). Similarly, there were main effects of dose found in LE rats on ethanol intake when analyzing both: all subjects [$F(3,24) = 18.411$, $p< 0.001$] and only those that that gained access to the sipper tube [$F(3,23) = 21.426$, $p< 0.001$]. Post hoc analyses signified that, in both instances, all doses of U50,488H attenuated ethanol consumption compared to saline and that 10.0 mg/kg decreased intake relative to 2.5 and 5.0 mg/kg of U50,488H in LE rats (see Figure 4).

Among rats reinforced with sucrose (ml/kg), there were main effects of dose of U50,488H observed in P rats when examining: all subjects [$F(3,21) = 29.597$, $p< 0.001$] and only those that that gained access to the sipper [$F(3,18) = 22.790$, $p< 0.001$]. Follow-up tests determined that in both cases 5.0 and 10.0 mg/kg decreased sucrose intake relative to saline and 2.5 mg/kg and that administration of 10.0 mg/kg further decreased consumption relative to 5.0 mg/kg (see Figure 5). Likewise, there was a main effect of dose found in LE rats on sucrose intake in: all subjects [$F(3,24) = 32.165$, $p< 0.001$] and in only those that gained access to the sipper [$F(3,22) = 30.817$, $p< 0.001$]. Post hoc analyses found that in LE rats, all doses significantly reduced sucrose drinking relative to saline (see Figure 6), but that 10.0 mg/kg decreased sucrose intake versus 2.5 mg/kg of U50,488H only in the analysis where all subjects were included (see Figure 6a).

The following analyses were done using the more conservative approach of including only those rats as subjects that actually gained access to the sipper tube in order to better assess the actual effects of line and dose on reinforcer consumption. Two-way mixed ANOVAs were done to examine the effects of line and dose and whether an interaction between the two was found on both ethanol (g/kg) and sucrose (ml/kg) intakes. A two-way mixed ANOVA failed to find an effect of line [$F(1,38) = 0.0161$, $p = 0.901$] on ethanol intake (g/kg), though there was a main effect of dose [$F(1,38) = 26.160$, $p< 0.001$] and a marginally significant dose*line interaction [$F(3,38) = 2.615$, $p = 0.065$]. Contrastingly, a two-way mixed ANOVA revealed that there were main effects of line [$F(1,40) = 13.931$, $p = 0.002$] and dose [$F(3,40) = 45.622$, $p< 0.001$] as well as a significant line*dose interaction [$F(3,40) = 10.410$, $p< 0.001$] on sucrose intake (ml/kg).

P rats drank significantly more sucrose than LE rats following the administration of saline, 2.5, and 5.0 mg/kg of U50,488H.

One-way RM-ANOVAs were performed to assess whether or not the effects of U50,488H dissipated 24 hours post injection. Results revealed that there were no main effects of dose on ethanol either in P [$F(3,21) = 0.102$, $p = 0.958$] or LE [$F(3,24) = 1.331$, $p = 0.288$] rats. Likewise, there were no main effects of dose of U50,488H on sucrose intake in either P [$F(3,21) = 1.508$, $p = 0.242$] or LE [$F(3,24) = 1.049$, $p = 0.389$] rats 24 hours post injection. Further, paired T-tests indicated that, in all groups, the saline and post-saline intakes did not differ from one another (see Figures 7 & 8).

Appetitive Phase

A series of one-way RM-ANOVAs were employed to assess the effects of dose of U50,488H on appetitive responding on both the reinforcer-associated "active" and non-associated "inactive" levers. Table 7 includes all the reinforcer-associated, inactive, and total lever responses for each subgroup across each dose of U50,488H administered. Results revealed that there was a main effect of dose on active lever responding in P rats reinforced with ethanol: [$F(3,21) = 35.334$, $p< 0.001$]. Post hoc analyses revealed that all doses of U50,488H significantly decreased responding on the ethanol-associated lever relative to saline. Further, while there was a main effect of dose on inactive lever responding [$F(3,21) = 3.437$, $p = 0.035$], post hoc tests failed to find any significant differences among doses (see Figure 9a). Similarly, there was a main effect of dose found on ethanol-associated [$F(3,24) = 12.503$, $p < 0.001$] but not inactive lever responding: [$F(3,24) = 2.942$, $p = 0.053$] in LE rats. Post hoc analyses revealed that only the 5.0 and 10.0 mg/kg doses were capable of attenuating responding on the ethanol-associated lever in LE rats and that at 10.0 mg/kg, responding was significantly decreased compared to the 2.5 mg/kg dose of U50,488H (see Figure 10a).

For P rats reinforced with sucrose, there was a main effect of dose of U50,488H on both active [$F(3,21) = 36.763$, $p < 0.001$] and inactive [$F(3,21) = 9.142$, $p< 0.001$] lever responding. Post hoc analyses reveal that all doses of U50,488H significantly attenuated responding on the sucrose-associated lever, and that administration of 5.0 and 10.0 mg/kg significantly decreased responding compared to the 2.5 mg/kg dose.

Similarly, responding on the inactive lever was decreased by all three doses relative to saline (see Figure 9b). For LE rats reinforced with sucrose, there was a main effect of dose on active [$F(3,24) = 17.728$, $p < 0.001$] but not inactive [$F(3,24) = 1.428$, $p = 0.259$] lever responding. Post hoc analyses revealed that both the 5.0 and 10.0 mg/kg doses of U50,488H significantly decreased responding relative to saline and the 2.5 mg/kg dose of U50,488H (see Figure 10b).

Two-way mixed ANOVAs were done to examine the effects of line, solution, and dose and any resulting interactions on both reinforcer-associated (active) and inactive lever responding. A two-way mixed ANOVA revealed that there were main effects of solution [$F(1,42) = 6.433$, $p = 0.024$] and dose [$F(3,42) = 69.145$, $p < 0.001$] as well as a significant solution*dose interaction [$F(3,42) = 3.239$, $p < 0.001$] on responding for the reinforcer-associated lever in P rats. P rats made more lever responses for sucrose than ethanol following the administration of saline, 2,5, and 5.0 mg/kg U50,488H. Further, U50,488H lessened lever responding in rats reinforced with ethanol and sucrose across all doses relative to saline. In contrast, while there was a main effect of dose [$F(3,48) = 28.874$, $p < 0.001$], neither a main effect of solution [$F(1,48) = 0.389$, $p = 0.541$] nor a significant solution*dose interaction [$F(3,48) = 1.228$, $p = 0.310$] was found among LE rats. A two-way mixed ANOVA failed to find a main effect of line [$F(1,45) = 0.052$, $p = 0.823$], but did reveal a main effect of dose [$F(3,45) = 40.951$, $p < 0.001$] and a significant line*dose interaction [$F(3,45) = 3.875$, $p = 0.015$] in rats responding on the ethanol-associated lever. Results revealed that following saline administration, P rats made significantly more (82.0 ± 6.6) responses on the ethanol-associated lever than did LE rats (61.0 ± 6.2). Similarly, responding on the sucrose-associated lever failed to reveal a main effect of line [$F(1,45) = 2.487$, $p = 0.136$], but did yield a main effect of dose [$F(3,45) = 52.340$, $p < 0.001$] and a significant line*dose interaction [$F(3,45) = 5.240$, $p = 0.003$]. Post hoc analyses revealed that P rats responded significantly more (104.3 ± 7.1) following saline administration than did LE rats (59.9 ± 6.7) on the sucrose-associated lever.

Latencies

A series of one-way RM-ANOVAs examining the effects of dose on latencies among P rats reinforced with ethanol found that injection of U50,488H resulted in a main effect of dose on latency to first lever press [($F(3,15) = 3.424$, $p = 0.045$], but only a marginal one on latency to first lick [$F(3,15) = 2.804$, $p = 0.076$] during the consummatory phase. Additionally, a main effect of dose on latency to first lever press [$F(3,21) = 4.865$, $p = 0.010$] was found during the appetitive phase. Further, while post hoc analyses failed to reveal any dose differences among latency to lever press during the consummatory phase, the time it took to initiate the first lever response during the appetitive phase significantly differed following administration of saline and the high (10.0 mg/kg) dose of U50,488H (see Table 8).

Analyses assessing the effects of dose on latencies among LE rats reinforced with ethanol found that injection of U50,488H failed to find main effects of dose on latency to first lever press: [$F(3,24) = 2.785$, $p = 0.063$] and latency to first lick [$F(3,23 = 1.453$, $p = 0.253$] during the consummatory phase. In contrast, there was a main effect of dose on latency to first lever press [$F(3,24) = 6.304$, $p = 0.003$] during the appetitive phase, the effects of which were due to the high (10 mg/kg) dose differing significantly from the saline and low (2.5 mg/kg) doses (see Table 8).

Analyses investigating the effects of U50,488H administration on latencies to lever press and lick among P rats reinforced with sucrose found that during the consummatory phase, there was a main effect of dose on latency to first lever press [$F(3,20) = 7.645$, $p = 0.001$] but not latency to first lick [$F(3,18) = 2.351$, $p = 0.106$]. Post hoc analyses revealed that this main effect was driven by the high dose (10.0 mg/kg) of U50,488H, administration of which resulted in a significantly longer time to initiate the first lever response than it did following administration of all other doses. Likewise, analyses revealed there was a significantly main effect of dose on latency to first lever press during the appetitive phase [$F(3,21) = 6.617$, $p = 0.003$]. Post hoc analyses revealed that this effect was also driven by the high (10.0 mg/kg) dose of U50,488H differing from saline and the low (2.5 mg/kg) dose (see Table 8).

Lastly, it was determined that among LE rats reinforced with sucrose, injection of U50,488H found a marginal, though not significant main effect of dose on latency to first lever press [$F(3,22) = 2.994$, $p = 0.053$] and a significant main effect on latency to first lick [$F(3,22) = 9.250$, $p < 0.001$] during the consummatory phase. Post hoc analyses revealed that it took significantly longer following administration of the high (10 mg/kg) dose to initiate licking than it did following administration of either saline or 2.5 mg/kg U50,488H. Likewise, there was a main effect of dose on latency to lever press [$F(3,24) = 8.807$, $p < 0.001$] during the appetitive phase with post hoc tests finding that it took longer for rats to make a response following administration of 10 mg/kg U50,488H than it did following the administration of all other doses (see Table 8).

U50,488H Discussion

Summary of Results of U50,488H on Reinforcer Consumption

Unexpectedly, the results from the consummatory phase reveal that Long Evans rats were more sensitive to the effects of the kappa agonist, U50,488H than P rats. Also of interest, within each rat population, U50,488H did not differentially attenuate the intakes of ethanol and sucrose as a function of dose such that among LE rats, the lowest dose of 2.5 mg/kg was able to decrease both sucrose and ethanol intake. However, the magnitude of intake suppression did vary as a function of dose with the highest dose (10.0 mg/kg) resulting in the greatest suppression of ethanol intake while all doses of U50,488H decreased sucrose intake in a similar manner. In contrast, among P rats, the lowest dose capable of eliciting an attenuation of sucrose and ethanol intake was 5.0 mg/kg, and unlike LE rats, P rats reinforced with sucrose and not ethanol displayed a dose-dependent decrement in consumption with maximal suppression occurring following treatment with 10.0 mg/kg of U50,488H.

Summary of Results of U50,488H on Reinforcer Seeking

In regard to the appetitive phase, however, while again there remained no difference among reinforcer type to the sensitivity of U50,488H pretreatment, P rats, collectively, were more sensitive than LE rats to the ability of U50,488H to attenuate responding for a reinforcer. Among P rats, the lowest dose of 2.5 mg/kg was able to significantly decrease responding for both ethanol and sucrose. As noted in the consummatory phase, P rats reinforced with sucrose showed a dose-dependent decrement in magnitude of responding while all doses of U50,488H decreased responding for ethanol to a similar degree. Among LE rats, the least effective dose capable of decreasing responding for a reinforcer was 5.0 mg/kg. Among LE rats reinforced with sucrose, the least effective dose did not differ from the highest dose in degree of response suppression while LE rats treated with U50,488H showed a greater magnitude of response attenuation following treatment with 10 mg/kg (see Table 9a for a summary of the results).

Pharmacotherapeutic Potential of U50,488H

The findings of this study are consistent with findings that have found kappa agonists to be effective in decreasing ethanol drinking (Lindholm et al., 2001; Nestby et al., 1999; Logrip et al., 2008; Cosgrove & Carroll, 2002). What was surprising were the differential effects of U50,488H on intake and responding such that LE rats were more sensitive to the effect of U50,488H on consumption and P rats more sensitive to the effects of the drug on responding. Further, there were no differences in the ability of the kappa receptor to differentially affect ethanol or sucrose reinforcement among P and LE rats, suggesting that the role of the kappa receptor system is more general. The role of the kappa system is one of dysphoria and displeasure (O'Brien et al., 1996; O'Malley, 1996; Walsh et al., 2001). In humans, treatment with U50,488H has been shown to induce altered pain sensation, hypothermia and/or flushing and hallucinations while use in animals has resulted in altered motor behavior, sedation, diuresis, and malaise. As such, it is not surprising that the kappa system could be attenuating reinforcer-reward not only by eliminating the positive reinforcing effects but also by possibly inducing negative ones. For example, kappa agoinsts have been shown to produce strong conditioned place aversions when administered alone (Iwamoto, 1985; Mucha & Herz, 1985; Shippenberg & Herz, 1986; Bals-Kubik et al., 1989) or in combination with other drugs (Glick et al., 1995; Logrip et al., 2009), and pretreatment with the kappa antagonists, such as nor-BNI, can block these effects (Glick et al., 1995).

The kappa system has also been implicated in mediating the negative reinforcing effects of ethanol when induced through dependence (Hölter et al., 2000; Koob & Walker, 2008; Walker et al., 2010), usually through the use of a kappa antagonist, such as nor-BNI. In contrast to the role of the kappa system in mediating the positive reinforcing effects of a drug (as in the present studies), when mediating the negative reinforcing effects, such as through the stress pathway, treatment with the kappa agonist U50,488H is reinforcing. For example, pretreatment with U50,488H potentiated a CPP to cocaine following exposure to stressors, such as the forced swim test and fear conditioning (McLaughlin et al., 2003, 2006a), and pretreatment with nor-BNI blocks these stress-induced CPP enhancements (McLaughlin et al., 2003, 2006a, 2006b). Therefore, the role

of the kappa system in mediating positive reinforcement may be acting through some sort of general dysphoric-type mechanism that may demonstrate more selectivity during times of stress or following dependence. However, in these present studies, the goal was to assess the effects of various receptor-selective drugs on the positive reinforcing effects of ethanol and sucrose reinforcement with stress kept to a minimum.

The lack of selectivity through the use of U50,488H for either ethanol or sucrose could also be a function of dose whereby the doses utilized in the current study were not low enough to detect any discernable differences in selectivity. In the current study, the doses tested were 2.5, 5.0, and 10.0 mg/kg. These selection of these doses were derived primarily from a study by Lindholm and colleagues (2001) that found single (IP) injections of either 2.5 or 5.0 mg/kg of U50,488H in Male Lewis rats failed to alter ethanol intake. In contrast, there was a significant decrease in ethanol intake seen following pretreatment with 10.0 mg/kg. However, 2.5 and 5.0 mg/kg were able to alter ethanol intake following 3 days of daily U50,488H treatment. In contrast, in DBA/2 mice, while 1.0 and 3.0 mg/kg of U50,488H were unable to induce a CPP alone, both doses were able to block a CPP to ethanol (Logrip et al., 2009). Likewise, Bal-Kubik and colleagues (1989) found doses as low as 1.0 and 2.0 but not 0.5 mg/kg (SC) of U50,488H induced a conditioned place aversion in Sprague-Dawley rats. Therefore, it is possible that the utilization of a lower dose range of U50,488H (such as 0.3, 1.0, and 3.0 mg/kg) may be produced more discernible results in terms of selectivity.

Overall, the findings from this experiment reveal that while 5.0 mg/kg of U50,488H was able to effectively attenuate ethanol intake and responding, it was equally efficacious at attenuating sucrose intake and responding. In fact, at this dose, which was minimally effective for decreasing ethanol reinforcement, there was actually a greater decrease seen in sucrose reinforcement. Therefore, the results indicate that U50,488H, at least at the doses tested, would not be a viable option to selectively reduce ethanol as it had a greater effect on the more palatable sucrose reinforcer, and may be a line of research that should be further pursued in studies that focus more on the role of the opioid system on mediating negative reinforcement and alcohol dependence.

NALTRINDOLE

Experiment 2: Effects of the Selective Delta Antagonist Naltrindole

Following training, rats in experiment 2 were given IP injections of vehicle (distilled water), 2.5, 5.0, and 10.0 mg/kg of the delta antagonist naltrindole 15 minutes prior to testing. Rats were then assessed for ethanol (10% v/v) or sucrose (2% w/v) consumption during the consummatory phase and responding on the reinforcer-associated (active) and inactive levers during the appetitive phase. No rats were excluded from either the initial (all subjects) consummatory or appetitive analyses (see Table 3). The exclusion of all 'zero' values that resulted from failure to initiate the intake session (i.e., only subjects that gained access to the reinforcer) resulted in inclusion of the following subjects for each naltrindole dose (vehicle, 2.5, 5.0, and 10.0 mg/kg, respectively) across the following groups: P ethanol (8,8,6,1); LE ethanol (9,8,9,2); P sucrose (8,7,7,2); LE sucrose (9,9,9,6) rats.

Consummatory Phase

A series of one-way RM-ANOVAs were performed to assess the effects of naltrindole on ethanol (g/kg) and sucrose (ml/kg) intakes in P and LE rats. Regardless of whether these subjects were removed from the analyses or whether a zero was assigned for total intake, a main effect of dose of naltrindole was found on ethanol intake in P rats: in all subjects [$F(3,21) = 36.046$, $p < 0.001$] and in only those that gained access to the sipper tube [$F(3,12) = 13.176$, $p < 0.001$]. Post hoc analyses revealed that when all subjects were included, 5.0 and 10.0 mg/kg of naltrindole significantly decreased ethanol consumption relative to vehicle and 2.5 mg/kg, and that the administration of 10.0 mg/kg further lowered drinking relative to 5.0 mg/kg of naltrindole (see Figure 11a). Likewise, in subjects that gained access to the sipper tube, 5.0 and 10.0 mg/kg effectively reduced ethanol consumption relative to vehicle and 2.5 mg/kg, and 10 mg/kg further attenuated

intake compared to 5.0 mg/kg of naltrindole (see Figure 11b). Additionally, because only one P rat successfully gained access to the sipper tube following administration of the highest (10.0 mg/kg) dose of naltrindole, another one-way RM ANOVA was run examining the effects of vehicle and only the two lower doses of naltrindole (2.5 and 5.0 mg/kg) on ethanol intake in P rats. The results illustrated a main effect of dose [$F(2,12) = 6.360$, $p = 0.013$]; post hoc analyses showed that 5.0 mg/kg significantly decreased intake versus both saline and 2.5 mg/kg of naltrindole. In contrast, while there was a main effects of dose found among LE rats on ethanol consumption when analyzing: all subjects [$F(3,24) = 9.038$, $p< 0.001$]; no main effect of dose was found when looking at only those LE rats that that gained access to the sipper tube [$F(3,16) = 0.617$, $p< 0.614$]. Post hoc analyses initially signified that the highest dose of naltrindole significantly attenuated ethanol consumption compared to saline, 2.5, and 5.0 mg/kg of naltrindole (see Figure 12a); however, when looking at only those rats that gained access to the sipper tube, there was no longer a main effect of dose and no difference among intakes (see Figure 12b).

Among rats reinforced with sucrose (ml/kg), there was again a main effect of dose observed in P rats when examining all subjects [$F(3,21) = 9.332$, $p< 0.001$; see Figure 13a], but this effect was no longer significant when examining only those P rats that that gained access to the sipper [$F(3,13) = 1.940$, $p = 0.173$; see Figure 13b]. Post hoc tests revealed that 10.0 mg/kg significantly reduced sucrose consumption compared to saline, 2.5, and 5.0 mg/kg of naltrindole when all subjects were included in the analyses. As with P rats reinforced with sucrose, analyses revealed that again, while there was a main effect of dose among all LE subjects reinforced with sucrose [$F(3,24) = 3.559$, $p = 0.029$; see Figure 14a]; this main effect of dose no longer remained significant when including only those LE rats that gained access to the sipper tube [$F(3,21) = 0.960$, $p = 0.430$; see Figure 14b). While an initial main effect of dose was found on sucrose drinking, post hoc analyses failed to reveal any significant differences among doses when all subjects were included in the analyses.

The following analyses were done using the more conservative approach of including only those rats as subjects that actually gained access to the sipper tube in order to better assess the actual effects of line and dose on reinforcer consumption. Two-way

mixed ANOVAs were done to examine the effects of line and dose and whether an interaction between the two was found on both ethanol (g/kg) and sucrose (ml/kg) intakes. A two-way mixed ANOVA revealed that there was no main effect of line [$F(1,28) = 0.022$, $p = 0.883$], but that there was a main effect of dose [$F(3,28) = 7.457$, $p < 0.001$] and only a marginally significant line*dose interaction [$F(3,28) = 2.812$, $p = 0.058$] on ethanol intake (g/kg). While a two-way mixed ANOVA found a main effect of line [$F(1,34) = 9.742$, $p = 0.006$] on sucrose intake, there was neither a main effect of dose [$F(3,34) = 2.535$, $p = 0.073$] nor a significant dose*line interaction [$F(3,34) = 0.386$, $p = 0.764$]. Results revealed that, overall, P rats drank significantly more sucrose (38.9 ± 4.3) than LE rats (24.3 ± 3.0).

One-way RM-ANOVAs were performed to assess whether or not the effects of naltrindole dissipated 24 hours post injection. Results revealed that there were no main effects of dose on ethanol among either P [$F(3,21) = 1.704$, $p = 0.197$] or LE rats [$F(3,24) = 1.761$, $p = 0.181$] 24 hours post injection. Likewise, there were no main effects of dose of naltrindole on sucrose intakes among either P [$F(3,20) = 0.883$, $p = 0.467$] or LE [$F(3,23) = 0.113$, $p = 0.952$] rats. Further, paired T-tests also indicated that, in all groups, the vehicle and post-vehicle intakes did not differ from one another (see Figures 15 and 16).

Appetitive Phase

A series of one-way RM-ANOVAs were run to assess the effects of dose of naltrindole on appetitive responding on both the reinforcer-associated "active" and non-associated "inactive" levers. Table 10 includes all the reinforcer-associated, inactive, and total lever responses for each subgroup across each dose of naltrindole administered. Results revealed that there was a main effect of dose on both active [$F(3,21) = 10.747$, $p < 0.001$] and inactive [$F(3,21) = 3.904$, $p = 0.023$] lever responding in P rats reinforced with ethanol. Post hoc analyses revealed that all doses of naltrindole significantly decreased responding on the ethanol-associated lever relative to vehicle (distilled water; see Figure 17a). As for the inactive lever, only the highest dose (10.0 mg/kg) of naltrindole decreased responding relative to vehicle. In contrast, analyses revealed that there was no main effect of naltrindole on responding for either the active [$F(3,24) =$

1.995, $p = 0.142$] or inactive [$F(3,24) = 2.062$, $p = 0.132$] lever in LE rats reinforced ethanol (see Figure 18a).

Among P rats reinforced with sucrose, the analyses revealed that there was a main effect of naltrindole on the sucrose-associated [$F(3,18) = 9.145$, $p< 0.001$] but not inactive [$F(3,18) = 1.756$, $p = 0.192$] lever. Post hoc tests indicated that both the 5.0 and 10.0 mg/kg doses decreased responding on the sucrose-associated lever relative to vehicle and that 10.0 mg/kg further attenuated responding relative to 2.5 mg/kg of naltrindole (see Figure 17b). Likewise, there was a main effect of dose of naltrindole on active [$F(3,24) = 6.720$, $p = 0.002$] but not inactive [$F(3,24) = 3.008$, $p = 0.050$] lever responding in LE rats reinforced with sucrose. Post hoc analyses showed that 10 mg/kg decreased responding on the sucrose-associated lever relative to vehicle and 2.5 mg/kg of naltrindole (see Figure 18b).

Two-way mixed ANOVAs were done to examine the effects of line, solution, and dose and any resulting interactions on both reinforcer-associated (active) and inactive lever responding. A two-way mixed ANOVA revealed that there was a marginal but non significant main effect of solution [$F(1,39) = 4.486$, $p = 0.054$] and a significant main effect of dose [$F(3,39) = 19.516$, $p< 0.001$], but no solution*dose interaction [$F(3,39) = 0.355$, $p = 0.786$] on responding for the reinforcer-associated lever in P rats. Results revealed that there was a main effect of naltrindole on both solution [$F(1,48) = 12.159$, $p = 0.003$] and dose [$F(3,48) = 7.420$, $p< 0.001$], but no significant solution*dose interaction [$F(3,48) = 1.589$, $p = 0.204$] in LE rats. Overall, LE rats responded significantly more on the sucrose- (39.0 ± 3.8) than ethanol-associated (20.3 ± 3.8) lever. A two-way mixed ANOVA failed to find a main effect of line [$F(1,45) = 0.014$, $p = 0.909$] but did reveal a main effect of dose [$F(3,45) = 8.879$, $p< 0.001$] and a significant line*dose interaction [$F(3,45) = 3.220$, $p = 0.031$] in rats responding on the ethanol-associated lever. Post hoc analyses revealed that following the administration of saline, that P rats responded significantly more on the ethanol-associated lever (57.4 ± 8.6) than did LE rats (25.0 ± 8.1). Responding on the sucrose-associated lever failed to reveal a significant main effect of line [$F(1,42) = 0.087$, $p = 0.772$] or a line*dose interaction [$F(3,42) = 0.542$, $p = 0.656$], but did yield a main effect of dose [$F(3,42) = 14.925$,

$p<0.001$]. Post hoc analyses revealed that, overall, all doses of naltrindole significantly reduced responding compared to saline on the sucrose-associated lever.

Latencies

A series of one-way RM-ANOVAs examining the effects of dose on latencies among P rats reinforced with ethanol found that naltrindole administration resulted in main effects of dose on latency to first lever press [($F(3,13) = 3.852$, $p = 0.036$], and latency to lick [$F(3,12) = 1962409.011$, $p< 0.001$] during the consummatory phase, and a main effect of dose on latency to first lever press [$F(3,21) = 10.293$, $p< 0.001$] during the appetitive phase. Further, while post hoc analyses failed to reveal any dose differences among latency to lever press during the consummatory phase, the time it took to initiate licking following sipper access took significantly longer following the administration of 10 mg/kg of naltrindole that it did following all other doses. Additionally, it took longer to initiate licking following the administration of 5.0 mg/kg of naltrindole than it did following the administration of either vehicle or 2.5 mg/kg. Regarding latency to first lever response in the appetitive phase, it took significantly longer for a response to be initiated following the administration of either 5.0 or 10.0 mg/kg of naltrindole than it did following the administration of vehicle and 2.5 mg/kg of naltrindole (see Table 11).

Analyses assessing the effects of dose on latencies among LE rats reinforced with ethanol found that injection of naltrindole during the consummatory phase failed to produce a main effect of dose on either latency to first lever press [$F(3,16) = 3.024$, $p = 0.060$] or latency to first lick [$F(3,16) = 0.692$, $p = 0.570$]. In contrast, there was a significant main effect of dose on latency to first lever response during the appetitive phase [$F(3,24) = 10.035$, $p< 0.001$]. Post hoc analyses revealed that this effect was driven by the high dose in that it took significantly longer for rats to emit a lever response following the administration of 10 mg/kg of naltrindole than it did after receiving all other doses (see Table 11).

Analyses investigating the effects of naltrindole administration on latencies to lever press and lick among P rats reinforced with sucrose found that during the consummatory phase, there were neither main effects of dose on latency to lever press [$F(3,14) = 2.068$, $p = 0.151$] nor latency to first lick [$F(3,13) = 0.226$, $p = 0.877$].

Contrastingly, there was a significantly main effect of dose on latency to first lever press during the appetitive phase [$F(3,18) = 4.849$, $p = 0.012$], the effects of which were driven by the high (10.0 mg/kg) dose in that it took significantly longer for rats to emit a lever response following the administration of 10 mg/kg than it did after receiving either vehicle or 2.5 mg/kg naltrindole (see Table 11).

Similar to above, examining the effects of naltrindole administration on latencies to lever press and lick among LE rats reinforced with sucrose found no main effects of dose on either [$F(3,20) = 1.398$, $p = 0.272$; $F(3,20) = 0.206$, $p = 0.891$] during the consummatory phase, but did find a main effect of dose on latency to first lever response during the appetitive phase [$F(3,24) = 22.223$, $p< 0.001$]. Post hoc analyses revealed that this effect was driven by the administration of 10.0 mg/kg of naltrindole, which resulted in a longer latency to make an initial lever response than it did following all other doses (see Table 11).

Naltrindole Discussion

Summary of Results of Naltrindole on Reinforcer Consumption

As predicted, P rats reinforced with ethanol showed a greater sensitivity to the effects of the selective delta opioid receptor antagonist naltrindole on consumption than did any other population/reinforcer combination. However, what was unexpected was that P rats reinforced with ethanol were the only group to show *any* alterations in reinforcer intake as a function of naltrindole treatment. That is, while naltrindole was able to dose-dependently decrease ethanol intake among P rats, there were no doses of naltrindole capable of altering consumption patterns either of ethanol among LE rats or, of interest clinically, sucrose among P or Long Evans rats. These results would seem to suggest that naltrindole is selective for its attenuation of ethanol only and among a population of rats that has been selected to confer a familial history of high alcohol drinking.

Summary of Results of Naltrindole on Reinforcer Seeking

During the appetitive phase, P rats reinforced with ethanol were again more sensitive to the effects of naltrindole than all other population/reinforcer groups, with doses as low as 2.5 mg/kg capable of decreasing responding for ethanol. However, in contrast to the intake studies, naltrindole was able to dose dependently decrease responding for sucrose among both P and LE rats. Among the sucrose-reinforced rats, P rats again revealed a greater sensitivity to the effects of naltrindole dose as 5.0 mg/kg was able to demonstratively decrease sucrose responding in P but not LE rats (as summarized in Table 9).

Pharmacotherapeutic Potential of Naltrindole

The results of this experiment are consistent with other studies that have found naltrindole to be effective at reducing ethanol consumption in the alcohol preferring (P) rats (Krishnan-Sarin et al., 1995a, 1995b), the High Alcohol Drinking (HAD) rats (Froehlich et al., 1991) and in inbred (C57BL/6) mice that have been shown to consume large volumes of ethanol (Lê et al., 1993; Kim et al., 2000). In contrast, only one study

has examined the effect of systemic naltrindole on LE rats, and the results varied as a function of alcohol paradigm employed. For example, when LE rats were given 24 hour, continuous access to 10% ethanol and water in the home cage, both 5.0 and 10.0 mg/kg of naltrindole effectively reduced ethanol intake (Nielsen et al., 2008). In this same study, however, when LE rats were given intermittent access to 20% ethanol for 24 hours, 3 times/week, naltrindole had no effect on ethanol intake (Nielsen et al., 2008). Among Wistar rats, systemic administration of both 5.0 and 15.0 mg/kg naltrindole failed to alter intake of a 6% ethanol solution during 60 minutes of limited access (Stromberg et al., 1998).

