
SCHOOL *of* GRADUATE STUDIES
EAST TENNESSEE STATE UNIVERSITY

East Tennessee State University
Digital Commons @ East Tennessee State University

Electronic Theses and Dissertations | Student Works

8-2007

# Nicotine Sensitization in β-Arrestin 2 Knockout Adolescent Mice.

Jennifer A. Correll
*East Tennessee State University*

Follow this and additional works at: https://dc.etsu.edu/etd

 Part of the Molecular and Cellular Neuroscience Commons

## Recommended Citation

Correll, Jennifer A., "Nicotine Sensitization in β-Arrestin 2 Knockout Adolescent Mice." (2007). *Electronic Theses and Dissertations.* Paper 2050. https://dc.etsu.edu/etd/2050

This Thesis - Open Access is brought to you for free and open access by the Student Works at Digital Commons @ East Tennessee State University. It has been accepted for inclusion in Electronic Theses and Dissertations by an authorized administrator of Digital Commons @ East Tennessee State University. For more information, please contact digilib@etsu.edu.

# Nicotine Sensitization in β-Arrestin 2 Knockout Adolescent Mice

---

A thesis

presented to

the faculty of the Department of Psychology

East Tennessee State University

In partial fulfillment

of the requirements for the degree

Master of Arts and Sciences in Clinical Psychology

---

by

Jennifer Aileen Correll

August 2007

---

Russell Brown, PhD, Chair

Alan Shields, PhD

Michael Woodruff, PhD

Keywords: β-arrestin 2, C57/B6, Nicotine Sensitization

ABSTRACT

Nicotine Sensitization in β-Arrestin 2 Knockout Adolescent Mice

by

Jennifer Aileen Correll

β arrestin-2 is a protein involved in signaling of D2 receptors and plays a mediating role in sensitization to psychostimulants and the opiate morphine. In this study, 3-4 week old BA-2 KO and wild type C57/B6 mice received nicotine tartarate (s.c, 0.5 mg/kg free base) for 7 or 14 consecutive days followed by a drug-free period. An acute nicotine challenge followed the drug-free period. Results indicated that the absence of β-arrestin-2 reduced sensitization to nicotine in Experiment 1. BA-2 KOs eventually demonstrated sensitization in Experiment 2. However, absence of β-arrestin-2 blocked expression of sensitization on the challenge. After the challenge, brain tissue was removed and the nucleus accumbens was dissected and analyzed for brain-derived neurotrophic factor (BDNF). Results showed that BDNF positively correlated with behavioral results. These results appear to indicate the importance of the β-arrestin 2 protein in locomotor sensitization and that dopamine signaling is related to BDNF.

CONTENTS

LIST OF FIGURES

Figure Page

# CHAPTER 1

## INTRODUCTION

The neurotransmitter dopamine (DA) is responsible for many functions including reward, motivation, mood, and motor control (Bibb, 2005; Di Chiara, 1999). Motivation is defined by Di Chiara (1999) as the process by which an organism responds to a stimulus in relation to its survival value. Furthermore, the mesolimbic DA pathway is commonly associated with many psychiatric disorders, including schizophrenia, attention-deficit/hyperactivity disorder, obsessive-compulsive disorder, and drug addiction (Bibb; Brown et al., 2004; Collins, Wade, Ledon, & Izenwasser, 2004; Le Foll & Goldberg, 2005). Although other neurotransmitter systems are involved, the DA system has been implicated in the relationship between reward pathways and addiction (Bibb; Fonder et al., 2005). All addictive drugs act, at least in part, by increasing DA release primarily in the nucleus accumbens (NAcc), but it has been shown that DA plays different roles in behavior in different areas of the brain (Di Chiara et al, 2004; Fonder et al.).

The drug reward pathway originates with DA cell bodies in the ventral tegmental area (VTA), located in the midbrain. Rewarding drugs increase signaling in the DA system by stimulating dopaminergic as well as glutamatergic receptors located on cell bodies in the VTA that send their projections to the NAcc (Traynor & Neubig, 2005). Additionally, areas in the striatum and frontal cortex have also been shown to play a role in the addictive properties of drugs. Dopamine neurons have been shown to play a particularly important role in the increase of neuronal responding to repeated administration of psychostimulant drugs and to environmental stimuli that are associated with the positive reinforcing effects of drugs (Marinelli, Rudick, Hu, & White, 2006).

## Dopamine Receptors

Dopamine binds to two families of receptors, the D1 and the D2. The D1 and D2 families of receptors are both classified as metabotropic receptors (Kolb & Whishaw, 2001). Metabotropic receptors are coupled to guanyl nucleotide-binding proteins (G proteins) that are responsible for beginning a cascade of events to open an ion channel and for changing metabolic activity in the cell. Three separate subunits make up the G protein: the alpha, beta, and gamma. When a neurotransmitter binds to a metabotropic receptor, the alpha-subunit separates from the beta and gamma subunits and is free to bind to other proteins in the cell or possibly to an ion channel to open this channel. Binding to an ion channel creates a change in the flow of ions by either opening a closed ion channel or, in some instances, closing an open channel. This change in turn causes the cell's electrical potential to change in the cell, which can lead to either depolarization or hyperpolarization of the cell's potential (Kolb & Whishaw).

## β-Arrestin 2 and Dopamine

The proteins β-arrestin 1 and β-arrestin 2 have been shown to be co-localized with D1 and D2 receptors, respectively, and have shown to be associated with G-protein signaling at each receptor. These two proteins have been shown to be responsible for receptor internalization and signal termination in both D1 and D2 receptors (Beaulieu et al, 2005; Bohn, Gainetdinov, & Caron, 2004; Zhang, et al., 2005). Both β-arrestins are expressed in neuronal tissues and are primarily concentrated in postsynaptic regions (Zuo, 2005). β-arrestin 1 and β-arrestin 2 perform some of the same functions, and are similar in that they are isoforms that share structural similarities and similar amino acids (Zhang et al). Both arrestins have also been shown to hinder G-protein coupling by inhibiting the hydrolysis of guanosine triphosphate (GTP) to guanosine diphosphate (GDP), which is the first step in the G-protein coupling signal cascade. For

example, when a receptor is exposed to an agonist, the β-arrestins are ultimately involved in the desensitization of the receptor (Dewire, Ahn, Lefkowitz, & Shenoy, 2007). Therefore, the β-arrestin molecule associates with receptors that are activated in order to block continued signaling (Grady, 2007). β-arrestins have been shown to be involved with a number of G-protein coupled receptors. Of particular importance to this study, β-arrestin-2 has been shown to be primarily involved in G-protein signaling at the D2 receptor as well as the μ opiate receptor. The opiate and dopaminergic neurotransmitter systems have been strongly implicated in mediating addiction to several addictive drugs.

Recent work has also shown that β-arrestin-2 plays a role in signal transduction, and can phosphorylate receptors on its own (Lefkowtiz & Shenoy, 2005). This is depicted below in Figure 1. β-arrestin-2 plays a role in both receptor desensitization that is involved in regulating second messenger signaling as well as regulating signal transduction that is mediated by, for example, MAP kinases and tyrosine kinases. Thus, it appears that β-arrestin-2 plays a multifaceted role in cell signaling that in general is regulatory but can also be involved in phosphorylation of the ion channel itself. Therefore, it can play a direct in the cell response.

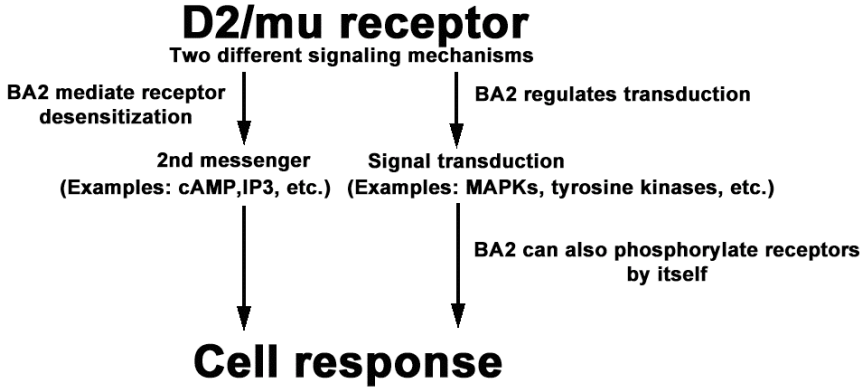


*Figure 1.* Depiction of the role of β-arrestin-2 in the cell response from the dopamine D2 and μ opioid receptor.

## β-Arrestin 2 and Addiction

Research has examined the role β-arrestin 2 plays in cocaine and morphine locomotor sensitization, using a genetic knockout for β-arrestin-2 in the mouse (Bohn et al., 2004; Hack, Bagley, Chieng, & Christie, 2005; Raehal, Walker, & Bohn, 2005; Zuo, 2005). A genetic knockout is an animal that is lacking a particular gene, and knockouts have been found to be particularly useful in gaining information about how a particular system operates when it is lacking an important component. Not only can information be gathered surrounding the function of the particular gene, but also it is possible to better understand brain plasticity when the particular gene is absent (Bohn et al., 2004; Zuo). For example, tolerance to morphine has been shown though enhanced and prolonged analgesic effects of morphine in β-arrestin 2 knockout mice (BA-2 KO; Zuo). However, whereas wild type controls became tolerant to acute and chronic morphine administration, BA-2 KOs continued to sensitize to morphine (Zuo). This result appears to indicate that the presence of β-arrestin-2 plays a role in tolerance to opiate drugs, and when not present, G-protein coupling that occurs at the μ opioid receptor continues to increase with continued administration of the drug. Essentially, knockout of the β-arrestin 2 protein increased sensitivity of μ opioid receptors. In further support of this notion, mice were also monitored for changes in responsiveness to pain when morphine was administered. To test pain responsiveness to morphine, mice were tested on a hot-plate test of antinociception, and BA-2 KOs exhibited a slower paw withdrawal after they have been exposed to morphine as compared to controls (Bohn et al.). This is actually congruent with locomotor sensitization results to morphine, in that BA-2 KOs are showing increases in sensitivity of the μ opiate receptor, which appears to be increasing their threshold to pain.

