



Theses and Dissertations

---

2020-08-03

## Aerobic Exercise Alters Opioid Receptors Following Chronic Alcohol Exposure

James N. Brundage  
*Brigham Young University*

Follow this and additional works at: <https://scholarsarchive.byu.edu/etd>



Part of the [Family, Life Course, and Society Commons](#)

---

### BYU ScholarsArchive Citation

Brundage, James N., "Aerobic Exercise Alters Opioid Receptors Following Chronic Alcohol Exposure" (2020). *Theses and Dissertations*. 8656.  
<https://scholarsarchive.byu.edu/etd/8656>

This Thesis is brought to you for free and open access by BYU ScholarsArchive. It has been accepted for inclusion in Theses and Dissertations by an authorized administrator of BYU ScholarsArchive. For more information, please contact [scholarsarchive@byu.edu](mailto:scholarsarchive@byu.edu), [ellen\\_amatangelo@byu.edu](mailto:ellen_amatangelo@byu.edu).

Aerobic Exercise Alters Opioid Receptors Following

Chronic Alcohol Exposure

James N. Brundage

A thesis submitted to the faculty of  
Brigham Young University  
in partial fulfillment of the requirements for the degree of

Master of Science

Jordan Yorgason, Chair  
Scott C. Steffensen  
David Thomson

Neuroscience Center  
Brigham Young University

Copyright © 2020 James N. Brundage

All Rights Reserved

## ABSTRACT

### **Aerobic Exercise Alters Opioid Receptors Following Chronic Alcohol Exposure**

James N. Brundage  
Neuroscience Center, Brigham Young University  
Master of Science

Opioid receptors have been a target of pharmacological manipulation in alcohol use disorder (AUD) recovery protocols for many years. Aerobic exercise, a common adjunct in AUD recovery, is known to modulate opioid receptors (ORs) both during both acute and long term exposure. The three subtypes of ORs: mu (MOR), delta (DOR), and kappa (KOR) are all expressed on neurons in the mesocorticolimbic circuitry. Kappa-opioid receptors are expressed directly on dopamine (DA) neuron terminals in the nucleus accumbens (NAc). Mu and Delta ORs are expressed on cholinergic interneurons (CINs) and GABA neurons in the NAc. In alcohol dependent rodents, KORs are hypersensitized. It is theorized that this hypersensitization contributes to EtOH seeking behavior. In contrast, aerobic exercise desensitizes the KORs. Given the high degree of pharmacological overlap between opioid receptors, it is also hypothesized that EtOH and aerobic exercise may have effects on MORs and DORs as well. Here, it is investigated whether a routine of voluntary aerobic exercise decreases EtOH induced changes to KOR modulation of dopamine (DA) release in the nucleus accumbens (NAc) along with possible mechanisms through which this might occur. The responsiveness of MORs and DORs in EtOH dependence, and how aerobic exercise modulates those effects is also investigated. Exercise attenuated EtOH induced hypersensitization of KORs in the NAc. Exercise decreases expression of KORs, which may account for the changes in KOR sensitization. The MOR agonist DAMGO decreased DA reuptake *ex vivo*, but not signal amplitude while DOR agonist DPDPE had no effect on either reuptake or signal amplitude.

Overall, dependent animals that were allowed to exercise, consumed less EtOH in a drinking in the dark model. These data suggest that exercise is a useful adjunct to AUD recovery protocols, and that its effects are likely mediated by KORs. The findings related to MORs and DORs suggest that MORs, but not DORs, may act through acetyl choline receptors to modulate DA reuptake in the NAc, however much more work is needed to characterize this effect.

Keywords: alcohol, aerobic exercise, opioid receptor, cholinergic interneuron, nucleus accumbens

## ACKNOWLEDGEMENTS

I must first thank my advisor, Dr. Jordan Yorgason, without whose mentorship and friendship I would not be the researcher I am today. I would also like to thank Dr. Scott Steffensen who has provided countless learning and growth opportunities for the past four years. I am extremely grateful to Dr. Kyle Bills, Dr. Daniel Obray and Andrew Payne for their scientific perspective, personal mentorship and friendship during my time at BYU. This project would certainly not have been completed without many dedicated undergraduate researchers. I would especially like to thank Alyssa Stockard, Brandon Richmond, Ben Williams, Charlton Bassett and Jared Baldrige. Watching their skills and understanding develop has been a highlight of my graduate studies. Finally, I must thank my ever supportive wife Lindsey, who has provided inspiration, encouragement and patience throughout this entire process.

## TABLE OF CONTENTS

Aerobic Exercise Alters Opioid Receptors Following.....	i
ABSTRACT.....	ii
ACKNOWLEDGEMENTS.....	iv
LIST OF FIGURES .....	vii
Aerobic Exercise Alters Opioid Receptors Following Chronic Alcohol Exposure.....	1
Alcohol and the Dopamine Hypothesis .....	1
Common Neuronal Substrates Underlying Alcohol and Opioid Reward and Dependence .....	2
Opioid Receptors in the Mesocorticolimbic System .....	3
Alcohol and Opioid Receptors.....	5
Aerobic Exercise, Endogenous Opioids, and AUD .....	6
Rationale and Hypotheses.....	8
METHODS .....	9
Animals.....	9
Fast Scan Cyclic Voltammetry .....	10
Immunohistochemistry .....	11
Drink-in-the-Dark Procedure .....	12
Statistical Analysis.....	12
RESULTS .....	15
Voluntary Exercise Alters KOR Sensitivity in the NAc.....	15

Voluntary Exercise Changes KOR Density in the NAc and VTA .....	17
Voluntary Exercise Decreases Seeking Behavior.....	17
MOR Activation Elevates Reuptake but Not Amplitude.....	18
DOR Activation has Little Direct Impact on DA Release in the NAc in Slice .....	19
Regional and Sex Differences in DA Transient Amplitude and Frequency .....	20
DISCUSSION.....	23
KORs Mediate Exercise Effects on Attenuation of EtOH Induced Hypersensitization	23
The Effects of MOR and DOR Activation on DA Release and Reuptake .....	24
Evidence for Regional Heterogeneity in CIN Effects on Striatal DA Release.....	26
REFERENCES .....	29

## LIST OF FIGURES

Figure 1: Overview of Experimental Procedures.....	9
Figure 2: Voluntary Exercise Alters KOR Sensitivity in the NAc .....	15
Figure 3: Exercise Lowers KOR Density in the NAc and VTA.....	16
Figure 5: Exercise Decreases Drug Seeking Behavior .....	18
Figure 6: MOR Activation Elevates Reuptake But Not Amplitude .....	19
Figure 7: DOR Activation Doesn't Alter Amplitude or Reuptake.....	20
Figure 8: Dopamine Transients Differ by Region and Sex.....	21



## **Aerobic Exercise Alters Opioid Receptors Following Chronic Alcohol Exposure**

### *Alcohol and the Dopamine Hypothesis*

Alcohol addiction affects the lives of millions of people worldwide. In the US alone, an estimated 28 million are currently undergoing treatment to recover from the effects of alcohol abuse disorder (AUD) at a cost of over 249 billion dollars annually (Sacks, Gonzales, Bouchery, Tomedi, & Brewer, 2015). The disease has dire consequences for those affected, as well as their families and communities. Despite many advances in treatment options, the one-year relapse rate remains around 50% (SAMHSA, 2016). Understanding the mechanisms underlying addiction is vital for preventing relapse and speeding the healing process in recovering addicts.

One common theory on how addiction functions in the brain focuses on changes to dopamine (DA) release in the mesocorticolimbic system. The nucleus accumbens (NAc) and the ventral tegmental area (VTA) are two relevant brain regions within this circuit. Dopamine is synthesized in the cell bodies of DAergic neurons in the VTA whose axons extend into the NAc, where DA is released (Koob & Volkow, 2010). A variety of neuron subtypes including  $\gamma$ -aminobutyric acid (GABA)ergic neurons, medium spiny neurons (MSN), glutamatergic neurons and cholinergic neurons further regulate release (Nolan et al., 2020). Dysregulation of the mesocorticolimbic system is a hallmark of drugs of abuse (Koob & Volkow 2010). One theory proposes that DA release in the NAc is a scalar index of reward (Wise, 2008). Dopamine release in the striatum may encode reward timing and/or salience (Berridge & Robinson, 1998; Phillips, Atkinson, Blackburn, & Blaha, 1993; Schultz, Apicella, Ljungberg, Romo, & Scarnati, 1993). There is some evidence that reward timing and salience are encoded in different striatal subregions (Cerri, Saddoris, & Carelli, 2014; Sackett, Saddoris, & Carelli, 2017; Saddoris, Cacciapaglia, Wightman, & Carelli, 2015). Regardless of how DA release actually translates to motivation and

behavior, it is clear that DA is a central feature in the pathophysiology of addiction and thus a relevant area of study for understanding drug abuse along the continuum of reward to dependence.

*Common Neuronal Substrates Underlying Alcohol and Opioid Reward and Dependence*

The prevailing theory posits that the addiction cycle is composed of three stages:

‘binge/intoxication’, ‘withdrawal/negative affect’, and ‘preoccupation/anticipation’, with discrete neuro-circuits mediating each of these stages (Koob & Volkow, 2010). The development of addiction starts with the ‘binge/intoxication’ stage that is mainly driven by the reinforcing and rewarding properties of drugs. Both animal and human studies have identified key elements within the mesolimbic dopamine (DA) system, including the midbrain ventral tegmental area (VTA) and nucleus accumbens (NAc) of the ventral striatum, that constitute brain substrates underlying reward. Some maintain that DA neuron activation and release in the mesolimbic DA system originating in the VTA and projecting to the NAc is literally a scalar index of reward (Wise, 2008). This system is known to be involved in reward from natural behaviors such as feeding (Ahn & Phillips, 2002, 2003; Phillips, Ahn, & Howland, 2003), drinking (Agmo, Federman, Navarro, Padua, & Velazquez, 1993; Agmo, Galvan, & Talamantes, 1995), and others such as intracranial self-stimulation (Gratton & Wise, 1983; Tzschentke, 2000; Wise, 2002). It has also been implicated in the habit-forming actions of several addictive drugs (Blackburn, Phillips, Jakubovic, & Fibiger, 1986; G.F. Koob, 1992; Wise, 1996; Wise, 2004; Wise & Bozarth, 1987). The dogma is that any drug or behavior that increases mesolimbic DA neuron activity and release will be reinforcing and potentially addictive (Kalivas, Churchill, & Klitenick, 1993; Kalivas & Volkow, 2005; Nestler, 2001). The prevailing view is that people consume drugs for their rewarding properties, which are mediated by this system. Addicts continue their cycle of abuse, in part, as a result of maladapted or depleted DA levels, with its accompanying

dysphoria, as well as anxiety and stress mediated by the extended amygdala that accompany ‘withdrawal/negative affect’. Ultimately, continued abuse results in neuroadaptations of cortical inputs to the mesolimbic system. These adaptations constitute the ‘preoccupation/anticipation’ stage and play an important role in the processing, modulation, and emotional linking (Mitsi & Zachariou, 2016) that drive subsequent craving and impulse behavior and ultimately dependence upon the reinforcer. The emerging view is that the dysregulated homeostasis that accompanies the development of drug addiction may result from experience-dependent neuroadaptations that hijack normal synaptic transmission in this system (Hyman & Malenka, 2001; Hyman, Malenka, & Nestler, 2006; Kauer & Malenka, 2007; Nugent & Kauer, 2008). The compulsion to consume EtOH and opioids stems from their positive reinforcing properties, including their anxiolytic and euphoric effects, as well as their negative reinforcing properties, including their aversive withdrawal symptoms that result from abstinence (Koob, Rassnick, Heinrichs, & Weiss, 1994). Symptoms of dependence accumulate with repeated drug taking and withdrawal (Overstreet, Knapp, & Breese, 2002).