Of interest, is the observation that naltrindole seemed to have, particularly at the 10.0 mg/kg dose in P rats attenuated the intake of ethanol consumption not through a significant reduction of intake behavior, but by proxy by preventing P rats from initiating the start of the consummatory session. This was accomplished by preventing the completion of the single appetitive response (RR1) that subsequently results in presentation of the reinforcer and begins initiates the 20, uninterrupted minutes of free access the rat earns to the reinforcer. This presented an initial complication in attempting to interpret the data because to include the consumption as an arbitrary ‘0’ (arguable by the fact that none of the reinforcer was consumed and that this was a product of drug treatment), would artificially increase the efficacy of a drug presumed to be decreasing ethanol reinforcement by decreasing intake, and further would more likely misattribute an effect to the drug that is really not there (essentially make a Type I error). Therefore, this observation provided a vulnerability in the sipper tube model not previously encountered. As such, it was determined that because this model is attempting to separately assess the effects of a drug on the seeking and consuming aspects of reinforcement, that during the consuming phase, the only intakes that would be included in the final analyses were those that resulted from making the RR1 response and were given the opportunity to consume the reinforcer. As the appetitive phase of the model takes in to account any locomotor effects that may be misattributed to drug efficacy, the consummatory phase only included analyses for those rats that gained the access to consume the reinforcer.

That said, the results from this study were all the more interesting considering how selective the locomotor effects of naltrindole were for P rats, especially those getting ethanol. However, what is even more intriguing and somewhat confusing, is that the ethanol-reinforced rats that were given naltrindole experienced the effects of the drug so severely that they were, with one exception, incapable of initiating the single RR response required to initiate the onset of the consummatory phase -indicating that the observed effects in P rats were independent of ethanol as it was never on board. In contrast, while this observation was noted to somewhat of a lesser degree in LE rats reinforced with ethanol and P rats reinforced with sucrose, neither of these groups showed such profound deficits in their ability to respond such that most of the them were able to initiate the onset of the consummatory phase. And further, once the access to the reinforcer was gained, naltrindole was ineffective at implementing any sort of attenuation in reinforcer consumption at doses in these groups that rendered rats in the P/ethanol group utterly immobile. These results suggest that a history of ethanol exposure, independent of dependence, in P rats may be all that is required in order to show such an adverse reaction to naltrindole.

Physical observations of the P rats following administration of the 10 mg/kg dose at the completion of the session showed them to be lethargic and sluggish. Therefore, the effects of the drug at the high dose on ethanol attenuation are likely attributed to the locomotor effects and the high dose, which only had one rat included in the analyses, was disregarded and analyses were then reperformed using only saline, 2.5, and 5.0 mg/kg naltrindole. However, in prior studies, doses of naltrindole up to 30 mg/kg have been used in some rats with minimal effects on locomotion reported. Further, a study conducted by Froehlich and colleagues (1998) investigating the effects of naltrindole on the development of a conditioned taste aversion (CTA) in P rats, the same rat line utilized in the current study, determined that doses of 0, 5.0, and 10.0 mg/kg were unable to induce a CTA to a banana-flavored solution. In fact, as much as 20 mg/kg of naltrindole was required for a CTA to develop. These results suggest that the P rats, presumably ethanol naïve, failed to experience any aversions that would be strong enough to attribute to the novel tastant following even the 10.0 mg/kg dose of naltrindole. Additionally, in

this same study, IP injections of 0.5 g/kg of ethanol –a dose so low that it is actually sometimes considered rewarding when given alone, when paired with 5.0 or 10.0 mg/kg of naltrindole, (both previously ineffective at inducing a CTA to banana flavored solution) induce a very strong CTA (Froehlich et al., 1998). These results add support to the current findings that ethanol and naltrindole interact to produce strong effects in P rats and further, that very little ethanol was required to facilitate this interaction, be it a history of voluntary ethanol consumption (as in the current study) or experience with low dose injections of 0.5 g/kg of ethanol (as just previous described).

These findings could lead to the initial conclusion that perhaps it is the delta opioid system that is facilitating the observed differences in those with histories of high alcohol drinking to have more efficacious responses to naltrexone. That is, naltrexone is capable of blocking both delta and mu opioid receptors, and this evidence suggests that selective blockade of the delta receptors is mediating the differences observed among selected lines. However, other evidence suggests that naltrindole is not as 'efficacious' in all selected lines, particularly the AA rats, which seem to be relatively immune to the effects observed in P rats following naltrindole administration in those with a history of ethanol familiarity (Honkanen et al., 1996). In contrast, the results of other studies seem to support the finding that the effects of naltrindole are more selective to P rats as no effect was found in Wistar (Stromberg et al., 1998) or Long Evans rats (Higley & Kiefer, 2006), though it is difficult to assess the effects in those studies that did show effects as none of them reported having any impaired locomotor responses. Therefore, it seems likely, for now given the research with AA rats and the fact that P rats reinforced with sucrose showed limited impairment such that consumption was not affected, that the effects of naltrindole observed on P rats were specific for P rats with a history of ethanol exposure and point to some underlying sensitivity that synergistically was activated when low doses of naltrindole interacted with a prior history of ethanol experience.

Given the findings of the current experiment, the most effective dose can be concluded as 5.0 mg/kg as it was selective for decreasing ethanol though only in P rats, which implicates that naltrindole use may demonstrate more efficacious results in

individuals displaying a phenotype for a predisposition towards high ethanol intake, as indicated through a strong history of familial alcoholism.

NALTREXONE

Experiment 3a: Effects of Naltrexone at Doses Specific for Mu Antagonism

Following training, rats in experiment 3a were given SC injections of saline, 0.1, 0.3, and 1.0 mg/kg of low doses (specific for the mu receptor) of the antagonist naltrexone 30 minutes prior to testing. Rats were then assessed for ethanol (10% v/v) or sucrose (2% w/v) consumption during the consummatory phase and responding on the reinforcer-associated (active) and inactive levers during the appetitive phase. No rats were excluded from either the initial (all subjects) consummatory or appetitive analyses (see Table 4). The exclusion of all 'zero' values that resulted from failure to initiate the intake session (i.e., only subjects that gained access to the reinforcer) resulted in inclusion of the following number of subjects for each naltrexone dose (saline, 0.1, 0.3 and 1.0 mg/kg, respectively) across the following groups: P ethanol (8,8,8,7); LE ethanol (9,8,9,9); P sucrose (8,8,8,8); LE sucrose (9,9,9,9) rats.

Consummatory Phase

A series of one-way RM ANOVAs were performed to assess the effects of dose of naltrexone on reinforcer intakes in P and LE rats. Regardless of whether these subjects were removed from the analyses or whether a zero was assigned for total intake, a main effect of dose of naltrexone was found on ethanol intake (g/kg) in P rats: in all subjects [$F(3,21) = 21.782$, $p < 0.001$] and in only those that gained access to the sipper tube [$F(3,20) = 19.015$, $p < 0.001$]. Post hoc analyses revealed that when all subjects were included, all doses of naltrexone decreased ethanol consumption versus saline, and that the administration of 0.3 and 1.0 mg/kg significantly lowered intakes relative to 0.1 mg/kg of naltrexone (see Figure 19a). Similarly, in subjects that gained access to the sipper tube, all three doses of naltrexone again reduced ethanol intakes compared to saline, but only 1.0 mg/kg attenuated drinking relative to 2.5 mg/kg of naltrexone (see

Figure 19b). Similarly, there were main effects of dose found in LE rats on ethanol consumption when analyzing both all subjects [$F(3,24) = 7.263$, $p < 0.001$] and only those that gained access to the sipper tube [$F(3,23) = 7.400$, $p = 0.001$]. Post hoc analyses signified that across all subjects, all doses of naltrexone significantly decreased intake compared to saline (see Figure 20a). However, among those rats that attained ethanol access, only 0.3 and 1.0 mg/kg of naltrexone significantly attenuated drinking relative to saline (see Figure 20b).

As all P and LE rats attained sucrose (ml/kg) access following each injection, only one RM ANOVA was run on each rat population (P and LE) in which all rats were included. Results revealed there were main effects of dose of naltrexone observed in both P [$F(3,21) = 25.228$, $p < 0.001$] and LE [$F(3,24) = 5.174$, $p = 0.007$] rats. Post hoc analyses revealed that among P rats, all doses of naltrexone decreased sucrose consumption relative to saline, and that 1.0 mg/kg further lowered intake versus 0.1 and 0.3 mg/kg of naltrexone (see Figure 21a). In contrast, among LE rats, only 0.3 mg/kg was capable of significantly attenuating sucrose intake compared to saline and 0.1 mg/kg of naltrexone (see Figure 21b).

The following analyses were done using the more conservative approach of including only those rats as subjects that actually gained access to the sipper tube in order to better assess the actual effects of rat population and dose on reinforcer consumption. Two-way mixed ANOVAs were done to examine the effects of line and dose and whether an interaction between the two was found on both ethanol (g/kg) and sucrose (ml/kg) intakes. A two-way mixed ANOVA revealed that while there was a main effect of dose [$F(3,43) = 24.320$, $p < 0.001$], there was neither a main effect of line [$F(1,43) = 0.220$, $p = 0.646$] or a significantly line*dose interaction [$F(3,43) = 1.552$, $p = 0.215$] of naltrexone on ethanol intake. In contrast, results revealed that there were main effects of both dose [$F(3,45) = 28.355$, $p < 0.001$] and line [$F(1,45) = 29.894$, $p < 0.001$] and a significantly line*dose interaction [$F(3,45) = 13.368$, $p < 0.001$] on sucrose intake in rats administered low doses of naltrexone. Follow-up analyses revealed that P rats drank significantly more sucrose than LE rats following the administration of saline, 0.1, 0.3, and 1.0 mg/kg of naltrexone. Similarly, the main effect of dose was driven almost

entirely by the effect of naltrexone on P rats, which were much more sensitive to the effects of naltrexone than were the LE rats (see Figure 21).

One-way RM-ANOVAs were performed to assess whether or not the effects of naltrexone dissipated 24 hours post injection. Results revealed that there were no main effects of dose on ethanol intake in either P [$F(3,21) = 0.912$, $p = 0.452$] or LE [$F(3,23) = 0.615$, $p = 0.612$] rats 24 hours post injection . Likewise, there were no main effects of dose of naltrexone on sucrose intakes in either P [$F(3,21) = 2.073$, $p = 0.134$] or LE [$F(3,23) = 0.290$, $p = 0.832$] rats. Further, paired T-tests also indicated that, in all groups, the saline and post-saline intakes did not differ from one another (see Figures 22 & 23).

Appetitive Phase

A series of one-way RM-ANOVAs were run to assess the effects of dose of naltrexone on appetitive responding on both the reinforcer-associated "active" and non-associated "inactive" levers. Table 12 includes all the reinforcer-associated, inactive, and total lever responses for each subgroup across each dose of naltrexone administered. Results revealed that there was a main effect of dose of naltrexone on responding on the ethanol-associated [$F(3,18) = 13.271$, $p< 0.001$] but not the non-associated [$F(3,18) = 1.518$, $p = 0.244$] in P rats. Post hoc analyses revealed that all doses of naltrexone were able to attenuate P responding on the ethanol-associated lever relative to saline (see Figure 24a). Similarly, there was a main effect of naltrexone on active [$F(3,21) = 5.585$, $p = 0.006$] but not inactive [$F(3,21) = 2.906$, $p = 0.059$] lever responding in LE rats reinforced with ethanol. Post hoc analysis revealed that the 0.1 and 1.0 (but not 0.3) mg/kg doses of naltrexone significantly reduce responding on the ethanol-associated lever relative to saline (see Figure 25a).

Among rats reinforced with sucrose, one-way RM-ANOVAs revealed that among P rats, there was a significant main effect of dose of naltrexone on active lever responding [$F(3,21) = 6.917$, $p = 0.002$] with follow-up tests revealing that only the high (1.0 mg/kg) dose of naltrexone was capable of attenuating responding on the sucrose-associated lever relative to saline. In contrast, there was no main effect of dose on inactive lever responding [$F(3,21) = 0.904$, $p = 0.456$] in P rats reinforced with sucrose

(see Figure 24b) or on either active [$F(3,24) = 2.737$, $p = 0.066$] or inactive [$F(3,24) = 0.764$, $p = 0.525$] lever responding in LE rats reinforced with sucrose (see Figure 25b).

Two-way Mixed ANOVAs were done to examine the effects of line, solution, and dose and any resulting interactions on both reinforcer-associated (active) and inactive lever responding. A two-way mixed ANOVA revealed that there were main effects of solution [$F(1,39) = 7.029$, $p = 0.020$] and dose [$F(3,39) = 10.072$, $p< 0.001$] and a marginally, but not statistically, significant solution*dose interaction [$F(3,39) = 2.324$, $p = 0.090$] on responding for the reinforcer-associated lever in P rats. Overall, P rats made more lever responses for sucrose (51.5 ± 3.8) than ethanol (36.7 ± 4.1). Further, the main effect of dose was driven by the ability of all doses of naltrexone to, overall, decrease responding across reinforcer relative to saline. In contrast, while there was a main effect of dose [$F(3,45) = 7.240$, $p< 0.001$], neither a main effect of solution [$F(1,45) = 0.000$, $p = 0.984$] nor a significant solution*dose interaction [$F(3,45) = 0.670$, $p = 0.575$] was found among LE rats. A two-way mixed ANOVA failed to find a main effect of line [$F(1,39) = 0.005$, $p = 0.946$], but did reveal a main effect of dose [$F(3,39) = 19.107$, $p< 0.001$] and a marginally significant line*dose interaction [$F(3,39) = 2.816$, $p = 0.052$] in rats responding on the ethanol-associated lever. Similarly, responding on the sucrose-associated lever failed to reveal a significant main effect of line [$F(1,45) = 3.984$, $p = 0.064$] or a line*dose interaction [$F(3,45) = 1.823$, $p = 0.156$], but did yield a main effect of dose [$F(3,45) = 8.130$, $p< 0.001$]. Post hoc analyses revealed that, overall, all doses of naltrexone significantly reduced responding compared to saline on both the ethanol- and sucrose-associated levers.

Latencies

A series of one-way RM-ANOVAs examining the effects of dose on latencies among P rats reinforced with ethanol found that treatment with low doses of naltrexone failed to produce either a main effect of dose on latency to first lever press [($F(3,21) = 0.912$, $p = 0.452$] or latency to first lick [$F(3,20) = 1.949$, $p = 0.154$] during the consummatory phase. Likewise, no main effect of dose on latency to first lever press [$F(3,17) = 1.104$, $p = 0.373$] was found during the appetitive phase. Analyses assessing the effects of dose on latencies among LE rats reinforced with ethanol found that

naltrexone failed to produce main effects of dose on latency to first lever press [$F(3,24) = 0.969$, $p = 0.424$] and latency to first lick [$F(3,23) = 0.782$, $p = 0.516$] during the consummatory phase or latency to first lever press [$F(3,21) = 0.874$, $p = 0.470$] during the appetitive phase (see Table 13)

Analyses investigating the effects of naltrexone administration on latencies to lever press and lick among P rats reinforced with sucrose found that during the consummatory phase, there was no main effects of dose on latency to first lever press [$F(3,21) = 0.621$, $p = 0.609$] or latency to first lick [$F(3,21) = 1.201$, $p = 0.334$]. In contrast, there was a main effect of dose on latency to first lever press: [$F(3,21) = 3.329$, $p = 0.039$] during the appetitive phase. However, post hoc tests failed to reveal any doses that significantly differed from each other. Lastly, it was determined that among LE rats reinforced with sucrose, treatment with low doses of naltrexone failed to have an effect on latency to first lever press [$F(3,24) = 1.091$, $p = 0.372$] and latency to first lick [$F(3,24) = 0.969$, $p = 0.424$] during the consummatory phase nor on latency to first lever press [$F(3,24) = 1.280$, $p = 0.304$] during the appetitive phase of testing (see Table 13).

Experiment 3b: Effects of Naltrexone as a Non-Selective Opioid Antagonist

Following training, rats in experiment 3b were given SC injections of saline, 1.0, 3.0, and 10.0 mg/kg of higher doses of the non-selective opioid antagonist naltrexone 30 minutes prior to testing. Rats were then assessed for ethanol (10% v/v) or sucrose (2% w/v) consumption during the consummatory phase and responding on the reinforcer-associated (active) and inactive levers during the appetitive phase. No rats were excluded from either the initial (all subjects) consummatory or appetitive analyses (see Table 5). The exclusion of all ‘zero’ values that resulted from failure to initiate the intake session (i.e., only subjects that gained access to the reinforcer) resulted in inclusion of the following number of subjects for each naltrexone dose (saline, 1.0, 3.0 and 10.0 mg/kg, respectively) across the following groups: P ethanol (8,7,7,6); LE ethanol (9,9,9,8); P sucrose (8,8,8,8); LE sucrose (7,7,7,7) rats.

Consummatory Phase

A series of one-way RM ANOVAs assessed the effects of higher doses of naltrexone (1.0, 3.0, and 10.0 mg/kg) on ethanol (g/kg) and sucrose (ml/kg) intakes in both P and LE rats. Regardless of whether these subjects were removed from the analyses or whether a zero was assigned for total intake, a main effect of dose of naltrexone was found on ethanol intake among P rats in all subjects [$F(3,21) = 23.190$, $p < 0.001$] and in only those that gained access to the sipper tube [$F(3,17) = 20.017$, $p < 0.001$]. Post hoc analyses revealed across both instances that all doses of naltrexone significantly decreased ethanol intake compared to saline (see Figure 26). Similarly, there were main effects of dose found among LE rats on ethanol consumption when analyzing both all subjects [$F(3,24) = 13.939$, $p < 0.001$] and only those that gained access to the sipper tube [$F(3,23) = 17.373$, $p < 0.001$]. Again, post hoc analyses signified that across both instances, all doses of naltrexone significantly decreased ethanol intake compared to saline (see Figure 27)

As all P and LE rats attained sucrose (ml/kg) access following each injection, only one RM-ANOVA was run on each rat population (P and LE) in which all rats were included. Results revealed there were main effects of dose of naltrexone observed in both P [$F(3,21) = 96.754$, $p < 0.001$] and LE [$F(3,18) = 7.827$, $p = 0.001$] rats. Post hoc analyses revealed that among P rats, all doses of naltrexone decreased sucrose consumption relative to saline and that 3.0 and 10.0 mg/kg further lowered intake versus 1.0 mg/kg of naltrexone (see Figure 28a). In contrast, among LE rats, only the 3.0 and 10.0 mg/kg doses of naltrexone were capable of significantly attenuating sucrose intake compared to saline (see Figure 28b).

The following analyses were done using the more conservative approach of including only those rats as subjects that actually gained access to the sipper tube in order to better assess the actual effects of rat population and dose on reinforcer consumption. Two-way mixed ANOVAs were done to examine the effects of line and dose and whether an interaction between the two was found on both ethanol (g/kg) and sucrose (ml/kg) intakes. A two-way mixed ANOVA revealed that while there was not a main effect of line [$F(1,40) = 0.733$, $p = 0.405$], there was both a main effect of dose [$F(3,40)$

= 39.168, $p < 0.001$] and a significant line*dose interaction [$F(3,40) = 5.152$, $p = 0.004$] of naltrexone on ethanol intake. Post hoc analyses revealed that following the administration of saline, P rats drank significantly more ethanol (0.961 $\pm$ 0.052) than LE rats (0.652 $\pm$ 0.049). Across both rat populations, all doses of naltrexone significantly decreased ethanol intake relative to saline. Similarly, results revealed that while there was no main effect of line [$F(3,39) = 0.861$, $p = 0.370$], there was a main effect of dose [$F(3,39) = 78.301$, $p < 0.001$] and a significantly line*dose interaction [$F(3,39) = 27.462$, $p < 0.001$] on sucrose intake in rats reinforced with higher doses of naltrexone. Follow-up analyses revealed that P rats drank significantly more sucrose (51.6 $\pm$ 1.8) than LE (28.0 $\pm$ 1.9) rats following the administration of saline (see Figure 28).

One-way RM-ANOVAs were performed to assess whether or not the effects of naltrexone dissipated 24 hours post injection. Results revealed that there were no main effects of dose on ethanol consumption in either P [$F(3,21) = 1.839$, $p = 0.171$] or LE [$F(3,23) = 2.036$, $p = 0.137$] rats 24 hours post injection. Likewise, there were no main effects of dose of naltrexone on sucrose drinking in either P [$F(3,21) = 1.181$, $p = 0.341$] of LE [$F(3,18) = 01.710$, $p = 0.201$] rats. Further, paired T-tests also indicated that, in all groups, the saline and post-saline intakes did not differ from one another (see Figures 29 & 30).

Appetitive Phase

A series of one-way RM-ANOVAs were run to assess the effects of dose of Naltrexone on appetitive responding on both the reinforce-associated "active" and non-associated "inactive" levers. Table 14 includes all the reinforcer-associated, inactive, and total lever responses for each subgroup across each dose of naltrexone administered. Results revealed that there was a main effect of dose of naltrexone on responding on the ethanol-associated [$F(3,18) = 59.655$, $p < 0.001$] but not the non-associated [$F(3,18) = 1.178$, $p = 0.346$] lever in P rats. Post hoc analyses revealed that all doses of naltrexone significantly reduced responding on the ethanol-associated lever relative to saline (see Figure 31a). There was also a main effect of dose of naltrexone among LE rats reinforced with ethanol on both active [$F(3,21) = 26.786$, $p < 0.001$] and inactive [$F(3,21) = 3.905$, $p = 0.023$] lever responding. Follow-up analyses revealed that all doses significantly

reduced responding on the ethanol-associated lever relative to saline, and that the 1.0 and 10.0 mg/kg (but not 3.0) doses significantly reduced responding on the non-sucrose associated lever relative to saline (see Figure 32a).

Among P rats reinforced with sucrose, there was a main effect of dose of naltrexone on both active [$F(3,21) = 18.338$, $p < 0.001$] and inactive [$F(3,21) = 4.667$, $p = 0.012$] lever responding with post hoc analyses revealing that all doses of naltrexone significantly decrease responding on the sucrose-associated lever relative to saline and that 1.0 and 3.0 (but not 10.0) mg/kg of naltrexone significantly reduce responding on the inactive lever relative to saline (see Figure 31b). Likewise, there was a significant main effect of dose on active [$F(3,45) = 8.999$, $p < 0.001$] and inactive [$F(3,24) = 8.337$, $p < 0.001$] lever responding. In both instances, follow-up tests found that all doses of naltrexone significantly decreased responding relative to saline (see Figure 32b).

Two-way mixed ANOVAs were done to examine the effects of line, solution, and dose and any resulting interactions on both reinforcer-associated (active) and inactive lever responding. A two-way mixed ANOVA revealed that there were main effects of solution [$F(1,39) = 8.382$, $p = 0.013$] and dose [$F(3,39) = 48.397$, $p < 0.001$] but no significant solution*dose interaction [$F(3,39) = 0.603$, $p = 0.617$] on responding for the reinforcer-associated lever in P rats. Overall, P rats made more lever responses for sucrose (35.7 ± 3.9) than ethanol (20.4 ± 3.9). Further, the main effect of dose was driven by the ability of all doses of naltrexone (1.0, 3.0, and 10.0 mg/kg) to, overall, decrease responding on the reinforcer-associated lever relative to saline. In contrast, while there was a main effect of dose [$F(3,45) = 29.922$, $p < 0.001$], neither a main effect of solution [$F(1,45) = 1.524$, $p = 0.236$] nor a significant solution*dose interaction [$F(3,45) = 1.079$, $p = 0.368$] was found among LE rats. A two-way mixed ANOVA found a main effect of line [$F(1,39) = 5.942$, $p = 0.030$] and dose [$F(3,39) = 68.261$, $p < 0.001$] but not a significant line*dose interaction [$F(3,39) = 1.171$, $p = 0.333$] of naltrexone on rats responding on the ethanol-associated lever. LE rats responded, collectively, more on the ethanol-associated lever (30.8 ± 2.9) than did P rats (20.4 ± 3.1). Responding on the sucrose-associated lever failed to reveal a significant main effect of line [$F(1,45) = 0.301$, $p = 0.591$] or line*dose interaction [$F(3,45) = 2.554$, $p = 0.067$], but did yield a main

effect of dose [$F(3,45) = 25.378$, $p < 0.001$]. Post hoc analyses revealed that, overall, all doses of naltrexone significantly reduced responding compared to saline on both the ethanol- and sucrose-associated levers.

Latencies

A series of one-way RM-ANOVAs examining the effects of dose on latencies among P rats reinforced with ethanol found that high doses of naltrexone failed to produce either a main effect of dose on latency to first lever press [($F(3,18) = 0.751$, $p = 0.536$] or latency to first lick [$F(3,15) = 1.219$, $p = 0.337$] during the consummatory phase or a main effect of dose on latency to first lever press [$F(3,18) = 1.411$, $p = 0.272$] during the appetitive phase. Among LE rats reinforced with ethanol, naltrexone administration resulted in a significant main effect of dose on latency to first lever press [$F(3,23) = 3.353$, $p = 0.036$] during the consummatory phase. However, post hoc analyses failed to reveal any significant differences among doses. In contrast, there were no main effects of dose following naltrexone administration on either latency to first lick [$F(3,22) = 0.699$, $p = 0.563$; consummatory phase] or latency to first lever press [$F(3,24) = 1.126$, $p = 0.358$; appetitive phase (see Table 15)].

Analyses investigating the effects of naltrexone administration on latencies to lever press and lick among P rats reinforced with sucrose found that during the consummatory phase, there were main effects of dose on both latency to first lever press [$F(3,21) = 4.078$, $p = 0.020$] and latency to first lick [$F(3,21) = 4.144$, $p = 0.019$]. While post hoc analyses failed to reveal any significant differences among doses in latency to first lever press, they did reveal that it took longer following administration of the middle (3 mg/kg) dose to initiate licking than it did following administration of saline. In contrast, there was no main effect of latency to first lever press during the appetitive phase [$F(3,21) = 1.898$, $p = 0.161$. Lastly, it was determined that among LE rats reinforced with sucrose, injection of naltrexone failed to find a main effect of dose on latency to first lever press [$F(3,18) = 0.346$, $p = 0.793$; consummatory phase], latency to first lick [$F(3,18) = 0.999$, $p = 0.416$; consummatory phase], or latency to first to lever press [$F(3,24) = 1.581$, $p = 0.220$; appetitive phase (see Table 15)].

Naltrexone Discussion

Experiment 3a: Results on Intake and Responding for Mu-Selective Doses

The results from the consummatory phase reveal that P rats were more sensitive to the effects of lower doses of naltrexone acting as a selective mu antagonist than LE rats. Among P rats, the lowest dose of naltrexone (0.1 mg/kg) was able to attenuate both sucrose and ethanol intake and the highest dose (1.0 mg/kg) showed a further decrement in intake relative to the other doses. Among LE rats, naltrexone decreased ethanol consumption at doses of both 0.3 and 1.0 mg/kg. Interestingly, 0.3 mg/kg but not 1.0 mg/kg of naltrexone was able to decrease sucrose intake among LE rats.

In contrast, naltrexone was able to selectively reduce responding for ethanol in both the P and LE rat populations. Naltrexone (0-1.0 mg/kg) failed to suppress sucrose responding among LE rats while only the highest (1.0 mg/kg) dose was capable of retarding responding for sucrose among P rats (see Table 9 for a summary of the results).

Experiment 3b: Results on Intake and Responding for Non-Selective Doses

As predicted, during the consummatory phase, all doses of naltrexone, when acting as a non-selective opioid antagonist, were able to decrease intake of ethanol in both P and LE rats. All doses were able to attenuate intake of ethanol to the same degree, creating a lack of a dose-response. Additionally, all doses of naltrexone were able to decrease sucrose intake among P rats though the middle and highest doses (3.0 and 10.0 mg/kg) were able to dose dependently decrease intake relative to the low (1.0 mg/kg) dose but did not differ in degree of attenuation from each other. In contrast, among LE rats reinforced with sucrose, the lowest dose of naltrexone administered, 1.0 mg/kg, was ineffective at reducing intake though the higher doses of 3.0 and 10.0 mg/kg of naltrexone did significantly attenuate sucrose intake.

In regards to the appetitive phase, all doses of naltrexone were able to decrease responding for ethanol and sucrose across both P and LE rats. There were no differences, however, across dose as all doses attenuated responding to the same degree relative to saline.

Modifications to Naltrexone Use as a Pharmacotherapy

Taken together, the results examining the effects of naltrexone on ethanol consumption support previously shown notions that doses of naltrexone < 1.0 mg/kg are likely selective for the mu receptor subtype. This is demonstrated by the findings that lower doses of naltrexone are more selective for both genotype and reinforcer than doses ≥ 1.0 mg/kg, which, with few exceptions, appear to blunt reinforcement in general. This is evident as there are no selective effects among the higher doses of naltrexone for either reinforcer and/or drinking phenotype. Further, the maximally effective doses seem to range between 1-3 mg/kg as doses of 10.0 mg/kg show no further decrement in drinking or seeking attenuation, suggesting that perhaps doses of 3.0 mg/kg are capable of maximally saturating the available receptors or that behavior has been 100% suppressed, either way indicating that there is no need for the utilization of these larger doses if the efficacy is the same.

The results of this study confirm prior findings with other experiments utilizing naltrexone as well as other studies selectively examining the effects of selective mu receptor antagonists, including beta-funaltrexamine (Stromberg et al., 1998; Krishnan-Sarin et al., 1998; Lê et al., 1993) and CTOP (Hyytiä, 1993; Kim et al., 2000). Further, naltrexone at lower doses (< 1.0 mg/kg), which have been demonstrated to have a selectivity for binding at the mu opioid receptor (Childers et al., 1979; Paterson et al., 1984; Takemori & Portoghese, 1984; Emmerson et al., 1994; Mhatre & Holloway, 2003; Wang et al., 2001; 2007) is not the ideal drug choice to utilize in this paradigm. This is especially true given the current attempt to compare it against higher doses of naltrexone, in the hopes of more specifically uncovering the role of the mu receptor in selectively mediating the effects of reinforcer consumption and seeking as a function of rat sample or reinforcer subtype.

