Introduction

Cocaine is a highly addictive drug, with increasing use seen in recent years in the United States (SAMHSA, 2016). In particular, females report greater pleasurable and rewarding effects following cocaine use and display a harder time abstaining from cocaine use as compared to males (Robbins et al., 1999; Becker and Koob, 2016). The high lifetime rates of cocaine use and low rates of cessation suggests females experience greater rewarding effects and are at greater vulnerability for cocaine addiction. There is a current disparity in cocaine use therapy, with a greater number of females reporting an unmet need in cessation treatment and an even smaller number entering and successfully completing rehabilitation programs. This disparity in successful rehabilitation may be contributed to the unilateral treatments given in clinical settings that do not take into account the biological sex differences and differential needs between females and males. Clinical and preclinical studies found sex differences in neural structures and mechanisms that mediate drug reward and suggests drugs of abuse function through distinct biological mechanisms in females and males. The NIH has mandated for sex to be treated as a biological variable (McCullough et al., 2014), recognizing the importance of including females into research and the existence of the distinct neural mechanisms that make females more susceptible to drug addiction. With females at greater vulnerability to the addictive effects of cocaine, it is imperative to develop tailored treatments to increase cocaine cessation rates in females and males (SAMHSA, 2016).

Drug addiction is a complex disorder with distinct neural mechanisms that mediate the rewarding and reinforcing properties (Corbit and Balleine, 2005) that lead to enduring synaptic, molecular, and morphological changes in the brain (Radley et al., 2015; Calipari et al., 2017). Chronic drug use alters the normal functioning of the mesocorticolimbic system, resulting in increased hedonic value to drugs, salience to drug-linked cues and compulsive drug-seeking behavior (Ostlund and Halbout, 2017). The pathway originates in the ventral tegmental area (VTA) and projects to the nucleus accumbens (NAc), and the prefrontal cortex (PFC), neural structures critical in motivation, reward and executive planning and function (Penberthy et al., 2010; Hyman and Malenka 2001).

Conditioned place preference (CPP) is a behavioral paradigm designed to investigate the rewarding effects of a drug by assessing the rewarding properties of a stimulus in the absence of the stimulus itself and the strength of its associability with environmental cues (Prus et al., 2009). Drugs of abuse such as psychostimulants (cocaine, methamphetamine, nicotine) that function through the mesocorticolimbic system have been shown to produce CPP (Bardo et al., 1995). Additionally, drugs that produce CPP results in the emergence of persistent and long-lasting dysfunctional circuitry as following extinction, challenge injections, contextual and drug-cues reinstate CPP (Mueller and Stewart, 2000; . Importantly, CPP adds to our understanding of the strength of contextual cues and drug reward as females form place preference at lower drug doses and require lower cocaine doses to reinstate CPP as compared to males (Bobzean et al., 2010; Quinines-Jenab and Jenab, 2012). Thus, CPP can be used to investigate sex differences in the acquisition and maintenance of drug taking.

Although there has been proposed treatments for the cessation for cocaine addiction, there has been limited efficacy as relapse rates with current treatments is disconcerting, with nearly a quarter of cocaine addicts relapsing within the first week of being discharged from cocaine therapy programs (DATOS, 2001; Simpson et al., 1999). The low success rate indicates that current treatments have limited efficacy on the cognitive dysfunctions and do not restore normal functioning to dysregulated neural circuitry.

One of the more promising pharmacological treatments for cocaine addiction is N-acetylcysteine (NAC). NAC is effective in inhibiting reinstatement of compulsive cocaine-seeking behavior, cue-induced reactivity (Moussawi et al., 2009), and successfully reverses the cocaine-induced metaplasticity, increases in membrane-bound NMDA and G-protein glutamate receptors (McClure et al., 2014; Reichel and See et al., 2012). NAC has been shown to restore glial glutamate transporter (GLT-1), normalizing extracellular glutamate levels in the nucleus accumbens, decreasing extrasynaptic glutamate and blocking activation of metabotropic glutamate receptor 5 (mGluR5) (Reissner et al., 2014), a receptor implicated in cocaine-seeking behaviors (Schmidt et al., 2013). Studies have demonstrated NAC reduces elevated

glutamate (Schmaal et al., 2012) and glutathione levels in astrocytes, and reverses excitotoxicity (Badisa et al., 2015), suggesting that NAC can reduce impulsivity associated with increased glutamate levels and restore normal glutamatergic transmission in the mesocorticolimbic pathway.