#### *Opioid Receptors in the Mesocorticolimbic System*

Opioid receptors (ORs) are expressed both directly on VTA DA neurons as well as local striatal GABA and acetyl choline (ACh) neurons, making them a strong potential target for modulating DA transmission in the dorsal striatum (Karkhanis, Holleran, & Jones, 2017). There are three traditionally recognized ORs: mu (MOR), delta (DOR), and kappa (KOR). Although some other types have been proposed, their standing as distinct receptor subtypes is still in question because they are not pharmacologically sensitive to naloxone. Therefore this manuscript will focus on the universally recognized three. Each receptor is an inhibitory G-coupled protein receptor whose activation at many doses leads to subsequent inhibition of firing rate and neurotransmission via inhibition of adenylate cyclase, reduction of calcium currents, and

activation of inwardly rectifying potassium channels (Attali, Saya, & Vogel, 1989; Henry, Grandy, Lester, Davidson, & Chavkin, 1995; Konkoy & Childers, 1989; Prather et al., 1995; Tallent, Dichter, Bell, & Reisine, 1994). Each receptor has a variety of ligands, many of which are cross reactive between receptor subtypes. The most common endogenous ligands for these receptors include dynorphins (DYN), which mainly bind KORs, enkephalins, which mainly bind MORs and DORs, and endorphins, which mainly bind MORs (Aldrich & McLaughlin, 2009).

Although all ORs are largely inhibitory, their expression on different cell types leads to differential effects on behavior, especially in reward circuitry. As depicted in Figure 1, KORs are expressed on DAergic neurons of the VTA that project into the NAc (Mansour, Burke, Pavlic, Akil, & Watson, 1996; Mansour, Khachaturian, Lewis, Akil, & Watson, 1987; Spanagel, Herz, & Shippenberg, 1992). These neurons are responsible for the release of DA into the NAc, and thus KOR activation leads to their inhibition, decreasing accumbal DA (Spanagel et al., 1992). In contrast, cholinergic interneurons and GABAergic feedback neurons express MORs and DORs (Hirose et al., 2005; Murakawa et al., 2004; Okutsu et al., 2006; Yoshida et al., 1999). Mesocorticolimbic GABA neurons are generally involved in the inhibition of VTA DA neurons, thus activation of MORs and DORs leads to disinhibition of DA neurons and a subsequent increase in DA in the NAc (Bonci & Williams, 1996; Johnson & North, 1992; Steffensen et al., 2006). Cholinergic interneurons participate in presynaptic excitation of DA terminals in the NAc (Nolan et al., 2020). However, there are still many unanswered questions about the relationship between DA release and cholinergic firing in the NAc. Speaking generally at the behavioral level, activation of MORs and DORs produce feelings of euphoria (Devine & Wise, 1994), while KORs produce dysphoria (Shippenberg, Zapata, & Chefer, 2007).

### *Alcohol and Opioid Receptors*

Direct access to the mesocorticolimbic system is a hallmark of drugs of abuse such as cocaine, methamphetamine, heroine (Koob & Volkow, 2010). This access enables them to directly modulate DA release and is thought to explain their potential for addiction. Unlike other drugs of abuse, the molecular target of EtOH is still unclear, though there is some evidence suggesting low dose EtOH may bind nicotinic acetylcholine receptors (Unpublished findings from our lab). One way alcohol it can affect relevant reward circuitry neurons is through activation of the endogenous opioid system (Gianoulakis, 1993). Interactions between ORs and EtOH are well-known. In fact, one of the three FDA-approved drugs to treat AUD is the MOR antagonist naltrexone (e.g., Common brands Revia and Vivitrol), which act to decrease the craving associated with AUD and opioid use disorder (OUD).

Alcohol triggers the release of endogenous opioids in mesocorticolimbic circuitry (Seizinger, Bovermann, Maysinger, Hollt, & Herz, 1983). Given that MORs are associated most with euphoria, it has been proposed that MORs mediate the pleasurable attributes of alcohol. This is supported by evidence that a common mutation of the CNS derived MOR, A118G polymorphism, is associated with increased ‘liking’ of alcohol in humans (Beyer, Koch, Schroder, Schulz, & Hollt, 2004; Bond et al., 1998; Crist & Berrettini, 2014; Ray et al., 2013; Ray & Hutchison, 2004; van den Wildenberg et al., 2007), although there is some disagreement (Hendershot, Claus, & Ramchandani, 2016; Sloan et al., 2018). Further support for the role of ORs in the mesocorticolimbic circuit demonstrates that a blockade of opioid receptors in the VTA prior to EtOH administration prevents DA release in the NAc (Acquas, Meloni, & Di Chiara, 1993; Benjamin, Grant, & Pohorecky, 1993; Lee et al., 2005; Tanda & Di Chiara, 1998; Yoshida et al., 1999). Similarly, MOR knockout mice do not self-administer EtOH (Roberts et

al., 2000). In contrast, mice that lack DORs show increased EtOH seeking behavior (Roberts et al., 2001), suggesting that they may play a role in preventing seeking behavior.

Chronic EtOH use changes OR sensitivity in various mesocorticolimbic brain regions. Mu-opioid receptors are hypersensitized in the NAc of mice when exposed to chronic EtOH conditions, while they are desensitized in other brain regions such as the substantia nigra pars reticulata (Cowen, Rezvani, Jarrott, & Lawrence, 1999). However, they also demonstrate a decreased binding affinity for specific agonist [D-Ala<sup>2</sup>, N-MePhe<sup>4</sup>, Gly-ol]-enkephalin (DAMGO) (Chen & Lawrence, 2000). Kappa opioid receptors are hypersensitized by chronic EtOH conditions in the NAc, VTA and parts of the amygdala (Melchior & Jones, 2017; Rose et al., 2016; Sirohi, Bakalkin, & Walker, 2012). Hypersensitization of the KORs is particularly interesting because it has been proposed that KORs contribute to the “dark side” of addiction (Karkhanis et al., 2017; Shippenberg et al., 2007; Walker & Koob, 2008). Their hypersensitization may contribute to increased dysphoria in the anhedonic state (Walker & Koob, 2008). Indeed, in many animal models, a pharmacological blockade of KORs results in decreased seeking behavior, though this has yet to be demonstrated in humans (Walker & Koob, 2008). Clearly, ORs play a major role in EtOH’s modulation of mesocorticolimbic circuitry.

The OR system has therapeutic potential. Recovery protocols already utilize opioid based pharmacological approaches which target MORs, and KOR drugs are currently being tested for their use in AUD and OUD recovery in humans (Walker & Koob, 2008). However, other non-pharmacological methods can also target ORs.

#### *Aerobic Exercise, Endogenous Opioids, and AUD*

A large body of evidence suggests that exercise is well suited to supplement AUD recovery protocols. Many studies have shown that exercise leads to molecular and psychological changes

beneficial to recovery (Lipowski, Szulc, & Bulinski, 2015; Piazza-Gardner & Barry, 2012; D. Wang, Wang, Wang, Li, & Zhou, 2014). Aerobic exercise, like acute ethanol consumption, has been shown to increase levels of tyrosine hydroxylase, the rate limiting enzyme in DA synthesis, in the NAc (Droste, Schweizer, Ulbricht, & Reul, 2006; Greenwood et al., 2011). It has also been associated with burst activation of DA neurons in the VTA (S. S. Wang, Wei, Liu, & Ren, 2011). Further, 6-weeks of voluntary wheel running in rats increased D2 auto receptor density in the NAc (Greenwood et al., 2011); a modification that has been associated with increased risk of addictive behavior (Martinez et al., 2004; Morgan et al., 2002; Voisey et al., 2012). D2 like receptors lead to inhibition of DAergic firing, and they are also downregulated in long term exercise (Greenwood et al., 2011). Along with the molecular data, exercise changes affective symptoms of addiction. It increases self-efficacy while decreasing depression (Leasure, Neighbors, Henderson, & Young, 2015; D. Wang et al., 2014).

Despite the myriad of evidence supporting exercise as a potential tool in AUD recovery, it's results on both animal and human behavior are mixed (Leasure et al., 2015; D. Wang et al., 2014). Alcohol preferring mice given access to a running wheel decreased drinking behavior (Darlington et al., 2016; Ehringer, Hoft, & Zunhammer, 2009; Gallego, Cox, Funk, Foster, & Ehringer, 2015). Other studies showed that mice in withdrawal consumed less alcohol when given *ad libitum* access to a running wheel, however mice that were already accustomed to running, ran less when given access to EtOH (Ozburn, Harris, & Blednov, 2008). While these data suggest that aerobic exercise may play a role in hedonic substitution, it also begs the question of why it only substitutes for EtOH seeking behavior during withdrawal (Ozburn et al., 2008). Exercise's role is further complicated by differences in sex, strain of mouse used, and age,

suggesting that genetic background plays a significant role in determining how exercise will affect drinking behavior (Leasure et al., 2015; Ozburn et al., 2008; D. Wang et al., 2014).

Meta-analysis of human studies on aerobic exercise in AUD recovery show that while exercise decreases EtOH induced depressive symptoms, it does not significantly decrease drinking behavior (D. Wang et al., 2014). The discrepancy between studies may be due to a lack of uniformity in the severity of AUD as well as differences in intensity of aerobic exercise, however this has not been confirmed. As the animal studies suggest, timing of the implementation of the exercise protocol may play a role in determining its usefulness. Exercise during withdrawal may act as a substitute for drinking while exercise during other phases of recovery may only ameliorate affective symptoms. Although it has not been thoroughly investigated, animal studies suggest that both sex and genetic background may also play a significant role in determining the efficacy of aerobic exercise in AUD recovery in humans. Clearly, exercise exhibits molecular effects on addiction in many ways. A better understanding of exactly how exercise affects specific, reward circuitry relevant molecular systems is needed to effectively determine when and how exercise should be used in AUD recovery protocols.

### *Rationale and Hypotheses*

**This thesis investigates whether and how aerobic exercise modulates the mesocorticolimbic system via opioid receptors in alcohol dependent animals.** Unique to this study on aerobic exercise and ORs is that it focuses directly on how exercise directly modulates striatal DA release. By better understanding how non-pharmacological stimuli modulate the striatal microcircuitry during dependence, we can better use these tools to help addicts. One of the main advantages of exercise as a treatment adjunct is the relative ease of implementation. Further, because exercise acts on the same systems as the pharmacological tools used in AUD



recovery, implementation of exercise protocols may alter normal pharmacological effects. By understanding how non-pharmacological tools act on opioid receptors, we may be able to enhance current AUD recovery protocols.