It is also of interest to note that at doses greater than 1.0 mg/kg, there was little evidence of the ability of higher doses of naltrexone to further suppress the seeking and/or intake of ethanol and/or sucrose. Given that doses less than 1.0 mg/kg have been deemed as “selective for the mu opioid receptor,” it would seem that doses greater than 1.0 mg/kg (which should therefore be capable of blocking delta and kappa opioid

receptors in addition to mu opioid receptors) would provide evidence of an even further attenuation than the lower doses. In the current study, 3.0 and 10.0 mg/kg of naltrexone showed no greater decrement of seeking responses and/or intake than 1.0 mg/kg. One reason for this could be that perhaps the higher doses of naltrexone utilized were not high enough to facilitate the antagonisms of the delta and/or kappa receptors based on binding studies that have found naltrexone to bind to the mu opioid receptor with a 10.75 and 14.33 greater affinity than to the kappa and delta receptors (Paterson et al., 1984).

However, even if the higher doses of naltrexone were able to antagonize the kappa opioid receptor, the results of the present investigation would be in line with previous studies that have found blockade of the kappa opioid receptor (though the use of selective kappa antagonists) to have no effect on either sucrose or ethanol intake in non-dependent animals (Doyon et al., 2006; Williams & Woods, 1998). In contrast, it would be interesting to see if simultaneous administration of a low doses of naltrexone (blocking the mu opioid receptors) and of U50,488H (activating the kappa receptors) would result in a further attenuation of seeking and/or intake than either alone. Likewise, given that the doses of naltrindole required to see a significant effect on drinking and intake were rather high in the present experiment, it is unlikely that, were the naltrexone doses utilized capable of blocking the delta opioid receptor, that they were high enough to facilitate any discernible effect at the delta receptor. As such, higher concentrations of naltrexone might be necessitated to show any further decrement in reinforcement through further activation of the delta receptor.

Unfortunately, there were no other antagonists that could effectively be utilized within the study design. For example, CTOP can only be administered centrally, and given that this series of studies is taking a treatment-based approach, it was essential to utilize a drug that could be administered peripherally and thus capable of crossing the blood brain barrier in order to exert its effects centrally. In contrast, while beta-funaltrexamine can be given systemically and cross the blood brain barrier, the effects are long lasting as the antagonist is considered irreversible, and therefore cannot work in a study design that utilized a within subjects design. As such, naltrexone at these lower

doses was the best option for comparing the effects for best attempting to accurately assess the role of the mu receptor in the current design.

Further, results of the current study support previous studies that have found that lower doses of naltrexone are more selective at attenuating ethanol than sucrose (Czachowski & DeLory, 2009; Kuzmin et al., 2007; Goodwin et al., 2001; Ji et al., 2008) though only during the appetitive phase of the experiment. While LE reinforced with ethanol were slightly more sensitive to the effects of lower doses of naltrexone than sucrose-reinforced LE rats during the consummatory phase, the efficacy of naltrexone did not differ during the consummatory phase in P rats as a function of reinforcer. Similarly, the results of the current study also support the clinical notion that naltrexone may be more beneficial in those that display a family history positive phenotype, as naltrexone treatment was more efficacious in attenuating ethanol intake in P rats at doses as low as 0.1 mg/kg relative to LE rats (but see Czachowski & DeLory, 2009). These findings also support the notion in the clinical literature that perhaps the effects of drinking phenotype are specific for naltrexone-mediated drinking only. That is, that P rats are more sensitive to the effects of ethanol only when ethanol in concurrently on board as P showed less sensitivity to the effects of naltrexone relative to LE rats during the appetitive phase (i.e., when the ethanol was not on board).

The results of the studies on naltrexone reveal that for those showing a potential phenotypic predisposition towards alcoholism (FH^+), 0.1 mg/kg was shown to be the most effective dose as 0.1 mg/kg naltrexone effectively reduced ethanol seeking in both LE and P rats without affecting sucrose. Additionally, if access to the ethanol was gained, 0.1 mg/kg effectively attenuated intake in the P rat though 0.3 mg/kg was needed to effectively attenuate intake in the outbred (FH^-) LE rats. Therefore, the most efficacious dose of naltrexone capable of decreasing ethanol-associated behaviors is 0.1 mg/kg in those displaying a predisposition towards a propensity for high ethanol intake. In contrast, in those that do not demonstrate a phenotype towards a predisposition towards high ethanol intake, or in those where familiar history is unclear or unknown, 0.3 mg/kg

is the most effective dose that should be utilized in attenuating these behaviors. Likewise, in all situations, 1.0 mg/kg of naltrexone should be considered the maximally effective dose.

GENERAL DISCUSSION

The general goal of these experiments was to investigate whether the use of drugs selective for various receptor subtypes of the endogenous opioid system could provide a potential alternative to naltrexone as a more efficacious pharmacotherapeutic in the maintenance of alcohol use disorders and dependency. Overall, the results from the present experiments support the hypothesis that the endogenous opioid system plays a significant role in modulating ethanol self-administration and reinforcement, and further, that drinking phenotype may confer a sensitivity to some receptor-selective drugs presenting the potential for better treatment outcomes. Table 9 provides a complete breakdown of the various drugs examined in each experiment: (1) the kappa agonist U50,488H; (2) the delta antagonist naltrindole; (3) mu-selective and non-selective doses of the opiate antagonist naltrexone and the ability of each dose to independently attenuate the consumption and responding for either sucrose or ethanol among samples of rats that were either selectively bred for a propensity to drink high amount of ethanol (P) or unselected, moderately drinking (LE) rats.

Overall, the results of the present experiments support the hypotheses that selective opioid receptor agonists and antagonists may differentially influence reinforcer self-administration and also that P and LE rat populations differ in their sensitivity to the effects of the various drugs. In experiment 1, while 5.0 mg/kg of U50,488H effectively attenuated ethanol intake and responding, it was equally efficacious at attenuating sucrose intake and responding. In fact, at this dose, which was minimally effective for decreasing ethanol reinforcement, there was a greater decrease seen in sucrose reinforcement. Therefore, the results indicate that U50,488H would not be a viable option to selectively reduce ethanol, at least at the current doses tested, as it had a greater effect on the more palatable sucrose reinforcer. In experiment 2, the most effective dose of naltrindole was

5.0 mg/kg as it was selective for decreasing ethanol drinking, though only in P rats, which implies that naltrindole use may demonstrate more efficacious results in individuals displaying a phenotype for a predisposition towards high ethanol intake, as indicated through a strong history of familial alcoholism. In experiment 3, the most effective dose of naltrexone was determined to vary as a function of line. For those showing a potential phenotypic predisposition towards alcoholism (FH^+), 0.1 mg/kg was shown to be the most effective dose because this dose effectively reduced ethanol seeking in both LE and P rats without affecting sucrose. Additionally, if access to the ethanol was gained, 0.1 mg/kg attenuated intake in the P rat though 0.3 mg/kg was required to attenuate intake in the outbred (FH^-) LE rats. Therefore, the most efficacious dose of naltrexone capable of decreasing ethanol-associated behaviors is 0.1 mg/kg in those displaying a predisposition towards a propensity for high ethanol intake. In contrast, in those that do not demonstrate a phenotype towards a predisposition towards high intake, or in those where familial history is unknown, 0.3 mg/kg is the most effective dose that should be utilized in attenuating these behaviors. As the results of each experiment have already been briefly discussed, the remainder of the discussion will focus on the broader implications and limitations of the collective findings from these studies.

Binge Drinking versus Dependency

In the current study, the roles of various drugs selective for the mu, delta, and kappa opioid receptor subtypes were examined to determine their efficacy on attenuating responding for and intake of sucrose and ethanol. The results of these studies determined that an acute administration of at least one dose of each drug tested (U50,488H, naltrindole, and naltrexone) was able to significantly attenuate some aspect of reinforcement among each combination of rat sample/reinforcer examined. The results of these studies are in line with previous ones that have found administration of a mu (Mhatre et al., 2004; Gardell et al., 1996, 1997a, 1997b; Goodwin et al., 2001; Stromberg et al., 1998, 2001, 2004; Liseman, 1989; Ji et al., 2008; Parkes & Sinclair, 2000; Koistinen et al., 2001; Perfumi et al., 2003; Ciccocioppo et al., 2007; Lê et al., 1993; Kim et al., 2004; Kamdar et al., 2007; Kuzmin et al., 2007, 2008; Bienkowski et al., 1999; Williams & Broadbridge, 2009; Walker & Koob, 2008; Czachowski & DeLory, 2009;

June et al., 1998; Gilpin et al., 2008; Sabino et al., 2006; Middaugh et al., 1999, 2000; Heidbreder et al., 2007; Escher & Mittleman, 2006; Stromberg et al., 1998; Kim et al., 2000; Honkanen et al., 1996; Hyytiä, 1993; Krishnan et al., 1998) or delta (Hyytiä & Kiianmaa, 2001; Kim et al., 2000; Froehlich et al., 1991; Krishnan-Sarin et al., 1995a; Lê et al., 1993; Higley & Kieffer, 2006) antagonist or kappa agonist (Lindholm et al., 2001; Nestby et al., 1999; Logrip et al., 2008; Cosgrove & Carroll, 2002) to decrease ethanol intake or self-administration in a limited access paradigm. That said, one major concern with the use of limited access paradigms, such as the sipper-tube model, is that they fail to induce and/or model "alcohol-dependence," and therefore are less likely to resemble the human condition, which tends to be characterized by chronic cycles of drug use, cessation, and relapse. However, while this may seem a critical flaw in the study design, it is important to mention that no animal model is a true depiction of the human condition of alcoholism, and that animal models are a means by which to more directly assess selective aspects of alcoholism while circumventing the ethical barriers and limitations of human experimentation.

In recent years, there has been a trend within alcohol research to move away from animal models utilizing the two-bottle choice, continuous access paradigm when assessing alcohol dependence. This is in part due to the characterization of human alcoholism as being punctuated with periods of heavy, episodic drinking bouts (i.e., a series of binges). Further, recent reports on the rates of binge drinking have found that among 10^{th} and 12^{th} graders, 21% and 28.1% (respectively) have engaged in binge drinking in the past 30 days. These numbers jump to 36.3% for those aged 18-24 (Johnston et al., 2006a, 2006b). While some individuals in this age group initiate drinking to cope with stress and to alleviate negative affect (Cooper et al., 2000), the majority of these individuals engage in drinking due to the positive expectancies they have about alcohol and for reasons deemed "positive" or celebration-worthy (Schulenberg & Maggs, 2002; Read et al., 2003).

In fact, binge or excessive drinking (defined as having 5 or more drinks at one time) is permitted, and in some cases, encouraged in the college environment (Arnett, 2005). Unfortunately, this developmental period, known as "emerging adulthood"

(typically ages 18-25) represents a time of increased alcohol use and, not surprisingly, coincides with a greater onset of alcohol use disorders than any other point across the lifespan (Arnett, 2000; Brown et al., 2009). Alcohol related problems that emerge during this period have been shown to be detrimental to the psychological and physical well-beings of afflicted individuals years later (Schulenberg et al., 2003). Studying the effects of potential pharmacotherapeutics on models of binge drinking prior to the development of dependency may seem futile considering that clinical treatment is often not sought until an individual has been diagnosed with full blown alcoholism. However, if binge drinking does mediate the transition to alcohol dependence and is facilitated mostly by the positive reinforcing effects of alcohol, then better understanding of the various mechanisms regulating those reinforcing effects may be crucial to developing an effective treatment for the maintenance of dependence.

Validity of the Sipper-Tube Model

Sipper-Tube Model and Predictive Validity

The sipper-tube model is used as a means to induce binge-like drinking, and while the use of the sipper-tube model may not appear to have much face validity, it has predictive validity. Predictive validity is more accurately what the model is used to assess in terms of the ability to develop potential pharmacotherapeutics that may be relevant to lessening the severity of alcohol dependence clinically. In addition, the use of a limited access model, such as the sipper tube model, is better suited to assess the role of the endogenous opioid system in mediating ethanol use, as this system has been heavily implicated in regulating the rewarding and positive effects of ethanol. In order to assess the role of the selective opioid receptor antagonists and agonist, it is important to use a model that facilitates the intake of pharmacologically relevant BELs, to ensure that the animals are experiencing the rewarding effects of the ethanol, and that the motivation for and consumption of ethanol is driven by the experience with the pharmacological effects as opposed to some other underlying factor, such as hunger or thirst.

In order to accomplish this, except where noted during the first week of training, rats were given *ad libitum* access to food and water, ensuring that neither hunger nor

thirst was driving their motivation to seek and/or consume ethanol. Further, many paradigms utilizing limited access to ethanol, especially in the homecage, do so during the initial hours of the dark cycle. As demonstrated by Zucker (1971), Sprague-Dawley rats consumed approximately 90% of their daily water and 75% of their daily food intake during the dark cycle. While some studies have observed a steady level of ethanol intake across the dark cycle in selected lines of mice (HAP; cHAP; Matson & Grahame, 2011), other have found an examination of ingestive behaviors to reveal that not only does the majority of intake occur during the dark phase, but that it tends to occur in bouts. Of these bouts, the largest one reportedly occurs within the first two hours of the dark cycle while two smaller ones occur approximately midway in to and during the last hour of the dark cycle (Rosenwasser et al., 1981). In fact, utilization of this observation resulted in the use of the current "drinking in the dark" (DID) paradigm by which increased consumption of ethanol, particularly in mice, is facilitated by scheduling limited access to high concentration of ethanol (~20%) during the first 2-3 hours of the dark cycle to correlated with their initial first bout (Allan et al., 2003; Rhodes et al., 2005, 2007).

The results of these experiments, which were performed a few hours into the light cycle, were therefore especially likely to be facilitated by the motivation to consume ethanol for its pharmacological effects as a final bout prior to the start of the light cycle indicates that the rats were likely sated prior to the initiation of the ethanol self-administration studies. Further, the results of these studies are all the more impressive considering that the ethanol intakes generated ranged from 0.9-1.2 g/kg in P rats and 0.6-1.0 g/kg in LE rats and produced pharmacologically relevant BELs (see Figure 2). These results are consistent both with studies that have found that rats can correctly discriminate ethanol from saline $\geq$ 80% of the time at BELs at or greater than 0.68 g/kg (Hodge et al., 2001) as well as those that have demonstrated pharmacology relevant BELs indicative of binge-like drinking, in both P (Verplaetse et al., 2011) and LE (Henderson & Czachowski, 2012) rats using the sipper-tube model.

The results of the current studies were able to validate the use of the sipper-tube model as an effective means by which to tease apart different aspects of ethanol (or sucrose) reinforcement, and how potential treatment options may differentially affect

these aspects. For example, in the current study, it was determined that the use of naltrindole, for example, was only effective in reducing ethanol intake in P rats at doses of 5.0 and 10.0 mg/kg. However, during the appetitive phase, results revealed that doses as low as 2.5 mg/kg, while ineffective at reducing intake, were able to disrupt responding on a lever associated with ethanol reinforcement in P rats. Further, while none of the naltrindole doses tested were able to attenuate sucrose intake in either P or LE rats or ethanol intake in LE rats, selective doses of naltrindole were able to attenuate responding on the reinforcer-associated lever in these same animals. Likewise, U50,488H was revealed to be more efficacious at attenuating reinforcer intake in LE rats (with the lowest dose of 2.5 mg/kg capable of significantly decreasing intake) while P rats were more sensitive to the appetitive phase such that 2.5 mg/kg U50,488H significantly decreased responding on the reinforcer-associated lever at a dose that was ineffective at mediating intake during the consummatory phase. These results are consistent with others studies that have found the sipper-tube model to effectively tease apart the seeking and consumption of reinforcers examined with other pharmacotherapeutics. For example, when comparing the effect of the serotonin 1_A agonist 8-OH-DPAT in the same model, the results revealed that while infusion of 1.0, 2.5, and 5.0 μg into the nucleus accumbens core was able to significantly reduce responding on the ethanol-associated lever, none of these doses were able to selectively attenuate ethanol intake (Czachowski, 2006).

Additional Advantages Over Other Operant Models

Aside from serving as a model capable of producing pharmacologically relevant BELs equivalent to those of a binge-like drinking episode both in rats that are selected for high amounts of ethanol consumption as well as in outbred rats, the sipper-tube model provides additional benefits over other operant self-administration paradigms. The main advantage of the sipper-tube model is that by dissociating the seeking from consuming components of reinforcement, this model avoids the confound of the pharmacological effects of ethanol interfering with an animal's physical ability to emit a response. Additionally, through this same mechanism, the model helps to identify whether observed decrements in the ability of a drug are likely to be mediating the rewarding effects of a reinforcer as opposed to substituting for them through the examination of the

appetitive phase of the model. For example, a general decrease in overall consumption could be the result of a drug interfering with the rewarding effects of the reinforcer. However, it is also possible that a drug could be substituting for and/or potentiating the effects of a reinforcer, both of which would also result in attenuated intake as less of the reinforcer would be required to achieve the same subjective 'high.' Unfortunately, any paradigm in which the reinforcer is on board would be ineffective at dissociating the two. Through the utilization of the appetitive phase of the sipper-tube model, however, this distinction can be more easily determined. For instance, if a drug is interfering with the rewarding effects of a reinforcer, the appetitive phase would likely show an attenuation in the motivation to "seek" reinforcer access (i.e., through a decrement in responding on the reinforcer-associated lever). Contrastingly, if a drug substituted or potentiated the rewarding effects of a particular reinforcer, the appetitive phase would likely show an increase in the motivation to "seek" and gain access to the reinforcer (resulting in an increase in responding on the reinforcer-associated lever).

Limitations of the Sipper Tube Model

Each model, however, is not without its limitations. For example, because it takes a significant amount of time to train the animals to increase responding on the reinforcer-associated lever from a RR10 to RR20, rats are almost always initially tested in the consummatory and subsequently the appetitive phase. This procedure is rarely counterbalanced. However, this is a limitation of the current study only. The consummatory and appetitive phases can be counterbalanced, though this would require an additional three weeks of initial training to an RR20. The disadvantage to utilizing the model without counterbalancing across testing phase is that rats always have a greater history of ethanol experience when tested during the appetitive versus the consummatory phase. However, given that rats are limited to reinforcer access for only 20 minutes a day and have already had 6 weeks of exposure to ethanol prior to the start of consummatory injections, it is unlikely that any additional changes or potential neuroadaptations occur in the brain during the weeks between consummatory and appetitive testing.

A second flaw of the model was exposed during the experiment examining the effects of naltrindole during the consummatory phase. In this study, there were a

significant number of rats, especially P rats, reinforced with ethanol that failed to emit the single response requirement necessitated to gain access to the reinforcer. Normally, the emission of this one response would not interfere with the consummatory phase. Presentation of the lever serves as a cue that the trial has started, enabling the rats to have exactly 20 minutes from making the RR1 to drinking as much of a reinforcer as they want. However, if the drug being tested, as in the case of naltrindole, is interfering with the ability of the rats to initiate the response through either sedative effects or by completely reducing the motivation to emit the response to '0,' there is no way to accurately assess the influence of the drug on intake. For example, in the current study, in those instances where the rats failed to make the RR1, intake had to be discounted because the utilization of a '0' as the reinforcer intake artificially decreased consumption, rendering it impossible to determine whether a decrease seen in consumption was a result of the drug or of arbitrary use of an inclusion of '0' as a measure of intake, increasing the likelihood of making a Type I error. As such, great effort was taken to analyze the consumption of a reinforcer utilizing only those instances where the rat made the requisite RR1 response. In hindsight, it would have been possible to rerun these rats at these doses in the model where the RR was manually overridden to allow the rats reinforcer access to more accurately assess the effect of a specific drug on reinforcer intake. However, overriding the RR would have resulted in some rats gaining reinforcer access after only the initial pretreatment time (i.e., naltrindole = 15 minutes) whereas other rats would have had an additional 20 minutes to wait prior to gaining reinforcer access. Given that we are looking at receptor-selective drugs that act through G-protein coupled receptors (i.e., have a slower onset), differences in the amount of time the drug has been on board prior to reinforcer access could potentially confound intake patterns.

Selectivity for Ethanol Reinforcement

Across experiments, the ability of the various receptor agonist/antagonists to selectively attenuate ethanol versus sucrose reinforcement varied as a function of receptor subtype and reinforcement (appetitive versus consummatory) phase. For example, as has been shown, U50,488H failed to reveal any differences across reinforcers. That is, U50,488H attenuated ethanol and sucrose reinforcement to the same degree across line

and dose. In contrast, the effects of naltrindole were much more selective for ethanol. In the consummatory phase, naltrindole only attenuated ethanol intake in P rats only. During the appetitive phase, in contrast, naltrindole was selective for ethanol attenuation at the lowest dose only in the P rats. Similarly, utilization of lower doses of naltrexone revealed that no dose more selectively attenuated ethanol versus sucrose in P rats. In contrast, across the appetitive phase, the lowest doses, 0.1 and 0.3 mg/kg, selectively attenuated ethanol responding in both LE and P rats. These results would suggest that the mechanism mediating the positive reinforcing effects of the endogenous opioid system, implicating both the mu and delta receptor systems, may be acting through a pathway that tends to mediate general reinforcement. In contrast, it is difficult to appropriately assess the role of the endogenous kappa opioid system in mediating reinforcement in this situation because the kappa system, which acts in opposition to the mu and delta systems, appears to be recruited during times of stress. As discussed previously, utilizing the current model, the goal was to avoid any unnecessary stress induction to circumvent the activation of the hypothalamic-pituitary-adrenal (HPA) axis thus avoiding any potential confounds involved though the initiation of a cortisol response. As such, while the kappa opioid system lacks any evidence of reinforcer selectivity when the effects of alcohol and sucrose appear to be mediated via positive reinforcement, the findings could alter drastically when ethanol (or sucrose) is used for its potentially negative reinforcing effects.

Roles of the Selective Receptor Subtypes in Mediating Reinforcement

The Role of Positive Reinforcement

Previous reports indicate that naltrexone attenuates the “subjective high” often associated with the alcohol use. Reports examining naltrexone and placebo treatment determined naltrexone to be more efficacious at attenuating relapse rates. For example, of those patients that experienced a “slip” of alcohol, only 17% versus 65% experienced a full relapse when being treated with naltrexone versus placebo, respectively. Of those that did “slip,” over half of the naltrexone-treated patients reported that the subjective high they usually attained from alcohol use was less than usual compared to only 11% of

placebo-treated patients (Volpicelli et al., 1992, 1995; O'Malley et al., 1992). The observation that the ability of naltrexone to regulate alcohol drinking seems contingent upon the availability to consume alcohol (Volpicelli, et al., 1995; O'Malley et al., 1996; Heinälä et al., 2001) led to the theory that naltrexone administration concurrent with alcohol consumption may generate the best treatment outcomes in human alcoholics (Sinclair, 2001). This is thought to occur via reinforcer devaluation through pharmacological extinction (Sinclair, 1996) with the proposed mechanism being that naltrexone decreases craving/intake by extinguishing the association of positive reinforcement and alcohol through repeated pairings.

Positive reinforcement is defined by the increased probability that a behavior will occur given the presentation of an appetitive stimulus, like a drug effect. For example, people that drink alcohol because they enjoy the subjective "high" and/or feelings of relaxation that result from alcohol use are drinking it for its positive reinforcing properties. However, the pairing of alcohol and naltrexone has been proposed to decrease alcohol consumption by interfering with the reinforcing effects of alcohol. Therefore, according to Sinclair (1996), repeatedly pairing alcohol and naltrexone will no longer produce those desirable feelings, and the association between the behavior and the desired effect(s) is weakened over time. These pairing ultimately result in a decreased likelihood of the participant engaging in the behavior, in this case, drinking. Given that the delta opioid system has also been implicated in mediating the rewarding effects in a similar manner to that of the mu opioid system, it is likely that both naltrexone and naltrindole are able to attenuate ethanol-reinforced behaviors by disrupting the rewarding effects of ethanol.

Mechanisms by which Rewarding Effects May Be Blunted

In addition to decreasing ethanol intake through extinguishing the positive reinforcing effects of ethanol, both naltrindole and naltrexone have been popularly theorized to decrease drinking by interfering with the palatability of ethanol by making it more aversive taste-wise. To test this theory, a variety of different strategies have been employed, including those looking at the ability of both naltrindole and naltrexone to induce a conditioned taste aversion (CTA) and the effects of both in paradigms that

assess taste reactivity (TR). Results revealed that Parker and Rennie (1992) were able to induce a CTA to a sucrose solution following the administration of both 1 and 10 mg/kg of naltrexone. Contrastingly, Froehlich and colleagues (1998) found that naltrindole, when given alone, was unable to produce a conditioned taste aversion to a banana flavored solution at doses of 2.5, 5.0, and 10.0 mg/kg (all the doses tested in the current study). In fact, the only dose tested capable of inducing a CTA to a preferred banana flavored solution in P rats was a 20.0 mg/kg dose of naltrindole. Interestingly, while injection of 0.5 g/kg of ethanol was found to be non aversive, paring it with injections of 5.0 and 10.0 mg/kg of naltrindole (previously non aversive doses) results in a significant conditioned taste aversion (Froehlich et al., 1998).

Additionally, both naltrexone and naltrindole were assessed for their effects in a taste reactivity procedure. First developed by Grill and Norgren (1978), tests of taste reactivity measure the orofacial and somatic responses that rats have in response to flavors that are infused directly into their mouths via an intraoral fistulae. Responses can include those termed favorable, or ingestive, responses and include tongue protrusions, mouth movements, and paw licks; neutral, or passive, responses which include passive dripping into the mouth, and non favorable, or aversive, responding and include chin rubs, gapes, head shakes, forelimb flairs, fluid expulsion, and paw treads. Interestingly, while naltrexone injections were able to decrease sucrose intake in the homecage, there was no effect of naltrexone on aversive responding. In contrast, the 10.0 mg/kg dose of naltrexone increased the amount of passive drips (neutral responding) relative to the saline dose but did not differ significantly from the 0.01 and 1.0 mg/kg naltrexone doses (Parker & Rennie, 1992).

Subsequent studies examining the effects of naltrexone on intraoral ethanol infusion have found mixed results. Coonfield and colleagues (2004) found that 3.0 mg/kg of naltrexone was ineffective at altering palatability in either Alko-Alcohol preferring (AA) or alcohol-preferring (P) rats but that the 7.5 mg/kg dose of naltrexone was able to decrease the palatability by decreasing ingestive responses and increasing aversive responses of 10% ethanol in AA but not in P rats. Hill and Kiefer (1997) found that naltrexone doses of 1.0 and 3.0 mg/kg were capable of altering the taste reactivity of

ethanol in male Long Evans rats by increasing aversive responses and reducing ingestive responses. Higley and Kiefer (2006) found that 3 mg/kg of naltrexone was capable of increasing aversive responses without altering ingestive ones in male Long Evans rats. Similarly, Ferraro and colleagues (2002) found that 3 mg/kg of naltrexone decreased ingestive responses for both ethanol and sodium chloride while increasing aversive responses in Long Evans rats. Further, the study found that naltrexone was able to increase aversive responding for sucrose without affecting ingestive responses and had no effect on either type of response for quinine. Additionally, Kiefer and colleagues (2005) examined the differences in taste reactivity in ethanol-naïve versus ethanol-familiar male Long Evans rats and found that 3.0 mg/kg of naltrexone was able to decrease ingestive and increase aversive responses in both groups of rats. However, the ethanol-familiar group made both significantly more ingestive and aversive responses than the naïve group. Regardless of the effects or lack of effects on taste reactivity in the different rats, naltrexone was uniformly able to attenuate ethanol consumption in all the cited studies in subsequent limited access homecage drinking evaluations (Hill & Kiefer, 1997; Ferraro et al., 2002; Coonfield et al., 2005; Kiefer et al., 2005; Higley & Kiefer, 2006).

In contrast to the multitude of studies examining the effects of taste reactivity of naltrexone on ethanol ingestion, only one study (Higley & Kiefer, 2006) examined the effects of naltrindole in this same paradigm. The results revealed that while neither the 2.0 nor 4.0 mg/kg doses of naltrindole were able to alter either taste reactivity or ethanol intake, 8.0 mg/kg of naltrindole was able to significantly increase aversive responding though it failed to alter ingestive responding unaltered. The fact that in all of these experiments drinking immediately returned to baseline levels following the termination of naltrexone and/or naltrindole treatment suggests that taste is not the primary mechanism by which naltrexone and/or naltrindole are decreasing ethanol intake. Additionally, studies assessing the role of these drugs on attenuating ethanol-associated responding further support this notion as there is no ethanol on board during the appetitive phase with which these drugs can interfere, yet these drugs still attenuate responding. Finally, studies that look at different routes of self-administration that bypass oral administration (and therefore taste) and naltrexone administration have found that

naltrexone is still able to reduce ethanol intake. For example, studies in rhesus monkeys with very tightly titrated ethanol intakes via intravenous self-administration (IVSA) have found 1.0, 3.0, and 5.0 mg/kg of naltrexone capable of reducing ethanol self-administration across four hour sessions (Altshuler, 1979). And, while studies utilizing the intragastric self-administration (IGSA) method of ethanol self-administration have not examined the effects of naltrexone on ethanol intake, results using the similar, but shorter-acting opiate antagonist naloxone found that naloxone reduced ethanol infusions in male Wistar rats at 2 but not at 24 hours (Sinden et al., 1983). The effects of other routes of administration using naltrindole have yet to be examined.

While the above evidence can effectively rule out taste as the primary mechanism by which naltrexone and its derivatives are mediating the rewarding effects of ethanol, it does not preclude the notion that perhaps naltrexone and its derivatives are mediating its effects by inducing a general malaise which blunts the reinforcing effects of ethanol (as well as other reinforcers). U50,488H, in part through its ability to mediate the rewarding effects of drugs including morphine, heroin, ethanol, and cocaine as well as its ability to induce a conditioned place aversion when given alone, is generally considered to create a dysphoric and unpleasant state (for a review, see Wee and Koob, 2010). Therefore, it is probable that mu and delta antagonists, such as naltrexone and naltrindole, can be acting through this same mechanism, especially at higher doses of these drugs.

Clinically, the reported negative side-effects of naltrexone, including headaches, nausea, and fatigue (Croop et al., 1997) mirror those that would be indicative of a general dysphoric state that would interfere with treatment compliance (Bouza et al., 2004). This would, in part, explain why in the present investigation, the seeking and consuming of both sucrose and ethanol following treatment with U50,488H and higher doses of naltrexone are attenuated and return to baseline levels when the drug is no longer on board. Unlike a conditioned taste aversion, the animals are first exposed to the drug treatment and therefore do not associate the resulting dysphoric state with the reinforcer. Further, the induction of a general malaise helps explain why the clinical application of naltrexone is effective in treating other conditions independent of alcohol or opiate addiction (Bostwick & Bucci, 2008; Daniel et al., 1992; Kosten et al., 1992; Marrazzi et

al., 1995; Kim et al., 2001; Raymond et al., 2002; Grant & Kim, 2005; Symons et al., 2004; Carrion, 1995). Contrastingly, the finding that extremely low doses of naltrexone and low doses of naltrindole may be more selective for attenuating the seeking and intake, respectively, of ethanol relative to sucrose, may suggest that there are sub-threshold doses that may be less likely to induce a general malaise and instead are more selective for attenuating ethanol reinforcement.