Acamprosate is a centrally-acting agent that has had success as a treatment for alcohol use disorder (Wiktkiewitz et al., 2012). Alcohol dependence is a complex neurobiological disorder with disruptions in dopamine, glutamate and GABA neurotransmitter systems (Brodie et al., 1990; Koob et al. 2006; Lima-Landman et al., 1980). Alcohol induces plastic changes in the glutamatergic system and induces hyperexcitability and NMDA overactivation. Research has shown acamprosate acts as a partial-agonist at the NMDA glutamate receptor with biphasic effects. At low levels, it is seen to enhance NMDA receptor activation, but at high levels, it has an inhibitory effect at the same receptors, reducing excess glutamate activity and reducing alcohol consumption (Naassila et al., 1998; al Qatari et al., 1998). Acamprosate has also been shown to induce the release of taurine, a compound that binds and decreases NMDA hyperexcitability and may contribute to Acamprosate's therapeutical effect (Dahchour et al., 2000).

Taurine is a sulfur-containing compound and is the most abundant amino acid in animal tissues (Brosnan and Brosnan, 2006). Although not traditionally classified as a neuromodulator, taurine displays several neuropsychopharmacological roles, which include its action as a neuromodulator, neurotrophic agent, and osmomodulator (Wu and Prentice, 2010). Taurine is mainly obtained by humans through their diet, and has been found to be concentrated in the brain and retina (Laidlaw et al., 1990). Taurine has been shown to be effective in reducing the neurotoxicity typically induced by drugs of abuse such as methamphetamine and cocaine through activation of antioxidant and mTOR signaling pathways (Li et al., 2012; Banerjee et al., 2013). It has been found that following chronic cocaine infusion, both glutamate and taurine levels in the striatum increase, suggesting taurine may also play a neuroprotective role in reducing cell death induced by glutamate-mediated neurotoxicity (Yablonsky-Alter et al., 2009). Preliminary studies have shown co-administration of taurine and cocaine reduces cocaine-induced locomotor activity and conditioned place-preference through direct interactions with the NMDA receptor (Banerjee et al., 2013; Chan et al., 2013).

The objective of this study is to assess the potential of taurine as an intervention for substance abuse and to address if sex difference plays a role in the efficacy of taurine in reducing cocaine preference. Moreover, we also investigated whether gonadal hormones regulate taurine's efficacy for reducing cocaine reward by including ovariectomized (OVX) female and gonadectomized (GDX) male rats in the study.

Methods

Subjects

Female and male Sprague-Dawley rats were obtained from an outbred stock of animals (Charles River). Thirty-six intact female and male are divided into four groups. An additional thirty-six OVX female and GDX male rats were divided into four groups (see table below). All rats were housed in a climate-controlled facility with a 12-hr light/dark cycle with *ad libitum* access to food and water. Housing and care were conducted in accordance with 1996 Guide for the Care and Use of Laboratory Rats (Institute of Laboratory Animal Resources on Life Sciences, National Research Council, 1996). All protocols and experimental procedures were approved by the City College of New York Institutional Animal Care and Use Committee (IACUC).