Cocaine is a drug in the psychostimulant class and has now been well characterized in its mode of action in the brain. Cocaine blocks the DA transporter with high affinity, meaning that it is a very effective drug at blocking reuptake of DA at the synapse. Increases in DA activity are typically positively correlated with increases in overall locomotor activity. When exposed to cocaine, mice were examined for increases in locomotor activity because increases in these behaviors are most commonly associated with psychostimulant administration in rodents (Bohn et al., 2004). When cocaine was administered to BA-2 KO mice, there was not an increase in locomotor activity but, in fact, a slight, but nonsignificant, decrease in response to acute cocaine administration (Bohn et al.; Gainetdinov et al., 2004). However, BA-2 KO mice demonstrated normal sensitization to chronic cocaine administration that was not significantly different from wild type controls (Gainetdinov et al.). The reduction in activity to initial cocaine administration could imply that knockout of β-arrestin 2 blocks the rewarding effects of acute cocaine; however, there is no current research on any other psychostimulant with BA-2 KOs.

The Dopamine Reward System: The Role of Dopamine Receptors

The pathway between the VTA and the NAcc is critical for behavioral responses to psychostimulant drugs (Di Chiara, 1999; Vezina, 2004). Studies have shown that the VTA/NAcc pathway mediates locomotor activity and drug self-administration of the psychostimulants nicotine, cocaine, amphetamine, and methamphetamine (Vezina, 2004). Additionally, ablation of either of these areas has been found to create a disruption in self-administration behavior of these drugs in animals (Jones & Wonnacott, 2004). It has been shown that the D1 receptor plays the more important role in locomotor sensitization to psychostimulant drugs. Vezina and Stewart (1989a) demonstrated that the D1 receptor antagonist SCH-23390 blocked sensitization to amphetamine but not morphine, while the D2 antagonist pimozide

blocked sensitization to morphine but not amphetamine. Bilateral injections of SCH-23390 into the VTA also blocked sensitization to amphetamine, resulting in the hypothesis that the D1 receptor plays the more important role in sensitization to psychostimulant drugs (Vezina & Stewart, 1989b). Other studies have shown the importance of the D1 receptor in locomotor sensitization to other psychostimulants, with the general conclusion being that the D1 receptor plays the more important role in locomotor sensitization to psychostimulant drugs (Harrod et al., 2004).

## Dopamine Reward System: Dopaminergic Pathways

The VTA sends a prominent dopaminergic projection to the NAcc which is comprised of two distinct subareas: the shell and the core (Di Chiara & Bassareo, 2007). The shell forms a crescent moon-shaped brain area around the anterior commisure in the basal forebrain and has also been referred to as the extended amygdala (Di Chiara, 2000). The amygdala is the emotional center in the brain, and the shell has been hypothesized to be associated with the emotional aspects of positive reinforcement, as this area sends and receives prominent projections to and from the amygdala and the prefrontal cortex. The core is located directly around the anterior commisure and is histologically similar to the ventral striatum. Based on the fact the striatum is an area that plays an important role in motor function, the core has been hypothesized to mediate the locomotor activating effects of positive reinforcement, including the locomotor activating effects of psychostimulant drugs.

Microdialysis studies examining differences in DA transmission in the shell versus the core have shown that the psychostimulant nicotine increases DA selectively in the shell, but to a reduced extent in the core (Di Chiara, 1999; Di Chiara, 2002). It has been hypothesized that the repeated stimulation of DA transmission in the NAcc shell enhances reward-based learning (Di

Chiara, 1999), and that DA transmission in the NAcc core mediates locomotor sensitization to psychostimulant drugs, which could signify that nicotine has stronger associative effects.

The prefrontal cortex (PFC) is a brain area that has been associated with knowledge and cognition and has been hypothesized to play a role in drug craving (Robinson & Berridge, 2003; Shu-Peng, Moon, Kim, & Myeong, 2004). The PFC, amygdala, and hippocampus are often referred to as NAcc-related circuitry and play an important role in modulating behavior relating to natural rewards (Robinson & Berridge). There have been several hypotheses surrounding the change from recreational drug use to addiction, and it is likely that the PFC plays a vital role in this transition. The *hedonic homeostasis hypothesis* centers on drug use as a means to achieve a "high" and continued use after addiction as a means to avoid withdrawal (Robinson & Berridge). It is important to consider that drug exposure over time becomes associated with particular cues because of its positive reinforcing characteristics. Researchers have posited the *aberrant learning hypothesis* which focuses more heavily on natural rewards. The roles played by NAcc-related circuitry in motivation and behavior could be increased by drugs of abuse and the learning mechanisms involved in addiction could contribute to impulsivity and relapse (Di Chiara & Bassareo, 2007; Robinson & Berridge). Interestingly, doses of nicotine that successfully stimulate DA transmission in the NAcc shell have not been shown to increase DA transmission in the medial PFC, an area where DA neurons terminate (Di Chiara, 1999).

## The Opiate System and its Role in Drug Reward

Three different types of opiate receptors have been discovered, and each of these receptors binds to different opiates (Feldman, Meyer, & Quenzer, 1997). Particularly relevant to this study is the μ-receptor, which has a high affinity for morphine. Binding to this receptor occurs in a variety of brain areas, many of which have been implicated in drug reward, including

the striatum, NAcc, amygdala, and hippocampus (Feldman et al.). The μ-receptor plays a major role in morphine analgesia and positive reinforcement associated with opiates.

## The Opiate System and its Effects on DA Release

Opiate receptors play a role in DA release by altering DA cell firing or acting in terminal areas (Feldman, Meyer, & Quenzer, 1997). Dopamine release from the VTA into the NAcc can be stimulated through DAMGO, a μ-opioid receptor agonist or DPDDE a δ-receptor agonist. Other opioid receptor agonists were showed to have no influence on DA levels (Feldman et al., 1997). Opiates can affect DA levels in the terminal areas when an agonist is infused directly into the NAcc. The μ-agonist have been shown to have no effect when infused directly into the NAcc, however, δ-receptor agonists did stimulate DA flow (Feldman et al.).

## Nicotine

Nicotine is an agonist at the nicotinic receptor. There are various types of nicotinic receptors in the central and peripheral nervous system (Pierce & Kumaseran, 2007). These subtypes are divided into two ligand binding sites, alpha (α) and beta (β), and receptors are made up of a combination of these subunits with the exception of the α7, which has no β subunit (Pierce & Kumaseran). In the brain, there are a high number of nicotinic receptors in the VTA, hippocampus, and frontal cortex. It is apparent that the high concentration of nicotinic-acetylcholine receptors (nAChRs) on the cell bodies of the VTA may contribute to dopaminergic neurotransmission in the NAcc, which has been implicated in the addictive properties of nicotine.

## The Effects of Nicotine on Dopaminergic Transmission

Laviolette and van der Kooy (2003) have shown that nicotine infusion directly into the VTA produces a wide range of effects on the rewarding properties of nicotine. Studies by Wonnacott (2000) and colleagues have shown that blockade of nAChR subtypes in the VTA via intrabrain infusion of nAChR antagonists blocks DA release in the NAcc and, in turn, blocks increases in locomotor sensitization to nicotine. Nicotine has also been shown to produce an increase in mood and arousal, decreased anxiety and appetite, improved attention, and cognitive enhancement (Cook, Spring, & McCharque, 2007; Pierce & Kumaseran, 2007; Rusted, Trawley, Heath, Kettle, & Walker, 2005). Like other addictive drugs, nicotine has effects on several different neurotransmitters. The nicotinic, dopaminergic, and serotonergic systems have all been implicated in nicotine addiction (Dani, 2003). In addition, nicotine also causes an increase in the number of nAChRs in several different brain areas (Hernandez & Terry, 2005; Ungless & Cragg, 2006). This increase in nAChRs in the brain could likely affect neurotransmission, as nAChRs are located both pre- and post-synaptically and have been shown to affect release of several different neurotransmitters, including DA, acetylcholine, serotonin (5-HT), glutamate, and GABA (Hernandez & Terry).

Nicotine is highly addictive because of the vast brain areas that are affected by this drug. Several major systems have been implicated in nicotine addiction (Dani & De Biasi, 2001). One system originates in the basal forebrain and projects to the cortex and hippocampus, whereas a second system originates in the tegmentum and projects to the thalamus, substantia nigra. A third system projects from the VTA to the NAcc and is affected by nicotine because it is an area rich in DA. (Dani & De Biasi). One of the primary pathways in which nicotine affects several neurotransmitters is through increasing calcium ionic influx in presynaptic terminals through its

action on presynaptically located nicotinic receptors. Nicotinic receptors have been shown to be presynaptically located on DA, 5-HT, and norepinephrine (NE) neurons. Thus, by acting on presynaptically located nicotinic receptors, it is thought this may be a primary mechanism through which nicotine acts to influence mood elevation and positive reinforcement

## Nicotine and the Opiate System

Opiates and psychostimulants act by either stimulating DA cell bodies or by stimulating DA synapses (Wise, 1987). Studies have examined the interaction between nicotine and the opiate system in animals and in humans. Krishnan-Sarin, Rosen, and O'Malley (1999) examined how the opiate system of adult nicotine users responded to the opioid antagonist naloxone. Nine smokers and 11 non-smokers were administered various levels of naloxone and watched for opiate-like withdrawal, nicotine craving, and changes in cortisol levels. Results showed that in smokers naloxone generated dose dependent increases in withdrawal symptoms with low doses of naloxone increasing nicotine craving and tiredness. Non-smokers also exhibited lower levels of cortisol in response to the drug (Krishnan-Sarin et al.).

In animal studies, Watkins, Stinus, Koob, and Markou (2000) examined the nAChR and opiate receptor role in withdrawal from nicotine. Animals were implanted with a mini-pump which administered nicotine or saline and the nicotinic antagonists mecamylamine, chlorisondamine, or dihyro-β-erythroidine or the opiate antagonist, naloxone, were administered subcutaneously. Conditioned aversion and threshold levels were measured. Animals receiving naloxone showed physical signs of reward and exhibited reward threshold only at high levels compared to the nicotinic antagonists. In contrast, naloxone produced conditioned aversion at significantly lower levels than needed to produce threshold. Therefore, the opiate system

appears to play a larger role in conditioned aversion related to nicotine withdrawal (Watkins, et al.).

## Nicotine Sensitization

Sensitization occurs when repeated exposure to a stimulus causes an increase in responding (Traynor & Neubig, 2005). Repeated exposures of psychostimulants have been found to cause an increase in behaviors associated with the drug (Byrnes & Wallace, 1997). In studies examining the relationship between nicotine and behavioral sensitization, it has been found that repeated injections of nicotine produces sensitization in rats after an initial hypoactive response to the drug (Domino, 2001; Schoffelmeer, De Vries, Wardeh, van de Ven, & Vanderschuren, 2002). For example, Domino examined three doses (.1, .32, and 1.0 mg/kg) in adult female rats. Animals receiving .1 mg/kg and .32 mg/kg demonstrated statistically significant increases in activity compared to controls after six days of drug administration. Animals that received a pretreatment of dexamethasone, a steroid hormone, before receiving 1.0 mg/kg of nicotine showed statistically higher activity levels than controls (Domino). In addition, the rewarding effects of nicotine have been found to be persistent. Studies have shown that the enhancing effects of nicotine are still present after a 2-week abstinence period (Levin, 1992).