The Role of Negative Reinforcement

Negative reinforcement could also contribute to the efficacy of naltrexone in decreasing drinking. In contrast to positive reinforcement, negative reinforcement is defined as the increased likelihood that a behavior will occur given the discontinuation of some aversive stimulus. By eliminating an undesirable feeling or state, the behavior is more likely to be repeated and subsequently strengthened. For example, in contrast to individuals that drink for the positive reinforcing effects of ethanol, others drink because alcohol alleviates symptoms of withdrawal or other aversive affective states such as depression or anxiety. Although a formal theory concerning the relationship between naltrexone and negative reinforcement has yet to be proposed, the mediation of negative reinforcement in alcohol dependence through the endogenous opioid system would likely be via the kappa receptor system. This is especially likely considering that the role of the kappa opioid receptor system, as has been previous demonstrated, appears to act in direct opposition to that of mu and delta (Hartig & Opitz, 1983; Locke et al., 1982; Iwamoto, 1981; Mucha & Herz, 1985; Chen et al., 2005; Handler et al., 1992; Di Chiara & Imperato, 1988; Tao & Auerbach, 2002, 2005; see Wee & Koob, 2010 for a review). For example, activation of the kappa opioid system through use of selective kappa agonists has been shown to decrease drinking across a variety of paradigms (Lindholm et al., 2001; Nestby et al., 1999; Logrip et al., 2008; Cosgrove & Carroll, 2002). Further, giving that a kappa agonist alone is enough to a induce a significant CPA in both rats and mice, which can be attenuated through the use of a selective kappa antagonist, such as norbinaltorphamine, the effects induced by the application of a kappa agonist are dysphoric and rather unpleasant.

In contrast to the mu and delta opioid systems, the kappa system has also been suggested to play a role in mediating the stress response and to facilitate the release of cortisol/corticosterone by activating the hypothalamo-pituitary-axis (HPA). The activation of this stress response involves the release of corticotropin-releasing hormone/factor (CRH/F) from the hypothalamus, which stimulates the release of adrenocorticotropic hormone (ACTH) from the pituitary gland. ACTH release activates the release of glucocorticoid hormones from the adrenal glands and ultimately cortisol/corticosterone (Del Campo et al., 1994; Delitala et al., 1994; Torpy et al., 1993; Inder et al., 1995). Ethanol elevates ACTH levels following acute administration (Rivier & Vale, 1987), which subsequently stimulates corticosteroid release. Interestingly, rats will self-administer both corticosterone (Piazza et al., 1993) and voluntarily consume ethanol, suggesting a complicated relationship between stress and the mediation of ethanol reinforcement.

Further, while stress has been implicated in the clinical research as a reason for alcohol consumption and maintenance, and it is believed that alcohol is used to attenuate stress and anxiety (via mechanisms of negative reinforcement), research utilizing various animal models does not demonstrate a clear, consistent pattern or relationship between stress and alcohol such that stress can increase, decrease, or have no effects on ethanol consumption (Becker et al., 2011; Bertholomey, 2011). Thus, the degree to which the kappa system may be recruited during a stress response and facilitate ethanol consumption during times of dependence is even less clear. However, the literature has noted that following ethanol dependence via either vapor inhalation and/or liquid diet, nor-BNI, which has been reported to increase (Mitchell et al., 2005), decrease (Logrip et al., 2008), or have no effect on (Doyon et al., 2006; Williams & Woods, 1998; Henderson, 2011) mediating ethanol intake in nondependent animals, consistently attenuates ethanol reinforcement in dependent animals (Hölter et al., 2000; Walker & Koob, 2008; Walker et al., 2010). Further, the role the kappa agonist, which has been demonstrated to consistently decrease ethanol in nondependent animals (Lindholm et al., 2001; Nestby et al., 1999; Logrip et al., 2008; Cosgrove & Carroll, 2002), remains

unclear. In fact, only recently has the role of the kappa receptor begun to be implicated in the mediation of ethanol reinforcement, which, was for so long, thought to be controlled solely but the mu and delta receptor subtypes.

Drinking Phenotype and the Endogenous Opioid System

Interpretation of the Study Results

The results of these studies demonstrated that though the endogenous opioid system plays a role in mediating the reinforcing effects of ethanol (and sucrose) in both P and LE rats, there are subtle differences in how the different drugs tested affected sucrose and ethanol seeking and intake as a function of drinking phenotype. Among rats treated with naltrindole, P rats were especially sensitive to the effects on ethanol intake and responding. Further, P rats showed a greater response to lower doses of naltrexone compared to LE rats. These results are consistent with clinical studies that have found that FH^{+} individuals treated with naltrexone have more positive treatment outcomes than those that are not (King et al., 1997; Monterosso et al., 2001; Rohsenow et al., 2007; Tidey et al., 2008). Interestingly, these results conflict with a recent review of the literature that attempted to assess differences in naltrexone response between rats and mice that had a propensity for high ethanol intake versus outbred, nonselected rats and mice, which found that naltrexone was equally efficacious at attenuating ethanol intake in paradigms modeling positive reinforcement (Henderson, unpublished data). However, in that review, the results were derived by comparisons of various doses (0.01-30 mg/kg; 0-450 ug/kg) of naltrexone across studies that utilized different concentrations (6%-20%) of ethanol across assorted limited access paradigms (homecage, operant) with varying amount of ethanol access (15-180 minutes) via dissimilar routes of naltrexone administration (subcutaneous, intraperitoneal, oral, microinfusion). Additionally, within those studies, very few looked at more than one line/strain/population of rat/mouse, thus this series of experiments is one of the first to examine and directly compare the effects of not only naltrexone, but also other receptor-selective drugs across different rat populations using the same paradigm.

Theories Regarding the Endogenous Opioid System and Reinforcement

These studies confirm the prevailing thought in the clinical literature that there is perhaps a subpopulation of individuals that have an underlying predisposition, likely genetic in nature, that confers a greater sensitivity to ethanol reinforcement and naltrexone treatment that is influenced in some way by the endogenous opioid system (Rubio et al., 2005; Rohsenow et al., 2007; Tidey et al., 2008; King et al., 1997; Monterosso et al., 2001; Williams et al., 2007; Xuei et al., 2006; Zhang et al., 2008; Edenberg et al., 2008; Ray et al., 2011; Kiefer et al., 2007). For example, some studies have attempted to identify differences in the density of endogenous opioid receptors, especially in regions of the brain believed to be important for mediating reinforcement in rats that differ in their propensity to drink high amounts of ethanol. For example, comparisons among the brains of alcohol-preferring P and their non-preferring NP counterparts reveal differential distributions of mu opioid receptors in the nucleus accumbens, amygdala, and olfactory tubercle (all increased in P rats versus NP rats; McBride et al., 1998). The Sardinian preferring rats (sP), both alcohol naïve and experienced, have decreased opioid receptors in the caudate-putamen and nucleus accumbens shell compared to the Sardinian non-preferring (sNP) rat (Fadda et al., 1999). Likewise, the Alko-Alcohol preferring (AA) and non-preferring (ANA) rats differ in their densities of opioid receptors such that the AA rats have increased densities of mu opioid receptors in the nuclei of limbic regions, including the nucleus accumbens shell, and the prefrontal cortex than do the ANA rats (de Waele et al., 1995; Marinelli et al., 2000). In addition, inbred strains of mice also show differential distributions of brain opioid receptors, with the alcohol preferring C57BL/6 mice having increased densities of delta receptors in both the ventral tegmental area and nucleus accumbens and increased kappa receptors in the amygdala and ventral tegmental area but decreased kappa binding in the nucleus accumbens and septum compared to the non preferring DBA/2 mice (de Waele & Gianoulakis, 1997; Jamensky & Gianoulakis, 1997).

Despite the differences observed between many of the high and low selected lines, not all of them revealed differences in receptor densities. For example, the high alcohol drinking (HAD) and low alcohol drinking (LAD) rats failed to differ in mu

receptor distribution (Gong et al., 1997). Taken together, the results of various studies of opioid receptor densities differ as a function of line and history of ethanol exposure as well as endogenous ligand and methodology used. It would be interesting to compare the receptor densities of a selected line, such as the P, both pre- and post- dependence to an outbred rat, such as its distant progenitor, the Wistar rat, to determine whether there are differences in opioid receptor densities. Any observed differences might elucidate the differences in opioid specificity and help generate a more accurate depiction of how opiate receptors are differentially distributed throughout the "reinforcement" centers of the brain.

Also, outbred, moderately alcohol drinking rats seem to be a more appropriate model of a social, or FH^{-}, drinker than are lines that have been selected for little to no alcohol drinking (which are typically used in these comparison studies). Therefore, examining any changes or differences in receptor densities that might occur as a result of dependence could lead to a better understanding of the role of the opioid system in mediating alcohol drinking. For example, Fadda and colleagues (1999) showed that there were no differences between the receptor densities of alcohol naïve and experienced sP rats (though there were differences between sP and sNP rats). By further investigating the differences between changes in receptor densities in alcohol naïve, alcohol experienced (nondependent), and alcohol dependent outbred rats, a significant change as a result of ethanol experience could help explain differences in the selectively of endogenous opioid system to attenuate ethanol drinking in models of positive versus negative reinforcement. This would be particularly relevant if these alterations in receptor densities, etc were specific to either selected or nonselected lines without affecting the other, facilitating a more complete understanding of how receptor densities and their alterations as a function of ethanol experience may enhance our understanding of the role of the endogenous opioid system in mediating ethanol reinforcement.

In addition to differences in the densities of various receptor subtypes, another mechanism that has been proposed to mediating the reinforcing effects of ethanol through regulation of the endogenous opioid system is underlying differences in beta-endorphin. Given that the delta and mu receptor antagonists conferred a greater sensitivity to

blocking the reinforcing effects of ethanol in P rats relative to LE rats, but that the kappa agonist U50,488H did not, it is possible that individuals with a predisposition towards high alcohol drinking (those that are FH^+) show differential responses to ethanol-induced release of beta-endorphin, which is believed to be responsible for the opioid-mediated reinforcement of ethanol (Keith et al., 1986; Gianoulakis et al., 1989; Gianoulakis, 1990; de Waele et al., 1992; Gianoulakis & Barcomb, 1987; Jarjour et al., 2009) relative to those that are not/FH^-. In an attempt to determine whether humans with a predisposition to excessive alcohol intake inherited an enhanced sensitivity of the endogenous beta-endorphin system, Gianoulakis (1996) examined a group of individuals that were FH^+ (an individual whose father and paternal grandfather both had alcoholism) and FH^- (individuals without any evidence of alcoholism of family member in the previous two generations), all of whom engaged only in occasional social drinking, on alterations in beta-endorphin levels following a dose-response (0, 0.25, 0.50, and 0.75 g/kg) of orally administered alcohol. The results revealed that while the FH^- individuals had higher basal plasma levels of beta-endorphin, that following placebo, FH^- individuals showed a time-dependent decrease in beta-endorphin levels. Interestingly, following alcohol intake, these same FH^- individuals did not demonstrate any alteration in beta-endorphin levels. In contrast, while FH^+ individuals revealed no change in beta-endorphin levels following placebo intake, FH^+ individuals did show a clear time-dependent increase in beta-endorphin levels following alcohol consumption (Gianoulakis, 1996).

In line with this research, alcoholics have also been determined to have significantly lower basal beta-endorphin plasma levels relative to non-alcoholics (Aguirre et al., 1995; del Arbol et al., 1995; Vescovi et al., 1992) and ethanol administration has been shown to increase beta-endorphin levels in these individuals (Gianoulakis et al., 1989; Vescovi et al., 1992). Further, following abstinence from ethanol, basal beta-endorphin plasma levels remained significantly low and failed to recover to pre-alcohol levels even after 10 years of abstinence from alcohol, suggesting perhaps a role of beta-endorphin in mediating the “craving” aspect of reinforcement (Vescovi et al., 1992; Esel et al., 2001; Zaleksa-Kaszubska et al., 2005). Likewise, in the preclinical literature Zaleksa-Kaszubska and colleagues (2006) found that when comparing Warsaw High

Preferring (WHP) and Warsaw Low Preferring (WLP) rats, that prior to any experience with ethanol, the lines differed genetically in their basal levels of beta-endorphin (however this difference approached but was not significant (460 versus 290 pg/ml). Further, following a single treatment of ethanol, these levels were elevated in both lines to levels significantly greater than that of control. Similar differences in beta-endorphin plasma levels were observed in AA rats (de Waele et al., 1994).

Studies using genetically altered, knockout mice that displayed either 0% (-/-), 50% (-/+), or 100% (+/+) beta-endorphin expression determined that at low ethanol concentrations ($\leq$ 7%), beta-endorphin deficient mice (-/-) drank significantly more ethanol in both a continuous access, two-bottle choice (Grisel et al., 1999) and operant self-administration (Hayward et al., 2004) paradigm than (+/+) mice. Further, these same mice were found to consistently self-administer ethanol intravenously while their wildtype (+/+) counterparts did not (Grahame et al., 1998). However, one caveat when utilizing knock out mice is that the receptor knockout mice have been developed to examine the role of the various opiate receptors in mediating ethanol reinforcement, the results of which have been mixed. While the data from the beta-endorphin knock out mice are in alignment with the research cited here, mu knock out mice have been shown to not drink ethanol (Hall et al., 2000; Roberts et al., 2000) while, conversely, delta receptor knockout mice have been shown to significantly increase their intake of ethanol (Roberts et al., 2001). Kappa knockout mice have demonstrated a decreased ethanol intake and saccharin preference (Kovacs et al., 2005). However, caution must be exercised when utilizing knockout mice to determine a contribution of a gene's function to a particular behavior, especially when that gene, in the case of the endogenous opioid system, is mediating a plethora of behaviors, some of which are essential to the species' survival. If knocking out a gene (i.e., the mu opioid gene) that is vital to an organism's survival (i.e., by mediating pain and analgesia) results in a viable organism, there are likely compensatory responses/adaptations occurring during the development that may seek to overcome these vital deficits. Compensatory actions, therefore, could alter the interpretation of behaviors that are being observed during experimental conditions and the knocked out gene could be misconstrued in its function in regulating a particular

behavior. However, a better option by which to determine the selective effects of a particular gene is through the use of a conditional knock out. The use of a conditional knockout would allow for the gene to be turned "off," and then evaluated for a behavioral response. Subsequently, the gene is turned back "on," and the same behaviors re-evaluated. This provides a much better idea of the contribution of individual genes to the maintenance of a behavior without the worry of compensatory alterations occurring. The use of a conditional knockout for each of the endogenous opioid receptor subtypes could provide a valuable tool by which to separately assess the role of each receptor subtype in mediating various components of ethanol-associated behaviors.

Finally, yet another mean used to measure endogenous opioid activity is through the measurement of opioid stimulated cortisol-induced release, usually through the use of antagonists such as naloxone or naltrexone. Following the onset of stress, efferent fibers within the brain converge upon neurons mediating corticotropin releasing factors (CRF) in the hypothalamus. Induction of the stress response and release of CRF induces the release of ACTH from the pituitary gland which in turn ultimately facilitates the release of cortisol from the adrenal gland (Johnson et al., 1992). Activation of the hypothalamic-pituitary-adrenal (HPA) axis can be achieved through the use of naloxone via its ability to activate CRF neurons at the level of the hypothalamus (Del Campo et al., 1994; Delitala et al., 1994; Torpy et al., 1993; Inder et al., 1995). Therefore, an assessment of cortisol levels directly following naloxone injections can indicate the sensitivity of the endogenous opioid system in an individual. The results then indicating that those with less inhibitory tone directed at the CRF (lower opioid activity) neuron would show a greater cortisol response relative to those that have more inhibitory tone (i.e., greater opioid activity) following an injection of the same dose of naloxone. Results from an experiment examining the effect of a naloxone challenge on cortisol release in FH^+ versus FH^- social drinking individuals only, revealed that while there was no difference in cortisol response following administration of a low dose of naloxone (50 ug/kg), FH^+ individuals showed a greater cortisol response after doses of 125, 375, and 500 ug/kg of naloxone versus FH^- individuals (Wand et al., 1998, 2001)

The results from the present study indicate that rats with a phenotype consistent with a family history of alcoholism show a greater response to treatment with selective mu and delta opiate antagonists relative to nonselected rats. Many theories in both the clinical and preclinical literature have suggested a role for the endogenous opioid system in mediating this sensitivity, ranging from differences in the distribution of mu and delta opioid receptors in various reinforcement centers of the brain, to differential responses of the beta-endorphin system and cortisol. However, while many theories have been put forth and evidence presented, just as many arguments exist for the opposing theories with equally compelling evidence. As such, these remain potential avenues of research to be explored. It could be beneficial to utilize various drinking phenotypes, such as rats selected for high ethanol intake and unselected, moderately drinking lines, and compare them across a battery of physiological responses (cortisol response, beta-endorphin release/levels, receptor binding) as a function of various levels of ethanol exposure in attempts to better answer these questions.

Conclusions

Alcoholism is a complicated disease and multiple neurotransmitter systems, including dopamine, serotonin, GABA, glutamate, opioid, adenosine, norepinephrine, NPY, and CRF/CRH (Durand & Barlow, 2000; Meyer & Quenzer, 2005; Dohrman et al., 1997; Lovinger, 1997; Froehlich, 1997; Mihic & Harris, 1997; Valenzuela, 1997; Gonzales & Jaworski, 1997; Di Chiara, 1997) have been implicated in regulating some aspect contributing to alcohol use and/or maintenance. It has also been demonstrated that these various neurotransmitter systems mediate different aspects of alcoholism, with some of them, such as the opiate system, clearly implicated in contributing to the maintenance of alcohol use via positive reinforcement while others, such as CRF/CRH, norepinephrine, and glutamate have been implicated as generally contributing to maintaining alcohol use/dependence via negative reinforcement. Interestingly, despite the decades of preclinical studies that have demonstrated the efficacy of these different neurotransmitter systems in attenuating various aspects of ethanol reinforcement, very few have materialized into effective therapeutic treatments at the clinical level. Of these, naltrexone has been shown to hold the most promise for further study and development

despite its current limitations. As such, the endogenous opioid system provides a good springboard from which subsequent research should be developed in the seemingly elusive pursuit of more effective treatment options. Hopefully, through this continued line of research, not only can the mechanisms by which the endogenous opioid system may be mediating alcohol use and dependence be determined, but also those of the other neurotransmitter system that are so intricately connected, the results of which can be exploited in the design of better treatment options.

Pursuant to this notion, at present, a closer examination of the roles of the selective opioid receptors in their contribution in the maintenance of various aspects of ethanol reinforcement provide a good catalyst from while to facilitate further research. To date, this is the first study that has examined the effects of various agonist/antagonists selective for each endogenous opioid receptor subtype in the same paradigm across two rat samples with varying drinking phenotypes looking at multiple reinforcers in a paradigm that procedurally dissociates the seeking "craving" component from the actual consumption component in an operant self-administration model. While the efficacies of the individual drugs cannot be directly compared to one another owing to the pharmacological differences that would require an extensive dose-response curve and several binding assays to untangle, comparisons can directly be made within each drug across dose, line, and reinforcer. Additionally, within each drug, comparisons can be made concerning dose on line and reinforcer on both drinking and seeking behaviors. The results of this study have ultimately provided an encouraging launching pad from which a multitude of avenues can be further explored with the promise of better treatment developments in the continual fight against alcohol addiction.

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TABLES

Table 1. The distribution of P and LE rats reinforced with either EtOH or Sucrose across each of the four experiments based upon drug treatment administered. The first number represents the number of rats that were trained to completion and included in the final analyses of the consummatory phase in each experiment. The second number indicates the number of rats that were included for each group in the appetitive analyses.

Experiment/Group	P Rats 10E	LE Rats 10E	P Rats 2S	LE Rats 2S
U50,488H	8	9	8	9
Naltrindole	8	9	8/7	9
Naltrexone (mu)	8/7	9/8	8	9
Naltrexone (ns)	8/7	9/8	8	7/9

Table 2. Mean (± SEM) of Weights (g) of Rats used in Experiment 1 (U50,488H) upon arrival, the Monday before the initiation of consummatory testing, and the Monday prior to the initiation of Appetitive Testing. Below each set of weights, the N indicates the number of rats that completed each phase to merit inclusion.

U50,488H		**Arrival**	**Consummatory**	**Appetitive**
P 10E	**Wt (g)**	240(12)	456(16)	531(21)
	N	8	8	8
LE 10E	**Wt (g)**	281(5)	457(10)	532(12)
	N	9	9	9
P 2S	**Wt (g)**	240(12)	435(29)	532(12)
	N	8	8	8
LE 2S	**Wt (g)**	273(4)	439(12)	518(15)
	N	9	9	9

Table 3. Mean (± SEM) of Weights (g) of Rats used in Experiment 2 (Naltrindole) upon arrival, the Monday before the initiation of consummatory testing, and the Monday prior to the initiation of Appetitive Testing. Below each set of weights, the N indicates the number of rats that completed each phase to merit inclusion.

Naltrindole		**Arrival**	**Consummatory**	**Appetitive**
P 10E	**Wt (g)**	294(11)	476(18)	556(20)
	N	8	8	8
LE 10E	**Wt (g)**	226(5)	425(17)	506(16)
	N	9	9	9
P 2S	**Wt (g)**	314(6)	507(9)	590(8)
	N	8	8	7
LE 2S	**Wt (g)**	217(4)	404(15)	484(20)
	N	9	9	9

Table 4. Mean (± SEM) of Weights (g) of Rats used in Experiment 3a (Naltrexone -mu) upon arrival, the Monday before the initiation of consummatory testing, and the Monday prior to the initiation of Appetitive Testing. Below each set of weights, the N indicates the number of rats that completed each phase to merit inclusion.

Naltrexone	**(mu)**	**Arrival**	**Consummatory**	**Appetitive**
P 10E	**Wt (g)**	231(7)	455(15)	537(17)
	N	8	8	7
LE 10E	**Wt (g)**	270(4)	430(11)	508(13)
	N	9	9	8
P 2S	**Wt (g)**	234(10)	454(14)	539(15)
	N	8	8	8
LE 2S	**Wt (g)**	280(7)	457(14)	544(17)
	N	9	9	9

Table 5. Mean (± SEM) of Weights (g) of Rats used in Experiment 4b (Naltrexone -ns) upon arrival, the Monday before the initiation of consummatory testing, and the Monday prior to the initiation of Appetitive Testing. Below each set of weights, the N indicates the number of rats that completed each phase to merit inclusion.

Naltrexone	**(ns)**	**Arrival**	**Consummatory**	**Appetitive**
P 10E	**Wt (g)**	260(12)	495(11)	582(9)
	N	8	8	7
LE 10E	**Wt (g)**	270(4)	422(13)	503(17)
	N	9	9	8
P 2S	**Wt (g)**	251(14)	484(9)	576(10)
	N	8	8	8
LE 2S	**Wt (g)**	278(2)	458(12)	551(13)
	N	9	7	9

Table 6. Mean (± SEM) lever presses during the appetitive phase following administration of U50,488H (Experiment 1).

U50,488H	**Saline**	**2.5 mg/kg**	**5.0 mg/kg**	**10.0 mg/kg**
Active Lever Presses				
P 10E	82.00(10.90)	25.38(7.75)	3.88(1.62)	9.50(4.42)
LE 10E	61.00(10.16)	37.33(4.97)	22.78(9.37)	5.89(3.16)
P 2S	104.25(9.23)	59.63(9.11)	22.13(9.00)	3.00(1.46)
LE 2S	59.89(7.17)	56.78(10.05)	24.89(8.52)	3.11(2.27)
Inactive Lever Presses				
P 10E	4.63(2.00)	0.75(0.41)	0.88(0.44)	0.75(0.31)
LE 10E	5.56(3.05)	1.22(0.57)	0.22(0.22)	0.00(0.00)
P 2S	8.25(2.24)	2.00(0.66)	0.63(0.38)	0.25(0.16)
LE 2S	2.44(0.84)	1.67(0.76)	0.67(0.24)	1.11(0.56)
Total Lever Presses				
P 10E	86.63(12.08)	26.13(8.03)	4.75(1.94)	10.25(4.52)
LE 10E	66.56(10.79)	38.56(5.11)	23.00(9.36)	5.89(3.16)
P 2S	112.50(10.23)	61.63(8.99)	22.75(8.99)	3.25(1.56)
LE 2S	62.33(7.10)	58.44(10.08)	25.56(8.52)	4.22(2.42)

Table 7. Mean (± SEM) Latency Data (s) following the administration of U50,488H (Experiment 1). * =significantly different from saline; # = significantly different from 2.5 mg/kg; ^ = significantly different from 5.0 mg/kg.

U50,488H	**Saline**	**2.5 mg/kg**	**5.0 mg/kg**	**10.0 mg/kg**
Consummatory Phase: Latency to 1st Lever Press (s)				
P 10E	48.6(10.6)	115.4(78.2)	48.1(10.0)	418.5(219.5)
LE 10E	16.1(4.3)	11.6(2.0)	14.7(3.6)	192.3(106.4)
P 2S	22.3(5.3)	34.5(11.5)	156.5(111.7)	491.7(131.8)*#^
LE 2S	13.1(4.6)	11.6(3.3)	16.3(7.8)	132.8(70.6)
Consummatory Phase: Latency to 1st Lick (s)				
P 10E	1.6(0.2)	2.4(0.8)	12.2(10.1)	176.9(133.8)
LE 10E	1.5(0.2)	2.3(0.6)	4.5(2.7)	19.3(15.0)
P 2S	1.1(0.1)	1.2(0.1)	1.4(0.1)	4.4(5.5)
LE 2S	1.2(0.1)	1.3(0.1)	1.8(0.3)	2.6(0.4)*#
Appetitive Phase: Latency to 1st Lever Press (s)				
P 10E	38.2(9.0)	397.1(160.2)	392.0(185.5)	673.6(147.4)*
LE 10E	33.3(6.9)	22.0(7.5)	224.5(139.8)	611.7(194.4)*#
P 2S	22.3(6.0)	21.4(4.1)	267.7(129.1)	610.2(166.1)*#
LE 2S	10.7(3.1)	21.2(4.9)	250.1(153.4)	687.4(179.3)*#^

Table 8. Mean (± SEM) Ethanol Intakes (g/kg) and Mean (± SEM) Blood Ethanol Levels (BEL; mg%) in P and LE rats across Experiments.

	Intake (g/kg)	**BEL (mg%)**
P Rats		
U50,488H	1.010(0.169)	57.575(5.361)
Naltrindole	0.949(0.071)	39.600(8.122)
Naltrexone (mu)	0.785(0.090)	60.188(6.304)
Naltrexone (ns)	0.965(0.091)	72.300(7.918)
LE Rats		
U50,488H	0.857(0.084)	62.922(3.306)
Naltrindole	0.566(0.066)	37.333(4.961)
Naltrexone (mu)	0.874(0.104)	74.233(8.477)
Naltrexone (ns)	0.643(0.099)	56.756(9.024)
Combined (P + LE rats)		
U50,488H	0.929(0.090)	60.406(3.042)
Naltrindole	0.746(0.066)	38.400(4.492)
Naltrexone (mu)	0.832(0.068)	67.624(5.502)
Naltrexone (ns)	0.795(0.077)	64.071(6.183)

Table 9. The effects of (A) U50,488H, (B) Naltrindole, (C) Naltrexone (mu), (D) Naltrexone (ns) on reinforcer intakes (consummatory) and responding on the reinforcer-associated lever (appetitive) across each group in each experiment. Arrow indicates significantly direction of effect relative to saline/vehicle; * = significantly different from low dose; ** = significantly differs from low and middle doses.

A. U50,488H

Dose (mg/kg)	2.5	5.0	10.0	Dose (mg/kg)	2.5	5.0	10.0
Intakes:				Appetitive:			
P 10E		↓	↓	P 10E	↓	↓	↓
LE 10E	↓	↓	↓**	LE 10E		↓	↓*
P 2S		↓*	↓**	P 2S	↓	↓*	↓*
LE 2S	↓	↓	↓	LE 2S		↓*	↓*

B. Naltrindole

Dose (mg/kg)	2.5	5.0	10.0	Dose (mg/kg)	2.5	5.0	10.0
Intakes:				Appetitive:			
P 10E		↓*	↓**	P 10E	↓	↓	↓
LE 10E				LE 10E			
P 2S				P 2S		↓	↓*
LE 2S				LE 2S			↓**

C. Naltrexone (mu)

Dose (mg/kg)	0.1	0.3	1.0	Dose (mg/kg)	0.1	0.3	1.0
Intakes:				Appetitive:			
P 10E	↓	↓	↓*	P 10E	↓	↓	↓
LE 10E		↓	↓	LE 10E	↓		↓
P 2S	↓	↓	↓**	P 2S			↓
LE 2S		↓		LE 2S			

D. Naltrexone (ns)

Dose (mg/kg)	1.0	3.0	10.0	Dose (mg/kg)	1.0	3.0	10.0
Intakes:				Appetitive:			
P 10E	↓	↓	↓	P 10E	↓	↓	↓
LE 10E	↓	↓	↓	LE 10E	↓	↓	↓
P 2S	↓	↓*	↓*	P 2S	↓	↓	↓
LE 2S		↓	↓	LE 2S	↓	↓	↓

Table 10. Mean (± SEM) lever presses during the appetitive phase following administration of Naltrindole (Experiment 2).

Naltrindole	**Vehicle**	**2.5 mg/kg**	**5.0 mg/kg**	**10.0 mg/kg**
Active Lever Presses				
P 10E	57.38(14.88)	23.50(8.86)	2.50(1.56)	0.38(0.26)
LE 10E	25.00(9.02)	35.11(10.57)	10.67(3.95)	10.33(6.16)
P 2S	70.71(11.51)	47.43(11.44)	21.86(8.00)	7.57(6.92)
LE 2S	60.22(11.83)	53.78(8.31)	34.00(9.32)	8.00(6.04)
Inactive Lever Presses				
P 10E	4.63(1.76)	2.50(0.95)	0.63(0.26)	0.13(0.13)
LE 10E	2.22(0.98)	2.89(1.60)	0.56(0.29)	0.11(0.11)
P 2S	6.57(3.96)	3.57(1.07)	0.86(0.60)	1.43(1.27)
LE 2S	3.44(1.60)	3.67(1.03)	2.11(0.74)	0.11(0.11)
Total Lever Presses				
P 10E	62.00(15.95)	26.00(9.09)	3.13(1.77)	0.50(0.33)
LE 10E	27.22(9.38)	38.00(10.95)	11.22(4.17)	10.44(6.26)
P 2S	77.29(14.60)	51.00(12.04)	22.71(8.48)	9.00(8.18)
LE 2S	63.67(13.13)	57.44(9.11)	36.11(9.70)	8.11(6.15)

Table 11. Mean (± SEM) Latency Data (s) following the administration of Naltrindole (Experiment 2). * =significantly different from vehicle; # = significantly different from 2.5 mg/kg; ^ = significantly different from 5.0 mg/kg.