Table 1-Description of Pre-treatment and Co-treatment Paradigm for Cohorts

Sex-Hormonal Status	Pre-Treatment (# of animals)	Co-administration (# of animals)	Treatment on Rough Side	Treatment on Smooth Side
Females-Intact	Taurine (*n* = 27)	Cocaine+Taurine (*n* = 9) Cocaine+Saline (*n* = 9) Taurine+Saline (*n* = 9)	Coc+Tau Coc+Sal Tau+Sal	Sal Sal Sal
	Saline (*n* = 9)	Cocaine+Saline (*n* = 9)	Coc+Sal	Sal
Males-Intact	Taurine (*n* = 27)	Cocaine+Taurine (*n* = 9) Cocaine+Saline (*n* = 9) Taurine+Saline (*n* = 9)	Coc+Tau Coc+Sal Tau+Sal	Sal Sal Sal
	Saline (*n* = 9)	Cocaine+Saline (*n* = 9)	Coc+Sal	Sal
Females-OVX	Taurine (*n* = 27)	Cocaine+Taurine (*n* = 9) Cocaine+Saline (*n* = 9) Taurine+Saline (*n* = 9)	Coc+Tau Coc+Sal Tau+Sal	Sal Sal Sal
	Saline (*n* = 9)	Cocaine+Saline (*n* = 9)	Coc+Sal	Sal
Males-GDX	Taurine (*n* = 27)	Cocaine+Taurine (*n* = 9) Cocaine+Saline (*n* = 9) Taurine+Saline (*n* = 9)	Coc+Tau Coc+Sal Tau+Sal	Sal Sal Sal
	Saline (*n* = 9)	Cocaine+Saline (*n* = 9)	Coc+Sal	Sal

Drugs

The drugs used were: cocaine hydrochloride and taurine (Sigma Inc., St Louis, MO). Cocaine (15 mg/kg) and taurine (100 mg/kg) were dissolved in 0.9% sterile saline and administered intraperitoneal (IP). The volume is based on the animal's weight and was administered 1 mL/kg. We have chosen to

administer our drugs IP because a meta-analysis conducted by Bardo et al. (1993) demonstrated cocaine administered IP more reliably produces place preference but not when given subcutaneous (Mayer and Parker, 1993). Additionally, cocaine produces preference more readily when administered in the least preferred compartment (Nomikos and Spyraki, 1988). The range of cocaine and taurine were selected based on previous research showing this cocaine dose produces reliable place preference (Banerjee, et al 2013) and this taurine dose was used to produce maximal levels of taurine in the animal during pretreatment and conditioning.

Apparatus

The conditioning apparatus consists of three compartments (PanLab/Harvard Apparatus; 300 (W) x 300 (D) x 340 (H) mm; Corridor: 80 (W) x 100 (D) x 340 (H) mm; Doors: 100 (W) x 140 (H)). The outer compartments are made up of four black removable panel walls with plexiglass on the top side to allow for behavioral observation. The floors also consisted of black removable panels. One of the outer compartments had rough-textured panels and the other outer chamber had smooth-textured panels. The outer chambers that contained rough-textured and smooth-textured panels were counterbalanced throughout the experiment. The middle chamber had removable panel walls and a grid floor. Animals were brought up from the vivarium and allowed to acclimate 15 minutes to the testing room before conditioning began. Behavior was recorded by an overhead camera and was later quantified.

Pretreatment and conditioning procedures

Two weeks before CPP procedures began, animals were given pretreatment injections of either taurine or saline (vehicle) every day for two weeks. After two weeks of pretreatment, a ten-day CPP behavioral paradigm was employed. In addition, a pre-tau/tau+sal group is included to investigate if reductions of cocaine preference are due to the neuroprotective of taurine and not because of a competing rewarding stimulus. This study employed a biased CPP procedure consisting of three phases: an initial habituation test, 8 days of conditioning and a final preference test. On Day 1 of CPP, animals were habituated to the conditioning apparatus. Animals were placed into the middle chamber and were allowed to freely explore the entire apparatus for 15 minutes. During conditioning (day 2 through day 9), before being placed into the CPP chamber, certain animals received a combination of either cocaine, saline, or taurine and are placed into the rough texture chamber for 30 minutes (see table above). On alternate days, all treatment groups received two injections of saline on both sides of the torso and were placed into the smooth texture chamber for 30 minutes. The conditioning procedure continued for 8 days. On the tenth day, behavior was assessed. Animals were in a drug-free state and were placed into the middle chamber with dividers in place. The dividers were then removed and the animals were allowed to freely explore the entire apparatus for 15 minutes and behavior was recorded using a video camera. The cohorts of female and male rats were performed at different times to avoid phenomenal cues. Experiment 1 assessed taurine pre-treatment and conditioning in males and experiment 2 assessed the same in intact females. To investigate if taurine efficacy is hormonally-dependent, experiment 3 assessed taurine and cocaine conditioning in GDX males while experiment 4 assessed taurine efficacy in OVX females.