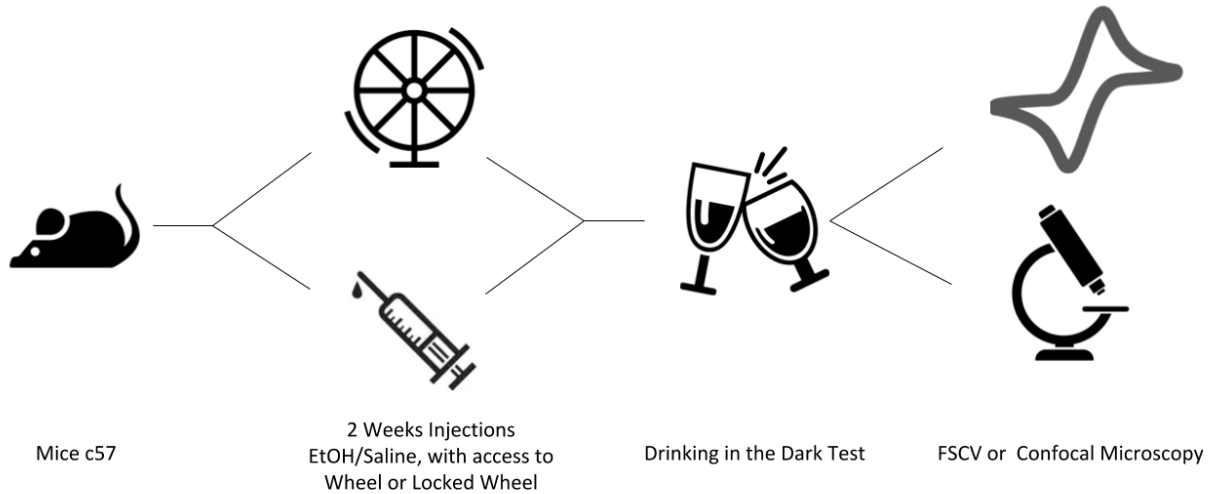


Figure 1: Overview of Experimental Procedures

## METHODS

### *Animals*

Male C57BL/6J and DBA/2J mice (Jackson Labs; aged 6-12 weeks) were given *ad libitum* access to food and water, and were maintained on a reverse 12:12-h light/dark cycle (lights on at 15:00 h). Mice were randomly assigned to one of three cohorts, ethanol without exercise, ethanol with exercise and saline with exercise. All mice were injected BID with ethanol (2.5g/kg; 16% w/v; IP) or an equivalent volume of saline for 14 days. Exercise groups were given *ad libitum* access to a running wheel during the 14 day period. Intoxication was visually verified with loss of consciousness or ataxia which decreased visually with dependence. All protocols and animal care procedures were in accordance with the National Institutes of Health Guide for the Care and Use

of Laboratory Animals and approved by the Brigham Young University Institutional Animal Care and Use Committee. All efforts were made to minimize animal suffering and number of animals used in the present study.

#### *Fast Scan Cyclic Voltammetry*

Isoflurane (Patterson Veterinary, Devens, MA) anesthetized mice were sacrificed by decapitation and brains were rapidly removed and transferred into ice-cold, pre-oxygenated (95% O<sub>2</sub>/5% CO<sub>2</sub>) artificial cerebral spinal fluid (aCSF) consisting of (in mM): NaCl (126), KCl (2.5), NaH<sub>2</sub>PO<sub>4</sub> (1.2), CaCl<sub>2</sub> (2.4), MgCl<sub>2</sub> (1.2), NaHCO<sub>3</sub> (25), glucose (11), L-ascorbic acid (0.4), pH adjusted to 7.4. Tissue was sectioned into 220  $\mu$ m-thick coronal slices containing the striatum with a vibrating tissue slicer (Leica VT1000S, Vashaw Scientific, Norcross, GA). Brain slices were placed in a submersion recording chamber, and perfused at 1 ml/min at 32 °C with oxygenated aCSF.

Fast scan cyclic voltammetry (voltammetry) recordings of dopamine signals were performed and analyzed using Demon Voltammetry and Analysis Software (Demon Voltammetry and Analysis; Yorgason, Espana, & Jones, 2011). The carbon fiber electrode (7  $\mu$ m X ~150  $\mu$ m) potential was linearly scanned as a triangular waveform from -0.4 to 1.2 V and back to -0.4 V (Ag vs Ag/Cl) at a scan rate of 400 V/s. Cyclic voltammograms were recorded at the carbon fiber electrode every 100 msec by means of a potentiostat (Dagan Corporation, Minneapolis, MN). Dopamine release was evoked every 2 min through a bipolar stimulating electrode. For input/output experiments examining baseline dopamine signals across increasing current stimulations, single pulse baseline dopamine signals were collected (4 msec, 350  $\mu$ A) until signals were stable for across 3 collections. Baseline single pulse responses were measured followed by concentration response for U50488 at 0.3  $\mu$ M and 1  $\mu$ M, DAMGO at 1  $\mu$ M and DPDPE 1 $\mu$ M

followed by a reversal dose of 1  $\mu$ M nor-BNI, 1  $\mu$ M naloxone or 1  $\mu$ M naltrexone depending on the receptor being tested; all drugs were bath applied. Each response was gathered from single-pulse stimulations given 2 min apart before introduction of the next drug concentration. In a separate set of experiments, spontaneous dopamine transients were measured in-between electrical stimulations. Dopamine transients were observed throughout the dorsal and ventral striatum in coronal and sagittal slices.

### *Immunohistochemistry*

Mice were anesthetized using isoflurane and underwent transcardial perfusion with 4% paraformaldehyde (PFA). Once perfused, brains were carefully removed and placed in 4% PFA for 24 hrs to facilitate continued fixation. After incubation in PFA, brains were placed in a solution of 30% sucrose in 1X PBS until the density of the brain matched that of the solution and the brains dropped to the bottom of the vial (~24-48 hrs). Brains were then flash frozen in dry ice and mounted on a cold microtome stage. Targeting the VTA and NAc, brains were sliced at 30  $\mu$ m on the microtome and slices were placed in cryoprotectant (30% ethylene glycol, 30% sucrose, 0.00002% sodium azide, in 0.1 M PB) and kept at -20°C until staining. Slices were washed 3 times in 1x PBS for 10 minutes on a rotator. They were then blocked with a blocking buffer comprised of 4% normal goat serum, 0.1% Triton-X 100 and 1x PBS. Slices were then washed another 3 times in 0.2% PBST on a rotator. Primary antibodies were applied and allowed to incubate for 20 hours. Following staining, the slices were washed 3 times in 0.2% PBST and secondary antibodies were applied. After a 2 hr incubation period they were washed another 3 times with 0.2% PBST and once with 1x PBS. Antibodies included Mouse anti-tyrosine hydroxylase (Novus, 1:1328), and Rabbit anti-KOR (LifeSpan, 1:200) as well as secondary Alexa Fluor 405 Donkey Anti-Sheep antibodies from (1:900) and Alexa Fluor 594 Goat anti-Rabbit (1:500). To mount slides, sections were placed on microscope slides and dried ~5 min. Once dried, a drop of vectashield (Vector

Laboratories) was placed on the tissue, and a cover slip was placed on the slide. Slides set overnight, and then they were kept at 4°C until imaging. An Olympus FluoView FV1000 confocal microscope was used to image mounted slices. Brain slices were mounted on microscope slides and imaged under oil immersion at 40X. To ensure consistent readings between samples, a constant photomultiplier tube voltage and gain were set between all acquired images.

#### *Drink-in-the-Dark Procedure*

To observe a behavioral correlate of alcohol dependence and validate our vapor chamber method, mice were trained and evaluated in a drink-in-the-dark (DID) two-bottle choice alcohol drinking test. Mice were injected with EtOH as described above. After a withdrawal period of 24 hours, animals were removed from home cages three hours into the dark cycle, and placed individually in cages with the bedding and food removed. They were given two sipper tubes, with one containing tap water and the other containing tap water and ethanol (20% v/v). Mice were allowed to drink from the tubes for 2 hours in the dark, and were then returned to their home cages. They repeated this test over 4 consecutive days with no injection exposure during that time.

#### *Statistical Analysis*

Dopamine release was analyzed using Demon Voltammetry software and measured at peak oxidation currents. Dopamine uptake was measured as the time constant ( $\tau$ ) from a single exponential fit between the peak dopamine current and its return to baseline. Custom software was written for detecting and analyzing spontaneous dopamine release events. The software performs a running subtraction on recordings to reduce drift and aliasing noise. Post-subtracted data is then compared across time against known cyclic voltammograms for dopamine, with a low threshold  $r^2$  value for initial detection ( $r^2 > 0.3$ ). The location of each putative event (legitimate and spurious) is captured in the software for subsequent manual verification. The

program reverts the data back to its non-subtracted form, and performs a new background subtraction (non-running) at the time point preceding the putative event. The resultant color plot around that time point, and cyclic voltammogram at the peak current are then examined, and compared against a known dopamine voltammogram to verify similar oxidation potentials. If signals are smaller than the limit of detection (calculated by multiplying the median standard deviation for each file by 3) they are automatically rejected. The events are simultaneously examined for evidence of a false positive caused by drift and aliasing noise coinciding with the oxidation potential. Dopamine concentrations were calculated from calibration values (at 1  $\mu$ M dopamine). Baseline frequency of dopamine transients was measured during a 10 minute period prior to drug application. Group data from experiments where a drug was applied was measured across a 3 minute period.

Statistics were performed using JMP 15 (JMP®, Version 15. SAS Institute Inc., Cary, NC, 1989-2019). Statistical significance was determined for groups of 2 variables using a two-tailed student t-test. Experiments with more than 2 groups, but only one factor were tested for significance using a one-way analysis of variance (ANOVA) or when data was from multiple time points from the same experiment a repeated measures ANOVA (RM-ANOVA) was performed. For experiments that examined multiple factors, and possible interactions, two-way ANOVA or RM-ANOVAs were used. Tukey's HSD and Bonferroni correction methods were used for post-ANOVA analysis.

For brain slice imaging, images were loaded into FIJI software. Images were duplicated to preserve the original settings while color thresholding and brightness contrast adjustments were made to determine the location of cells and create ROIs. ROIs were then projected back onto the unedited images where area and mean intensity were recorded for each channel. This

process was performed by three independent raters blinded to the hypothesis. To determine relative density of KORs, the ratio of mean fluorescence to area was determined.

## RESULTS

### *Voluntary Exercise Alters KOR Sensitivity in the NAc*

It was hypothesized that a routine of voluntary exercise would attenuate EtOH induced hypersensitization of KORs. Kappa opioid receptor sensitivity was measured as reactivity of KORs to the KOR agonist U50,488 and antagonist nor-BNI on DA release and reuptake in the NAc. Mice were randomly assigned to one of three cohorts, EtOH without exercise, EtOH with exercise, and saline with exercise. All mice were injected twice/day (BID) with EtOH (2.5g/kg; 16% w/v; IP) or an equivalent volume of saline for 14 days. Exercise groups were given *ad libitum* access to a running wheel during the 14 day period.