Naltrindole	**Saline**	**2.5 mg/kg**	**5.0 mg/kg**	**10.0 mg/kg**
Consummatory Phase: Latency to 1st Lever Press (s)				
P 10E	34.9(10.7)	29.3(8.4)	257.7(102.3)	235.0(0.0)
LE 10E	18.7(4.5)	88.0(51.2)	146.1(79.6)	546.5(534.5)
P 2S	18.3(3.5)	41.3(16.8)	151.6(76.1)	94.0(47.2)
LE 2S	141.8(89.7)	36.6(9.3)	37.8(11.6)	24.0(7.5)
Consummatory Phase: Latency to 1st Lick (s)				
P 10E	1.7(0.2)	1.5(0.04)	2.2(0.3)*#	1200.0(0.0)*#^
LE 10E	1.7(0.2)	1.4(0.1)	135.0(133.1)	3.0(1.1)
P 2S	1.5(0.5)	1.9(0.7)	1.4(0.1)	1.3(0.1)
LE 2S	1.6(0.3)	1.4(0.2)	1.4(0.1)	1.3(0.1)
Appetitive Phase: Latency to 1st Lever Press (s)				
P 10E	186.6(98.6)	80.0(25.6)	668.8(183.5)*#	957.2(162.2)*#
LE 10E	172.9(129.1)	96.4(35.3)	415.1(165.3)	938.4(142.0)*#^
P 2S	87.1(27.4)	231.9(164.1)	367.4(215.0)	801.4(206.2)*#
LE 2S	59.7(13.0)	32.3(7.9)	89.9(49.8)	873.5(167.7)*#^

Table 12. Mean (± SEM) lever presses during the appetitive phase following administration of Naltrexone (mu; Experiment 3a).

Naltrexone (mu)	**Saline**	**0.1mg/kg**	**0.3mg/kg**	**1.0 mg/kg**
Active Lever Presses				
P 10E	89.00(13.42)	24.57(7.14)	20.88(9.48)	12.29(6.57)
LE 10E	60.25(8.94)	31.75(9.95)	36.00(5.94)	20.50(4.15)
P 2S	78.13(8.84)	55.75(8.72)	46.88(7.79)	25.25(6.25)
LE 2S	56.33(10.05)	31.78(5.76)	28.22(7.09)	32.78(11.83)
Inactive Lever Presses				
P 10E	3.71(1.86)	3.00(1.57)	6.14(2.65)	1.43(1.10)
LE 10E	5.63(1.86)	3.00(1.57)	7.88(3.61)	0.88(0.64)
P 2S	4.50(1.48)	4.75(3.17)	11.75(8.51)	1.00(0.42)
LE 2S	1.00(0.37)	0.78(0.40)	2.00(0.67)	1.00(0.88)
Total Lever Presses				
P 10E	92.71(13.00)	27.57(7.98)	27.00(9.95)	13.71(7.63)
LE 10E	65.88(10.12)	34.75(10.74)	43.88(6.49)	21.38(4.41)
P 2S	82.63(8.61)	60.50(10.13)	58.63(12.17)	26.25(6.33)
LE 2S	57.33(10.25)	32.56(5.57)	30.22(7.44)	33.78(12.54)

Table 13. Mean (± SEM) Latency Data (s) following the administration of Naltrexone (mu; Experiment 3a).

Naltrexone (mu)	**Saline**	**0.1mg/kg**	**0.3mg/kg**	**1.0mg/kg**
Consummatory Phase: Latency to 1st Lever Press (s)				
P 10E	76.0(22.0)	69.6(12.2)	110.1(43.6)	67.3(17.4)
LE 10E	25.8(9.8)	52.7(32.9)	27.3(10.5)	23.6(8.9)
P 2S	30.8(8.5)	43.4(10.3)	32.1(6.1)	38.1(10.6)
LE 2S	22.1(8.6)	25.9(6.3)	84.2(62.7)	20.0(4.3)
Consummatory Phase: Latency to 1st Lick (s)				
P 10E	1.5(0.2)	1.4(0.1)	1.7(0.2)	2.2(0.5)
LE 10E	3.3(1.7)	1.7(0.2)	1.7(0.1)	1.8(0.2)
P 2S	1.0(0.1)	1.1(0.1)	1.2(0.1)	1.1(0.03)
LE 2S	1.5(0.2)	1.5(0.1)	1.4(0.1)	2.4(0.1)
Appetitive Phase: Latency to 1st Lever Press (s)				
P 10E	58.2(11.3)	431.6(163.1)	137.3(60.6)	62.1(14.6)
LE 10E	11.0(3.0)	197.2(144.7)	29.9(9.5)	178.9(146.6)
P 2S	17.1(4.7)	22.0(2.8)	22.4(6.3)	56.5(17.8)
LE 2S	29.9(12.4)	31.0(10.7)	65.0(29.6)	29.5(9.8)

Table 14. Mean (± SEM) lever presses during the appetitive phase following administration of Naltrexone (ns; Experiment 3b).

Naltrexone (ns)	**Saline**	**1.0mg/kg**	**3.0mg/kg**	**10.0 mg/kg**
Active Lever Presses				
P 10E	65.86(5.86)	4.14(2.08)	5.00 (2.62)	6.57 (2.34)
LE 10E	75.13(9.03)	20.88(5.10)	20.88(6.68)	6.25(3.23)
P 2S	84.25(13.22)	25.00(8.32)	10.00(3.38)	23.38(4.08)
LE 2S	70.11(11.67)	38.56(8.59)	33.00(8.66)	18.44(4.98)
Inactive Lever Presses				
P 10E	1.86(0.60)	2.43(1.78)	0.43(0.20)	1.00(0.49)
LE 10E	6.00(2.62)	0.50(0.33)	2.00(0.82)	0.25(0.16)
P 2S	3.00(0.60)	1.00(0.46)	0.50(0.50)	1.50(0.76)
LE 2S	4.22(1.02)	0.56(0.29)	1.11(0.61)	0.44(0.24)
Total Lever Presses				
P 10E	67.72(6.00)	6.57(2.51)	5.43(2.62)	7.57(2.62)
LE 10E	81.13(10.32)	21.38(5.17)	22.88(6.68)	6.50(3.22)
P 2S	87.25(13.41)	25.63(8.43)	10.50(3.22)	24.88(4.37)
LE 2S	74.33(10.25)	39.11(8.59)	34.11(8.98)	18.89(5.18)

Table 15. Mean (± SEM) Latency Data (s) following the administration of Naltrexone (ns; Experiment 3b) * =significantly different from saline

Naltrexone (ns)	**Saline**	**1.0mg/kg**	**3.0 mg/kg**	**10.0 mg/kg**
Consummatory Phase: Latency to 1st Lever Press (s)				
P 10E	62.9(32.8)	81.6(46.2)	195.0(131.9)	64.2(23.5)
LE 10E	32.9(6.9)	44.7(20.3)	217.3(101.9)	107.3(39.4)
P 2S	23.9(8.4)	27.8(6.4)	52.8(8.9)	53.4(9.0)
LE 2S	20.1(5.6)	31.1(13.0)	27.6(13.5)	35.4(12.7)
Consummatory Phase: Latency to 1st Lick (s)				
P 10E	2.5(0.9)	2.2(0.2)	5.9(3.6)	13.9(11.6)
LE 10E	9.4(6.1)	2.6(0.6)	16.4(14.5)	2.4(0.5)
P 2S	1.1(0.1)	1.3(0.1)	1.5(0.2)*	1.4(0.1)
LE 2S	1.1(0.1)	1.3(0.1)	172.5(171.2)	1.7(0.1)
Appetitive Phase: Latency to 1st Lever Press (s)				
P 10E	48.1(7.0)	366.4(180.8)	383.1(562.4)	429.9(202.0)
LE 10E	36.6(15.2)	236.3(144.2)	160.4(130.7)	328.3(166.8)
P 2S	15.3(3.6)	64.7(27.4)	136.6(68.9)	41.5(13.6)
LE 2S	13.7(4.8)	40.0(21.0)	168.3(129.9)	280.2(173.9)

FIGURES

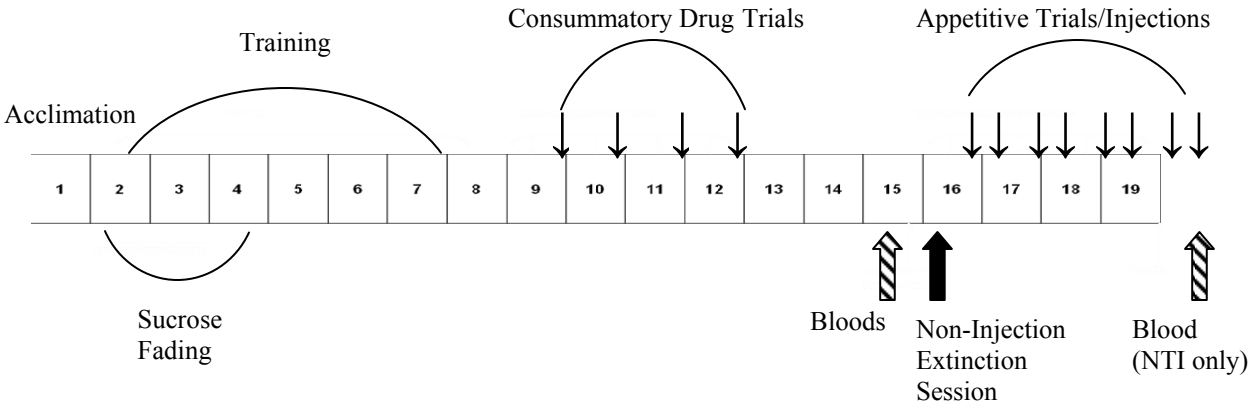

Figure 1. Timeline (by week) demonstrating the design of the four drug experiments included in the overall study. The time points at which rats underwent drug and/or saline injections are denoted with downward arrows. The solid upward arrow is indicative of the non-injection extinction and the striped upward arrows are indicative of where in the study blood ethanol levels were assessed following a session.

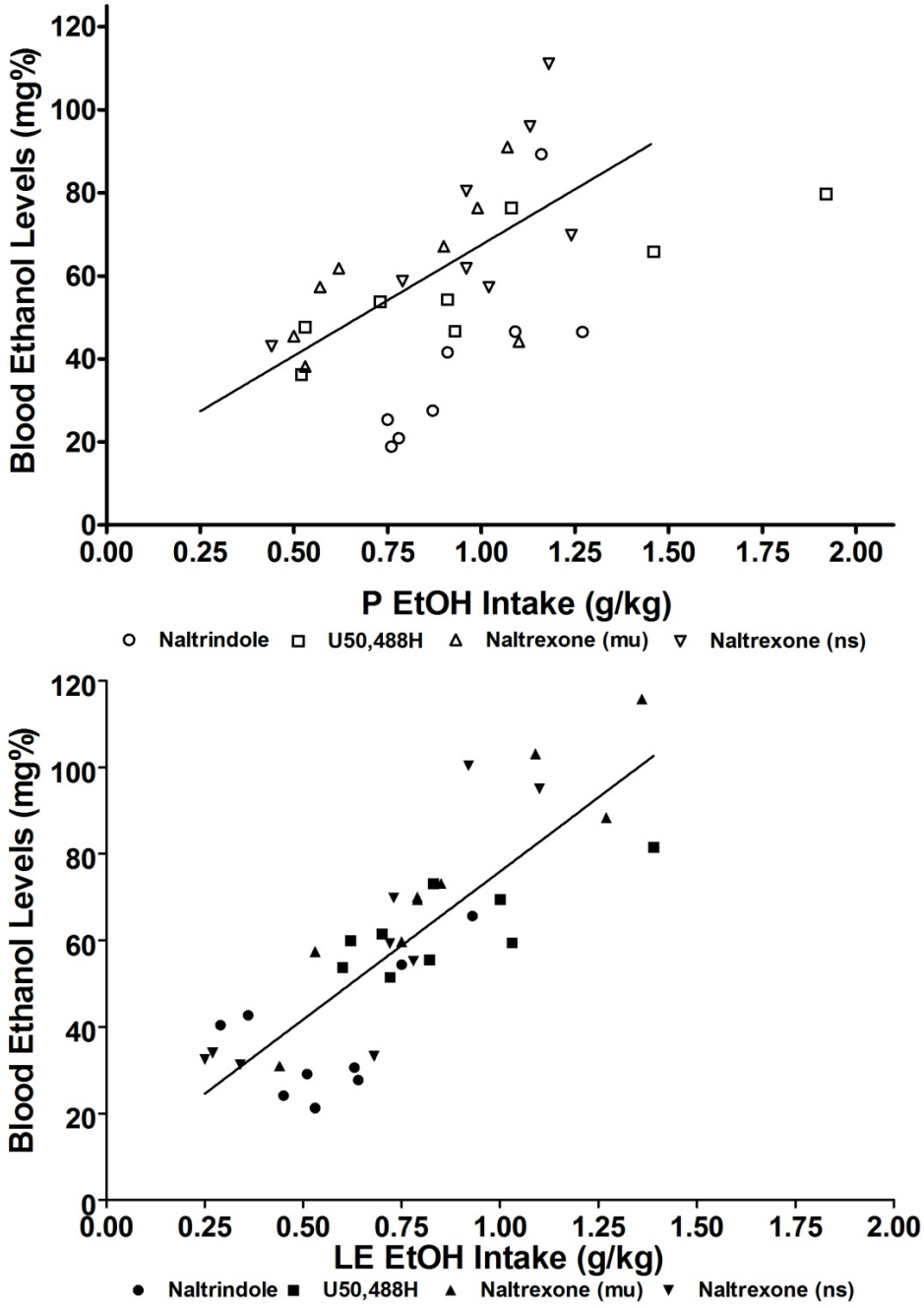

Figure 2. Scatterplots correlating the Blood Ethanol Levels (BELs; mg%) and EtOH Intakes (g/kg) in (a) P and (b) LE Rats across all Experiments.

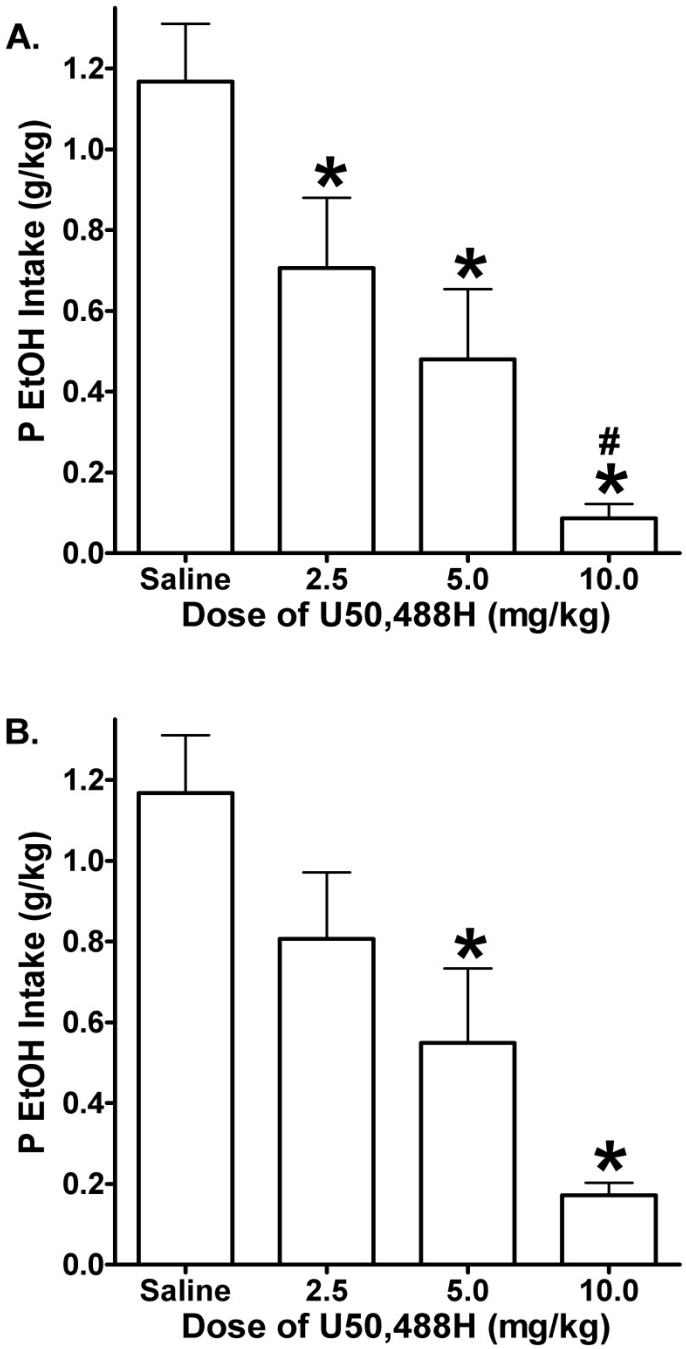

Figure 3. Experiment 1: Effect of the Kappa Agonist U50,488H on EtOH Consumption (g/kg) in P Rats (a) with a '0' included in instances of missing data (i.e., rat failed to make single RR necessary for reinforcer access) and (b) where any missing data has been excluded. * = significantly differs from Saline; # = significantly differs from the 2.5 mg/kg dose.

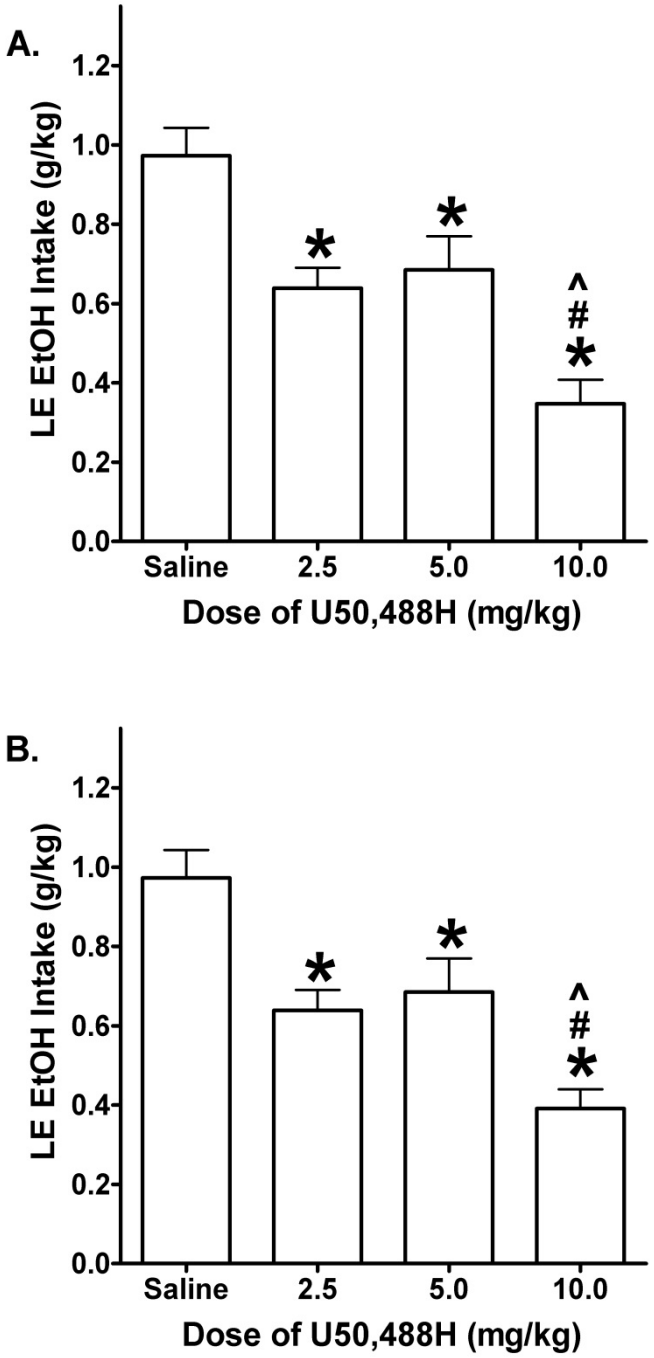

Figure 4. Experiment 1: Effect of the Kappa Agonist U50,488H on EtOH Consumption (g/kg) in LE Rats (a) with a '0' included in instances of missing data (i.e., rat failed to make single RR necessary for reinforcer access) and (b) where any missing data has been excluded. * = significantly differs from Saline; # = significantly differs from the 2.5 mg/kg dose; ^ = Significantly differs from 5.0 mg/kg dose.

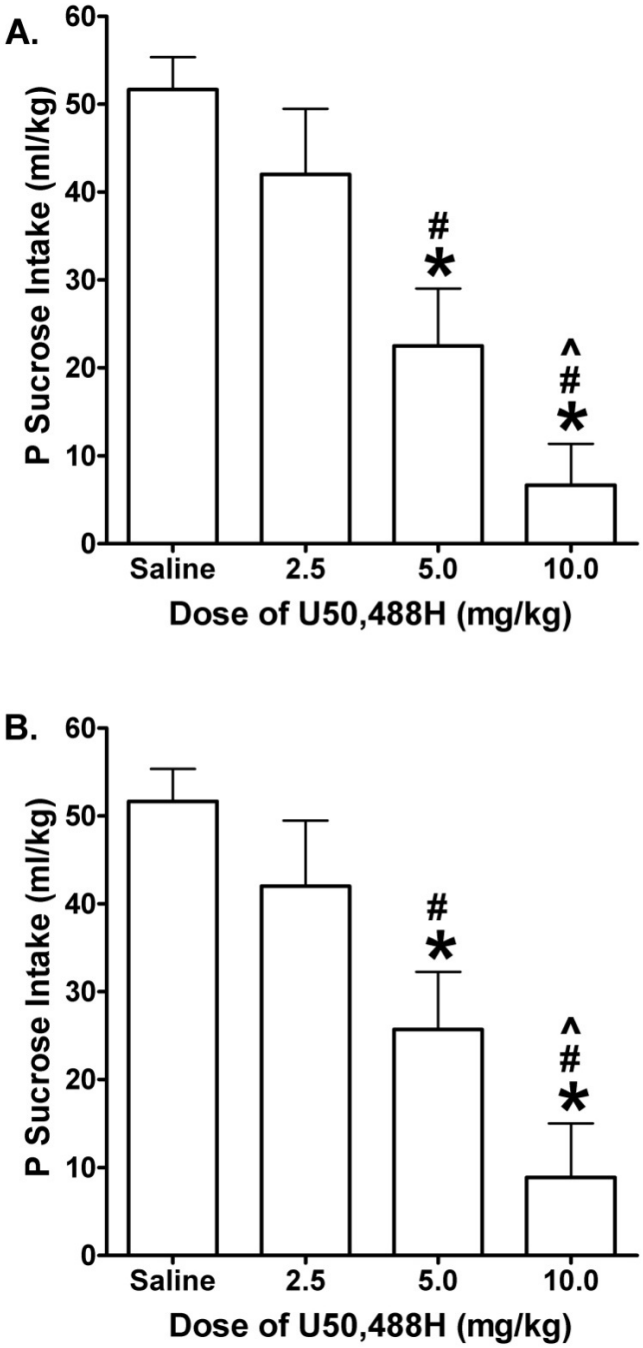

Figure 5. Experiment 1: Effect of the Kappa Agonist U50,488H on Sucrose Consumption (ml/kg) in P Rats (a) with a '0' included in instances of missing data (i.e., rat failed to make single RR necessary for reinforcer access) and (b) where any missing data has been excluded. * = significantly differs from Saline; # = significantly differs from the 2.5 mg/kg dose; ^ = Significantly differs from 5.0 mg/kg dose.

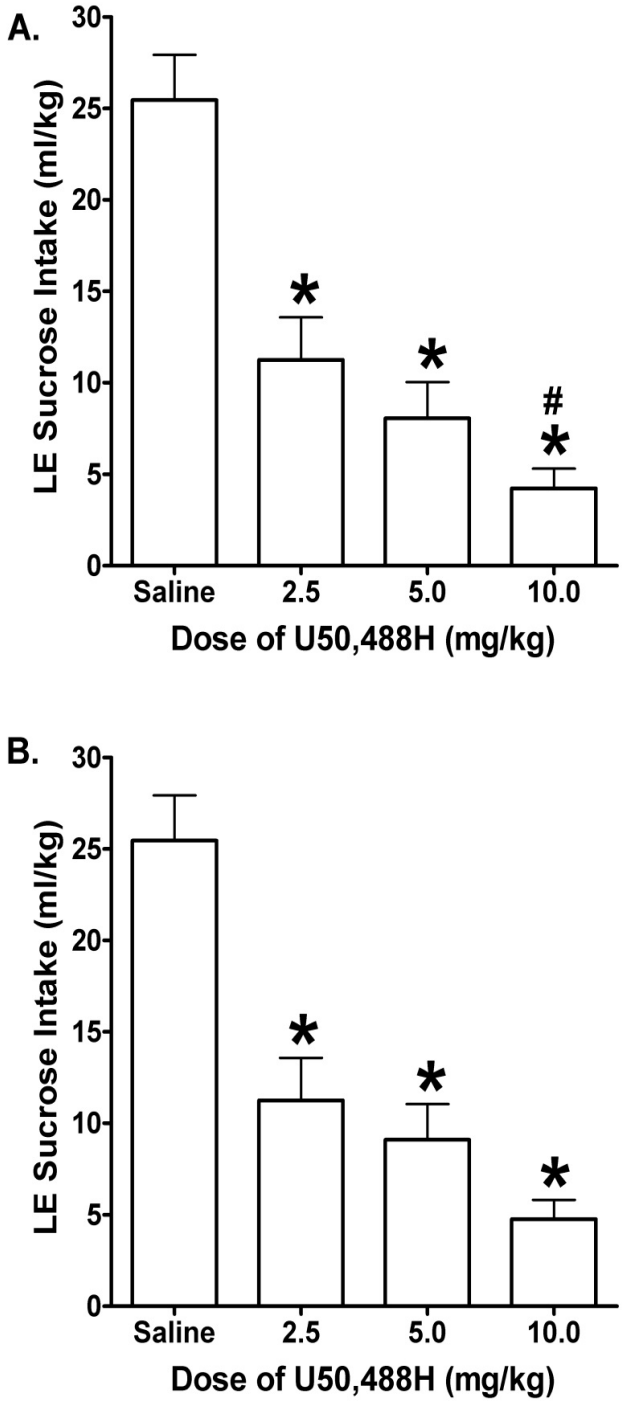

Figure 6. Experiment 1: Effect of the Kappa Agonist U50,488H on Sucrose Consumption (ml/kg) in LE Rats (a) with a '0' included in instances of missing data (i.e., rat failed to make single RR necessary for reinforcer access) and (b) where any missing data has been excluded. * = significantly differs from Saline; # = significantly differs from the 2.5 mg/kg dose.

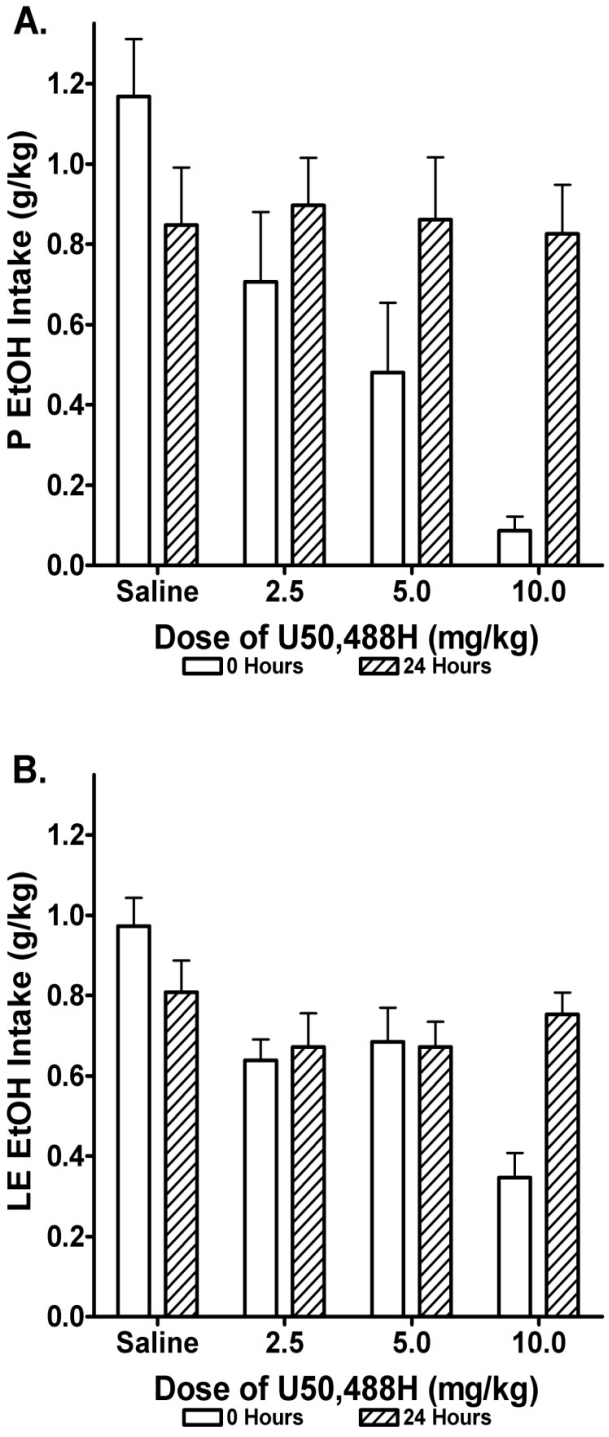

Figure 7. Experiment 1: Effects of the kappa agonist U50,488H on (A) EtOH intake (g/kg) and (B) Sucrose intake (ml/kg) in P Rats on the day of and 24 hours post injections.