Two-way ANOVAs were used to determine how treatment impacted time spent in the paired and unpaired chambers. Post-hoc comparisons using Fisher's PLSD were performed to determine significant differences between the paired and unpaired chambers within each treatment. Significance was determined by $p < 0.05$.

Results

Overall, pretreatment of taurine was effective in attenuating cocaine preference in male and female rodents. However, in females, its efficacy was dependent on whether taurine and cocaine were co-administered.

The control group of intact male rats conditioned to cocaine showed a significant preference to the cocaine-paired chamber (Figure 1; t-value = 2.35; p = 0.037) compared to the unpaired chamber. Intact males exposed to a pre-treatment of taurine and conditioned to cocaine show no significant preference towards either chamber. Pre-treatment of taurine and co-administration of taurine/cocaine further reduces cocaine-preference, showing no significant differences between time spent in paired and unpaired chambers. . Males that were pre-treated with taurine and conditioned to taurine did not show a significant preference to the taurine-paired chamber.

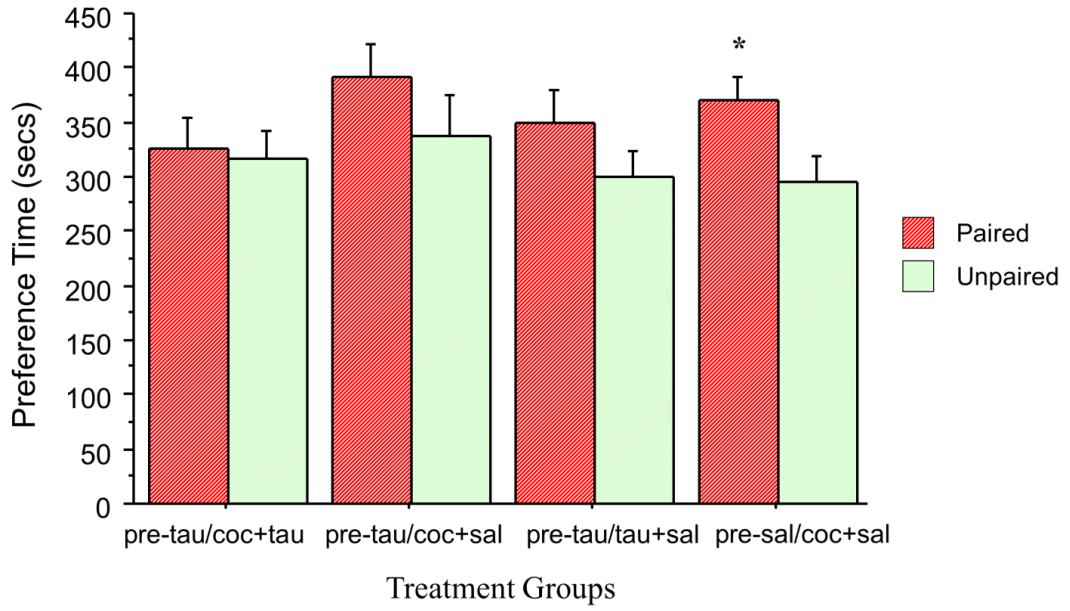

Figure 1: Taurine attenuates cocaine preference in intact male rats. Males conditioned to cocaine show a significant preference towards the cocaine-paired chamber *(*; $p \leq 0.05$). Taurine pre-treatment as well as taurine co-administration diminishes cocaine preference with no significant differences between time spent in the cocaine-paired and unpaired chambers. Males administered only taurine do not form a preference towards the taurine-paired chamber.

Figure 1 depicts the time spent in the drug-paired and unpaired chambers of intact males across experimental conditions. The statistical analyses revealed a main effect of preference ($F_{(1,54)}=5.35$, $*p=0.025$). Specifically, post-hoc comparisons revealed control male rats who received saline pre-treatment and cocaine administration showed a significant preference to the cocaine-paired chamber ($p \leq 0.05$). Male rats pre-treated with taurine and conditioned with cocaine did not display significant preference to the cocaine-paired chamber. Rats conditioned with taurine and cocaine displayed similar time spent in the cocaine-paired and unpaired chamber. Pre-treatment and conditioning with taurine does not induce preference for the taurine-paired chamber.