## Adolescent vs. Adulthood Nicotine Exposure

There have been studies analyzing age differences in nicotine use, especially comparing adolescent to adult use. The reason for this is that adolescence is an age at which nicotine use often begins, with 80% of smokers beginning before age 18 (Collins, Wade, Ledon, & Izenwasser, 2004; Faraday, Elliott, & Grunberg, 2001). Findings have shown that nicotine dependence beginning in adolescence causes higher rates of dependence and increases in daily tobacco use, while decreasing the likelihood of cessation in adulthood (Collins et al.; Elliott,

Faraday, Phillips, & Grunberg, 2005; Faraday et al.). Animal studies on the effects of nicotine in adolescence have shown that chronic nicotine administration in adolescent male rats produced long-term hyperactivity that was fundamentally different than adults (Faraday et al.). Furthermore, male rats that were exposed to nicotine during adolescence were more sensitive to the activating effects of nicotine when it was administered in adulthood (Faraday, Elliott, Phillips, & Grunberg, 2003). Unlike male rats, nicotine did not produce long lasting hyperactivity in female rats (Elliott et al.). Additionally, there was a dose-response difference, in that adolescent female rats responded to nicotine by increasing activity at two different doses, .5 mg/kg and 1.0 mg/kg, whereas adult females only demonstrated increased activity to the .5 mg/kg dose (Elliott et al.).

Trauth, Seidler, and Slotkin (2000) examined the effects of nicotine exposure in adolescent rats by administering nicotine though a minipump from postnatal day (PN) 30 to PN 47.5, which corresponds with early and late adolescence in the rat. Results showed that adolescent exposure produces behavioral and neurochemical changes in adulthood. Female rats displayed a reduction in grooming during adolescent exposure and a reduction in locomotor activity and rearing behaviors 2-weeks after cessation, while male rats showed no differences (Trauth, Seidler, & Slotkin). Additionally, levels of DA and NE were activated, or increased, after adolescent nicotine exposure (Trauth, Seidler, Ali, & Slotkin, 2001). However, two criticisms of these studies are that a minipump was used to deliver nicotine, which means that the drug was constantly delivered throughout adolescence; second, the nicotine dosage used far exceeded clinically relevant levels.

It has been shown previously that repeated exposure to nicotine produces marked increases in DA release, serotonin transporter binding, serotonin release and reuptake, (Collins,

et al., 2004). One hypothesis for the difficulties in smoking cessation for adults whose dependence began in adolescence could be explained by permanent alterations in neurotransmitters that occur during adolescence. Supporting this hypothesis is research performed by Collins and colleagues (2004) which shows that male adolescent rats exposed to nicotine showed increases in DA transporter densities in the caudate putamen and NAcc while adult rats did not show changes in DA transporter densities in these areas. Changes in dendritic spine length in the NAcc have also been found in male adolescent rats (McDonald, et al, 2005). Animals that received nicotine had significantly longer dendrites and a significant increase in the number of segments than controls (McDonald, et al.). These results suggest changes in brain plasticity in response to adolescent nicotine exposure that may be mediating the long-term and persistent effects of nicotine in adulthood. However, Brown and Kolb (2001) also showed this in adult rats, so changes may not be a result of age differences but of age similarity.

## Neurotrophic Factors

Neurotrophic factors have been shown to mediate the growth of cells in development and regulate plasticity and survival in adult cells (Shoval & Weizman, 2005). Previous studies have shown that chronic drug exposure generates changes in brain structure that could lead to stimulant addiction. Nicotine has been shown to increase both nerve growth factors (NGF) and brain-derived neurotrophic factors (BDNF) in several brain areas including the hippocampus and frontal cortex (French et al., 1999; Kenny et al., 2000). BDNF has also been shown to be activity-dependent and significant increases in cell activity typically produce significant increases in BDNF (Hennigan, O'Callaghan, & Kelly, 2007).

## Statement of the Problem

β-arrestin-2 is a regulatory molecule in two systems that are involved with and play primary roles in addiction: the DA and opiate systems. When β-arrestin-2 is not present, because it is a regulatory molecule, it should increase sensitivity of these receptors. Based on the fact that β-arrestin-2 is co-localized with and regulates μ opiate receptor and DA D2 receptors, its absence should increase sensitivity of these receptors and increase responding to drugs that act as agonists in these systems. Nicotine is an addictive drug in the psychostimulant class, and its additive properties are mediated by the DA system. Nicotine is known to significantly increase DA release from the presynaptic terminal. Additionally, there have been age differences in nicotine effects on behavior, in that nicotine exposure in adolescence produces different behavioral responses and appears to produce an increased propensity to use nicotine in adulthood. This study will be designed to produce nicotine sensitization in a BA-2 KO mouse model. Only male mice will be studied, and all animals will be tested between 3-5 weeks of age, which is congruent with adolescence in the mouse. It is hypothesized that adolescent BA-2 KO mice will show decreased locomotor activity after nicotine administration compared to adolescent wild type mice.

# CHAPTER 2

# METHOD

## Experiment One

Thirteen male BA-2 KO mice and 18 male wild-type C57/B6 mice that were 3-4 weeks old at the beginning of testing were used as subjects. Mice were socially housed (2-3 per cage) in a climate-controlled vivarium with a 12 h light/dark cycle. Food and water were provided ad libitum. The mice were divided into two groups with the experimental group receiving nicotine and the control group saline. All injections were given in 1 mg/kg dose. The East Tennessee State University Committee on Animal Care approved all procedures in this study.

Habituation occurred 1 day before beginning behavioral testing. For habituation, all mice were given a subcutaneous (s.c.) injection of saline and placed in the locomotor activity chamber for 30 minutes. A grid of black lines covered the floor of the arena, and horizontal activity was scored by the number of grid line crossings made by each mouse during each testing session. Sensitization training began 1 day after habituation was complete. During sensitization testing, mice were given an s.c. injection of 0.5 mg/kg nicotine tartarate (free base) or saline every day. After each injection, mice were immediately placed into a locomotor chamber for 10 minutes of behavioral testing. Behavioral sensitization lasted for 7 days with testing occurring daily over this period. After the completion of sensitization testing, there was a 1-week abstinence period during which mice received no drug or saline injections. On day 7 after sensitization testing was complete, mice in the nicotine group were given a nicotine challenge in which mice in the nicotine group received an s.c. injection of nicotine and animals assigned to the saline group received an s.c. injection of saline. They were then placed in the locomotor chamber for 10 minutes and activity was recorded. Tissue was removed the following day for analysis.

The rationale for a challenge performed 7 days after sensitization testing is based on work by Kalivas and colleagues. A primary concern in understanding the neural underpinnings of nicotine-induced sensitization is the time at which behavioral and neurochemical measurements are obtained after discontinuing long-term drug treatment. It has been suggested that measurements taken during the first week after drug withdrawal may mask some of the biochemical alterations underlying behavioral sensitization (Kalivas & Duffy, 1993). For this reason, it has been suggested that it is most appropriate to measure changes in neural function following a drug challenge administered a week or more after discontinuing the drug administration regimen (Pierce & Kalivas, 1997). Therefore, in this experiment, a nicotine challenge was performed 1 week after repeated nicotine administration has ceased.

Experiment Two

Eight male BA-2 KOs and 21 male wild-type C57/B6 mice that were 3-4 weeks old at the beginning of testing were used as subjects. Mice were socially housed (2-3 per cage) in a climate-controlled vivarium with a 12 h light/dark cycle. Food and water were provided ad libitum. The mice were divided into two groups with the experimental group receiving nicotine and the control group saline. All injections were given in 1 mg/kg dose. The East Tennessee State University Committee on Animal Care approved all procedures in this study.

Habituation occurred 1 day before beginning behavioral testing. For habituation, all mice were given a subcutaneous (s.c.) injection of saline and placed in the locomotor activity chamber for 30 minutes, and activity was analyzed across three consecutive 10 min sessions, which acted as the repeated measure. A grid of black lines covered the floor of the arena, and horizontal activity was scored by the number of grid line crossings made by each mouse during each testing session. Sensitization training began 1 day after habituation was complete. During sensitization

testing, mice were given an s.c. injection of 0.5 mg/kg nicotine tartarate (free base) or saline every day. After each injection, mice were immediately placed into a locomotor chamber for 10 minutes of behavioral testing. Behavioral sensitization lasted for 2-weeks with testing occurring daily over a 14-day period. After the completion of sensitization testing, there was a 1-week abstinence period during which mice received no drug or saline injections. On day 7 after sensitization testing was complete, mice in the nicotine group were given a nicotine challenge in which mice in the nicotine group received an s.c. injection of nicotine and animals assigned to the saline group received an s.c. injection of saline. They were then placed in the locomotor chamber for 10 minutes and activity was recorded. Tissue was removed the following day for analysis.

## BDNF Analysis

Twenty-four hours after the nicotine challenge, brains were harvested and flash frozen in isopentane before being stored at -80°c. The nucleus accumbens and occipital cortex were dissected and homogenized in a RIPA cell lysis buffer. Following homogenization, tissue samples were analyzed using a BDNF $E_{max}$ Immunoassay system (Promega, Madison, WI). Optical density was measured using a 96-well Bio-Rad plate reader.

# CHAPTER 3

# RESULTS

## Experiment 1

The primary statistic used was the ANOVA, and all post hoc comparisons were performed using Fisher's LSD post hoc tests. The independent variables were genetic mutation (wild type or BA-2 KO), drug treatment (nicotine or saline), and day of treatment (7 days). The dependent variable was number of horizontal line crosses. Three different time points were analyzed: The habituation period, the 7-day sensitization training sessions, and the nicotine challenge. For habituation, a 2 x 2 x 3, three-way repeated measures ANOVA revealed a significant main effect of repeated measure ($F(1,59)=86.33$, $p<.001$). Both the BA-2 KO and wild-type mice demonstrated significant decrease in activity over the 30 min period, but there were no significant differences between wild types and BA-2 KO mice.