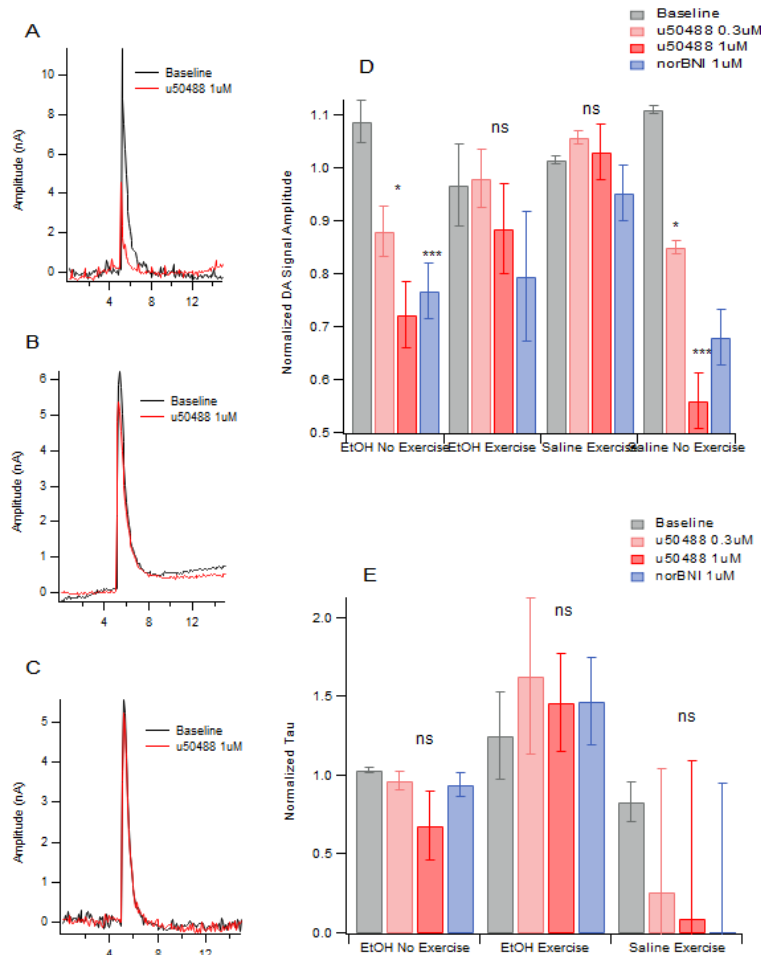
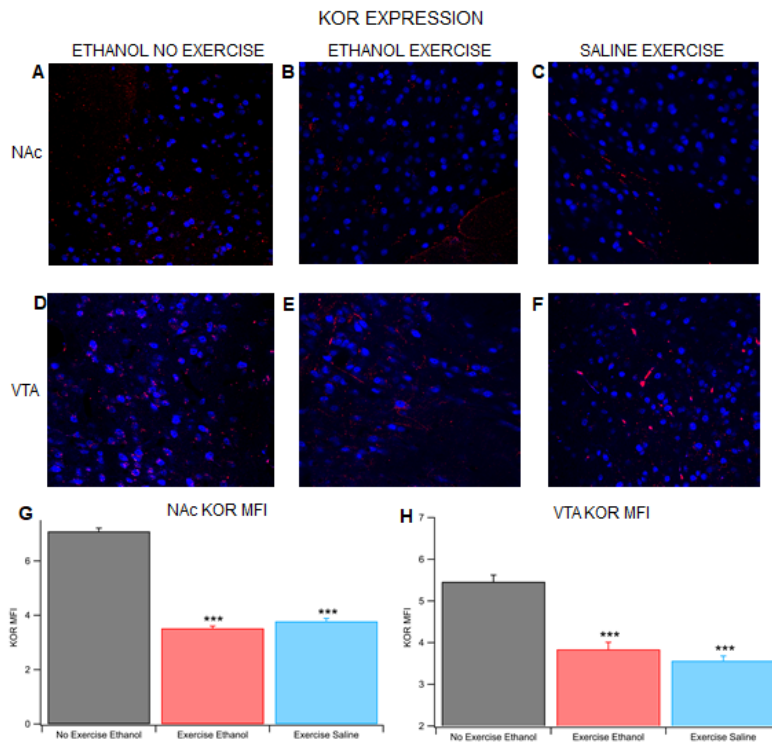


Figure 2: *Voluntary Exercise Alters KOR Sensitivity in the NAc*: Example traces of KOR sensitivity for the EtOH/No Exercise group (A), EtOH/Exercise groups (B) and the Saline/Exercise group (C). Quantification of group effects in terms of signal amplitude (D) and reuptake (E) with an n = 6 slices per group.

Chronic EtOH animals who did not have access to a running wheel showed a  $21\% \pm 5\%$  ( $p$ -value = 0.0329) drop in signal amplitude from baseline following administration of  $0.3 \mu\text{M}$  U50,488 and a  $36\% \pm 6\%$  ( $p$ -value = 0.0002) drop from baseline when the dose was increased to  $1 \mu\text{M}$  U50,488. Antagonist nor-BNI did not significantly increase peak height ( $p$ -value = 0.9302). In contrast, chronic EtOH animals that were allowed access to a running wheel did not demonstrate a statistically significant change from baseline following any of the drugs administered ( $F_{3,11} = 0.8299$ ;  $p$ -value = 0.5047). The saline and exercise group also showed no change from baseline ( $F_{3,12} = 1.39$ ;  $p$ -value = 0.2926).



Reuptake was *not* altered for mice in the EtOH without exercise group ( $F_{3,24} =$

*Figure 3: Exercise Lowers KOR Density in the NAc and VTA: Example images of KOR expression following EtOH and exercise procedures or controls in the NAc (A-C) and VTA (D-F). Quantification of mean fluorescence intensity of KOR fluorescence in the NAc (G) and VTA (H),  $n = 6$  animals per group.*

$1.65$ ;  $p$ -value = 0.2049), the EtOH with exercise group ( $F_{3,11} = 0.2029$ ;  $p$ -value = 0.8922) or the saline with exercise group ( $F_{3,12} = 0.8804$ ;  $p$ -value = 0.2204). These results indicate that exercise



attenuates and possibly prevents EtOH induced KOR hypersensitization and that KOR signaling does not significantly affect reuptake. The lack of responsiveness in the saline group may be the result of KOR desensitization due to exercise alone.

#### *Voluntary Exercise Changes KOR Density in the NAc and VTA*

After observing changes in EtOH induced KOR sensitization from voluntary exercise via FSCV, it was hypothesized changes to KOR density were responsible for the effects of chronic EtOH and the attenuation of those effects by exercise. Changes to KOR density in the NAc and VTA were investigated with immunohistochemistry (IHC).

The mean fluorescent intensity (MFI) in the NAc of the EtOH group without exercise was  $7.08 \pm 0.085$ , while MFI in the EtOH with exercise group and the saline with exercise group were  $3.25 \pm 0.125$  and  $3.77 \pm 0.11$  respectively. In the VTA, MFI followed a similar pattern with MFIs of  $5.46 \pm 0.15$ ,  $3.48 \pm 0.16$  and  $3.84 \pm 0.14$  for the EtOH no exercise, EtOH exercise and saline exercise groups respectively. All exercise groups were significantly lower than the non-exercise groups in the same region at the same level of significance ( $p$ -value  $< 0.0001$ ). These data indicate that changes to KOR density in both the NAc and VTA contribute to changes in KOR sensitivity in the NAc following EtOH and exercise.

#### *Voluntary Exercise Decreases Seeking Behavior*

It was also hypothesized that changes to KOR sensitivity and density are related to changes in EtOH seeking behavior. Significant differences were noted between the average of all

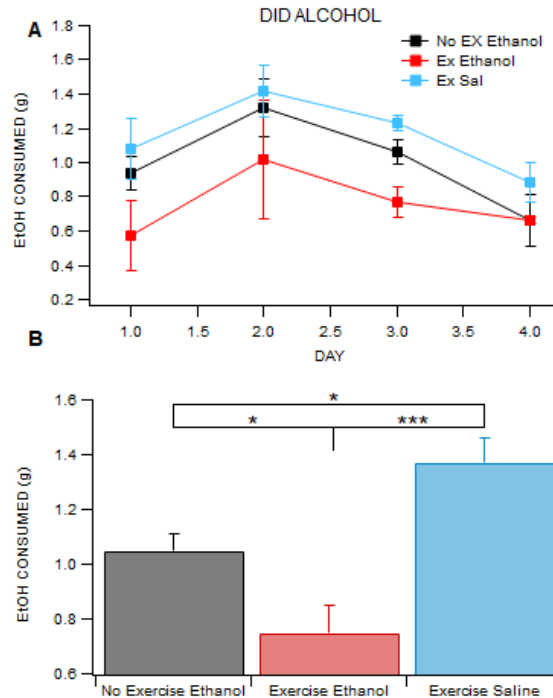


Figure 4: Exercise Decreases Drug Seeking Behavior: Daily break down of EtOH consumption(A) and quantification of average EtOH consumed (B)  $n = 6$  animals per group.

groups for all days ( $F_{2,43} = 11.2596$ ,  $p=0.0001$ ). The EtOH no exercise group drank on average  $1.05\text{g} \pm 0.063$  in the drink-in-the-dark (DID) procedure while the ethanol with exercise group drank  $0.747\text{g} \pm 0.098$ . Interestingly, the saline with exercise group drank more than either with an average consumption of  $1.37\text{g} \pm 0.089$ .

These data demonstrate that voluntary exercise decreases EtOH consumption in dependent animals in the DID paradigm. Surprisingly, the group that was non-dependent and exercised showed increased drinking behavior compared to both groups.

#### *MOR Activation Elevates Reuptake but Not Amplitude*

Mu opioid receptors are expressed on cholinergic interneurons and local GABA neurons which alter DA release in the accumbens. Given the high degree of pharmacological homology between ORs, it was hypothesized that MORs may also be changing in response to EtOH,

aerobic exercise, or both. In the slice preparation, the effects of DAMGO administration was previously unclear. Although other MOR agonists decrease basal CIN firing rate, they also increase burst firing in the presence of glutamate (Chieng & Bekkers, 2001). Changes to MOR sensitivity in terms of signal amplitude and reuptake were also investigated.

DAMGO (1  $\mu$ M) had no effect on signal amplitude in any of the treatment conditions ( $F_{5,59}=0.5775$ ; p-value = 0.7170). However, DAMGO elevated tau by  $52\% \pm 18\%$  (95% CI 11%-94%; p-value = 0.0055) while the tau

value following naloxone administration was not significantly different than baseline (p-value = 0.5959), indicating that naloxone restored tau to baseline levels. Neither EtOH nor exercise altered these effects, suggesting unexplored MOR effects on DA uptake are unaffected by chronic EtOH use or a routine of aerobic exercise.