Intact-females form a preference to the cocaine-paired chamber (Figure 2; t-value = 2.16; p = 0.05) when they are not pre-exposed to taurine. Overall, the two-way ANOVA determined that intact females do not form a preference to the taurine-paired chamber. Similar to intact males, taurine pre-treatment reduces cocaine preference in intact females, showing no significant difference between the time spent in the paired and unpaired chambers. Interestingly, taurine pre-treatment and co-administration of taurine/cocaine significantly enhances cocaine preference (t-value 2.85 = $p < 0.01$).

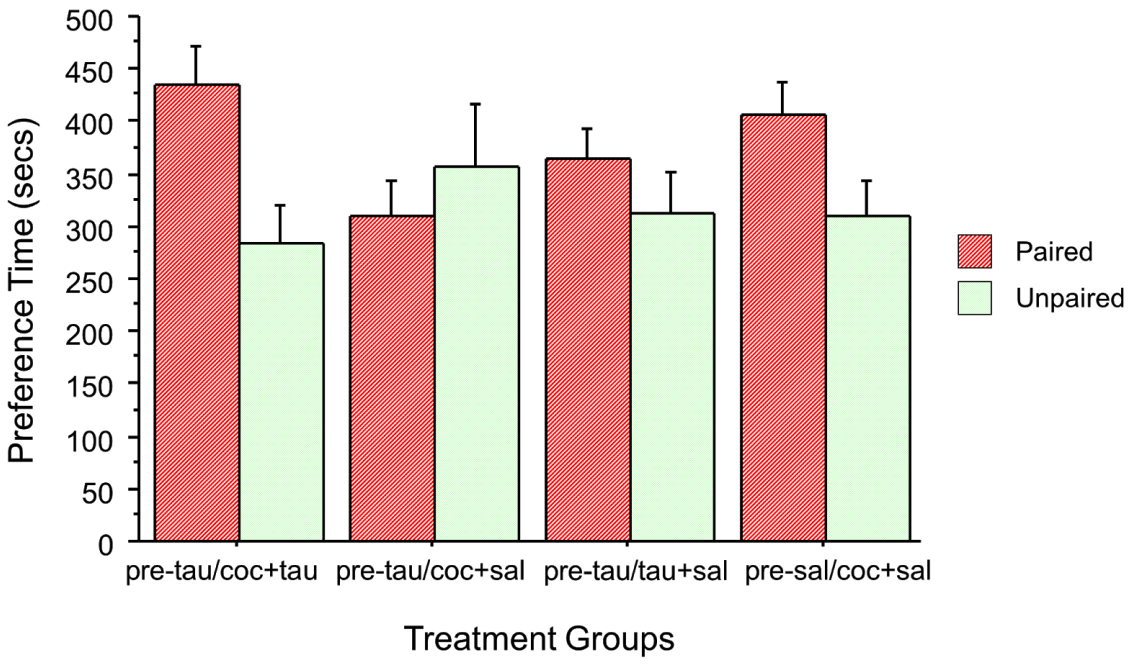

Figure 2: Cocaine and taurine co-administration enhances cocaine preference in intact females. Females conditioned to cocaine form a preference to the cocaine-paired chamber (*; $p \leq 0.05$) but taurine pre-treatment inhibits cocaine preference. Taurine pre-treatment and co-administration produces preference to the cocaine-paired chamber in female rats (**; $p \leq 0.05$). Females administered only taurine do not form a preference towards the taurine-paired chamber.