For sensitization training sessions (presented in Figure 2), a 2 x 2 x 7 three-way repeated measures ANOVA was used, with each of the 7 days of testing representing a separate level of the day of treatment repeated measures independent variable. This ANOVA revealed a significant main effect of both drug treatment ($F(1, 26) =4.65$, $p<.04$) and mutation ($F(1, 26) = 15.74$, $p<001$), and a significant two-way interaction of Drug Treatment x Day of Testing ($F(6, 26) =4.45$, $p<.001$). Nicotine produced an initial hypoactivity in the wild type controls, and over days produced a significant increased in horizontal activity in the wild type control mice to the levels of the saline-treated wild types. However, nicotine-induced hypoactivity in the BA-2 KO mice at Days 3 and 4 and BA-2 KOs demonstrated significant hypoactivity to wild types throughout testing. Thus, BA-2 KO mice demonstrate nicotine-induced decreases in locomotor activity.

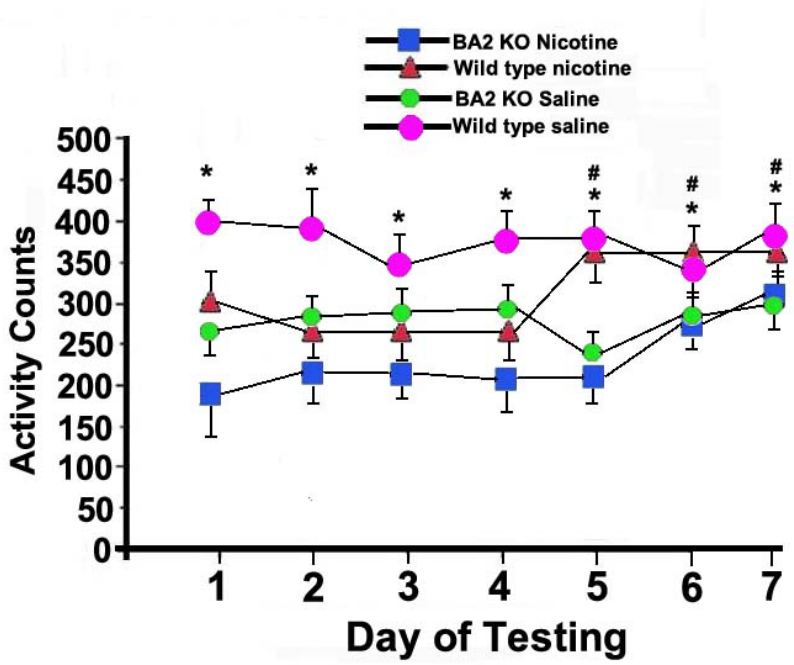


*Figure 2.* Seven-day drug training.

For the nicotine challenge (see Figure 3) a 2 x 2 two-way ANOVA revealed a significant main effect of mutation ($F(1, 27) = 17.771$, $p<.01$), and a significant interaction of Drug x Mutation ($F(1, 27) = 4.225$, $p<.04$). Nicotine induced hypoactivity in BA-2 KO mice relative to all other groups, replicating the nicotine-induced hypoactivity at Days 3 and 4 during sensitization training. Interestingly, nicotine induced a significant increase in activity in wild types on the challenge, whereas this did not occur during sensitization training. Finally, the BA-2 KO mutation induced an overall hypoactive response as compared to wild types.

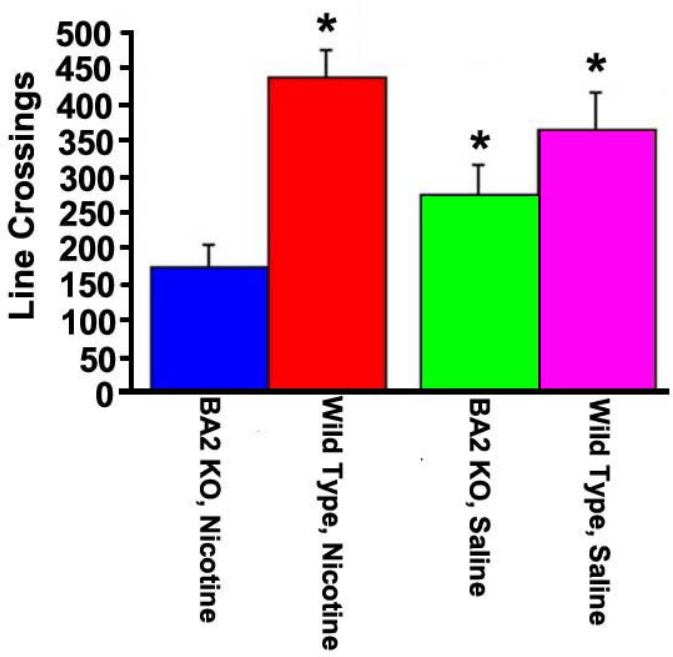


*Figure 3.* Experiment 1 nicotine challenge.

Experiment 2

The primary statistic used was the ANOVA, and all post hoc comparisons were performed using Fisher's LSD post hoc tests. The independent variables were genetic mutation (wild type or BA-2 KO), drug treatment (nicotine or saline), and day of treatment (14 days). The dependent variable was number of horizontal line crosses. Two different time points were analyzed: The 14-day sensitization training sessions and the nicotine challenge. For sensitization training, (see Figure 4) a 2x2x14 three-way repeated measures ANOVA revealed a significant main effect of day of testing ($F(13,21) = 3.93$, $p<.001$) and a significant two-way interaction of Genetic Mutation x Week of testing ($F(13,21) = 4.46$, $p<.001$). After 14 days of sensitization training, wild type controls did demonstrate significant increases in locomotor activity by days 13 and 14. Although BA-2 KOs did eventually sensitize to nicotine, this was not revealed until days 13 and 14 of sensitization training.

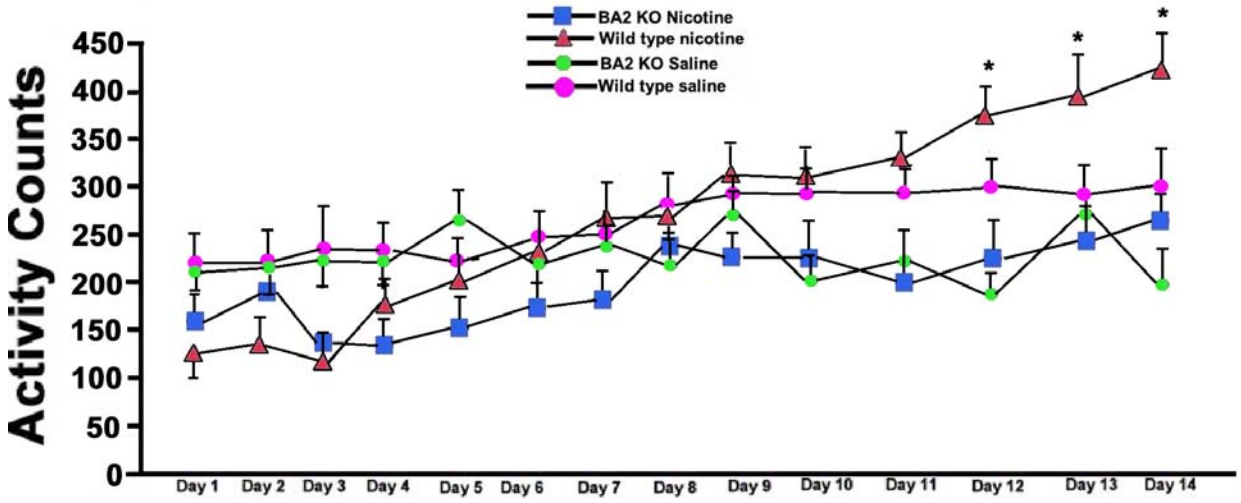


*Figure 4.* Fourteen-day sensitization training.

For the nicotine challenge (see Figure 5), a 2 x 2 ANOVA revealed a significant main effect of Genetic Mutation ($F(1, 22) = 15.95$, $p<.001$). BA-2 KO mice administered nicotine did not demonstrate a significant increase in activity and thus did not express sensitization to nicotine. Wild-type controls that were administered nicotine demonstrated a significant increase in activity after a 7-day abstention period, demonstrating that wild-type animals given nicotine demonstrate both induction and expression of nicotine sensitization.

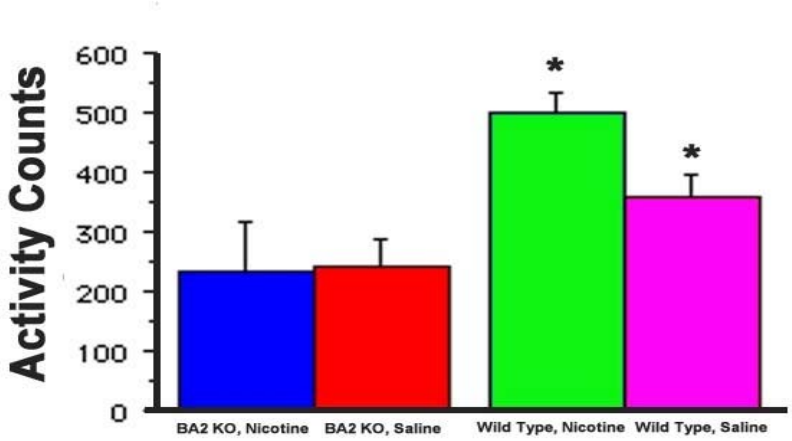


*Figure 5.* Nicotine challenge for 14-day sensitization training.

BDNF Analysis

In experiment one, a 2 x 2 ANOVA (see Figure 6) revealed a significant interaction of Genetic Mutation x Adolescent Drug Treatment ($F(1,13) = 5.82$, $p<.031$). Wild-type animals treated with nicotine demonstrated a significant increase of BDNF over all other groups. Nicotine appears to have produced a significant decrease of BDNF in the BA-2 KO group. Likewise, in Experiment two, a 2 x 2 ANOVA (see Figure 7) revealed a significant interaction of Genetic Mutation x Adolescent Drug Treatment ($F(1,13) = 5.82$, $p<.031$). Wild-type animals treated with nicotine demonstrated a significant increase of BDNF over all other groups. Nicotine appears to have produced a significant decrease of BDNF in the BA-2 KO group ($F(1, 15) = 4.73$, $p<.04$). Like Experiment one, wild-type controls treated with nicotine demonstrated a significant increase of BDNF over all other groups, and nicotine appears to have produced a significant decrease of BDNF in BA-2 KOs.