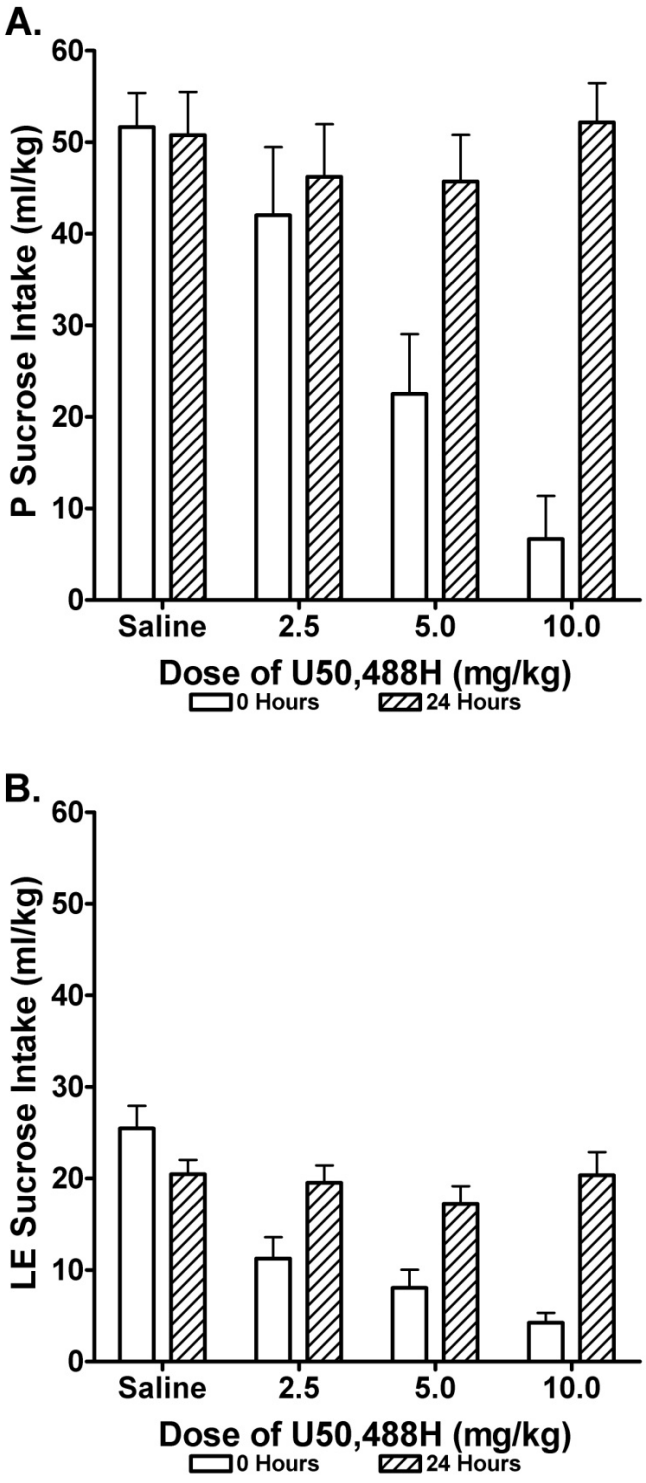

Figure 8. Experiment 1: Effects of the kappa agonist U50,488H on (A) EtOH intake (g/kg) and (B) Sucrose intake (ml/kg) in LE Rats immediately following and 24 hours post injections.

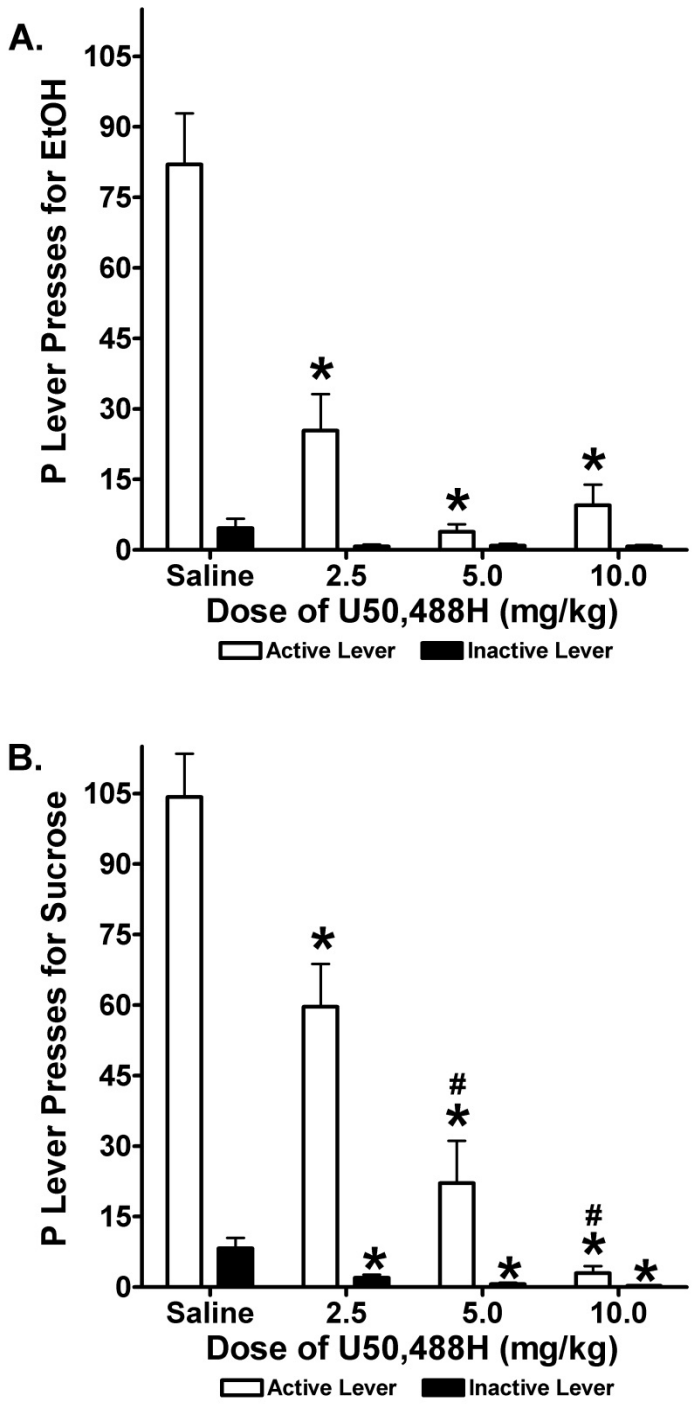

Figure 9. Experiment 1: Effects of the Kappa Agonist U50,488H on responding for (a) EtOH and (b) Sucrose on the active and inactive levers in P Rats. * = significantly differs from Saline; # = significantly differs from the 2.5 mg/kg dose.

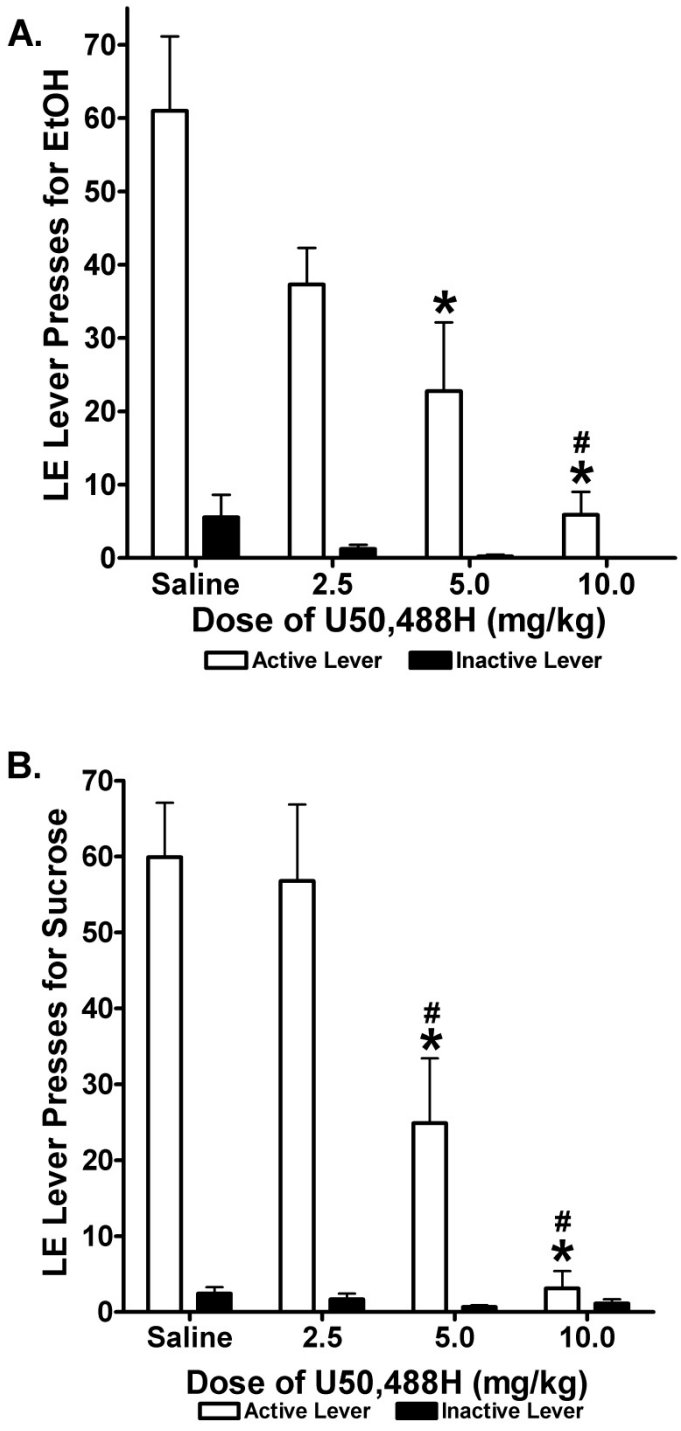

Figure 10. Experiment 1: Effects of the Kappa Agonist U50,488H on responding for (a) EtOH and (b) Sucrose on the active and inactive levers in LE Rats. * = significantly differs from Saline; # = significantly differs from the 2.5 mg/kg dose.

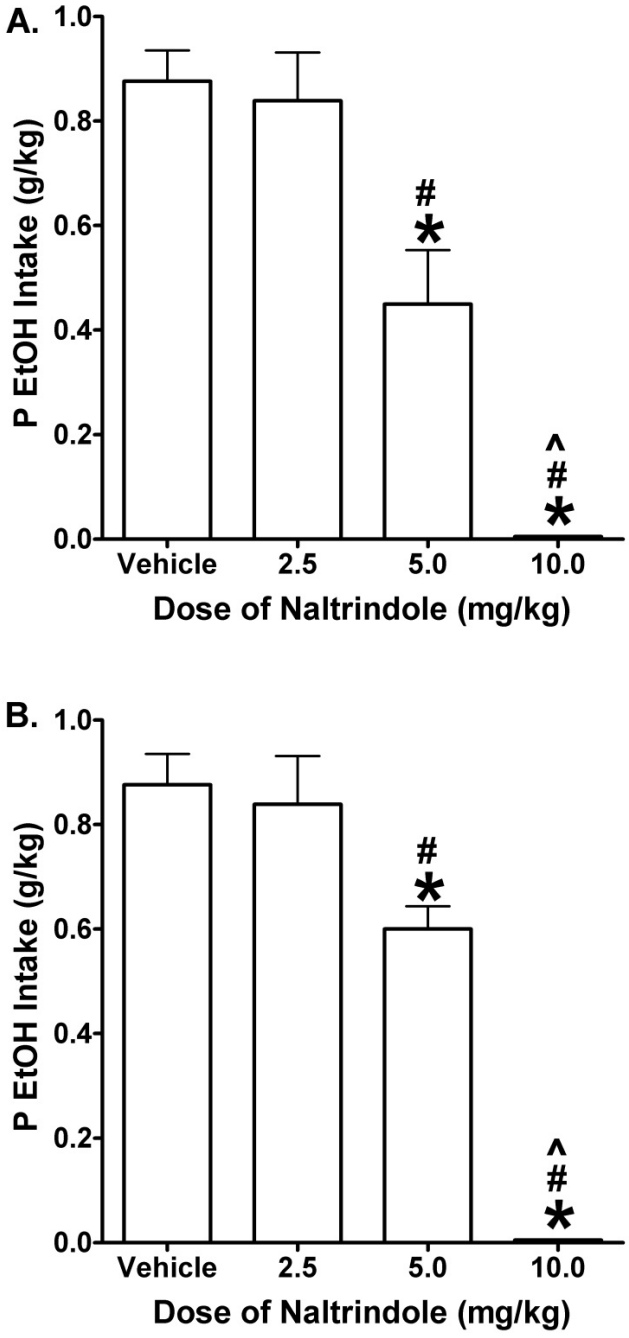

Figure 11. Experiment 2: Effect of the Delta Antagonist Naltrindole on EtOH Consumption (g/kg) in P Rats (a) with a '0' included in instances of missing data (i.e., rat failed to make single RR necessary for reinforcer access) and (b) where any missing data has been excluded. * = significantly differs from Vehicle; # = significantly differs from the 2.5 mg/kg dose; ^ = significantly differs from 5.0 mg/kg dose.

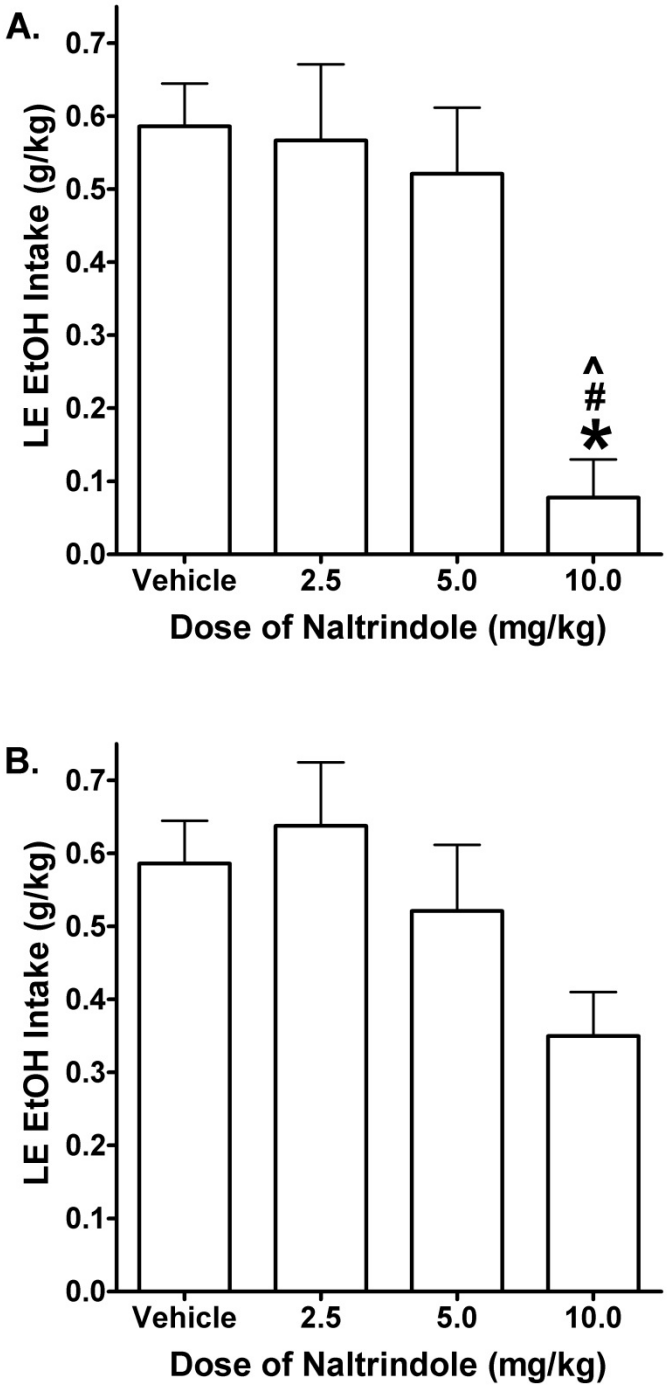

Figure 12. Experiment 2: Effect of the Delta Antagonist Naltrindole on EtOH Consumption (g/kg) in LE Rats (a) with a '0' included in instances of missing data (i.e., rat failed to make single RR necessary for reinforcer access) and (b) where any missing data has been excluded. * = significantly differs from Vehicle; # = significantly differs from the 2.5 mg/kg dose; ^ = significantly differs from 5.0 mg/kg dose.

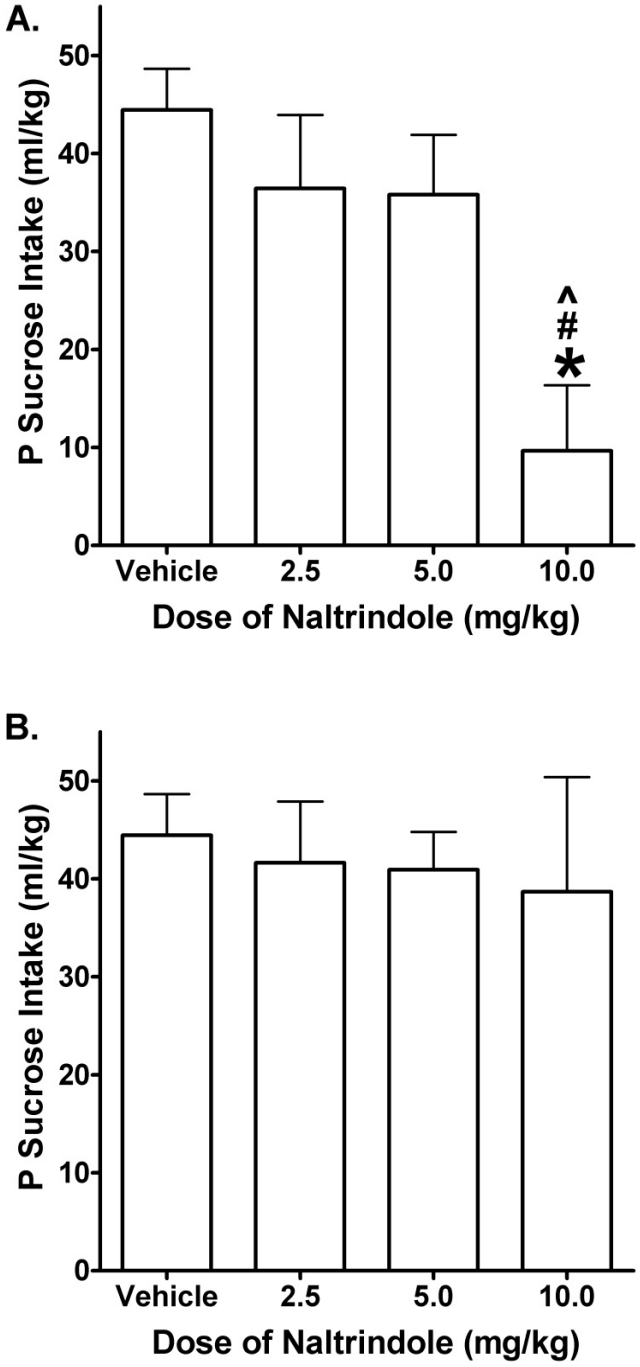

Figure 13. Experiment 2: Effect of the Delta Antagonist Naltrindole on Sucrose Consumption (ml/kg) in P Rats (a) with a '0' included in instances of missing data (i.e., rat failed to make single RR necessary for reinforcer access) and (b) where any missing data has been excluded. * = significantly differs from Vehicle; # = significantly differs from the 2.5 mg/kg dose; ^ = significantly differs from 5.0 mg/kg dose.

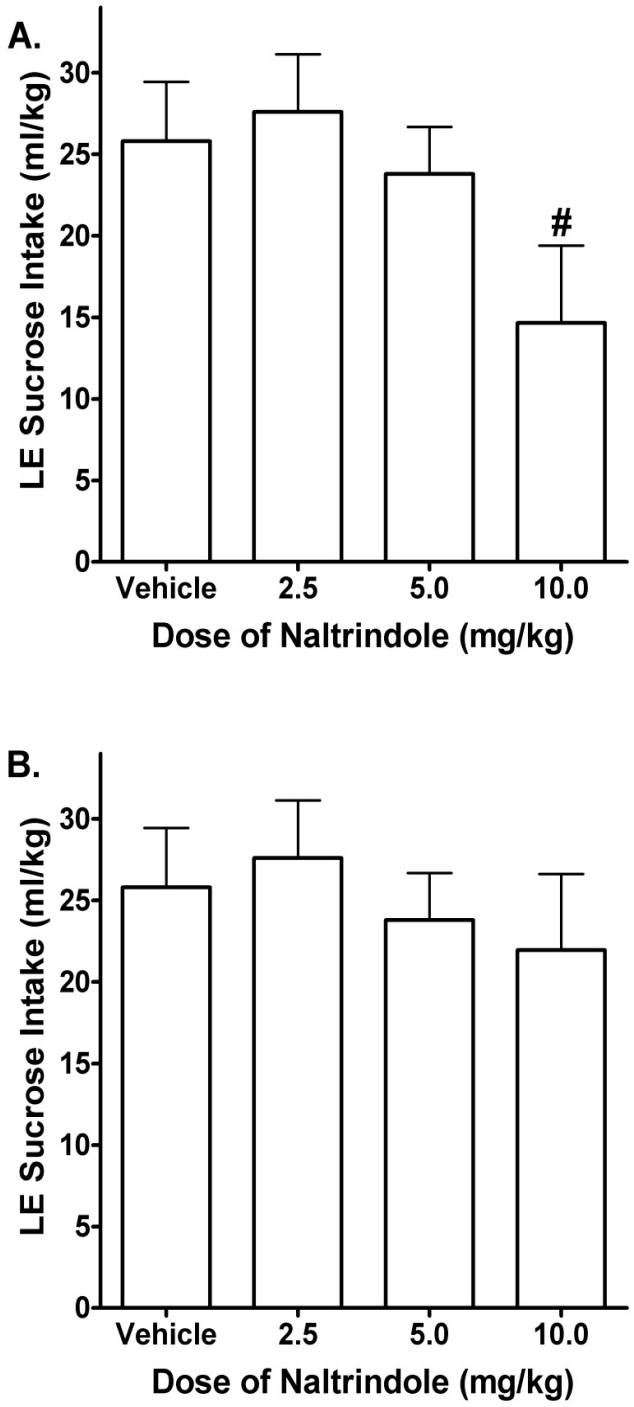

Figure 14. Experiment 2: Effect of the Delta Antagonist Naltrindole on Sucrose Consumption (ml/kg) in LE Rats (a) with a '0' included in instances of missing data (i.e., rat failed to make single RR necessary for reinforcer access) and (b) where any missing data has been excluded. # = significantly differs from the 2.5 mg/kg dose.

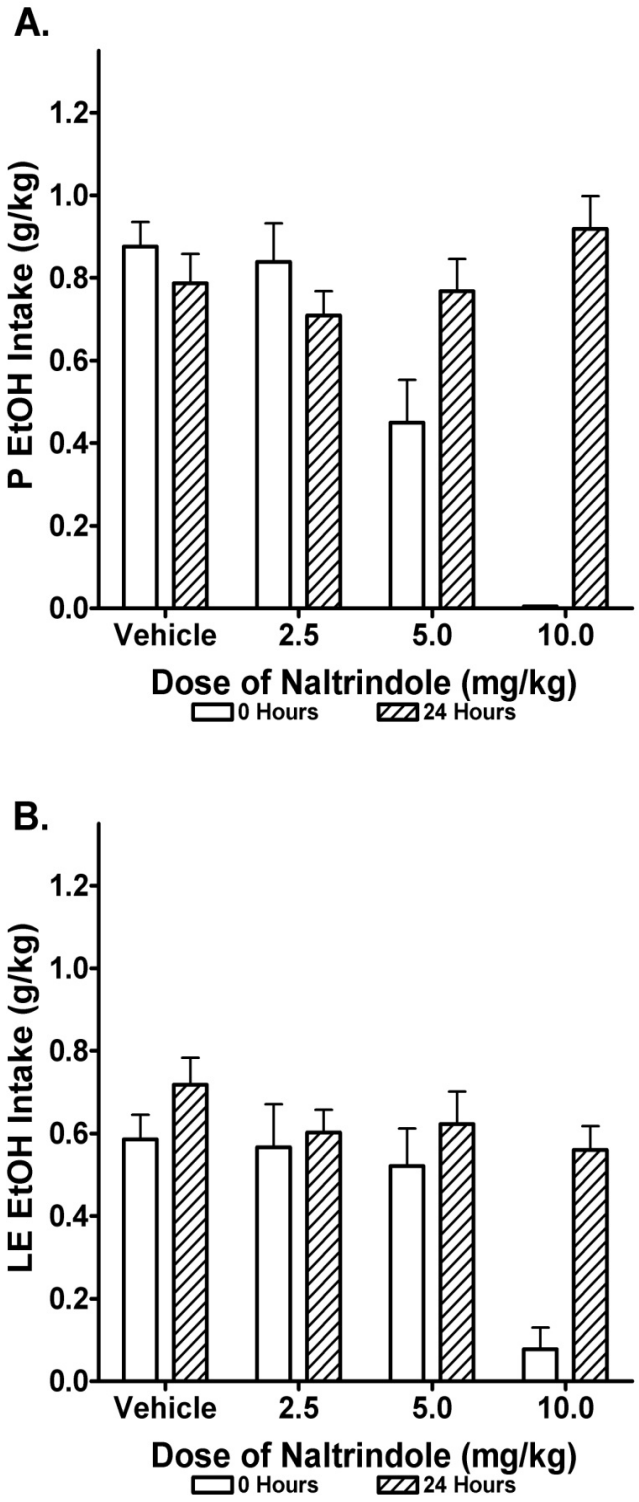

Figure 15. Experiment 2: Effects of the delta antagonist Naltrindole on (A) EtOH intake (g/kg) and (B) Sucrose Intake (ml/kg) in P Rats immediately following and 24 hours post injections.

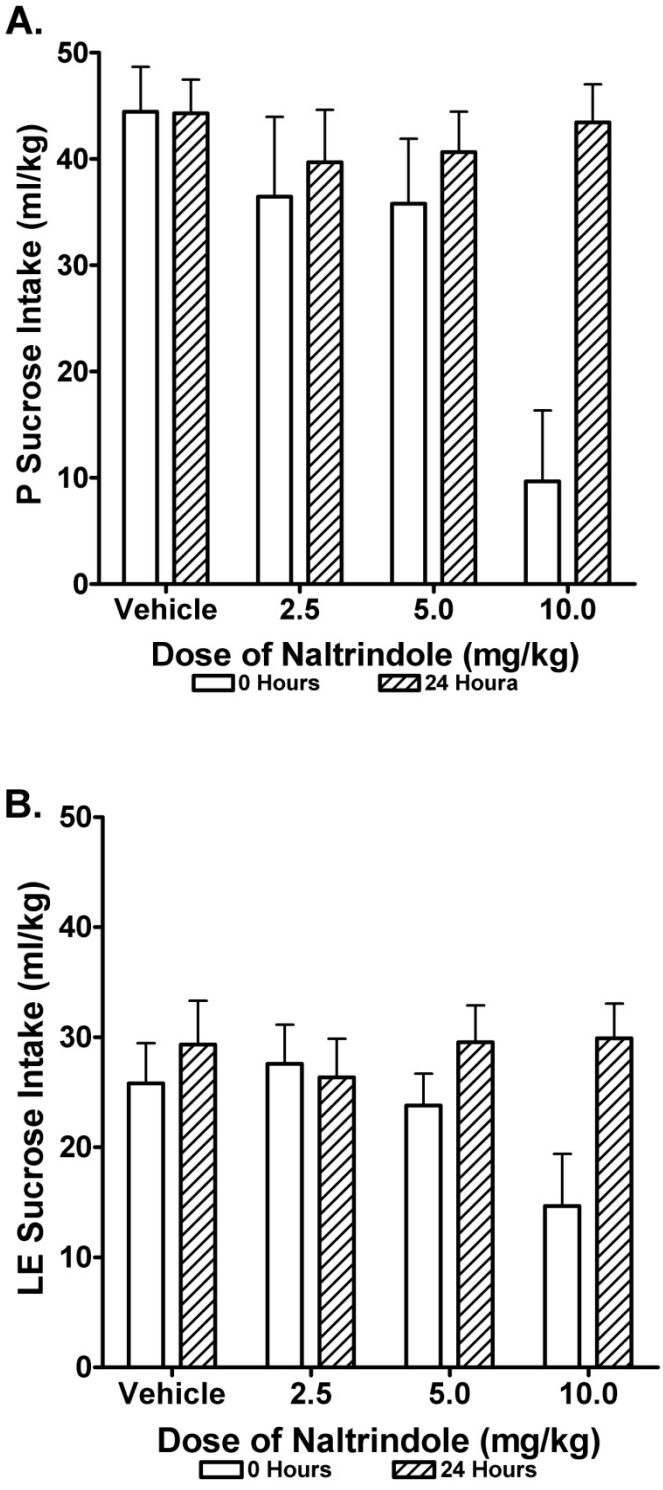

Figure 16. Experiment 2: Effects of the delta antagonist Naltrindole on (A) EtOH intake (g/kg) and (B) Sucrose Intake (ml/kg) in LE Rats immediately following and 24 hours post injections.

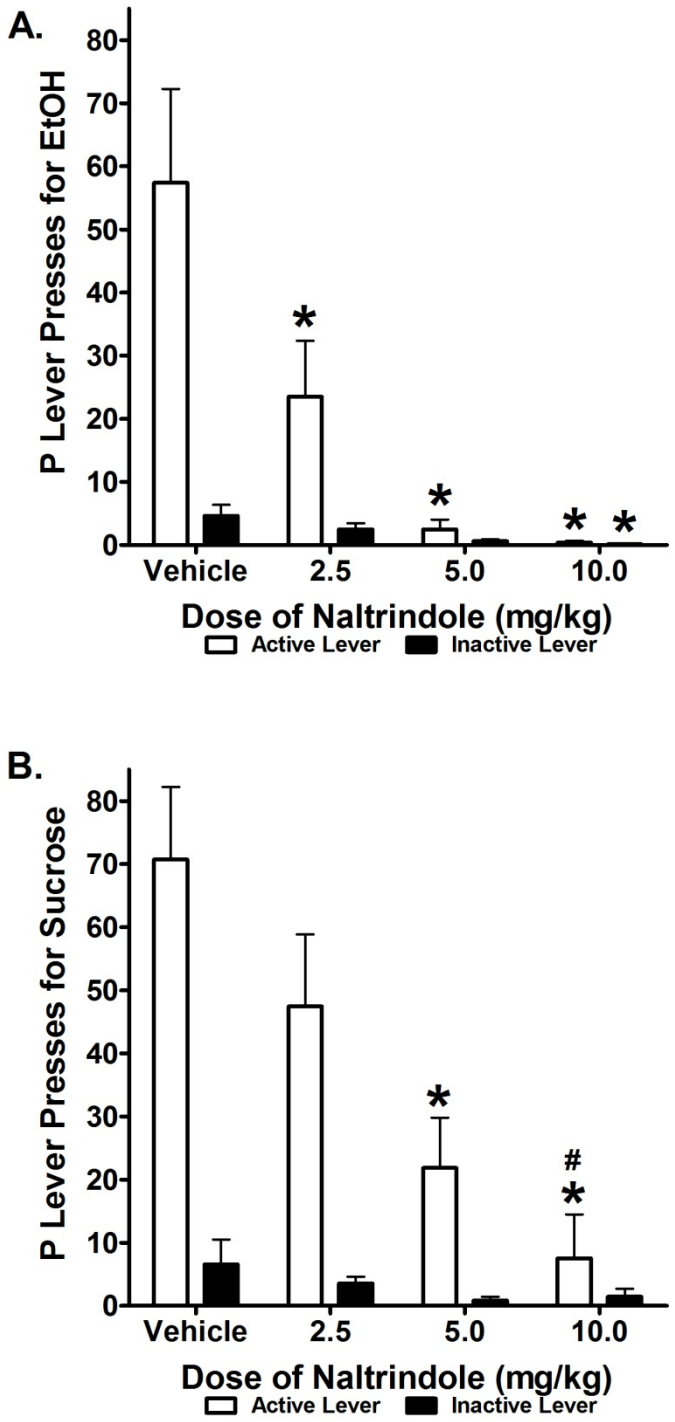

Figure 17. Experiment 2: Effects of the Delta Antagonist Naltrindole on responding for (a) EtOH and (b) Sucrose on the active and inactive levers in P Rats. * = significantly differs from Vehicle; # = significantly differs from the 2.5 mg/kg dose.