*DOR Activation has Little Direct Impact on DA Release in the NAc in Slice*

Along with the MORs, DORs are also expressed on CINs and local GABA neurons, and it was hypothesized that they may also contribute to the effects of EtOH and exercise on DA

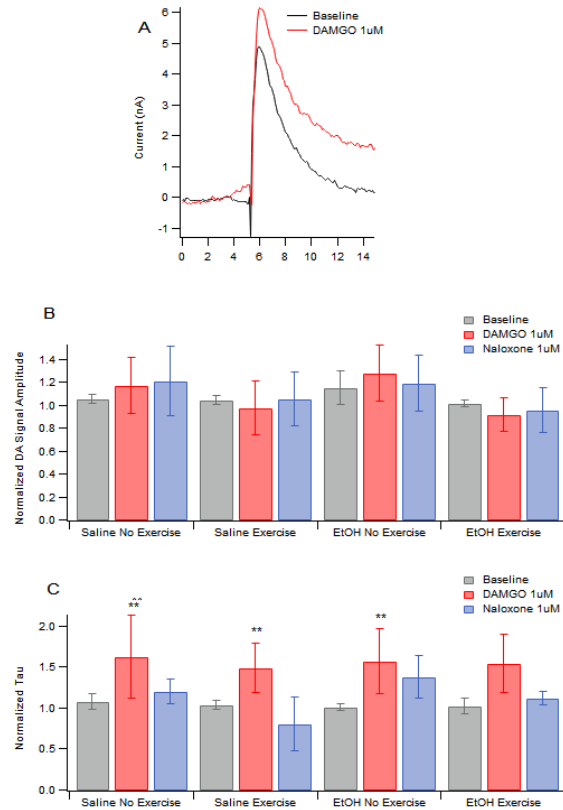
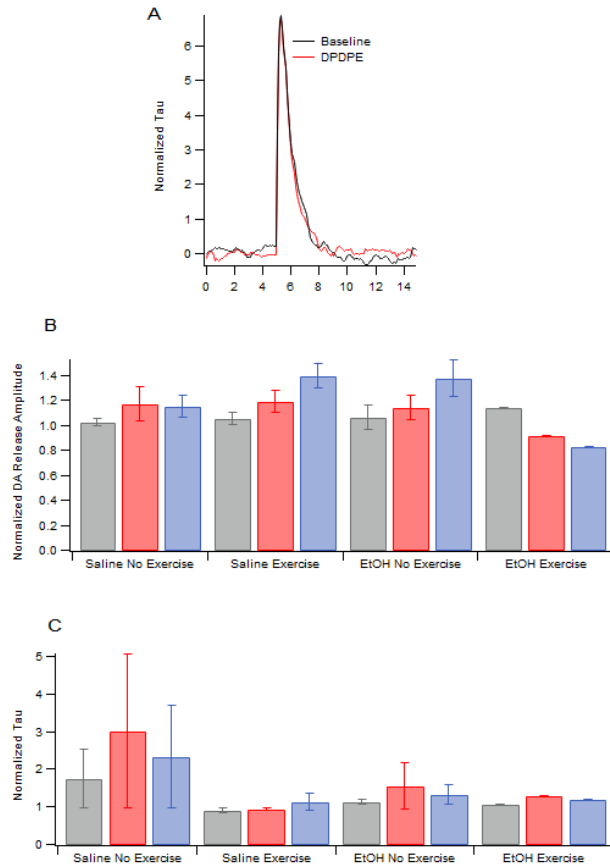


Figure 5: MOR Activation Elevates Reuptake But Not Amplitude: Representative trace showing response of DA release to DAMGO 1uM (A). Quantification of DAMGO 1uM effects on signal amplitude (B) and reuptake (C) with an n = 6 slices in each group.



*Figure 6: DOR Activation Doesn't Alter Amplitude or Reuptake:* Representative trace showing response of DA release to DPDPE 1 $\mu$ M (A). Quantification of DPDPE 1 $\mu$ M effects on signal amplitude (B) and reuptake (C) with an n = 6 slices in each group except EtOH Exercise, which has an n = 2 slices.

### *Regional and Sex Differences in DA Transient Amplitude and Frequency*

Cholinergic interneuron effects on DA release can be measured by investigating DA spontaneous DA release events known as dopamine transients. As the findings with MORs and DORs deviated from current literature, it was hypothesized that regional differences in cholinergic synapses may be responsible. It was also hypothesized that sex may also contribute to CIN effects in slice. In order to investigate whether CIN effects on DA release varied by

release in the NAc. Changes to DOR sensitivity in terms of signal amplitude and reuptake were also investigated.

Unlike MORs, DOR agonists did not significantly elevate signal amplitude ( $F_{3,8}=0.8531$ ; p-value = 0.5032) or tau ( $F_{3,8} = 0.4859$ ; p-value = 0.7015). Delta opioid receptor antagonists also failed to alter signal amplitude ( $F_{3,8} = 0.6643$ ; p-value = 0.5970) or tau ( $F_{3,8} = 0.4991$ ; p-value = 0.6932). These data indicate that DORs have little effect on DA release in the NAc when activated independently.

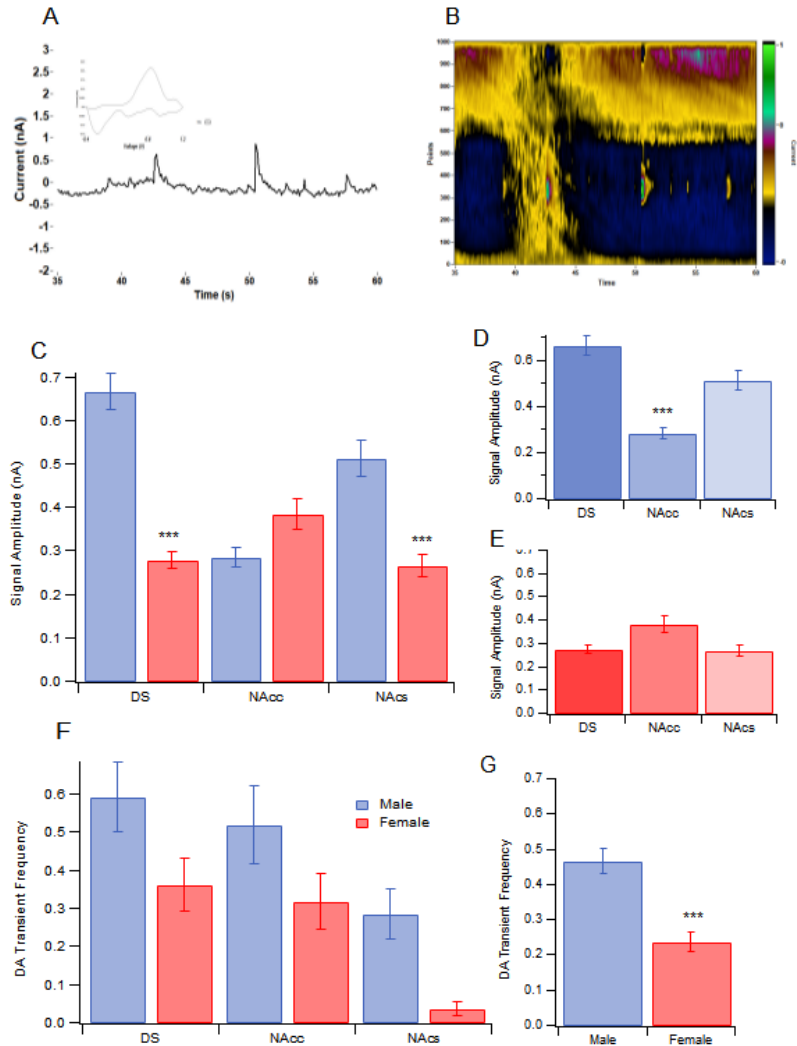


Figure 7: Dopamine Transients Differ by Region and Sex: Representative examples of spontaneous DA release events in a current/time plot (A) and a color plot (B). Quantification of DA transient amplitude (C) with regional comparisons by sex (D-E) and frequency (F) an overall sex comparison (G) with an n = 5-8 slices in each region and sex.

striatal subregion or by sex, transient DA release was recorded by fast scan cyclic voltammetry (FSCV).

For males, DA transient amplitude was  $112.96\% \pm 9.87\%$  larger in the DS than the NAc core (95% CI 57.95%, 187.14% ; Bonferroni adjusted p-value = 0.0009) and  $44.43\% \pm 12.9\%$  larger in the NAc shell and the NAc core (95% CI 18.31%, 62.2%; Bonferroni adjusted p-value = 0.0009). There is no significant difference in the amplitude of males between the DS and the NAc shell (95% CI 15.21% smaller, 65.15% larger; Bonferroni

adjusted p-value = 0.9891). In females there was no significant difference in transient amplitude between the DS and NAc core (95% CI 48.26%, 7.81% ; Bonferroni adjusted p-value = 0.1098), the DS and NAc shell (95% CI 63.72%, 160.89% ; Bonferroni adjusted p-value = 8.3646) or the NAc core and the NAc shell (95% CI 51.55%, 250.2% ; Bonferroni adjusted p-value = 3.5712). These results indicate that in males, amplitude varies by region similar to previous studies from DS to NAc core. However, in females there are no regional differences in DA transient amplitude.

Frequency of DA transients was also investigated. For the measurement of amplitude, the effects of sex were dependent on region, and thus the model was fit with an interaction term between the two. However, for frequency, the interaction term between sex and region is not significant (p-value = 0.84). This is unsurprising given that the same pattern of regional frequency appears in both males and females. For this reason, the model for frequency was fit without the interaction term.

Frequency in the DS is greater than in the NAc shell by  $18\% \pm 4\%$  (95% CI 9%, 27%; p-value < 0.0001) greater in the NAc core than in the NAc shell by a factor of  $14\% \pm 4\%$  (95% CI 5%, 24%; p-value = 0.0014). While the difference between DS and NAc core is not significant (p-value = 0.4389) it does follow a dorsal ventral gradient. The median male frequency is larger than in females by a factor of  $12\% \pm 3\%$  (95% CI 5%, 19%; p-value = 0.0006). Thus, there are clear regional differences in DA transient frequency in both males and females. The pattern of regional differences is the same for both sexes, but females have lower frequency than males across all regions.

## DISCUSSION

### *KORs Mediate Exercise Effects on Attenuation of EtOH Induced Hypersensitization*

Figures 1, 2 and 3 demonstrate that aerobic exercise modulates the KOR system. Animals exposed to EtOH alone were hypersensitive to U50,488, while animals that underwent a routine of aerobic exercise were not (Fig. 1A-D). Although there was no statistically significant change from baseline in EtOH exercise mice after U50,488 administration, the direction of the amplitude indicates that those slices may only be slightly responsive to that dose (Fig. 1D). Reuptake was unaffected by KOR drugs (Fig. 1E). Exercised animals also demonstrated lower levels of KOR density in both the NAc and VTA (Fig. 2). It is therefore proposed that aerobic exercise ameliorates the hypersensitization of KORs by downregulating receptor density in those regions. Exercise is a common adjunct to AUD recovery protocols with mixed success though no mechanism has explained its action on reward circuitry. While aerobic exercise has countless effects on the body, these data suggest that changes to the DYN/KOR system represent at least one pathway through which exercise directly modulates reward circuitry to affect EtOH seeking behavior. In line with this hypothesis, exercised animals drank significantly less than animals who were not allowed to exercise (Fig. 3).

These data also better inform the use of opioids in AUD disorder treatment. Pharmacological approaches to AUD recovery protocols include naltrexone, a partial MOR, DOR and KOR antagonist as well as more specific KOR antagonists. These data support the theory that KOR antagonism may help reduce seeking behavior. Because aerobic exercise acts to prevent EtOH induced hypersensitization, it may reduce the dose of KOR antagonist needed to effectively reduce seeking behavior, or enhance KOR antagonist results. Future studies of exercise on the DYN/KOR system should investigate whether aerobic exercise enhances the therapeutic effects of KOR antagonists.

While there is consistent evidence that KORs are hypersensitized in dependent animals, previous work has not characterized the mechanism of hypersensitization. The sensitization may be the result of changes to KOR affinity for DYN or through an increase in KORs at the plasma membrane. There is evidence that both sensitization and density change during this process (Arida et al., 2015). It is unclear why chronic EtOH causes these changes.

Ethanol elicits the release of DYN (Karkhanis et al., 2017). This chronic release may trigger changes in receptor density via changes to the b-arrestin family of downstream effectors. These changes may also be caused by a separate allosteric modulator of KORs. Generally speaking however, repeated stimulation of a receptor results in desensitization via an increase in receptor turnover at the plasma membrane. This is the opposite of the effect of repeated EtOH exposure demonstrated in Figure 1. While acute EtOH has been shown to increase DYN levels, no studies have investigated whether this effect persists throughout dependence. It is possible that EtOH stimulated DYN release decreases throughout the acquisition process, leading to KOR sensitization. Dynorphin release also occurs following acute stress (Karkhanis et al., 2017), and chronic stress results in KOR hypersensitization. It is possible that KOR sensitization due to stress and EtOH are occurring by the same mechanism.