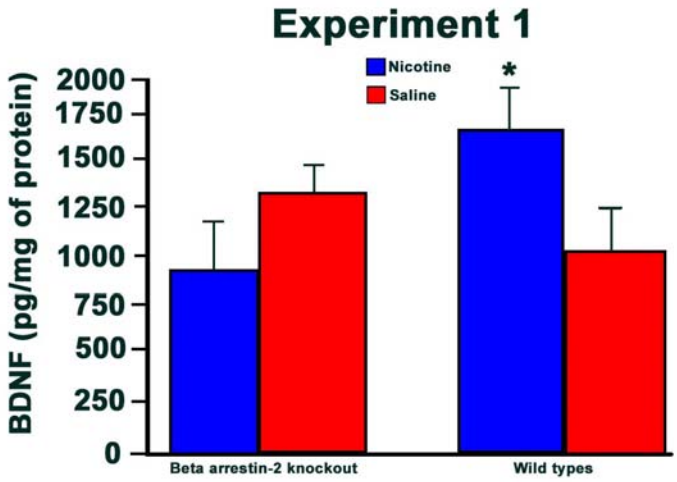


*Figure 6.* BDNF analysis for experiment 1.

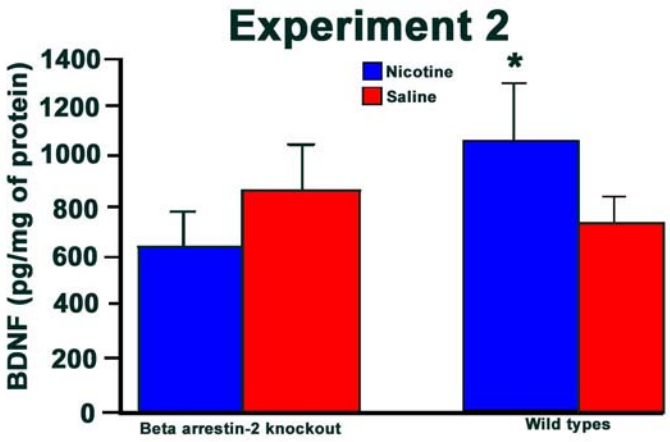


*Figure 7.* BDNF analysis for experiment 2.

# CHAPTER 4

# DISCUSSION

The purpose of the current study was to examine the effects of nicotine exposure on locomotor activity and NAcc BDNF in adolescent BA-2 KO mice. The results demonstrate that β-arrestin 2 is an important protein in nicotine locomotor sensitization because adolescent BA-2 KO mice administered nicotine did not reach activity levels of wild type controls administered nicotine. These results contrast with a past study examining the effects of cocaine on adult BA-2 KOs that showed no significant change in locomotor activity in response to cocaine, which is in the same drug family as nicotine (Bohn et al., 2004). The difference in results between the current study and that of Bohn and colleagues point to an important difference in the synaptic mechanism between cocaine and nicotine, as cocaine is primarily an inhibitor of the DA transporter, whereas nicotine increases presynaptic DA release and blocks the DA presynaptic autoreceptor (Wonnacott, Sidhpura, & Balfour, 2005). Based on the current results, it appears that β-arrestin-2 may play a more important role in sensitization to nicotine as compared to cocaine. Interestingly, BA-2 KOs did eventually sensitize to nicotine over a 14-day period, showing that absence of β-arrestin-2 *reduced* nicotine sensitization, but it did not *eliminate* sensitization to the drug. On the nicotine challenge in both experiments, wild-type controls given nicotine demonstrated expression of sensitization, whereas BA-2 KO mice did not express sensitization to nicotine. This result appears to show that although β-arrestin-2 plays a role in the induction of sensitization, but its absence blocks the expression of sensitization after an abstinence period. Finally, BDNF results showed that adolescent BA-2 KO mice receiving nicotine show a decrease in BDNF compared to all other groups, which appears to be congruent with their behavior on the nicotine challenge. This decrease in BDNF appears to support the

hypothesis that removal of β-arrestin 2 would produce decreased cellular response to nicotine, which may be due to one of several mechanisms. First, the lack of ability to remove the agonist from the receptor (receptor desensitization) could produce over-activity in the cell and eventually result in cell death; or it could be that the lack of β-arrestin 2 resulted in an overall decrease in cellular response due to its direct role in phosphorylation of the ion channel; or it could be that increasing activity of the D2 receptor, known to be an inhibitory receptor, may produce an overall decreased response to nicotine. At this point, clearly, much work is to be done to discover the mechanism underlying the effects of nicotine on BDNF in BA-2 KO mice. Impressively, nicotine did produce a significant increase in BDNF in both Experiment 1 and 2 in wild types even after a 7-day drug washout, and the effect of BDNF observed in BA-2 KOs was replicated across both experiments.

## Nicotine Sensitization

Nicotine sensitization occurs when an animal demonstrates an increased behavioral response to repeated administrations of nicotine. Nicotine is unique when analyzing locomotor sensitization in the psychostimulant drug class because an increase in the behavioral response to this drug occurs after an initial hypoactive response. Previous studies in both rats and mice have repeatedly shown that a period of hypoactivity is subsequently followed by a robust increase in locomotion that surpasses the activity levels of controls (Domino, 2001; Schoffelmeer, De Vries, Wardeh, van de Ven, & Vanderschuren, 2002). In the current study, it appears that mice demonstrated a more robust hypoactive response to nicotine than is typical in rats. In fact, wild-type mice receiving nicotine did not increase in activity to the levels of saline-treated wild-type controls until the 8th day of drug treatment in Experiment 2. Whether this is a species difference in locomotor behavior, or it reflects species differences in the dopaminergic system between

mice and rats is not known at this time. However, it can be assumed that the behavioral responses that are observed reflect neuronal changes in the brain in response to nicotine, particularly relating to the relationship between nicotine administration and increases in DA levels in the NAcc (Balfour, 2004; Di Chiara et al, 2004). Thus, we can infer that the persistent hypoactive response to nicotine may be due to a species difference in the way the NAcc responds to the drug. Additionally, a relatively high dose of nicotine was used in this study (0.5 mg/kg free base) and it may be that C57/B6 mice are more sensitive to nicotine than other strains of mice as well as rats, at least in the initial hypoactive response to the drug. There is evidence that mice respond to nicotine differently depending upon strain; however, these differences are not necessarily caused by differences in the nicotinic system (Overstreet, 1995; Robinson et al., 1996). Somewhat consistent with this hypothesis, C57BL/6 mice were the most sensitive to oral nicotine consumption (Robinson, Marks, & Collins); however, this study used a different route of administration than that used in the current study.

Pierce and Kalivas (1997) define the initiation of behavioral sensitization as events that lead to changes in the brain, whereas the expression of sensitization is the behavioral change from initiation that results in a behavioral response to the drug after a drug-free abstinence period. In Experiment 1, after 7 days of nicotine administration, both wild-types and BA-2 KOs receiving nicotine showed initiation of sensitization in that both groups increased activity from day 1 to day 7 of treatment. However, only wild types demonstrated expression of sensitization on the nicotine challenge. After 14 days of nicotine treatment, both wild types and BA-2 KOs again demonstrated initiation of sensitization in comparing day 1 to day 14 of activity, and both groups demonstrated an increase in activity relative to wild-type controls and BA-2 KOs that received saline. Results from the challenge showed that wild-type mice continued to express

sensitization to nicotine as in Experiment 1, but knockout mice did not. Therefore, it can be inferred that lacking β-arrestin 2 blocks expression of nicotine sensitization after an abstention period, indicating that the β-arrestin-2 protein is important in the persistent changes produced by nicotine in locomotor sensitization. This is particularly important because research has shown that nicotine receptor activation results in long-term expression of sensitization even after a relatively short period of nicotine administration (Miller et al., 2001). Therefore, it appears that the β-arrestin-2 protein plays an important role in the maintenance of sensitization to nicotine.

Based on the co-localization of β-arrestin-2 with D2 receptors, the current results also were informative relative to the role of the D1 and D2 receptors in nicotine sensitization. Past research has analyzed the role of the D1 and D2 receptor families and their involvement in nicotine locomotor sensitization. For example, systemic administration of the D1 antagonist SCH 23390 or the D2 antagonist spiperone has been shown to block the locomotor stimulant effect of nicotine infusions into the VTA or NAcc (Kita, Okamoto, & Nakashima, 1992). Additionally, both SCH 23390 and the D2 antagonist eticlopride have been shown to block nicotine sensitization when nicotine was administered intravenously (Sziraki, Sershen, Hashim, & Lajtha, 2002). However, there is very little information regarding DA antagonist effects on sensitization to systemic nicotine injections. Regarding other psychostimulants, the majority of evidence points to the importance of the D1, but not the D2 receptor, in sensitization to amphetamine (Vezina & Stewart, 1989a). However, in the current study, it appears that the D2 receptor plays a role in nicotine sensitization. Compared to wild-type controls administered nicotine, BA-2 KOs receiving nicotine demonstrated significantly lower levels of activity until they reached day 8 of treatment, but their level of activity did not increase any further throughout the 14 days of behavioral testing. Although BA-2 KOs demonstrated sensitization to nicotine, these animals did

not reach the activity levels of control animals as rapidly as wild types receiving nicotine, nor were they as active as wild-type controls by the end of testing. Because the D2 receptor has been shown to be an inhibitory receptor and β-arrestin 2 is a regulator of G-protein coupling at this receptor, it appears that absence of this protein may decrease the rewarding effects of nicotine, making it a possible clinical target for the treatment of nicotine addiction.

There have been other studies using different behavioral paradigms to study the roles of D1 and D2 receptors in nicotine's rewarding properties. For example, Spina and colleagues (2006) examined the roles of these receptors in nicotine CPP and used D1 and D2 antagonists by injecting them directly into the NAcc core and shell. Results showed that nicotine does generate a conditioned place preference using either a 0.4 or 0.6 mg/kg dose injected subcutaneously. The D1 antagonist SCH 39166 did not affect CPP when injected into the core but did alleviate nicotine-induced conditioned place preference when injected into the shell. The D2 antagonist L-sulpiride did not affect nicotine-induced CPP when injected into the shell. These results demonstrate the importance of the D1 receptors in the NAcc core and shell relative to the rewarding properties of nicotine.