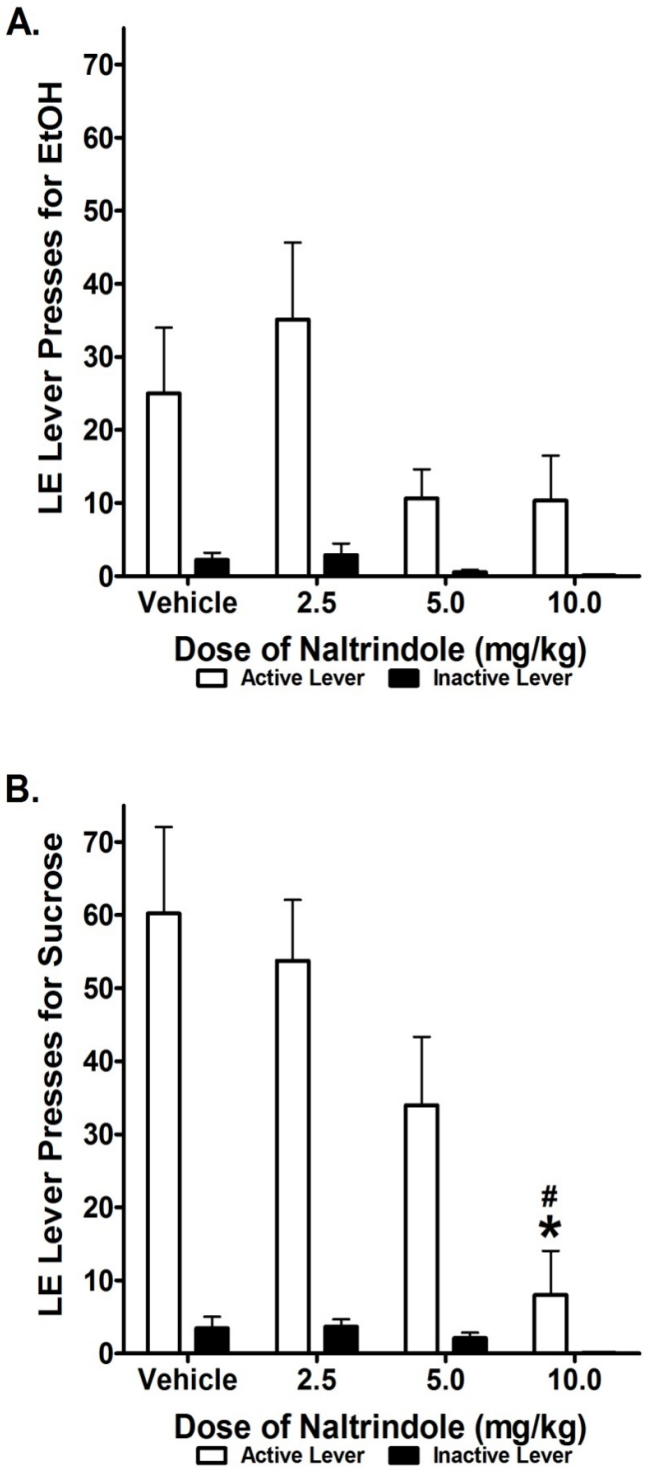

Figure 18. Experiment 2: Effects of the Delta Antagonist Naltrindole on responding for (a) EtOH and (b) Sucrose on the active and inactive levers in LE Rats. * = significantly differs from Vehicle; # = significantly differs from the 2.5 mg/kg dose.

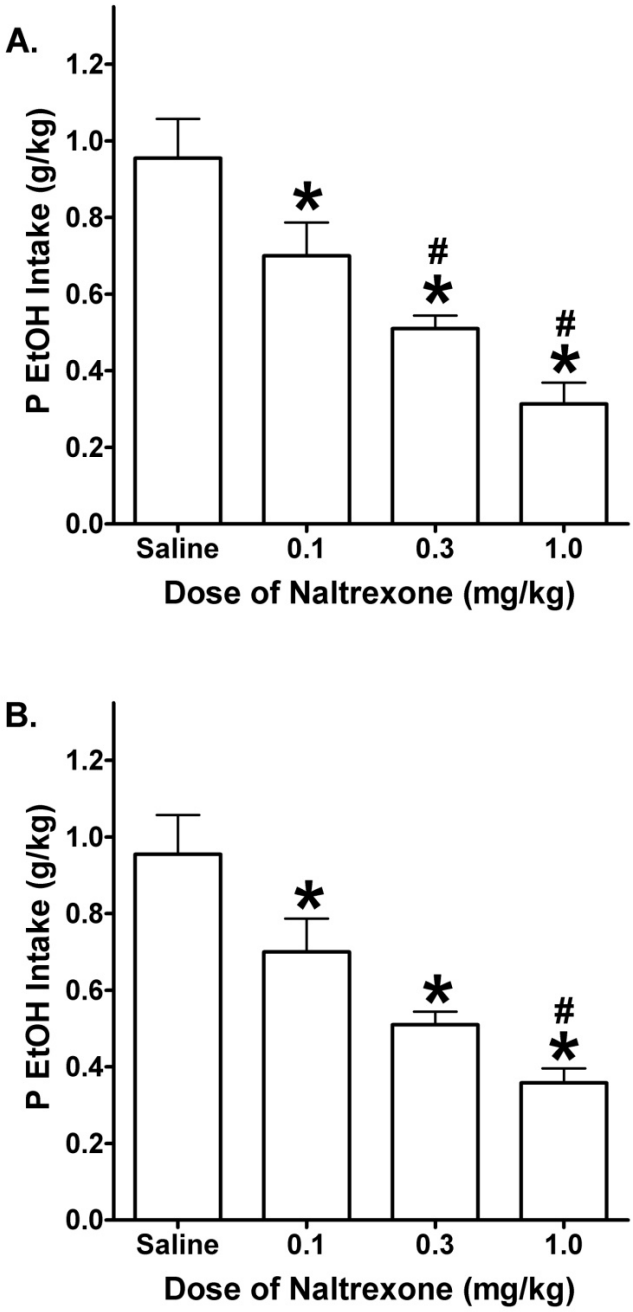

Figure 19. Experiment 3a: Effects of the Mu Antagonist Naltrexone on EtOH Consumption (g/kg) in P Rats (a) with a '0' included in instances of missing data (i.e., rat failed to make single RR necessary for reinforcer access) and (b) where any missing data has been excluded. * = significantly differs from Saline; # = significantly differs from the 0.1 mg/kg dose.

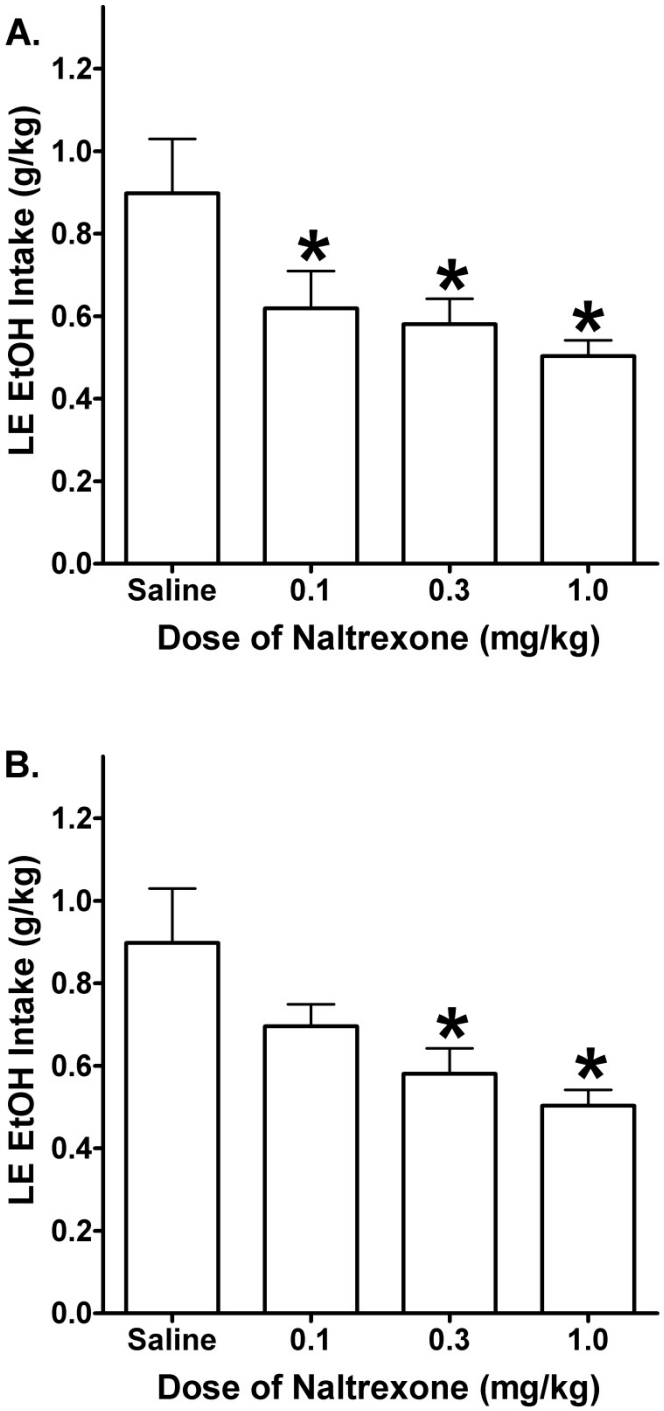

Figure 20. Experiment 3a: Effects of the Mu Antagonist Naltrexone on EtOH Consumption (g/kg) in LE Rats (a) with a '0' included in instances of missing data (i.e., rat failed to make single RR necessary for reinforcer access) and (b) where any missing data has been excluded. * = significantly differs from Saline.

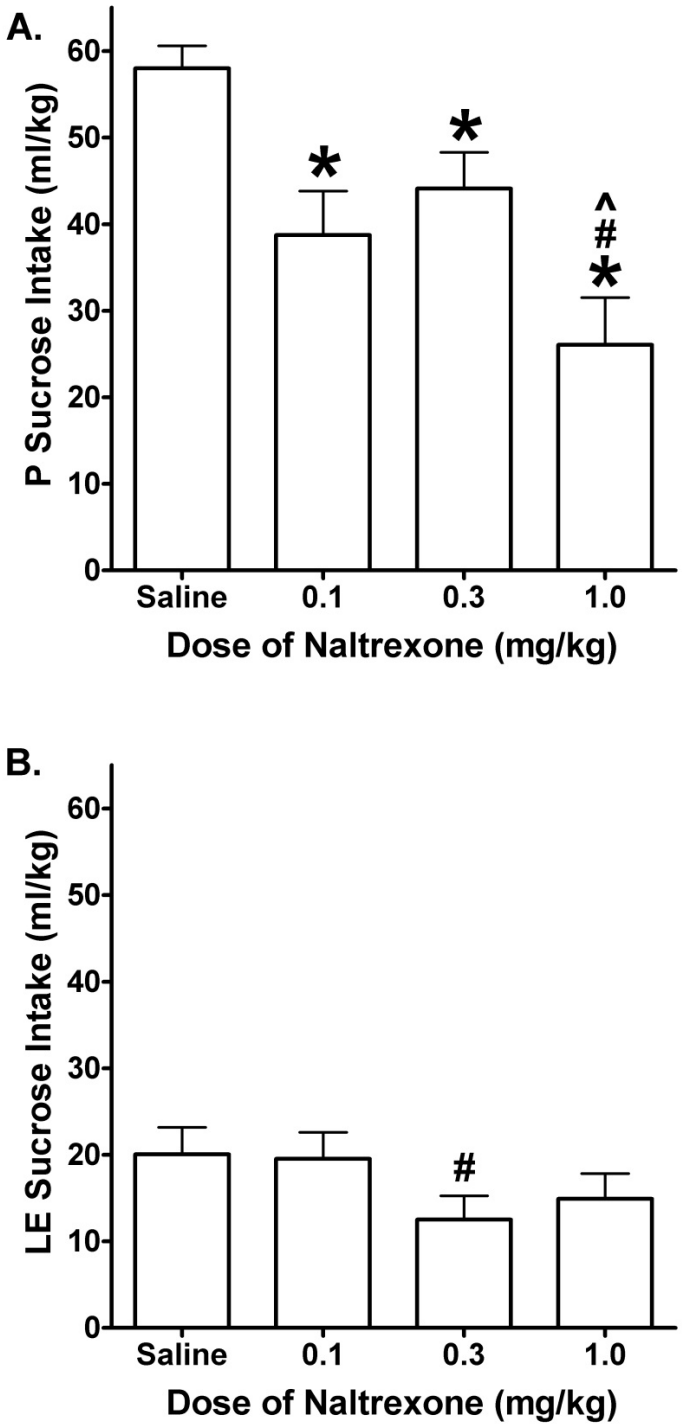

Figure 21. Experiment 3a: Effects of the Mu Antagonist Naltrexone on Sucrose Consumption (ml/kg) in (a) P and (b) LE Rats. * = significantly differs from Saline; # = significantly differs from 0.1 mg/kg; ^ = significantly differs from 0.3 mg/kg dose.

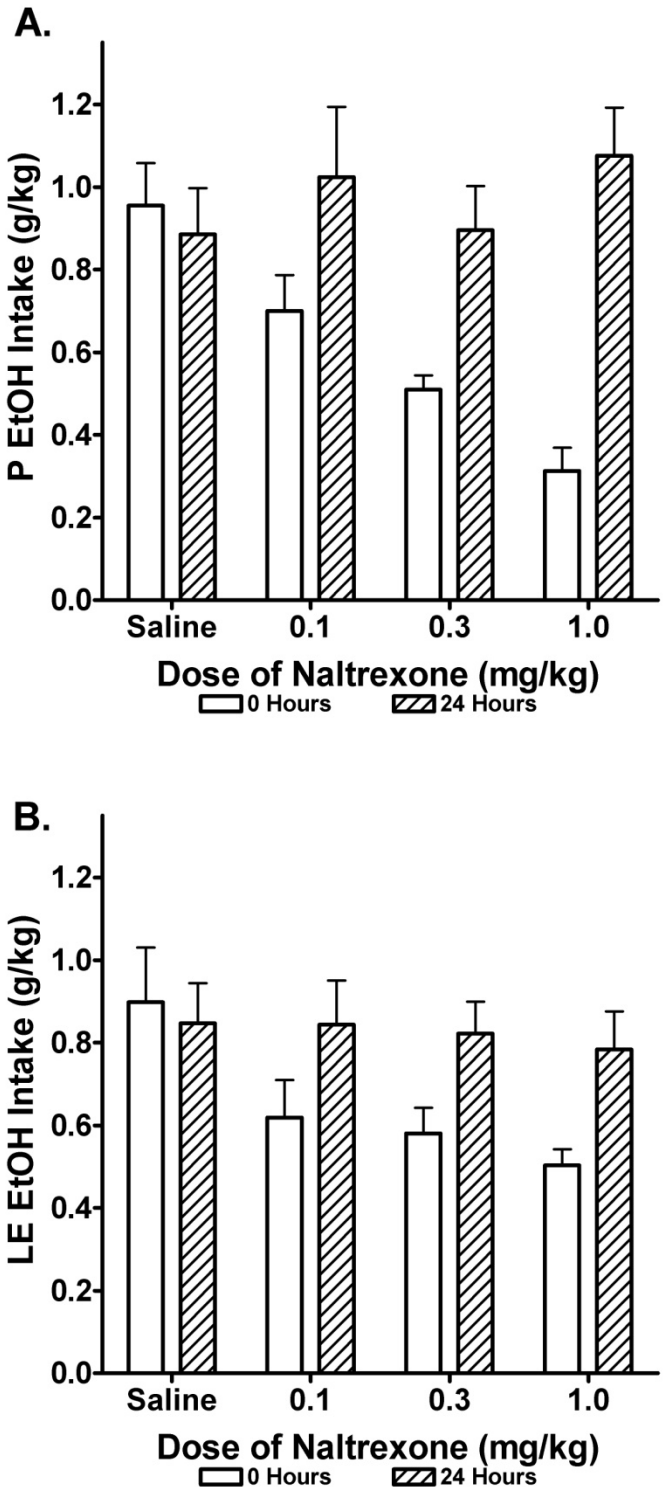

Figure 22. Experiment 3a: Effects of the mu antagonist Naltrexone on (A) EtOH intake (g/kg) and (B) Sucrose intake (ml/kg) in P Rats immediately following and 24 hours post injections.

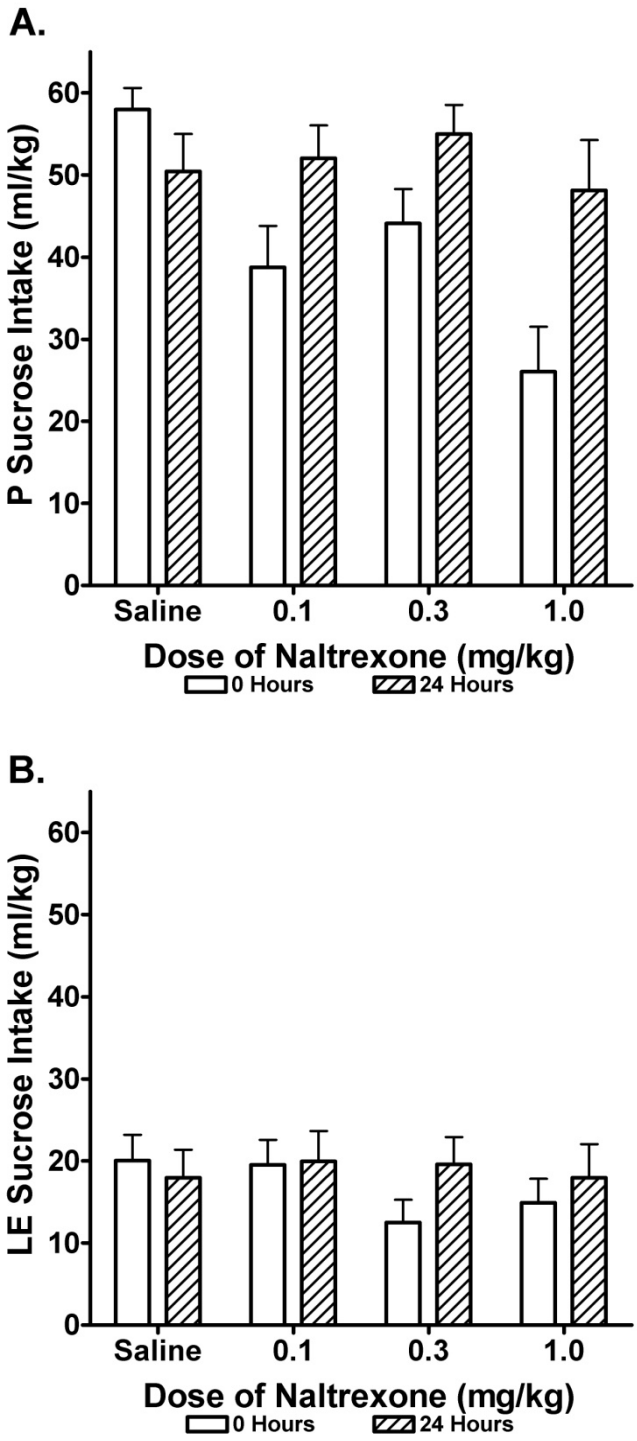

Figure 23. Experiment 3a: Effects of the mu antagonist Naltrexone on (A) EtOH intake (g/kg) and (B) Sucrose intake (ml/kg) in LE Rats immediately after and 24 hours post injections.

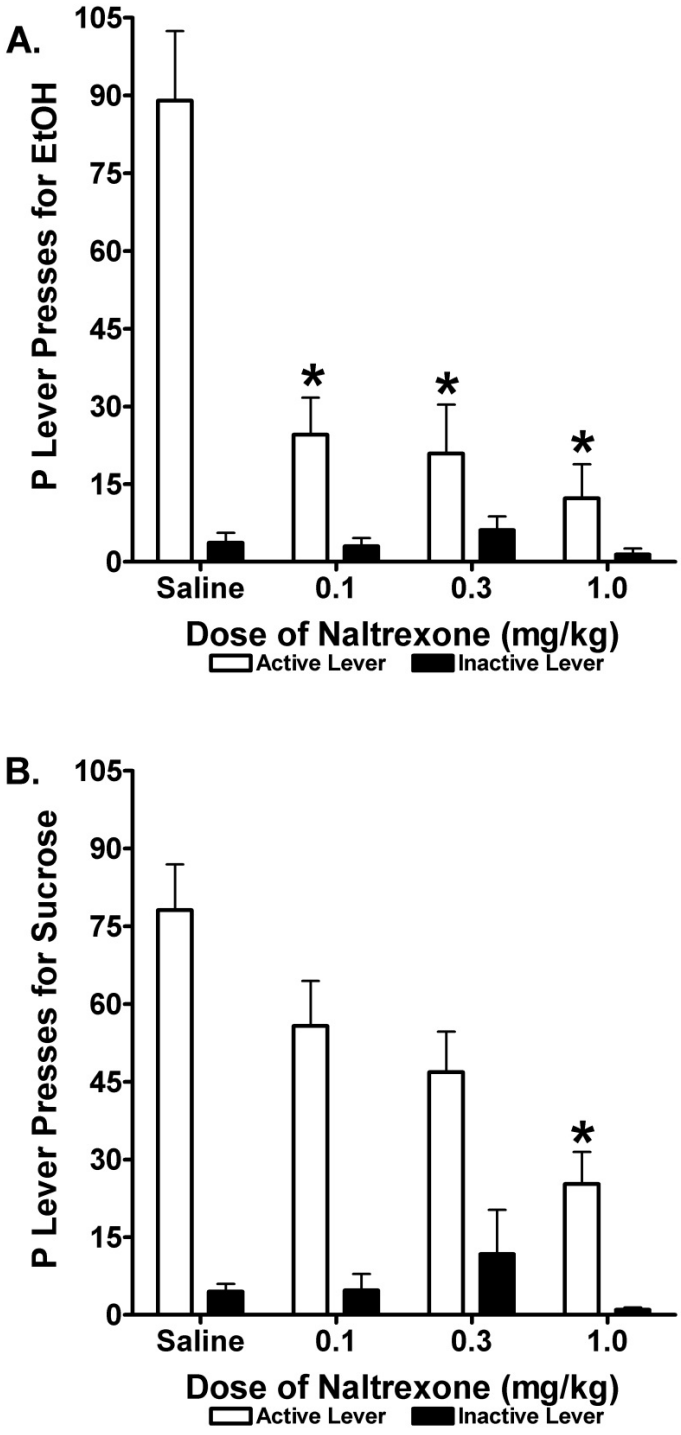

Figure 24. Experiment 3a: Effects of the Mu Antagonist Naltrexone on responding for (a) EtOH and (b) Sucrose on the active and inactive levers in P Rats. * = significantly differs from Saline.

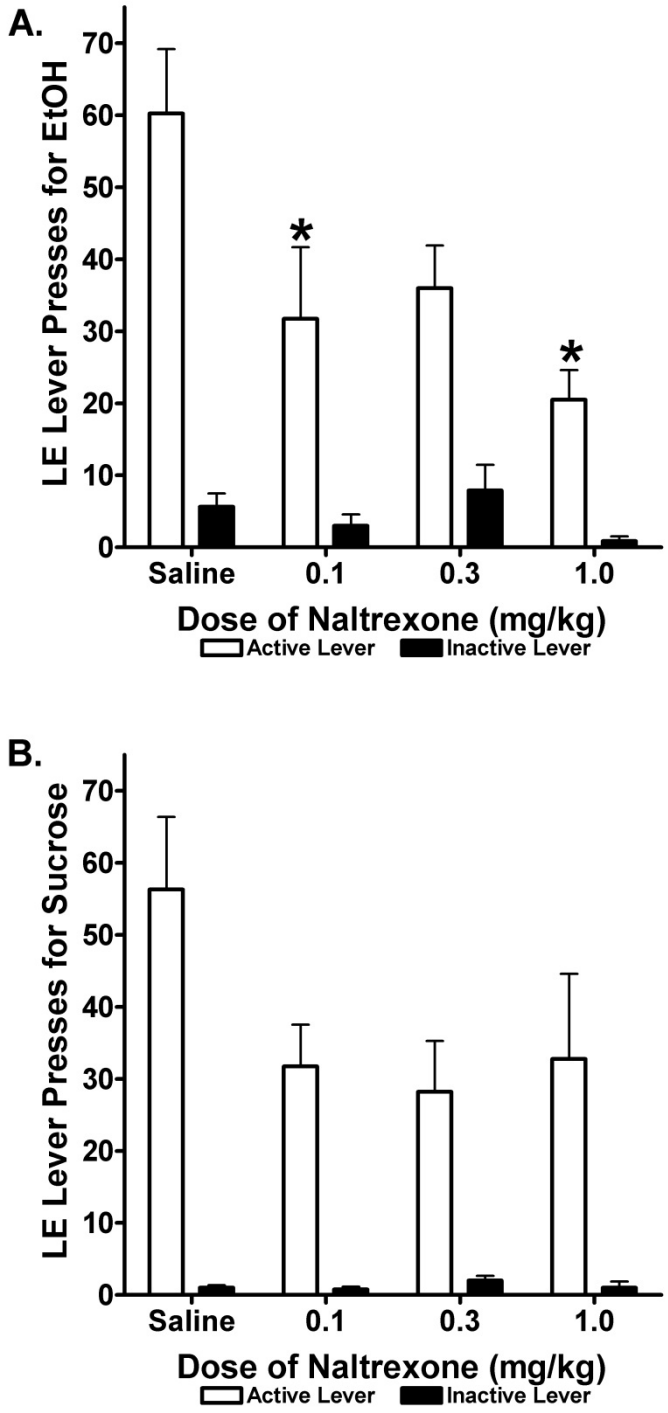

Figure 25. Experiment 3a: Effects of the Mu Antagonist Naltrexone on responding for (a) EtOH and (b) Sucrose on the active and inactive levers in LE Rats. * = significantly differs from Saline.

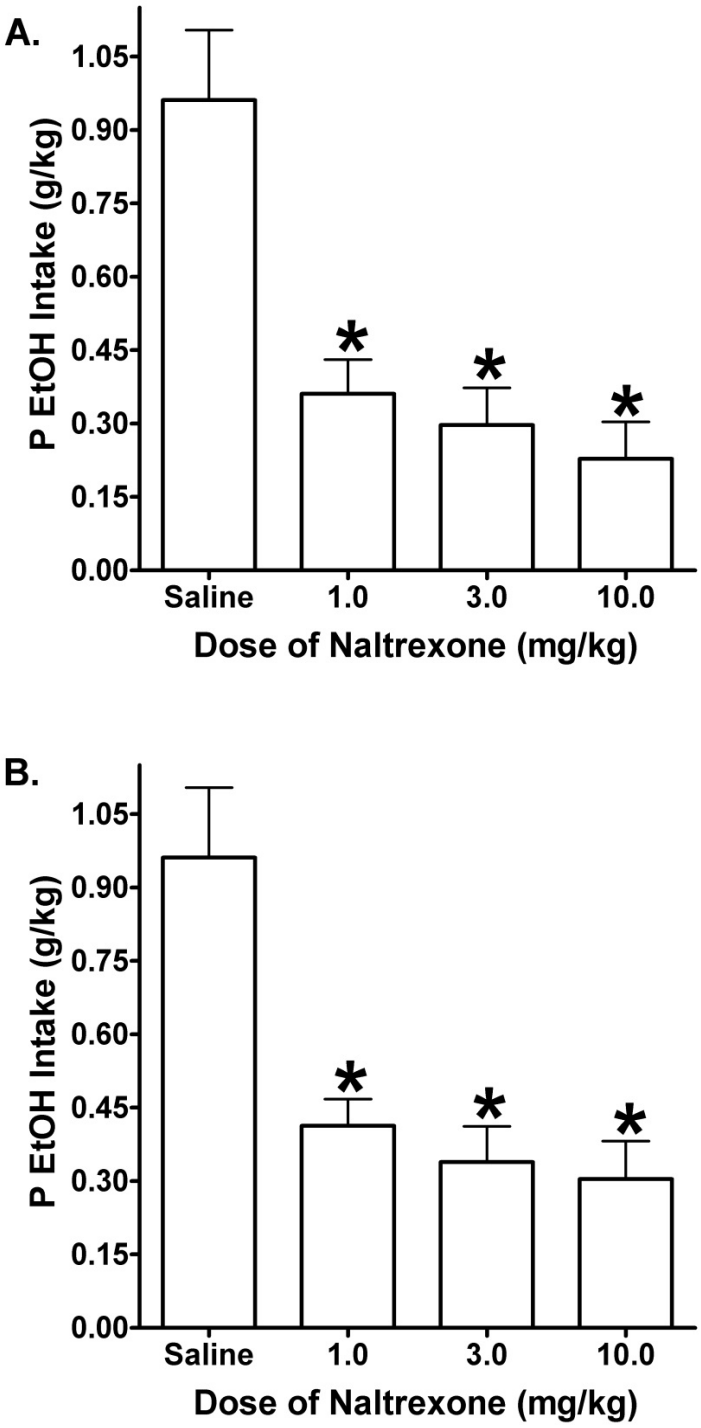

Figure 26. Experiment 3b: Effects of the Non-Selective Antagonist Naltrexone on EtOH Consumption (g/kg) in P Rats (a) with a '0' included in instances of missing data (i.e., rat failed to make single RR necessary for reinforcer access) and (b) where any missing data has been excluded. * = significantly differs from Saline.