#### *The Effects of MOR and DOR Activation on DA Release and Reuptake*

In addition to the effects on the KORs, exercise and EtOH effects on the MORs were also investigated. For rodents *in vivo*, MOR agonists clearly elevate DA release in the NAc; however, *ex vivo* effects of MOR and DOR agonism are less clear than those of the KORs in the literature. Some studies demonstrate that MOR agonists decrease signal amplitude (Britt & McGehee, 2008), though this was only successful in the dorsal region of the NAc shell. Other striatal regions showed less consistent effects of MOR agonists. Isolated from VTA inputs, MORs are



expressed on CINs and striatal GABA neurons; and it is theorized that MOR effects *ex vivo* can be largely attributed to the presynaptic effects of CINs on DA terminals via muscarinic and nicotinic acetylcholine receptors.

We found that DAMGO (1  $\mu$ M) had no effect on signal amplitude, but clearly slowed reuptake (Fig. 4A-C). Changes to reuptake were reversed to baseline by naloxone administration (Fig. 4C). The lack of effect on signal amplitude is not surprising given that recordings were performed primarily in the NAc core rather than the dorsal NAc shell. Previous literature did not report on reuptake in any regions, and we are therefore unsure whether our findings match the previous literature. Evidence that the muscarinic acetylcholine agonist oxotremorine decreases reuptake and is reversed by antagonist scopolamine supports the idea that muscarinic acetylcholine receptors modulate DAT function (Shin, Adrover, & Alvarez, 2017). In theory, elevated ACh transmission at muscarinic varicosities would also decrease reuptake.

Unfortunately, there is still much to be discovered about the relationship between MOR agonists and ACh transmission. There is evidence that MOR and DOR agonists decrease ACh transmission in the NAc (Kiguchi et al., 2016). Such evidence fits with the activity of MORs as a  $G_i$ -coupled receptor. In contrast, there is also evidence that while MOR agonists decrease CIN firing rate, they also increase burst firing (Hopf et al., 2007), especially in response to local glutamate (unpublished findings). The two possible effects of DAMGO on CINs are further complicated by the need to distinguish firing rate data collected at the cell body, and actual effects on transmission at varicosities. Because there is no data regarding the synaptic distribution of MORs, it is difficult to know the exact effects of MORs on presynaptic ACh release on DA terminals and whether they differ from effects at the cell body. Our findings indicate that DAMGO administration may increase ACh release, likely at muscarinic ACh

receptors, resulting in decreased DA reuptake. Further work needs to be done to determine whether DAMGO actually increases ACh in some instances and regions, and whether these effects are sufficient to modulate DAT activity.

In conjunction with the MORs, DORs are also expressed on CINs and local GABA neurons in the NAc. Our results indicate that DOR agonist DPDPE had no clear effect on DA amplitude or reuptake in the NAc (Fig. 3). This finding is surprising, given that many of the same studies indicating MOR responses also demonstrated similar findings for DORs. However, our investigations may be in different subregions of the striatum rather than targeted to the dorsal NAc shell. Striatal regional differences have been for a variety of different substrates and functions. These data highlight the importance of regional specificity in determining the effects of various receptors, especially those expressed on CINs. Recent work in the lab has demonstrated regional differences in spontaneous DA release shown here, a phenomenon driven by CINs in slice (Yorgason, Zeppenfeld, & Williams, 2017).

#### *Evidence for Regional Heterogeneity in CIN Effects on Striatal DA Release*

Transients were investigated in terms of signal amplitude and frequency. Frequency is a measure of the number of transient events detected and may indicate changes in likelihood of spontaneous release or the likelihood of spontaneous event detection. Given that DA neuron terminals are severed from their cell bodies in the VTA and our previous findings (Yorgason et al., 2017), it is believed that CINs underlie DA transient activity via presynaptic excitation.

In males, it was found that DA transient amplitude was highest in the DS, followed by the NAc shell and finally the NAc core (Fig. 6D). While a difference was detected between the DS and NAc core, no difference was detected between the DS and NAc shell (Fig. 6D). While

this is in slight contrast to previous findings that amplitude varies on a dorsal to ventral gradient, it is possible that the slight variability is due to separate factors affecting DA release amplitude.

Signal amplitude is a measure of the amount of DA released and changes to amplitude may reflect differences in the amount of DA stored per vesicle, the number of vesicles released in a spontaneous event or an increase in the number of DA synapses involved in release. During evoked DA release, a consistent depolarizing stimulus is given in the exact same spot for the course of the experiment. This means that the same number of terminals are likely involved in each signal, making the amplitude value simpler to interpret. Dopamine transients are likely affected by many of the same factors as evoked amplitude, but are also subject to increasing variability due to a potential host of other factors including uneven CIN connectivity and different numbers of terminals activating at different times. Despite more competing explanations, the DS still has the highest DA baseline and it is significantly larger than in the NAc core, the next most ventral region (Fig. 6F). The NAc shell amplitude is the only one that deviates from previous literature. This indicates the need for further exploration of factors that may separate the NAc shell from the other two regions.

Surprisingly, no regional differences in transient amplitude were found in females (Fig. F). To the best of the author's knowledge, no regional differences in FSCV DA release for females, evoked or spontaneous, have been previously investigated. These sex differences may be explained by a large host of substrates and processes including KORs, D2 autoreceptors, nitric oxide release and Ca<sup>2+</sup> channel variability (Calipari et al., 2017), and further work is needed to further elucidate the mechanisms behind sex differences. The lack of regional differences in female mice contributes to growing evidence that there are important differences between the

striatal circuitry of males and females. Further work should be done investigating mechanisms behind differences in regional distribution of DA release.

DA transient frequency also varied by region with the highest frequency in the DS and NAc core and a lowest frequency in the NAc shell (Fig. 6F). The same pattern was seen for males and females, though males had higher frequency in all brain regions (Fig. 6G). This demonstrates that likelihood of transient detection is highest in the DS and NAc core and lower in the NAc shell which likely reflects differential qualities of CIN synapses in different striatal subregions. Successful connectivity is related to either the number of synapses or the subunit makeup of nAChRs. One group has demonstrated striatal regional differences in nAChR subunit makeup (Threlfell et al., 2010), suggesting that nAChR subunit variance is a strong candidate for explaining regional differences in DA, though further work is needed to confirm. Investigation of DA transient reuptake is also required to rule out the possibility that differences in vesicle reuptake or packaging are responsible for these effects.

There remains a lot of work to characterize the relationship between MOR and DOR activation on CINs and their effects on striatal DA release. Previous work on MOR and DOR release has focused on only a small region where opioid effects were easily detectable in slice. Our studies indicate that it will be important moving forward to investigate these effects in various striatal subregions and both sexes in order to understand DA release in a more nuanced way.

## REFERENCES

- Acquas, E., Meloni, M., & Di Chiara, G. (1993). Blockade of delta-opioid receptors in the nucleus accumbens prevents ethanol-induced stimulation of dopamine release. *Eur J Pharmacol*, 230(2), 239-241.
- Agmo, A., Federman, I., Navarro, V., Padua, M., & Velazquez, G. (1993). Reward and reinforcement produced by drinking water: role of opioids and dopamine receptor subtypes. *Pharmacol Biochem Behav*, 46(1), 183-194.
- Agmo, A., Galvan, A., & Talamantes, B. (1995). Reward and reinforcement produced by drinking sucrose: two processes that may depend on different neurotransmitters. *Pharmacol Biochem Behav*, 52(2), 403-414.
- Ahn, S., & Phillips, A. G. (2002). Modulation by central and basolateral amygdalar nuclei of dopaminergic correlates of feeding to satiety in the rat nucleus accumbens and medial prefrontal cortex. *J Neurosci*, 22(24), 10958-10965.
- Ahn, S., & Phillips, A. G. (2003). Independent modulation of basal and feeding-evoked dopamine efflux in the nucleus accumbens and medial prefrontal cortex by the central and basolateral amygdalar nuclei in the rat. *Neuroscience*, 116(1), 295-305.
- Aldrich, J. V., & McLaughlin, J. P. (2009). Peptide kappa opioid receptor ligands: potential for drug development. *AAPS J*, 11(2), 312-322. doi: 10.1208/s12248-009-9105-4
- Arida, R. M., Gomes da Silva, S., de Almeida, A. A., Cavalheiro, E. A., Zavala-Tecuapetla, C., Brand, S., & Rocha, L. (2015). Differential effects of exercise on brain opioid receptor binding and activation in rats. *J Neurochem*, 132(2), 206-217. doi: 10.1111/jnc.12976
- Attali, B., Saya, D., & Vogel, Z. (1989). Kappa-opiate agonists inhibit adenylate cyclase and produce heterologous desensitization in rat spinal cord. *J Neurochem*, 52(2), 360-369.
- Benjamin, D., Grant, E. R., & Pohorecky, L. A. (1993). Naltrexone reverses ethanol-induced dopamine release in the nucleus accumbens in awake, freely moving rats. *Brain Res*, 621(1), 137-140.
- Berridge, K. C., & Robinson, T. E. (1998). What is the role of dopamine in reward: hedonic impact, reward learning, or incentive salience? *Brain Res Brain Res Rev*, 28(3), 309-369. doi: 10.1016/s0165-0173(98)00019-8
- Beyer, A., Koch, T., Schroder, H., Schulz, S., & Holtt, V. (2004). Effect of the A118G polymorphism on binding affinity, potency and agonist-mediated endocytosis, desensitization, and resensitization of the human mu-opioid receptor. *J Neurochem*, 89(3), 553-560. doi: 10.1111/j.1471-4159.2004.02340.x
- Blackburn, J. R., Phillips, A. G., Jakubovic, A., & Fibiger, H. C. (1986). Increased dopamine metabolism in the nucleus accumbens and striatum following consumption of a nutritive meal but not a palatable non-nutritive saccharin solution. *Pharmacol Biochem Behav*, 25(5), 1095-1100.
- Bonci, A., & Williams, J. T. (1996). A common mechanism mediates long-term changes in synaptic transmission after chronic cocaine and morphine. *Neuron*, 16, 631-639.
- Bond, C., LaForge, K. S., Tian, M., Melia, D., Zhang, S., Borg, L., . . . Yu, L. (1998). Single-nucleotide polymorphism in the human mu opioid receptor gene alters beta-endorphin binding and activity: possible implications for opiate addiction. *Proc Natl Acad Sci U S A*, 95(16), 9608-9613. doi: 10.1073/pnas.95.16.9608