## Nicotine and the μ-opioid Receptor

Although β–arrestin 2 has been shown to be co-localized with the D2 receptor, it has also been shown to be co-localized with the μ opiate receptor, which may be important in explaining the effects observed here. Past studies have shown an interaction between nicotine and opiate receptors, although nicotine is not in the opiate drug family. Nicotine has been shown to generate antinociception through activation of nAChRs, and it is believed that the μ-opioid receptor is involved in tolerance to nicotine (Galeote, Keiffer, Maldonado, & Berrendero, 2006). Because β-arrestin 2 is involved in both the DA and the opioid system, the μ-receptor may play a

role in other behavioral responses to nicotine. Galeote and colleagues examined this role in C57BL/6 mice as controls and μ-receptor knockout mice. Results showed that chronic nicotine treatment produced tolerance antinociception in both wild types and in knockouts; however, tolerance developed faster in the μ-receptor knockouts than in controls (Galeote et al), and μ receptors down regulated in the basal ganglia, demonstrating that the mu receptor plays a role in the development of tolerance to nicotine's antinociceptive effects, and opiate antagonists such as naloxone have been shown to block this effect (Campbell, Taylor, & Tizabi, 2007). Additionally, other studies have shown that pretreatment with the opiate antagonist naloxone can block nicotine-induced conditioned place preference (Zarrindast et al. 2003), and in μ opioid receptor knockout mice nicotine sensitization has been shown to be reduced (Yoo et al., 2004). It appears that the μ receptor may play a mediating role in nicotine sensitization, thus, knockout of the β-arrestin 2 protein may enhance μ opiate signaling. It remains that increases of μ opiate receptor signaling would produce an increased, not decreased, locomotor response to nicotine. On the other hand, it has been shown that β-arrestin-2 is important in signal transduction, meaning that β-arrestin 2 can be involved in direct phosphorylation of the ion channel in the signal transduction pathway, and this may be a key point in explaining the role of β-arrestin 2 in nicotine sensitization.

## BDNF Analysis

Research has shown that neurotrophic factors play a role in addiction and positively correlate with increased locomotor activity after either amphetamine or cocaine administration (Tsai, 2007), but there is not any information as to whether nicotine induces increases in BDNF that correlate with increases in activity produced by nicotine. As hypothesized, in the current study, adolescent BA-2 KO mice that received nicotine demonstrated a decrease in NAcc BDNF

compared to all other groups. Control animals receiving nicotine showed an increase in BDNF that is somewhat consistent with the literature examining BDNF in rats, as past research has shown an increase in the genetic expression of BDNF after chronic nicotine treatment, although this effect was shown within 48 hours of drug administration using higher doses than that used in the present study (French et al., 1999; Fumagalli et al., 2004). Research in rats has shown that even a single dose of cocaine can result in an increase in BDNF and chronic administration can produce increase in BDNF in the PFC (Le Foll, Diaz, & Sokoloff, 2005). Rats infused with BDNF in the NAcc or VTA have shown demonstrated an enhanced response to cocaine when compared to rats that were not infused with BDNF (Horger et al., 1999). This result points to the importance of the relationship between psychostimulants and BDNF. In the current study it can be inferred that there was a significant decrease in BDNF because the absence of the β-arrestin 2 protein prevented long-term reward from nicotine. Whether BDNF plays a direct or modulating role in the rewarding effects of nicotine is not known, but based on the fact that BDNF is important in synaptic maintenance and development and the changes observed were at least congruent with the behavioral response, certainly implies BDNF may play a role in nicotine drug reward.

## Adolescent Nicotine Exposure

There has been very little research performed on β-arrestin 2 and its role in psychostimulant sensitization. In the current study, adolescent C57/B6 mice appear to be different from adolescent rats in that the wild-type controls did not sensitize to nicotine during 7 days of nicotine administration, a finding that has been consistent in the literature examining the effects of nicotine in adolescent rats (Collins, Montano, & Izenwasser, 2004; Collins, Wade, Ledon, & Izenwasser, 2004; Domino, 2001; Wilmouth & Spear, 2006).

Research has shown that adolescent mice exposed to nicotine show differences in behavioral responses in early, middle, and late adolescent mice (Adriani et al., 2004). Adolescent CD-1 mice allowed access to oral nicotine showed a decrease in cortisol levels after nicotine consumption and showed a preference for nicotine when given free choice between water and nicotine (Adriani, Macri, Pacifici, & Laviola, 2002a). Furthermore, later research examining differences between early, late, and middle adolescent CD-1 mice demonstrated that mice showed a preference for nicotine in early adolescence, no greater preference for water or nicotine during middle adolescence, and an avoidance of nicotine during late adolescence. When nicotine concentrations were reduced, only early adolescent mice increased their nicotine consumption to compensate (Adriani, Marci, Pacifici, & Laviola, 2002b). Studies examining the effects of adolescent nicotine exposure on subsequent adult cocaine exposure have found that repeated nicotine administration causes a decrease in cocaine's rewarding effects as measured by CPP, but an increase in its locomotor activating effects (Kelley & Rowan, 2004). Thus, it appears that adolescent nicotine exposure can produce persistent changes in adult brain function that may be important in predicting addiction to psychostimulants in adulthood.

Klein and colleagues (2004) examined the effects of oral nicotine consumption in adolescent C57BL/6J mice. Six doses were used and mice were allowed access to saccharin solution and one of the six doses for 7 days. Males and females were assessed for weight gain, food consumption, fluid intake, and voluntary nicotine consumption. Results showed that animals receiving nicotine showed no gender differences in weight gain, food consumption, or fluid intake. However, female adolescent mice consumed more nicotine than males when oral nicotine consumption was taken into account as a percentage of fluid intake (Klein, et al). Although only males were used in the current study and nicotine was administered by the

experimenter and not administered voluntarily, it can be inferred that male and female mice would demonstrate different behavioral responses to nicotine when administered subcutaneously.

Kota and colleagues (2007) examined the effects of nicotine in adolescent and adult male IRC mice on analgesia, locomotor activity, CPP, and withdrawal. Results showed that adolescent and adult animals differed in all areas examined. Adolescent mice demonstrated CPP at lower doses than adults (.05 and .01 mg/kg as compared to .7 and 1.0 mg/kg), fewer physical signs of withdrawal, and lower activity levels than adults. Adolescents were also less sensitive to tail-flick testing and demonstrated a higher tolerance during hot plate testing than adults. These results suggest that adolescent IRC mice demonstrate a higher sensitivity to nicotine than adults. However, like BA-2 KOs receiving nicotine, adolescent IRC mice demonstrated lower levels of activity demonstrating that not only may there be species differences but strain differences as well.

## Limitations of the Current Study

One limitation of the current study is that wild-type mice receiving nicotine did not sensitize in 7 days of treatment, which has been shown in the literature (Collins, Montano, Izenwasser, 2004; Collins, Wade, Ledon, & Izenwasser, 2004; Domino, 2001; Wilmouth & Spear, 2006). However, it is unclear if this finding is related to strain differences because previous research has shown that this particular strain of mice is more sensitive to nicotine (Robinson, Marks, & Collins, 1996). Therefore, this finding could be related to sensitivity of this strain and dosing could have been too high or to low to induce sensitization in 7 days. Also, the current study used a relatively low number of BA-2 KOs and increasing the number of BA-2 KOs used in the study would be beneficial to learning more about locomotor behavior and protein expression in the brain in these animals. Furthermore, only males were used in the

current study and research has shown that males and females respond differently to nicotine (Klein et al., 2004). Examining sex differences in BA-2 KOs could provide insight into fundamental differences between males and females that can affect response to nicotine.

## Future Studies

In light of the limited amount of research examining the effects of nicotine on locomotor sensitization in adolescent mice, and particularly in BA-2 KO mice, future research could examine a wide range of areas. Examining dose-response in BA-2 KO mice would be beneficial as the current study only used one dose. A dose-response curve could provide valuable information surrounding how activity changes based on the amount of nicotine administered. Furthermore, various doses could be given during early, middle, and late adolescence to determine how sensitivity to nicotine changes throughout adolescence.

Self-administration of nicotine could also provide valuable information about the mechanisms surrounding DA transmission and nicotine dependence and withdrawal in the absence of β-arrestin 2. Self-administration could examine if BA-2 KOs would become dependent on nicotine and increase self-administration overtime. This is important to understanding more about how β-arrestin 2 contributes to reward because it can be inferred that BA-2 KOs would only self-administer nicotine if there was a positive benefit from the drug. Because previous research has shown that some strains of adolescent mice do not demonstrate withdrawal symptoms, it would be important to determine if symptoms of withdrawal would be present in BA-2 KOs if access to nicotine ceased.

## Conclusions

The current study provides valuable information about the role of β-arrestin 2 in nicotine sensitization. β-arrestin 2 is important in G-protein regulation, signal transduction, and receptor desensitization in both and DA and opiate function. Adolescent nicotine use generates not only nicotine dependence but also increases consumption and makes cessation in adulthood more difficult. The results of the current study point to β-arrestin 2 as playing an important role in the neural adaptations related to nicotine administration, and it appears that knocking out the β-arrestin 2 protein reduces the activating effects of nicotine in animals. This finding is important as it points to the importance of the β-arrestin 2 protein in nicotine addiction, and this study is the first in what hopefully will be a series of studies analyzing the role of this protein in addiction. Finally, it is clear there is an interaction between the β-arrestin 2 protein and brain plasticity via BDNF in the NAcc, and future studies will be designed to analyze the mechanisms of this important interaction.

REFERENCES

Adriani, W., Granstrem, O., Macri, S., Izykenova, G., Dambinova, S., & Laviola, G. (2004). Behavioral and neurochemical vulnerability during adolescence in mice: Studies with nicotine. *Neuropsychopharmacology, 29*, 869-878.

Adriani, W., Macri, S., Pacifici, R, & Laviola, G. (2002a). Peculiar vulnerability to nicotine oral self-administration in mice during early adolescence. *Neuropsychopharmacology, 27*, 212-224.

Adriani, W., Macri, S., Pacifici, R, & Laviola, G. (2002b). Restricted daily access to water and voluntary nicotine oral consumption in mice: Methodological issues and individual differences. *Behavioral Brain Research, 134*, 21-30.

Balfour, D.J. (2004). The neurobiology of tobacco dependence: A preclinical perspective on the role of the dopamine projections to the nucleus accumbens. *Nicotine and Tobacco Research, 6*, 899-912.

Barnes, M., Lawford, B.R., Burton, S.C., Heslop, K.R., Noble, E.P., Hausdorf, K, et al. (2006). Smoking and schizophrenia: Is symptom profile related to smoking and which antipsychotic medication is of benefit in reducing cigarette use? *The Australian and New Zealand Journal of Psychiatry, 40*, 575-580.