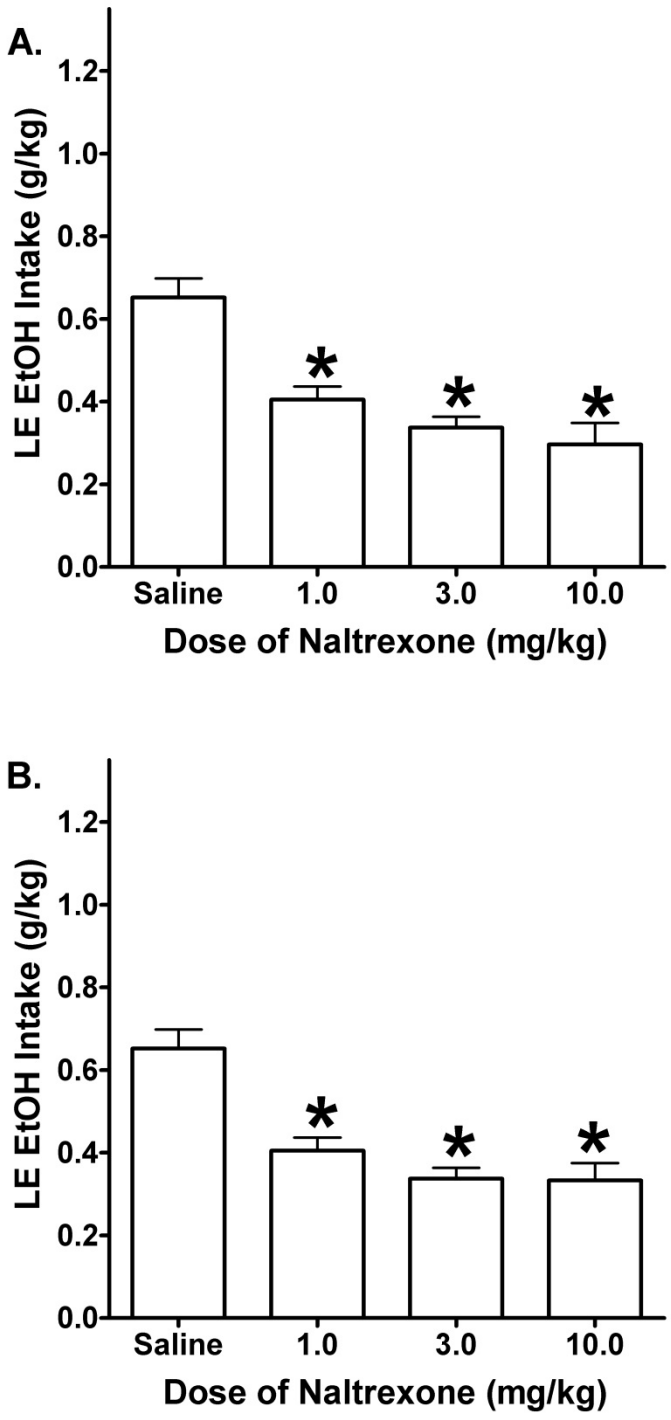

Figure 27. Experiment 3b: Effects of the Non-Selective Antagonist Naltrexone on EtOH Consumption (g/kg) in LE Rats (a) with a '0' included in instances of missing data (i.e., rat failed to make single RR necessary for reinforcer access) and (b) where any missing data has been excluded. * = significantly differs from Saline.

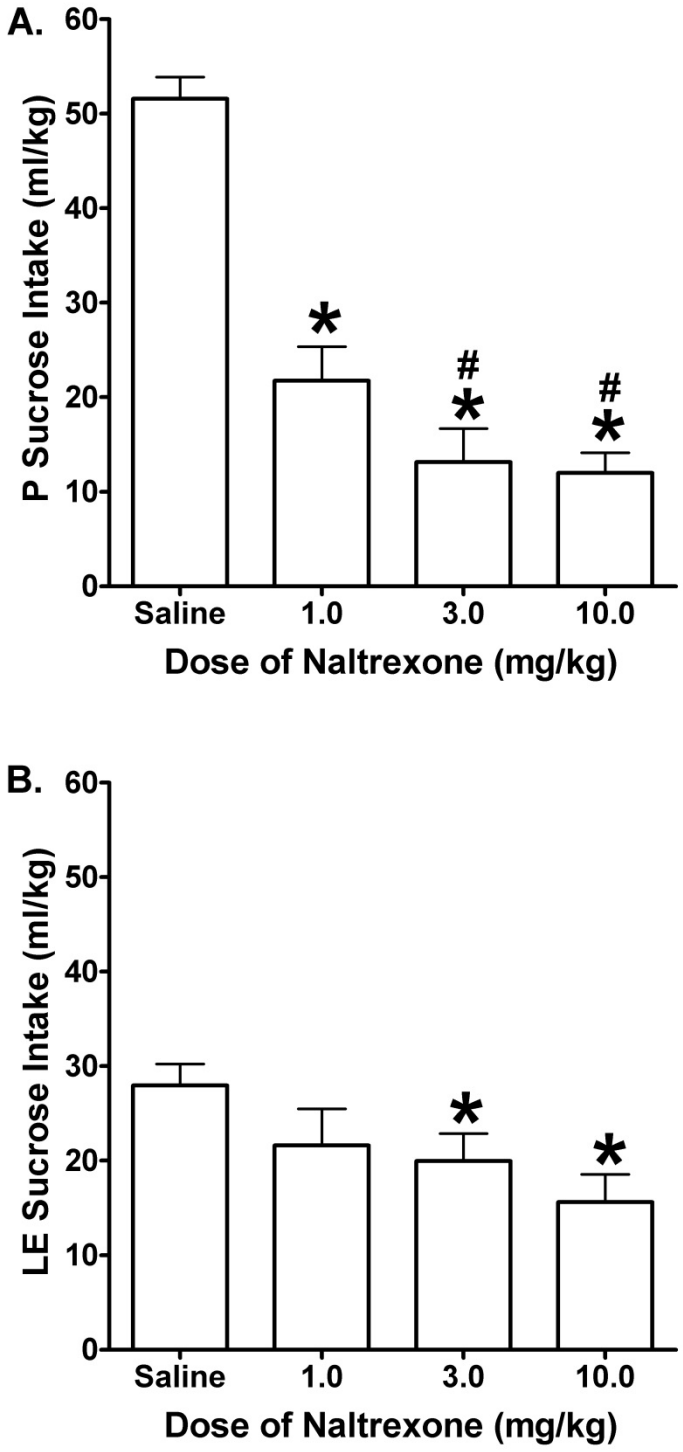

Figure 28. Experiment 3b: Effects of the Non-Selective Antagonist Naltrexone on Sucrose Consumption (ml/kg) in (a) P and (b) LE Rats. * = significantly differs from Saline; # = significantly differs from 1.0 mg/kg.

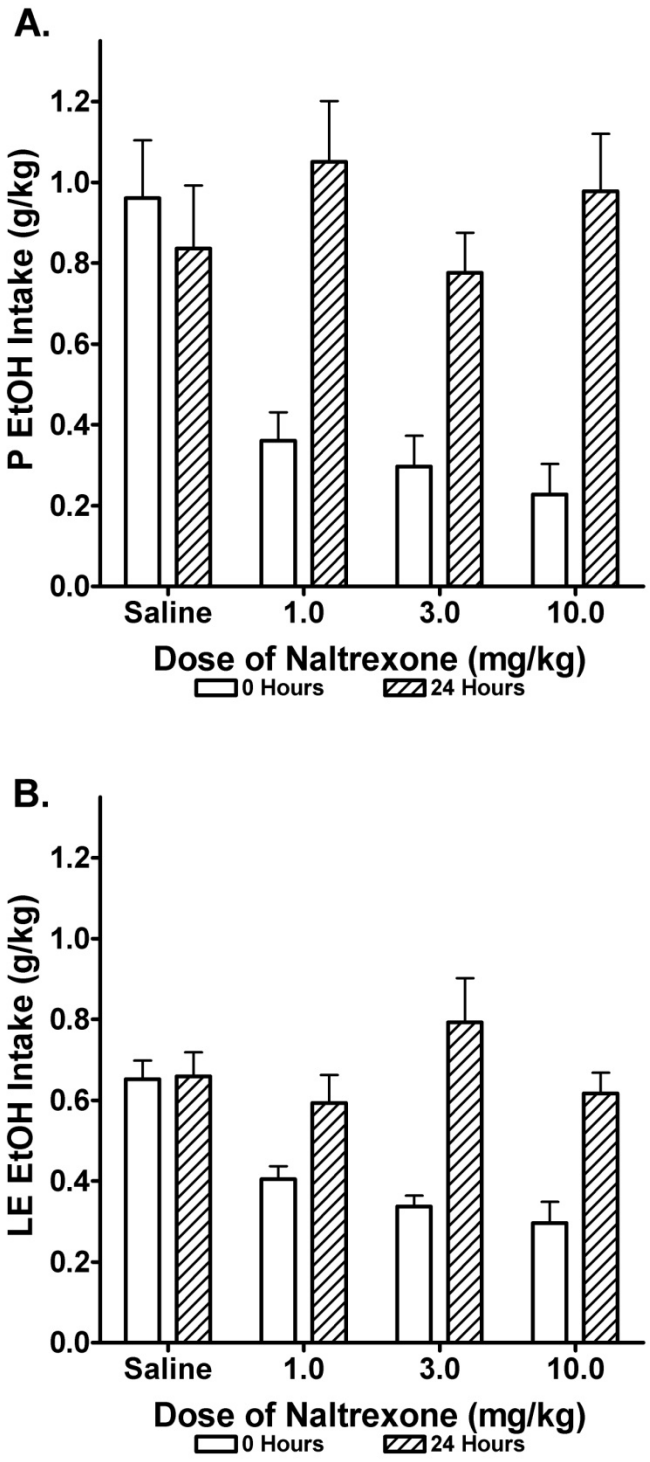

Figure 29. Experiment 3b: Effects of the Non-Selective Opioid Antagonist Naltrexone on (A) EtOH Intake (g/kg) and (B) Sucrose Intake (ml/kg) in P Rats immediately after and 24 hours post injections.

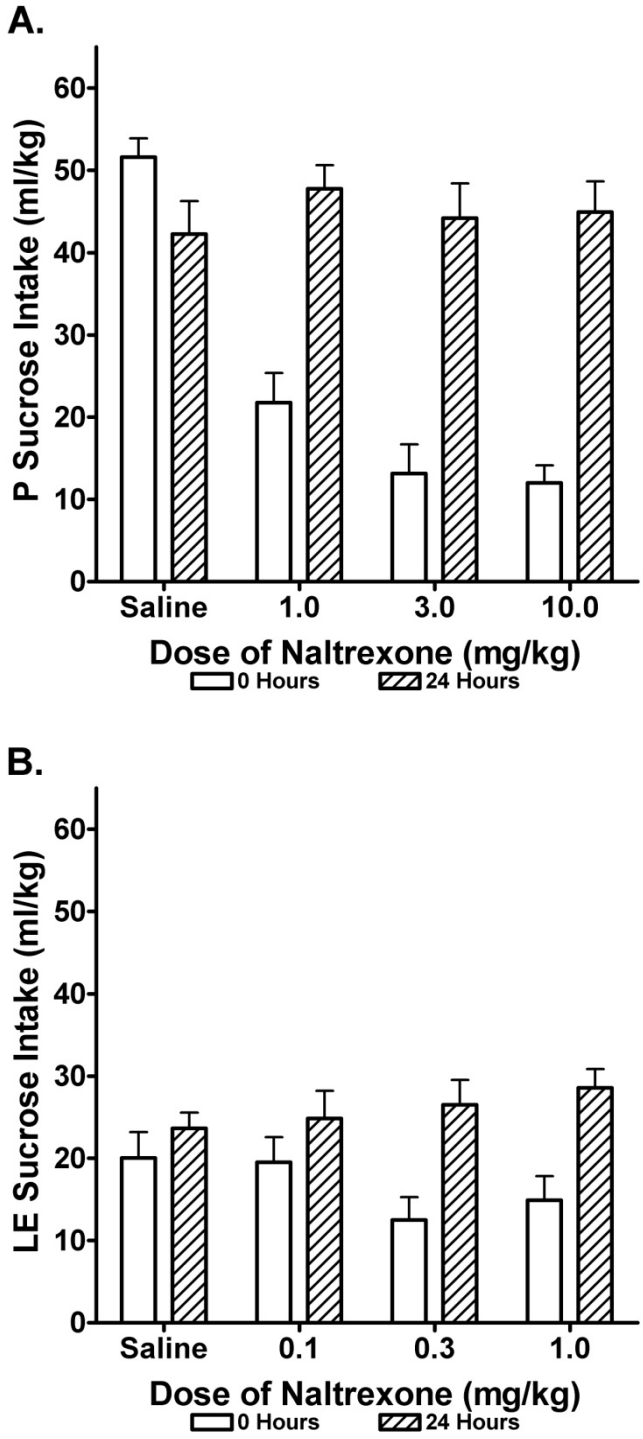

Figure 30. Experiment 3b: The Effects of the Non-Selective Opioid Antagonist Naltrexone on (A) EtOH Intake (g/kg) and (B) Sucrose Intake (ml/kg) in LE Rats immediately after and 24 hours post injections.

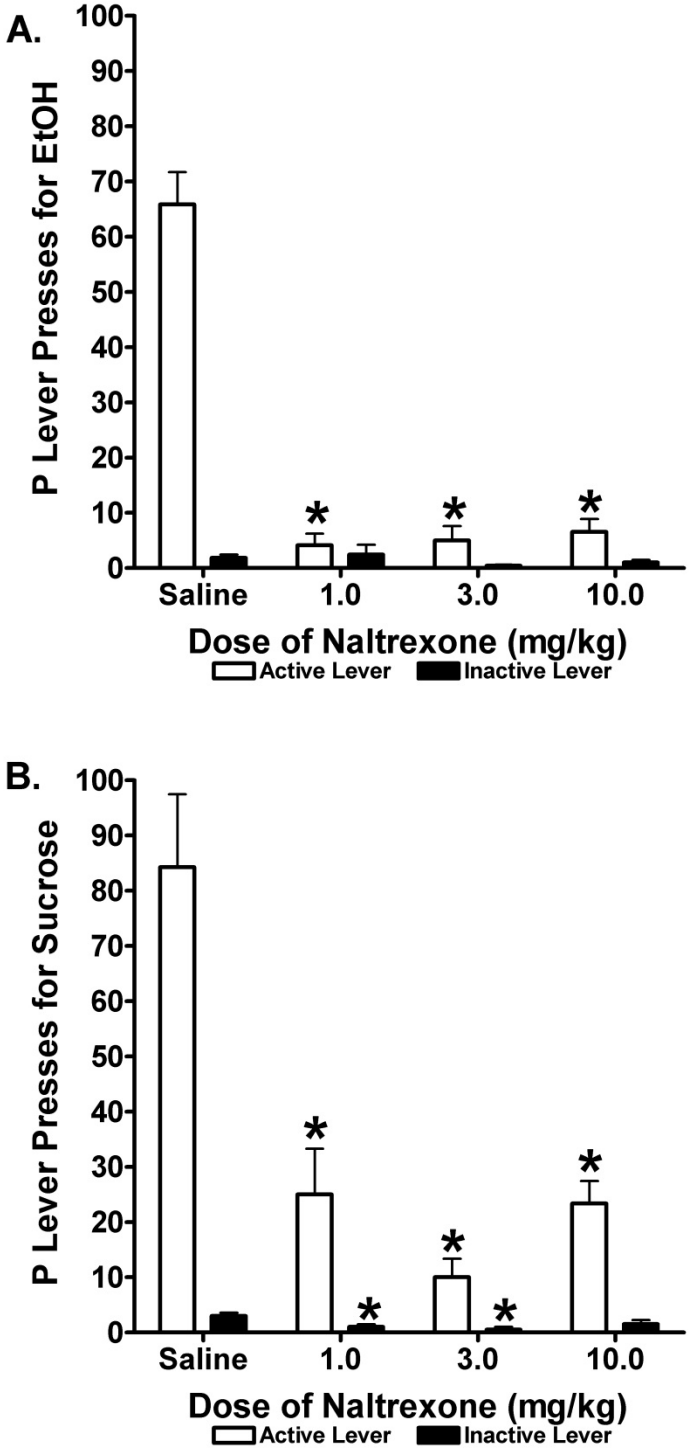

Figure 31. Experiment 3b: Effects of the Non-Selective Antagonist Naltrexone on responding for (a) EtOH and (b) Sucrose on the active and inactive levers in P Rats. * = significantly differs from Saline.

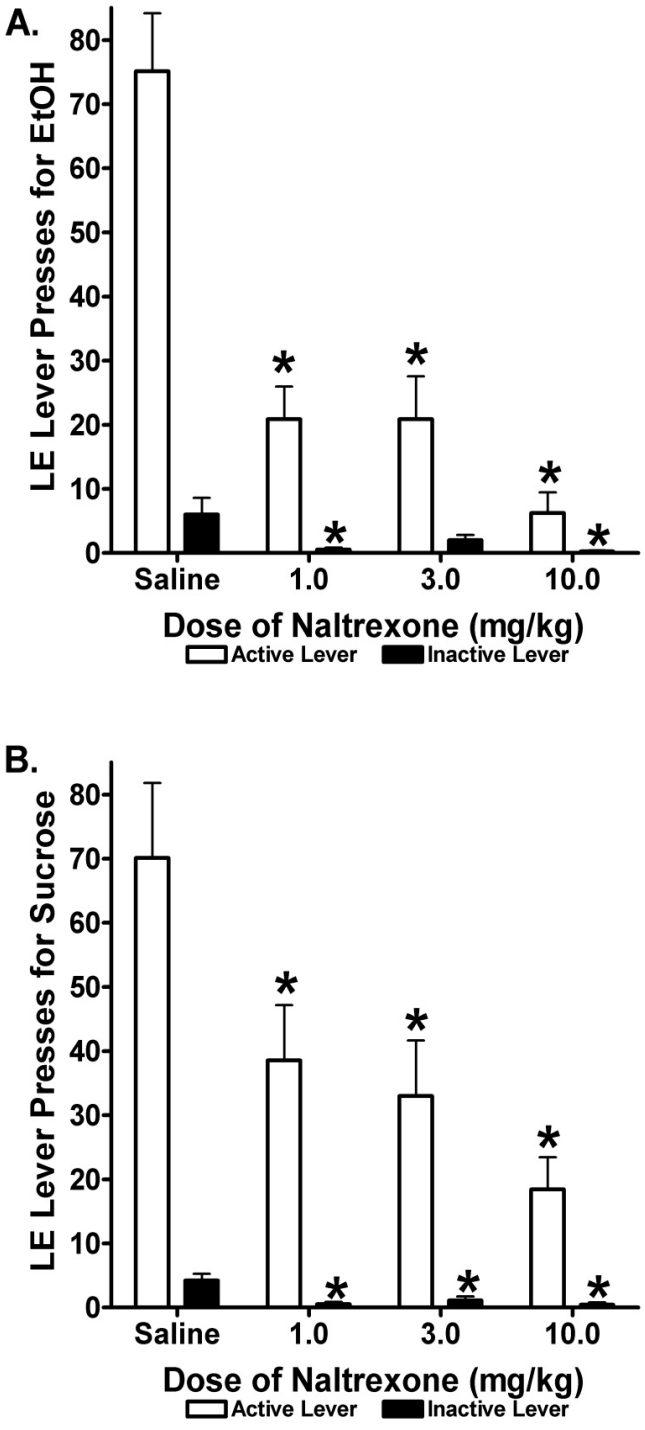

Figure 32. Experiment 3b: Effects of the Non-Selective Antagonist Naltrexone on responding for (a) EtOH and (b) Sucrose on the active and inactive levers in LE Rats. * = significantly differs from Saline.

VITA

VITA

Angela Nicole Henderson

Education

Purdue University, Indianapolis, IN	May 2012
Graduate Student in Doctoral Psychobiology Program	
Purdue University, Indianapolis, IN	August 2009
M.S., Psychobiology of Addictions	
Purdue University, Indianapolis, IN	May 2006
B.S., Psychology	
Indiana University, Bloomington, IN	May 2003
B.S., Biology *with Distinction*	
Minor, Psychology	

Honors/Awards/Fellowships

Research Society on Alcoholism (RSA) Travel Award	June 2009; June 2011
NIAAA Training Grant Trainee Award	May 2008-May 2012
Research Investment Fund (RIF) Fellowship	January 2007
GUZE Meeting Travel Award, IUPUI	March 2006
School of Science Scholar, IUPUI	Fall 2004-Spring 2006
Phi Beta Kappa Honor Society, Indiana University	Spring 2002
Golden Key Honor Society, Indiana University	Fall 2001
Elected to Alpha Chi Sigma, Indiana University	Fall 1999
Dean's List, Indiana University	Fall 1999-Spring 2003
Accepted into Indiana University Honors Program	Fall 1999
Indiana University Valedictorian Scholarship	Fall 1999

Memberships

Research Society on Alcoholism Member	Spring 2007–Current
Society for Neuroscience	Fall 2007

Research Experience

Graduate Research: Departments of Psychology and Psychiatry, Psychobiology of Addictions Graduate Program, Indiana University-Purdue University Indianapolis, 2008-Current (Doctoral Research Advisor: Dr. Cristine L. Czachowski).

Research Projects/Skills

- Examining the dose-dependent effects of various opiate receptor antagonist (naltrindole, naltrexone, and nor-BNI) and agonist (U50,488H) on the motivation to seek and consume ethanol and sucrose in an operant paradigm in both selected P rats and nonselected Long-Evans rats
- Gaining proficiency in performing bilateral surgeries (using pentobarbital) and in administering NPY drug infusions into the central nucleus of the amygdala in rats
- Taking and measuring blood ethanol levels using the analox machine and correlating them with drinking patterns in P and Long-Evans rats
- Developing the ability to do perfusions and brain extractions
- Exploring the consumption and seeking by various selected and nonselected rat lines for various reinforcers (including ethanol and sucrose) using a model that is procedurally able to separate the motivation to work for (i.e., craving/seeking) and consumption (intake) in an operant paradigm.

Graduate Research: Department of Psychology, Psychobiology of Addictions Graduate Program, Indiana University-Purdue University Indianapolis, 2006-2008, (Thesis Research Advisors: Drs. Rob B. Stewart and Nancy E. Badia-Elder).

Research Projects/Skills

- Examining the dose-dependent effects of consecutive daily ICV NPY infusions lasting for six days at three different stages of ethanol consumption (acquisition, maintenance, and reinstatement) in P rats.
- Understanding the role of Neuropeptide S (NPS) on ethanol consumption in P rats using a range of doses of NPS following continuous access to or a deprivation period from 10% (v/v) ethanol.
- Exploring the role of the NPY Y1 receptor in modulating NPY's suppressive effects of ethanol intake in P rats by administering a Y1 receptor agonist and Y1 receptor antagonist ICV in a dose-dependent manner..
- Analyzing the effects of repeated cycles of restraint stress, alcohol deprivation, and NPY administration on consequent ethanol consumption
- Investigating the effects of NPY administration into the Nucleus Accumbens on subsequent ethanol drinking in P rats
- Surveying the effects of a dose-dependent administration of Neuropeptide S (NPS) on anxiety, locomotor activity, and alcohol consumption in P and NP rats.
- Determining the extent to which P rats are able to discriminate between various doses of NPY and IP EtOH using a T-maze task.

Teaching Experience

- Teaching Assistant: *B311: Introductory Lab in Psychology,* Fall 2007-Spring 2008 Led labs designed to introduce students to creating and implementing sound scientific experiments as well as how to write completed, comprehensive research papers in correct APA format. Also instructed students using SPSS statistical software how to run appropriate statistical tests for various experimental designs and how to interpret their output (Instructors: Drs. Leslie Asburn-Nardo and Debbie Herold).
- Teaching Assistant: *B310: Lifespan Development,* Fall 2006-Spring 2007 (Supervisor, Dr. Kroupa) Fielded students' (230) questions, comments, and concerns as well as did all the grading of student quizzes, essays, exams, and papers.
- Teaching Assistant: *B103: Orientation to a Major in Psychology*, Spring 2005-Spring 2006, (Supervisor, Dr. Drew C. Appleby) Served as the Lead TA in Spring 2006. Spent a great deal of time communicating and assisting groups of students in "families" in writing a 9 chapter book in proper APA style/format.
- Indiana University Athletic Tutor, Bloomington, IN, Fall 2002-Spring 2003, tutored collegiate athletes in: Psychology, Biology, Chemistry, and Spanish.

Published Papers

Henderson AN; Czachowski CL. (2012). Neuropeptide Y (NPY) in the central nucleus of the amygdala (CeA) does not affect ethanol-reinforced responding in binge-drinking, nondependent rats. *Pharmacology, Biochemistry, and Behavior, 101,* 8-13.

Bertholomey ML; **Henderson AN;** Badia-Elder NE; Stewart RB. (2011). Neuropeptide Y (NPY) –induced reductions in alcohol intake during continuous access and following alcohol deprivation are not altered by restraint stress in alcohol-preferring (P) rats. *Pharmacology, Biochemistry, and Behavior, 97,* 453-461.

Gilpin NW; **Henderson AN;** Badia-Elder NE; Stewart RB. (2011). Effects of neuropeptide Y (NPY) and ethanol on arousal and anxiety-like behavior in alcohol-preferring (P) rats. *Alcohol, 45,* 137-145.

Oberlin BG; Best C; Matson LM; **Henderson AN;** Grahame NJ. (2011). Derivation and characterization of replicate high- and low- alcohol preferring lines of mice and a high-drinking crossed HAP line. *Behavioral Genetics, 41,* 288-302.

Badia-Elder NE; **Henderson AN;** Bertholomey ML; Dodge NC; Stewart, RB. (2008). The effects of Neuropeptide S (NPS) on ethanol drinking and other related behaviors in alcohol-preferring (P) and –nonpreferring (NP) rats. *Alcoholism: Clinical and Experimental Research, 32,* 1380-7.

Published Abstracts

Henderson AN; Czachowski CL. (2012). The effects of the opioid agonist U50,488H and antagonist naltrexone on the seeking and intake of sucrose and ethanol in selected and nonselected rats. *Alcoholism: Clinical and Experimental Research,* Accepted for Publication in June Edition.

Henderson AN; Czachowski CL. (2011). The effects of the kappa opioid antagonist Nor-BNI on the seeking and intake of sucrose and ethanol in alcohol preferring (P) rats. *Alcoholism: Clinical and Experimental Research, 35(*s1), 185A.

Henderson AN; Czachowski CL. (2010). The effects of the delta opioid antagonist naltrindole on the seeking and intake of sucrose and ethanol in selected and nonselected rats. *Alcoholism: Clinical and Experimental Research, 34(s*2), 144A.

Henderson AN; Czachowski CL. (2009). The effects of neuropeptide Y (NPY) in the CeA on the "motivated binge drinking" of ethanol and sucrose. *Alcoholism: Clinical and Experimental Research, 33*(s1), 94A.

Bertholomey ML; Stewart RB; **Henderson AN;** Badia-Elder NE. (2009). Tolerance to the effects of NPY on ethanol drinking but not food intake in alcohol-preferring (P) rats following repeated daily NPY infusions. *Alcoholism: Clinical and Experimental Research, 33(*s1), 94A.

Bertholomey ML; **Henderson AN;** Stewart RB; Badia-Elder NE. (2008). Neuropeptide Y Y1 receptor modulation of ethanol intake is alcohol preferring (P) rats. *Alcoholism: Clinical and Experimental Research, Supp32*(6), 216A.

Henderson AN; Bertholomey ML; Stewart RB; and Badia-Elder NE. (2007). Acquisition and maintenance of ethanol drinking following multiple infusions of Neuropeptide Y (NPY) in alcohol-preferring (P) rats. *Alcoholism: Clinical and Experimental Research, Supp31*(2), 198A.

Bertholomey ML; **Henderson AN;** Stewart RB; Badia-Elder NE. (2007). Effects of stress, repeated ethanol deprivation, and Neuropeptide Y (NPY) on ethanol intake in alcohol-preferring (P) rats. *Alcoholism: Clinical and Experimental Research, Supp31*(2), 198A.

Bertholomey ML; **Henderson AN;** Stewart RB; Badia-Elder NE. (2007). Effects of NPY in the nucleus accumbens on consummatory behaviors in alcohol-preferring (P) rats. *Alcoholism: Clinical and Experimental Research, Supp31*(2), 197A.

Oberlin BG; **Henderson AH;** Grahame NJ. (2007). High alcohol preferring mice are more impulsive than low alcohol preferring mice as measured by a delay discounting task. *Alcoholism: Clinical and Experimental Research, Supp31*(2), 215A.

Dodge NC; **Henderson AN;** Bertholomey ML; Stewart RB; Badia-Elder NE. (2006). Neuropeptide S: Effects on anxiety, locomotor activity, and ethanol consumption in alcohol-preferring (P) and non-preferring (NP) rats. *Alcoholism: Clinical and Experimental Research, Supp30*(6), 126A.

Gilpin NW; Stewart RB; Dodge NC; **Henderson AN;** Badia-Elder NE. (2006). Suppression of ethanol intake by Neuropeptide Y (NPY) in Wistar rats depends on intermittence of prior ethanol exposure. *Alcoholism: Clinical and Experimental Research, Supp30*(6), 126A.

Gilpin NW; Stewart RB; Dodge NC; **Henderson AN;** Badia-Elder NE. (2006). Neuropeptide Y (NPY) suppresses light-enhanced acoustic startle reflect in alcohol-preferring (P) rats. *Alcoholism: Clinical and Experimental Research, Supp30*(6), 126A.

Invited Talks

When Our Best Is Not Good Enough: Opioid Targets for Alcoholism Beyond Naltrexone. Speaker in the Entitled Symposium: Behavior: Addition and Recovery as a part of the Third Annual Ann E Daugherty Symposium, Tara Treatment Center, Franklin, IN, June 8, 2012

The Effects of New Pharmacotherapies on Curbing Binge Drinking. Guest Lecturer in Dr. Green's Neuropsychology Course. Presented at Hanover College, Hanover, IN, December 2011

Exploring Novel Pharmacotherapies in the Treatment of Alcoholism. Guest Lecturer in Dr. Green's Neuropsychology Course. Presented at Hanover College, Hanover, IN, April 2011

The Effects of Naltrindole on the Seeking and Intake of Sucrose and Ethanol in Selected and Outbred Rats. Presented at the Indianapolis Neuroscience Conference. Indiana University-Purdue University at Indianapolis, Indianapolis, IN, October 2010

Saccharin Drinking in cHAP, HAP1, and Hs/Ibg Mice. HAP/LAP Mini Conference. Indiana University-Purdue University at Indianapolis, Indianapolis, IN, June 2006