- Britt, J. P., & McGehee, D. S. (2008). Presynaptic opioid and nicotinic receptor modulation of dopamine overflow in the nucleus accumbens. *J Neurosci*, *28*(7), 1672-1681. doi: 10.1523/JNEUROSCI.4275-07.2008
- Calipari, E. S., Juarez, B., Morel, C., Walker, D. M., Cahill, M. E., Ribeiro, E., . . . Nestler, E. J. (2017). Dopaminergic dynamics underlying sex-specific cocaine reward. *Nat Commun*, *8*, 13877. doi: 10.1038/ncomms13877
- Cerri, D. H., Saddoris, M. P., & Carelli, R. M. (2014). Nucleus accumbens core neurons encode value-independent associations necessary for sensory preconditioning. *Behav Neurosci*, *128*(5), 567-578. doi: 10.1037/a0037797
- Chen, F., & Lawrence, A. J. (2000). Effect of chronic ethanol and withdrawal on the mu-opioid receptor- and 5-Hydroxytryptamine(1A) receptor-stimulated binding of [(35)S]Guanosine-5'-O-(3-thio)triphosphate in the fawn-hooded rat brain: A quantitative autoradiography study. *J Pharmacol Exp Ther*, *293*(1), 159-165.
- Chieng, B., & Bekkers, J. M. (2001). Inhibition of calcium channels by opioid- and adenosine-receptor agonists in neurons of the nucleus accumbens *Br J Pharmacol* (Vol. 133, pp. 337-344).
- Cowen, M. S., Rezvani, A. H., Jarrott, B., & Lawrence, A. J. (1999). Ethanol consumption by Fawn-Hooded rats following abstinence: effect of naltrexone and changes in mu-opioid receptor density. *Alcohol Clin Exp Res*, *23*(6), 1008-1014.
- Crist, R. C., & Berrettini, W. H. (2014). Pharmacogenetics of OPRM1. *Pharmacol Biochem Behav*, *0*, 25-33. doi: 10.1016/j.pbb.2013.10.018
- Darlington, T. M., McCarthy, R. D., Cox, R. J., Miyamoto-Ditmon, J., Gallego, X., & Ehringer, M. A. (2016). Voluntary wheel running reduces voluntary consumption of ethanol in mice: identification of candidate genes through striatal gene expression profiling. *Genes Brain Behav*, *15*(5), 474-490. doi: 10.1111/gbb.12294
- Devine, D. P., & Wise, R. A. (1994). Self-administration of morphine, DAMGO, and DPDPE into the ventral tegmental area of rats. *J Neurosci*, *14*(4), 1978-1984.
- Droste, S. K., Schweizer, M. C., Ulbricht, S., & Reul, J. M. (2006). Long-term voluntary exercise and the mouse hypothalamic-pituitary-adrenocortical axis: impact of concurrent treatment with the antidepressant drug tianeptine. *J Neuroendocrinol*, *18*(12), 915-925. doi: 10.1111/j.1365-2826.2006.01489.x
- Ehringer, M. A., Hoft, N. R., & Zunhammer, M. (2009). Reduced alcohol consumption in mice with access to a running wheel. *Alcohol*, *43*(6), 443-452. doi: 10.1016/j.alcohol.2009.06.003
- Gallego, X., Cox, R. J., Funk, E., Foster, R. A., & Ehringer, M. A. (2015). Voluntary exercise decreases ethanol preference and consumption in C57BL/6 adolescent mice: sex differences and hippocampal BDNF expression. *Physiol Behav*, *138*, 28-36. doi: 10.1016/j.physbeh.2014.10.008
- Gianoulakis, C. (1993). Endogenous opioids and excessive alcohol consumption. *J Psychiatry Neurosci*, *18*(4), 148-156.
- Gratton, A., & Wise, R. A. (1983). Brain stimulation reward in the lateral hypothalamic medial forebrain bundle: mapping of boundaries and homogeneity. *Brain Res*, *274*(1), 25-30.
- Greenwood, B. N., Foley, T. E., Le, T. V., Strong, P. V., Loughridge, A. B., Day, H. E., & Fleshner, M. (2011). Long-term voluntary wheel running is rewarding and produces plasticity in the mesolimbic reward pathway. *Behav Brain Res*, *217*(2), 354-362. doi: 10.1016/j.bbr.2010.11.005

- Hendershot, C. S., Claus, E. D., & Ramchandani, V. A. (2016). Associations of OPRM1 A118G and Alcohol Sensitivity with Intravenous Alcohol Self-Administration in Young Adults. *Addict Biol*, *21*(1), 125-135. doi: 10.1111/adb.12165
- Henry, D. J., Grandy, D. K., Lester, H. A., Davidson, N., & Chavkin, C. (1995). Kappa-opioid receptors couple to inwardly rectifying potassium channels when coexpressed by *Xenopus* oocytes. *Mol Pharmacol*, *47*(3), 551-557.
- Hirose, N., Murakawa, K., Takada, K., Oi, Y., Suzuki, T., Nagase, H., . . . Koshikawa, N. (2005). Interactions among mu- and delta-opioid receptors, especially putative delta1- and delta2-opioid receptors, promote dopamine release in the nucleus accumbens. *Neuroscience*, *135*(1), 213-225. doi: 10.1016/j.neuroscience.2005.03.065
- Hopf, F. W., Martin, M., Chen, B. T., Bowers, M. S., Mohamedi, M. M., & Bonci, A. (2007). Withdrawal from intermittent ethanol exposure increases probability of burst firing in VTA neurons in vitro. *J Neurophysiol*, *98*(4), 2297-2310. doi: 00824.2007 [pii] 10.1152/jn.00824.2007
- Hyman, S. E., & Malenka, R. C. (2001). Addiction and the brain: the neurobiology of compulsion and its persistence. *Nat Rev Neurosci*, *2*(10), 695-703. doi: 10.1038/3509456035094560 [pii]
- Hyman, S. E., Malenka, R. C., & Nestler, E. J. (2006). Neural Mechanisms of Addiction: The Role of Reward-Related Learning and Memory. *Annu Rev Neurosci*. doi: 10.1146/annurev.neuro.29.051605.113009
- Johnson, S. W., & North, R. A. (1992). Opioids excite dopamine neurons by hyperpolarization of local interneurons. *J Neuroscience*, *12*(2), 483-488.
- Kalivas, P. W., Churchill, L., & Klitenick, M. A. (1993). The circuitry mediating the translation of motivational stimuli into adaptive motor responses. In P. W. Kalivas & C. D. Barnes (Eds.), *Limbic Motor Circuits and Neuropsychiatry* (pp. 237-287). Boca Raton: CRC Press, Inc.
- Kalivas, P. W., & Volkow, N. D. (2005). The neural basis of addiction: a pathology of motivation and choice. *Am J Psychiatry*, *162*(8), 1403-1413. doi: 10.1176/appi.ajp.162.8.1403
- Karkhanis, A., Holleran, K. M., & Jones, S. R. (2017). Dynorphin/Kappa Opioid Receptor Signaling in Preclinical Models of Alcohol, Drug, and Food Addiction. *Int Rev Neurobiol*, *136*, 53-88. doi: 10.1016/bs.irn.2017.08.001
- Kauer, J. A., & Malenka, R. C. (2007). Synaptic plasticity and addiction. *Nat Rev Neurosci*, *8*(11), 844-858. doi: nrn2234 [pii] 10.1038/nrn2234
- Kiguchi, Y., Aono, Y., Watanabe, Y., Yamamoto-Nemoto, S., Shimizu, K., Shimizu, T., . . . Saigusa, T. (2016). In vivo neurochemical evidence that delta1-, delta2- and mu2-opioid receptors, but not mu1-opioid receptors, inhibit acetylcholine efflux in the nucleus accumbens of freely moving rats. *Eur J Pharmacol*, *789*, 402-410. doi: 10.1016/j.ejphar.2016.07.028
- Konkoy, C. S., & Childers, S. R. (1989). Dynorphin-selective inhibition of adenylyl cyclase in guinea pig cerebellum membranes. *Mol Pharmacol*, *36*(4), 627-633.
- Koob, G. F. (1992). Dopamine, addiction and reward. *Semin. Neurosci.*, *4*, 139-148.
- Koob, G. F., Rassnick, S., Heinrichs, S., & Weiss, F. (1994). Alcohol, the reward system and dependence. In B. Jansson, H. Jörnvall, U. Rydberg, L. Terenius, & B. L. Vallee (Eds.),

- Towards a Molecular Basis of Alcohol Use and Abuse* (pp. 103-114). Basel, Switzerland: Birkhäuser.
- Koob, G. F., & Volkow, N. D. (2010). Neurocircuitry of addiction. *Neuropsychopharmacology*, 35(1), 217-238. doi: 10.1038/npp.2009.110
- Leasure, J. L., Neighbors, C., Henderson, C. E., & Young, C. M. (2015). Exercise and Alcohol Consumption: What We Know, What We Need to Know, and Why it is Important. *Front Psychiatry*, 6, 156. doi: 10.3389/fpsy.2015.00156
- Lee, Y. K., Park, S. W., Kim, Y. K., Kim, D. J., Jeong, J., Myrick, H., & Kim, Y. H. (2005). Effects of naltrexone on the ethanol-induced changes in the rat central dopaminergic system. *Alcohol Alcohol*, 40(4), 297-301. doi: 10.1093/alcalc/agh163
- Lipowski, M., Szulc, M., & Bulinski, L. (2015). Physical activity among other health-related behaviors in treatment of alcoholism. *J Sports Med Phys Fitness*, 55(3), 231-240.
- Mansour, A., Burke, S., Pavlic, R. J., Akil, H., & Watson, S. J. (1996). Immunohistochemical localization of the cloned kappa 1 receptor in the rat CNS and pituitary. *Neuroscience*, 71(3), 671-690.
- Mansour, A., Khachaturian, H., Lewis, M. E., Akil, H., & Watson, S. J. (1987). Autoradiographic differentiation of mu, delta, and kappa opioid receptors in the rat forebrain and midbrain. *J Neurosci*, 7(8), 2445-2464.
- Martinez, D., Broft, A., Foltin, R. W., Slifstein, M., Hwang, D. R., Huang, Y., . . . Laruelle, M. (2004). Cocaine dependence and d2 receptor availability in the functional subdivisions of the striatum: relationship with cocaine-seeking behavior. *Neuropsychopharmacology*, 29(6), 1190-1202. doi: 10.1038/sj.npp.1300420
- Melchior, J. R., & Jones, S. R. (2017). Chronic ethanol exposure increases inhibition of optically targeted phasic dopamine release in the nucleus accumbens core and medial shell ex vivo. *Mol Cell Neurosci*, 85, 93-104. doi: 10.1016/j.mcn.2017.09.007
- Mitsi, V., & Zachariou, V. (2016). Modulation of pain, nociception, and analgesia by the brain reward center. *Neuroscience*, 338, 81-92. doi: 10.1016/j.neuroscience.2016.05.017
- Morgan, D., Grant, K. A., Gage, H. D., Mach, R. H., Kaplan, J. R., Prioleau, O., . . . Nader, M. A. (2002). Social dominance in monkeys: dopamine D2 receptors and cocaine self-administration. *Nat Neurosci*, 5(2), 169-174. doi: 10.1038/nn798
- Murakawa, K., Hirose, N., Takada, K., Suzuki, T., Nagase, H., Cools, A. R., & Koshikawa, N. (2004). Deltorphin II enhances extracellular levels of dopamine in the nucleus accumbens via opioid receptor-independent mechanisms. *Eur J Pharmacol*, 491(1), 31-36. doi: 10.1016/j.ejphar.2004.03.028
- Nestler, E. J. (2001). Psychogenomics: opportunities for understanding addiction. *The Journal of Neuroscience*, 21(21), 8324-8327.
- Nolan, S. O., Zachry, J. E., Johnson, A. R., Brady, L. J., Siciliano, C. A., & Calipari, E. S. (2020). Direct dopamine terminal regulation by local striatal microcircuitry. *J Neurochem*. doi: 10.1111/jnc.15034
- Nugent, F. S., & Kauer, J. A. (2008). LTP of GABAergic synapses in the ventral tegmental area and beyond. *J Physiol*, 586(6), 1487-1493. doi: jphysiol.2007.148098 [pii] 10.1113/jphysiol.2007.148098
- Okutsu, H., Watanabe, S., Takahashi, I., Aono, Y., Saigusa, T., Koshikawa, N., & Cools, A. R. (2006). Endomorphin-2 and endomorphin-1 promote the extracellular amount of accumbal dopamine via nonopioid and mu-opioid receptors, respectively. *Neuropsychopharmacology*, 31(2), 375-383. doi: 10.1038/sj.npp.1300804