Beaulieu, J.M., Sotnikova, T.D., Marion, S., Lefkowitz, R.J., Gainetdinov, R.R., & Caron, M.G. (2005). An Akt/β-arrestin 2/PP2A signaling complex mediates dopaminergic neurotransmission and behavior. *Cell, 122*, 261-273.

Bevins, R.A., Eurek, S., & Besheer, J. (2005). Timing of conditioned responding in a nicotine locomotor conditioning preparation: Manipulations of the temporal arrangement between context cues and drug administration. *Behavioral Brain Research, 159*, 135-143.

Bibb, J.A. (2005). Decoding dopamine signaling. *Cell, 122*, 153-155.

Bohn, L.M., Gainetdinov, R.R., & Caron, M.G. (2004). G protein-coupled receptor kinase/β-arrestin systems and drug abuse. *NeuroMolecular Medicine, 5*, 41-50.

Bohn, L.M., Gainetdov, R.R., Sotnikova, T.D., Medvedev, I.O., & Lefkowitz, R.J. (2003). Enhanced rewarding properties of morphine, but not cocaine, in βarrestin-2 knockout mice. *The Journal of Neuroscience, 23*, 10265-10273.

Breslau, N., & Peterson, E.L. (1996). Smoking cessation in young adults: Age at initiation of cigarette smoking and other suspected influences. *American Journal of Public Health, 86*, 214-220.

Brown, R.W., & Kolb, B. (2001). Nicotine sensitization increases dendritic length and spine density in the nucleus accumbens and cingulate cortex. *Brain Research, 899*, 94-100.

Brown, R.W., Thompson, K.D., Thompson, K.N., Ward, J.J., Thacker, S.K., Williams, M.T., et al. (2004). Adulthood nicotine treatment alleviates behavioural impairments in rats neonatally treated with quinpirole: Possible roles of acetylcholine function and neurotrophic factor expression. *European Journal of Neuroscience, 19*, 1634-1642.

Brynes, J.J., & Wallace, L.J. (1997). Amphetamine-induced sensitization and release of dopamine in slices from the ventral tegmental area of rats is enhanced following administration of cholera toxin into the ventral tegmental area. *Neuroscience Letters, 223*, 45-48.

Campbell, V.C., Taylor, R.E., & Tizabi, Y. (2007). Effects of selective opioid receptors receptor antagonists on alcohol-induced and nicotine-induced antinociception. *Alcoholism: Clinical and Experimental Research*,

Cohen, C., Perrault, G., Griebel, G., & Soubrie, P. (2005). Nicotine-associated cues maintain nicotine-seeking behavior in rats several weeks after nicotine withdrawal: Reversal by the cannabinoid (CB1) receptor antagonist, rimonabant (SR141716). *Neuropsychopharmacology, 30*, 145-155.

Collins, S.L., Montano, R., & Izenwasser, S. (2004). Nicotine treatment produces persistent increases in amphetamine-stimulated locomotor activity in periadolescent male but not female or adult male rats. *Brain Research. Developmental Brain Research, 153*, 175-187.

Collins, S.L., Wade, D., Ledon, J., & Izenwasser, S. (2004). Neurochemical alterations produced by daily nicotine exposure in periadolescent vs. adult male rats. *European Journal of Pharmacology, 502*, 75-85.

Cook, J.W., Spring, B., & McChargue, D. (2007). Influence of nicotine on positive affect in anhedonic smokers. *Psychopharmacology*, *192,* 87-95.

Dani, J.A. (2003). Roles of dopamine signaling in nicotine addiction. *Molecular Psychiatry*, *8*, 255-6.

Dani, J.A., & De Biasi, M. (2001). Cellular mechanisms of nicotine addiction. *Pharmacology, Biochemistry, & Behavior, 70*, 439-446.

Dewire, S.M., Ahn, S., Lefkowitz, R.J., & Shenoy, S.K. (2007). Beta-arrestins and cell signaling. *Annual Review of Physiology, 69*, 483-510.

Di Chiara, G. (1999). Drug addiction as dopamine-dependent association learning disorder. *European Journal of Pharmacology, 375*, 13-30.

Di Chiara, G. (2000).Role of dopamine in the behavioural actions of nicotine related to addiction. *European Journal of Pharmacology, 393*, 295-314.

Di Chiara, G. (2002). Nucleus accumbens shell and core dopamine: Differential role in behavior and addiction. *Behavioral Brian Research, 137*, 75-114.

Di Chiara, G., & Bassareo, V. (2007). Reward system and addiction: What dopamine does and doesn't do. *Current Opinion in Pharmacology*, *7*, 69-76.

Di Chiara, G., Bassareo, V., Fenu, S., De Luca, M.A., Spina, L., Cadoni, C., et al. (2004). Dopamine and drug addiction: The nucleus accumbens shell connection. *Neuropharmacology*, *47*, 227-241.

Domino, E.F. (2001). Nicotine induced behavioral locomotor sensitization. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, 25, 59-71.

Downey, K.K., & Kilbey, M.M. (1995). Relationship between nicotine and alcohol expectancies and substance dependence. *Experimental and Clinical Psychopharmacology, 3*, 174-182.

Elliott, B.M., Faraday, M.M., Phillips, J.M., & Grunberg, N.E. (2005). Adolescent and adult female rats differ in sensitivity to nicotine's activity effects. *Pharmacology, Biochemistry, and Behavior, 80*, 567-575.

Erhardt, S., Schwieler, L., & Engberg, G. (2002). Excitatory and inhibitory responses of dopamine neurons in the ventral tegmental area to nicotine. *Synapse, 43,*227-237.

Faraday, M.M., Elliott, B.M., & Grunberg, N.E. (2001). Adults vs. adolescents rats differ in biobehavioral response to chronic nicotine administration. *Pharmacology, Biochemistry, and Behavior, 70*, 475-489.

Faraday, M.M., Elliott, B.M., Phillips, J.M., & Grunberg, N.E. (2003). Adolescents and adults males differ in sensitivity to nicotine's activity effects. *Pharmacology, Biochemistry, and Behavior, 74*, 917-931.

Feldman, R.S., Meyer, J.S., & Quenzer. L.F. (1997). Catecholamines. *Principles of Neuropsychopharmacology.* Sunderland, MA: Sinauer Associates. 277-344.

Fonder, M.A., Sacco, K.A., Termine, A., Boland, B.S., Seyal, A.A., Dudas, M.M., et al. (2005). Smoking cue reactivity in schizophrenia: Effects of a nicotinic receptor antagonist. *Biological Psychiatry, 57*, 802-808.

French, S.J., Humby, T., Horner, C.H., Sofroniew, M.V., & Rattray, M. (1999). Hippocampal neurotrophin and trk receptor mRNA levels are altered by local administration of nicotine carbachol, and pilocarpine. *Brain Research. Molecular Brain Research, 67*, 124-136.

Fumagalli, F., Bedogni, F., Perez, J., Racagni, G., & Riva, M. (2004). Corticostrial brain-derived neurotrophic factor dysregulation in adult rats following prenatal stress. *European Journal of Neuroscience, 20*, 1348-1354.

Gainetdinov, R.R., Premont, R.T., Bohn, L.M., Lefkowitz, R.J., & Caron, M.G. (2004). Desensitization of G protein-coupled receptors and neuronal functions. *Annual Review of Neuroscience, 27*, 107-144.

Galeote, L., Keiffer, B.L., Maldonado, R., & Berrandero, F. (2006). Mu-opioid receptors are involved in tolerance to nicotine antinociception. *Journal of Neurochemistry, 97*, 416-423.

Grady, E.F. (2007). β-Arrestin a two fisted terminator. *Science, 315*, 605-606.

Gurpequi, M., Martinez-Ortega, J.M., Jurado, D., Aquilar, M.C., Diaz, F.J., & de Leon, J. (2007). Subjective effects and the main reason for smoking in outpatients with schizophrenia: A case-control study. *Comprehensive Psychiatry, 48*, 186-191.

Hack, S.P., Bagley, E.E., Chieng, B.C.H., & Christie, M.J. (2005). Induction of δ-opioid receptor function in the midbrain after chronic morphine treatment. *The Journal of Neuroscience, 25*, 3192-3198.

Harrod, S.B., Mactutus, C.F., Bennett, K., Hasselrot, U., Wu, G., Welch, M., et al. (2004). Sex differences and repeated intravenous nicotine: Behavioral sensitization and dopamine receptors, *Pharmacology, Biochemisty, and Behavior, 78*, 581-592.

Hennigan, A., O'Callaghan, R.M., & Kelly, A.M. (2007). Neurotrophins and their receptors: Roles in plasticity, neurodegeneration, and neuroprotection. *Biochemical Society Transactions, 35*, 242-7.

Hernandez, C.M., & Terry, A.V. (2005). Repeated nicotine exposure in rats: Effects on memory function, cholinergic markers, and nerve growth factor. *Neuroscience, 130*, 997-1012.

Horger, B.A., Iyasere, C.A., Berhow, M.T., Messer, C.J., Nestler, E.J., & Taylor, J.R., (1999). Enhancement of locomotor activity and conditioned reward to cocaine by brain-derived neurotrophic factor. *The Journal of Neuroscience, 19*, 4110-4122.

Jones, I.W., & Wonnacott, S. (2004). Precise localization of a7 nicotinic acetylcholine receptors on glutamatergic axon terminals in the rat ventral tegmental area. *The Journal of Neuroscience, 24*, 11244-11252.

Kalivas P.W., & Duffy P. (1993). Time course of extracellular dopamine and behavioral sensitization to cocaine: Dopamine axon terminals. *Journal of Neurosci*ence *13*, 266-275.

Kelley, B.M., & Rowan, J.D. (2004). Long-term, low-level adolescent nicotine exposure produces dose-dependent changes in cocaine sensitivity and reward in adult mice. *International Journal of Developmental Neuroscience, 22*, 339-348.

Kenny, P.J., File, S.E., & Rattray, M. (2000). Acute nicotine decreases and chronic nicotine

increases the expression of brain-derived neurotrophic factor mRNA in rat hippocampus. *Brain Research. Molecular Brain Research, 85*, 234-238.

Kita, T., Okamoto, M., & Nakashima, T. (1992). Nicotine-induced sensitization to ambulatory stimulant effect produced by daily administration into the ventral tegmental area and the nucleus accumbens in rats. *Life Science, 50*, 583-590.

Kolb, B., & Whishaw, I.Q. (2001). How do neurons communicate? *An Introduction to Brain and Behavior.* New York: Worth, 152-189.