- Overstreet, D. H., Knapp, D. J., & Breese, G. R. (2002). Accentuated decrease in social interaction in rats subjected to repeated ethanol withdrawals. *Alcohol Clin Exp Res*, 26(8), 1259-1268. doi: 10.1097/01.ALC.0000023983.10615.D7
- Ozburn, A. R., Harris, R. A., & Blednov, Y. A. (2008). Wheel running, voluntary ethanol consumption, and hedonic substitution. *Alcohol*, 42(5), 417-424. doi: 10.1016/j.alcohol.2008.04.006
- Phillips, A. G., Ahn, S., & Howland, J. G. (2003). Amygdalar control of the mesocorticolimbic dopamine system: parallel pathways to motivated behavior. *Neurosci Biobehav Rev*, 27(6), 543-554.
- Phillips, A. G., Atkinson, L. J., Blackburn, J. R., & Blaha, C. D. (1993). Increased extracellular dopamine in the nucleus accumbens of the rat elicited by a conditional stimulus for food: an electrochemical study. *Can J Physiol Pharmacol*, 71(5-6), 387-393. doi: 10.1139/y93-059
- Piazza-Gardner, A. K., & Barry, A. E. (2012). Examining physical activity levels and alcohol consumption: are people who drink more active? *Am J Health Promot*, 26(3), e95-104. doi: 10.4278/ajhp.100929-LIT-328
- Prather, P. L., McGinn, T. M., Claude, P. A., Liu-Chen, L. Y., Loh, H. H., & Law, P. Y. (1995). Properties of a kappa-opioid receptor expressed in CHO cells: interaction with multiple G-proteins is not specific for any individual G alpha subunit and is similar to that of other opioid receptors. *Brain Res Mol Brain Res*, 29(2), 336-346.
- Ray, L. A., Bujarski, S., MacKillop, J., Courtney, K. E., Monti, P. M., & Miotto, K. (2013). Subjective Response to Alcohol among Alcohol Dependent Individuals: Effects of the Mu-Opioid Receptor (OPRM1) Gene and Alcoholism Severity. *Alcohol Clin Exp Res*, 37(Suppl 1), E116-124. doi: 10.1111/j.1530-0277.2012.01916.x
- Ray, L. A., & Hutchison, K. E. (2004). A polymorphism of the mu-opioid receptor gene (OPRM1) and sensitivity to the effects of alcohol in humans. *Alcohol Clin Exp Res*, 28(12), 1789-1795. doi: 10.1097/01.alc.0000148114.34000.b9
- Roberts, A. J., Gold, L. H., Polis, I., McDonald, J. S., Filliol, D., Kieffer, B. L., & Koob, G. F. (2001). Increased ethanol self-administration in delta-opioid receptor knockout mice. *Alcohol Clin Exp Res*, 25(9), 1249-1256.
- Roberts, A. J., McDonald, J. S., Heyser, C. J., Kieffer, B. L., Matthes, H. W., Koob, G. F., & Gold, L. H. (2000). mu-Opioid receptor knockout mice do not self-administer alcohol. *J Pharmacol Exp Ther*, 293(3), 1002-1008.
- Rose, J. H., Karkhanis, A. N., Chen, R., Gioia, D., Lopez, M. F., Becker, H. C., . . . Jones, S. R. (2016). Supersensitive Kappa Opioid Receptors Promotes Ethanol Withdrawal-Related Behaviors and Reduce Dopamine Signaling in the Nucleus Accumbens. *Int J Neuropsychopharmacol*, 19(5). doi: 10.1093/ijnp/pyv127
- Sackett, D. A., Saddoris, M. P., & Carelli, R. M. (2017). Nucleus Accumbens Shell Dopamine Preferentially Tracks Information Related to Outcome Value of Reward. *eNeuro*, 4(3). doi: 10.1523/eneuro.0058-17.2017
- Sacks, J. J., Gonzales, K. R., Bouchery, E. E., Tomedi, L. E., & Brewer, R. D. (2015). 2010 National and State Costs of Excessive Alcohol Consumption. *Am J Prev Med*, 49(5), e73-79. doi: 10.1016/j.amepre.2015.05.031
- Saddoris, M. P., Cacciapaglia, F., Wightman, R. M., & Carelli, R. M. (2015). Differential Dopamine Release Dynamics in the Nucleus Accumbens Core and Shell Reveal

- Complementary Signals for Error Prediction and Incentive Motivation *J Neurosci* (Vol. 35, pp. 11572-11582).
- SAMHSA. (2016). Facing Addicton in America: The Surgeon Gernal's Report on Alcohol, Drugs, and Health: US Department of Health and Human Services.
- Schultz, W., Apicella, P., Ljungberg, T., Romo, R., & Scarnati, E. (1993). Reward-related activity in the monkey striatum and substantia nigra. *Prog Brain Res*, 99, 227-235. doi: 10.1016/s0079-6123(08)61349-7
- Seizinger, B. R., Bovermann, K., Maysinger, D., Holtt, V., & Herz, A. (1983). Differential effects of acute and chronic ethanol treatment on particular opioid peptide systems in discrete regions of rat brain and pituitary. *Pharmacol Biochem Behav*, 18 Suppl 1, 361-369. doi: 10.1016/0091-3057(83)90200-9
- Shin, J. H., Adrover, M. F., & Alvarez, V. A. (2017). Distinctive Modulation of Dopamine Release in the Nucleus Accumbens Shell Mediated by Dopamine and Acetylcholine Receptors *J Neurosci* (Vol. 37, pp. 11166-11180).
- Shippenberg, T. S., Zapata, A., & Chefer, V. I. (2007). Dynorphin and the pathophysiology of drug addiction. *Pharmacol Ther*, 116(2), 306-321. doi: 10.1016/j.pharmthera.2007.06.011
- Sirohi, S., Bakalkin, G., & Walker, B. M. (2012). Alcohol-induced plasticity in the dynorphin/kappa-opioid receptor system. *Front Mol Neurosci*, 5, 95. doi: 10.3389/fnmol.2012.00095
- Sloan, M. E., Klepp, T. D., Gowin, J. L., Swan, J. E., Sun, H., Stangl, B. L., & Ramchandani, V. A. (2018). The OPRM1 A118G polymorphism: converging evidence against associations with alcohol sensitivity and consumption *Neuropsychopharmacology* (Vol. 43, pp. 1530-1538).
- Spanagel, R., Herz, A., & Shippenberg, T. S. (1992). Opposing tonically active endogenous opioid systems modulate the mesolimbic dopaminergic pathway. *Proc Natl Acad Sci U S A*, 89(6), 2046-2050.
- Steffensen, S. C., Stobbs, S. H., Colago, E. E., Lee, R. S., Koob, G. F., Gallegos, R. A., & Henriksen, S. J. (2006). Contingent and non-contingent effects of heroin on mu-opioid receptor-containing ventral tegmental area GABA neurons. *Exp Neurol*, 202(1), 139-151. doi: 10.1016/j.expneurol.2006.05.023
- Tallent, M., Dichter, M. A., Bell, G. I., & Reisine, T. (1994). The cloned kappa opioid receptor couples to an N-type calcium current in undifferentiated PC-12 cells. *Neuroscience*, 63(4), 1033-1040.
- Tanda, G., & Di Chiara, G. (1998). A dopamine-mu1 opioid link in the rat ventral tegmentum shared by palatable food (Fonzies) and non-psychostimulant drugs of abuse. *Eur J Neurosci*, 10(3), 1179-1187. doi: 10.1046/j.1460-9568.1998.00135.x
- Threlfell, S., Clements, M. A., Khodai, T., Pienaar, I. S., Exley, R., Wess, J., & Cragg, S. J. (2010). Striatal muscarinic receptors promote activity dependence of dopamine transmission via distinct receptor subtypes on cholinergic interneurons in ventral versus dorsal striatum. *J Neurosci*, 30(9), 3398-3408. doi: 10.1523/jneurosci.5620-09.2010
- Tzschentke, T. M. (2000). The medial prefrontal cortex as a part of the brain reward system. *Amino Acids*, 19(1), 211-219.
- van den Wildenberg, E., Wiers, R. W., Dessers, J., Janssen, R. G., Lambrichs, E. H., Smeets, H. J., & van Breukelen, G. J. (2007). A functional polymorphism of the mu-opioid receptor

- gene (OPRM1) influences cue-induced craving for alcohol in male heavy drinkers. *Alcohol Clin Exp Res*, 31(1), 1-10. doi: 10.1111/j.1530-0277.2006.00258.x
- Voisey, J., Swagell, C. D., Hughes, I. P., van Daal, A., Noble, E. P., Lawford, B. R., . . . Morris, C. P. (2012). A DRD2 and ANKK1 haplotype is associated with nicotine dependence. *Psychiatry Res*, 196(2-3), 285-289. doi: 10.1016/j.psychres.2011.09.024
- Walker, B. M., & Koob, G. F. (2008). Pharmacological evidence for a motivational role of kappa-opioid systems in ethanol dependence. *Neuropsychopharmacology*, 33(3), 643-652. doi: 10.1038/sj.npp.1301438
- Wang, D., Wang, Y., Wang, Y., Li, R., & Zhou, C. (2014). Impact of physical exercise on substance use disorders: a meta-analysis. *Plos One*, 9(10), e110728. doi: 10.1371/journal.pone.0110728
- Wang, S. S., Wei, C. L., Liu, Z. Q., & Ren, W. (2011). [The influence of L-glutamate and carbachol on burst firing of dopaminergic neurons in ventral tegmental area]. *Sheng Li Xue Bao*, 63(1), 25-30.
- Wise, R. A. (1996). Addictive drugs and brain stimulation reward. *Annu. Rev. Neurosci.*, 19, 319-340.
- Wise, R. A. (2002). Brain reward circuitry: insights from unsensed incentives. *Neuron*, 36(2), 229-240.
- Wise, R. A. (2004). Dopamine, learning and motivation. *Nat Rev Neurosci*, 5(6), 483-494.
- Wise, R. A. (2008). Dopamine and reward: the anhedonia hypothesis 30 years on. *Neurotox Res*, 14(2-3), 169-183. doi: 10.1007/BF03033808
- Wise, R. A., & Bozarth, M. A. (1987). A psychostimulant theory of addiction. *Psychol. Rev.*, 94(1-24).
- Yorgason, J. T., Espana, R. A., & Jones, S. R. (2011). Demon voltammetry and analysis software: analysis of cocaine-induced alterations in dopamine signaling using multiple kinetic measures. *J Neurosci Methods*, 202(2), 158-164. doi: 10.1016/j.jneumeth.2011.03.001
- Yorgason, J. T., Zeppenfeld, D. M., & Williams, J. T. (2017). Cholinergic Interneurons Underlie Spontaneous Dopamine Release in Nucleus Accumbens. *J Neurosci*, 37(8), 2086-2096. doi: 10.1523/JNEUROSCI.3064-16.2017
- Yoshida, Y., Koide, S., Hirose, N., Takada, K., Tomiyama, K., Koshikawa, N., & Cools, A. R. (1999). Fentanyl increases dopamine release in rat nucleus accumbens: involvement of mesolimbic mu- and delta-2-opioid receptors. *Neuroscience*, 92(4), 1357-1365.