Krishnan-Sarin, S., Rosen, M.I., & O'Malley, S.S. (1999). Naloxone challenge in smokers: Preliminary evidence of an opioid component in nicotine dependence. *Archives of General Psychiatry, 56*, 663-8.

Laudet, A.B., Magura, S., Vogel, H.S., & Knight, E.L. (2004). Perceived reasons for substance misuse among persons with psychiatric disorder. *The American Journal of Orthopsychiatry, 74*, 365-375.

Laviolette, S.R., & van der Kooy, D. (2003). Blockade of mesolimbic dopamine transmission dramatically increases sensitivity to the rewarding effects of nicotine in the ventral tegmental area. *Molecular Psychiatry, 8*, 50-9.

Lazev, A.B., Herzog, T.A., & Brandon, T.H. (1999). Classical conditioning of environmental cues to cigarette smoking. *Experimental and Clinical Psychopharmacology, 7*,56-63.

LeDuc, P.A., & Mittleman, G. (1995). Schizophrenia and psychostimulant abuse: A review and re-analysis of clinical evidence. *Psychopharmacology (Berlin), 121*, 407-427.

Le Foll, B., & Goldberg, S.R. (2005). Control of reinforcing effects of nicotine by associated environmental stimuli in animals and humans. *TRENDS in Pharmacological Sciences, 26*, 287-293.

Le Foll, B., Diaz, J., & Sokoloff, P. (2005). A single cocaine exposure increases BDNF and D3 receptor expression: Implications for drug conditioning. *Neuroreport, 16*, 175-178.

Lefkowitz, R.J., & Shenoy, S.K. (2005). Transduction of receptor signals by β-arrestins. *Science, 308*, 512-517.

Levin, E.D. (1992). Nicotinic systems and cognitive function. *Psychopharmacology, 108*, 417-431.

Ma, L., & Pei, G. (2007). Beta-arrestin signaling and regulation of transcription. *Journal of Cell Science, 120*, 213-218.

McDonald, C.G., Dailey, V.K., Bergstrom, H.C., Wheeler, T.L., Eppolito, A.K., Smith, L.N., et al., (2005). Periadolescent nicotine administration produces enduring changes in dendritic morphology, of medium spiny neurons from nucleus accumbens. *Neuroscience Letters, 385*, 163-167.

Meyer, R.E. (1986). How to understand the relationship between psychopathology and addictive disorders: Another example of the chicken and the egg. In R.E. Meyer (Ed.) *Psychopathology and Addictive Disorders*, pp. 3-16. New York, NY: Guilford Press.

Miller, D.K., Wilkins, L.H., Bardo, M.T., Crooks, P.A., & Dwoskin, L.P. (2001). Once weekly administration of nicotine produces long-lasting locomotor sensitization in rats via a nicotinic receptor-mediated mechanism. *Psychopharmacology, 156*, 469-476.

Mogenson, G.J., & Yang, C.R. (1991). The contribution of basal forebrain to limbic-motor integration and the mediation of motivation to action. *Adv. Exp. Med. Biol*, 295, 267-290.

Overstreet, D.H. (1995). Differential effects of nicotine in inbred and selectively bred rodents. *Behavior Genetics, 25*, 179-185.

Parker, L.A. (1992). Place conditioning in a three-or four-choice apparatus: Role of stimulus novelty in drug-induced place conditioning. *Behavioral Neuroscience, 106*, 294-306.

Pierce R. C., & Kalivas P. W. (1997).A circuitry model of the expression of behavioral sensitization to amphetamine-like psychostimulants. *Brain Research Review, 25,* 192-216.

Pierce, R.C., & Kumaresan, V. (2007). The mesolimbic dopamine system: The final common pathway for the reinforcing effect of drugs of abuse? *Neuroscience and Behavioral Reviews*, *30*, 215-238.

Raehal, K.M., Walker, J.L., & Bohn, L.M. (2005). Morphine side effects in β-arrestin 2 knockout mice. *Journal of Pharmacology and Experimental Therapeutics, 314*, 1195-1200.

Robinson, T.E., & Berridge, K.C. (2003). Addiction. *Annual Review of Psychology*, *54*, 25-53.

Robinson, S.F., Marks, M.J., & Collins, A.C. (1996). Inbred mouse strains vary in oral self-selection of nicotine. *Psychopharmacology, 124*, 332-339.

Rusted, J.M., Trawley, S., Heath, J., Kettle, G., & Walker, H. (2005). Nicotine improves memory for delayed intentions. *Psychopharmacology, 182*, 355-365.

Schindler, C.W., & Carmona, G.N. (2002). Effects of dopamine agonists and antagonists on locomotor activity in male and female rats. *Pharmacology, Biochemistry, and Behavior, 72*, 857-863.

Schoffelmeer, A.M., De Vries, T.J., Wardeh, G., van de Ven, W.M., & Vanderschuren, J.M.J. (2002). Psychostimulant-induced behavioral sensitization depends on nicotinic receptor activation. *The Journal of Neuroscience, 22*, 3269-3276.

Shoval, G., & Weizman, A. (2005). The possible role of neurotrophins in the pathogenesis and therapy of schizophrenia. *European Neuropsychopharmacology: The Journal of the European College of Neuropsychopharmacology, 15,* 319-329.

Sorg, B.A., Li, N., & Wu, W.R. (2001). Dopamine D1 receptor activation in the medial prefrontal cortex prevents the expression of cocaine sensitization. *The Journal of Pharmacology and Experimental Therapeutics, 297*, 501-508.

Spina, L., Fenu, S., Longoni, R., Rivas, E, & Di Chiara, G. (2006). Nicotine-conditioned single trial place preference: Selective role of nucleus accumbens shell dopamine D1 receptors in acquisition. *Psychopharmacology, 184*, 447-455.

Sziraki, I., Sershen, H., Hashim, A., & Lajtha, A. (2002). Receptors in the ventral tegmental area mediating nicotine-induced dopamine release in the nucleus accumbens. *Neurochemical Research, 27*, 253-261.

Trauth, J.A., Seidler, F.J., Ali, S.F., & Slotkin, T.A. (2001). Adolescent nicotine exposure produces immediate and long-term changes in CNS noradrenergic and dopaminergic function. *Brain Research, 892*, 269-280.

Trauth, J.A., Seidler, F.J., & Slotkin, T.A. (2000). Persistent and delayed behavioral changes after nicotine treatment in adolescent rats. *Brain Research, 880*, 167-172.

Traynor, J.R., & Neubig, R.R. (2005), Regulators of protein signaling. *Molecular Invention, 5*, 30-41.

Tsai, S.J. (2007). Increased central brain-derived neurotrophic factor activity could be a risk for substance abuse: Implications for treatment. *Medical Hypotheses, 68*, 410-414.

Ungless, M.A., & Cragg, S.J. (2006). A choreography of nicotinic receptors directs the dopamine neuron routine. *Neuron, 50*, 815-816.

Vanable, P.A., Carey, M.P., Carey, K.B., & Maisto, S.A. (2003). Smoking among psychiatric outpatients: Relationship to substance use, diagnosis, and illness severity. *Psychology of Addictive Behaviors, 17*, 259-265.

Vezina, P. (2004). Sensitization of dopamine neuron reactivity and self-administration of psychomotor stimulant drugs. *Neuroscience and Biobehavioral Reviews, 27*, 827-839.

Vezina, P., & Stewart, J. (1989a). The effect of dopamine receptor blockade on the development of sensitization to the locomotor activating effects of amphetamine and morphine. *Brain Research, 499*, 108-120.

Vezina, P., & Stewart, J. (1989b). Microinjections of Sch-23390 into the ventral tegmental area and substantia nigra pars reticulata attenuate the development of sensitization to the locomotor activating effects of systemic amphetamine. *Brain Research, 495*, 401-406.

Watkins, S.S., Stinus, L., Koob, G.F., & Markou, A. (2000). Reward and somatic changes during precipitated nicotine withdrawal in rats: Centrally and peripherally mediated effects. *Journal of Pharmacology and Experimental Therapeutics, 292*, 1053-64.

Wilmouth, C.E., & Spear, L.P. (2006). Withdrawal from chronic nicotine in adolescence and adult rats. *Pharmacology, Biochemistry, and Behavior, 85*, 648-657.

Wise, R.A. (1987). The role of reward pathways in the development of drug dependence. Pharmacology Therapy, 35, 227-63.

Wonnacott, S., Kaiser, S., Mogg, A., Soliakov, L., & Jones, I.W. (2000). Presynaptic nicotinic receptors modulating dopamine release in the rat striatum. *European Journal of Pharmacology, 393*, 51-58.

Wonnacott, S., Sidhpura, N., & Balfour, D.J. (2005). Nicotine: From molecular mechanisms to behavior. *Current Opinion in Pharmacology, 5*, 53-59.

Yoo. J.H., Lee, S.Y., Loh, H.H., Ho, I.K., & Jang, C.G. (2004). Loss of nicotine-induced behavioral sensitization in μ-opioid receptor knockout mice. *Synapse, 51*, 219-223.

Zarrindast, M.R., Faraji, N., Rostami, P., Sahraei, H., & Ghoshouni, H. (2003). Cross-tolerance between morphine- and nicotine-induced conditioned place preference in mice. *Pharmacology, Biochemistry, and Behavior, 74*, 363-369.

Zhang, X., Wang, F., Chen, X., Li, J., Xiang, B., Zhang, Y.O., et al. (2005). β-arrestin 1 and β-arrestin 2 are differentially required for phosphorylation-dependent and –independent internalization of δ-opioid receptors. *Journal of Neurochemistry, 95*, 169–178.

Zuo, Z. (2005). The role of opioid receptor internalization and β-arrestins in the development of opioid tolerance. *Anesthesia and Analgesia, 101*, 728-734.

# VITA

## JENNIFER A. CORRELL

| | |
|---|---|
| Personal Data: | Date of Birth: October 22, 1983<br>Place of Birth: Columbia, South Carolina<br>Marital Status: Single |
| Education: | Heathwood Hall Episcopal School, Columbia, South Carolina<br>Columbia College, Columbia, South Carolina;<br>Psychology, B.A., 2005<br>East Tennessee State University, Johnson City, Tennessee;<br>Clinical Psychology, M.A., 2007 |
| Professional Experience: | Graduate Assistant, East Tennessee State University, College of Arts and Sciences, 2005-2007. |
| Honors and Awards: | Most Outstanding Psychology Student, Columbia College.<br>Honeycutt Award, Columbia College. |