Figure 2 depicts the time spent in the paired and unpaired chambers of intact females across experimental conditions. The statistical analyses revealed a main effect of preference ($F_{(1,58)}$=4.97, *p=0.030). Specifically, post-hoc comparisons revealed control female rats who received saline pre-treatment and conditioned with cocaine form a preference to the cocaine-paired chamber ($p \leq 0.05$). Females pre-treated with taurine and conditioned with cocaine do not show a significant preference to either the cocaine-paired or unpaired chamber. Interestingly, female rats who received taurine pre-treatment and conditioned with taurine and cocaine produced a potentiation of preference to the cocaine-paired chamber ($p \leq 0.05$). Pre-treatment and conditioning with taurine did not result in a preference to the taurine-paired chamber.

GDX-males that were not exposed to taurine form a significant preference to the cocaine-paired chamber (Figure 3; t –value = 2.78); p=0.024). A two-way ANOVA revealed that GDX males pre-treated with taurine did not show any significant preference to the cocaine, cocaine/taurine or taurine paired chambers when compared to the preference time for the unpaired chambers.

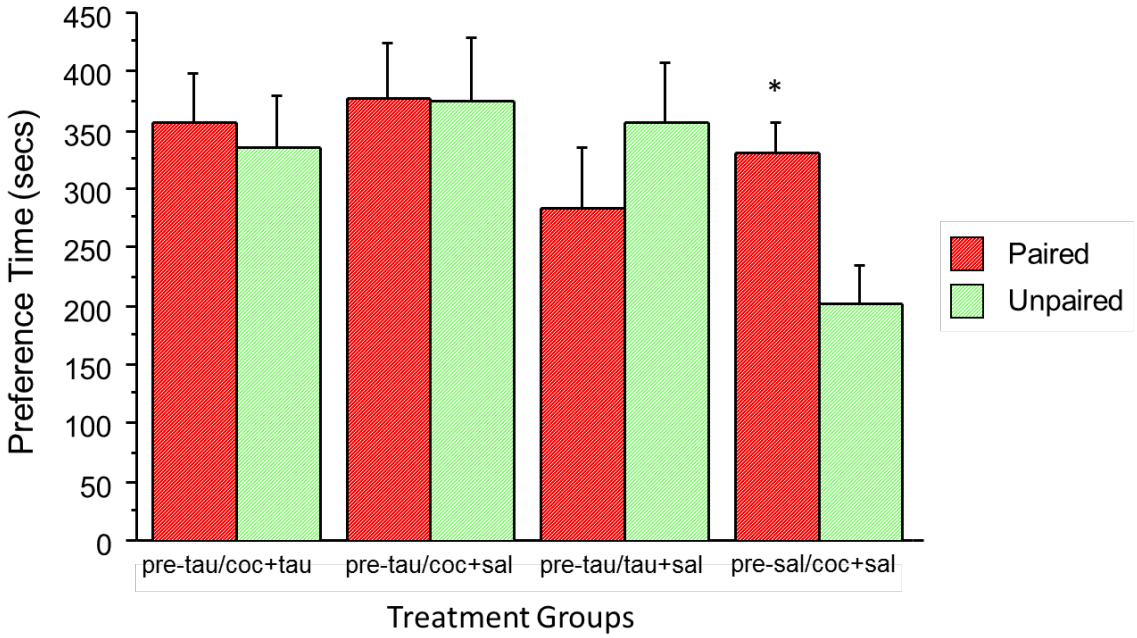

Figure 3: Cocaine preference and taurine efficacy is not testosterone dependent. GDX males conditioned to cocaine-only show a preference towards the cocaine-paired chamber. GDX males do not form a preference to the taurine-paired chamber.

Figure 3 depicts the time spent in the paired and unpaired chambers of GDX males across experimental conditions. The statistical analyses revealed no significant differences in treatment ($F_{(3,60)}$=1.88, p=ns) or preference ($F_{(1,60)}$=0.37, p=ns).

Finally, and interestingly, a two-way ANOVA revealed a significant interaction in OVX-females (Figure 4: F (3, 62) = 4.11; $p < 0.01$). OVX females did not show a significant preference to the cocaine paired chambers under any conditions. Interestingly, OVX females did establish a significant preference to the taurine-paired chamber, when pre-treated with taurine for two weeks (t-value = 3.20; $p < 0.0064$). These significant differences between the females conditioned to cocaine versus taurine are what are driving the significant interaction, as seen by the t-test comparisons.

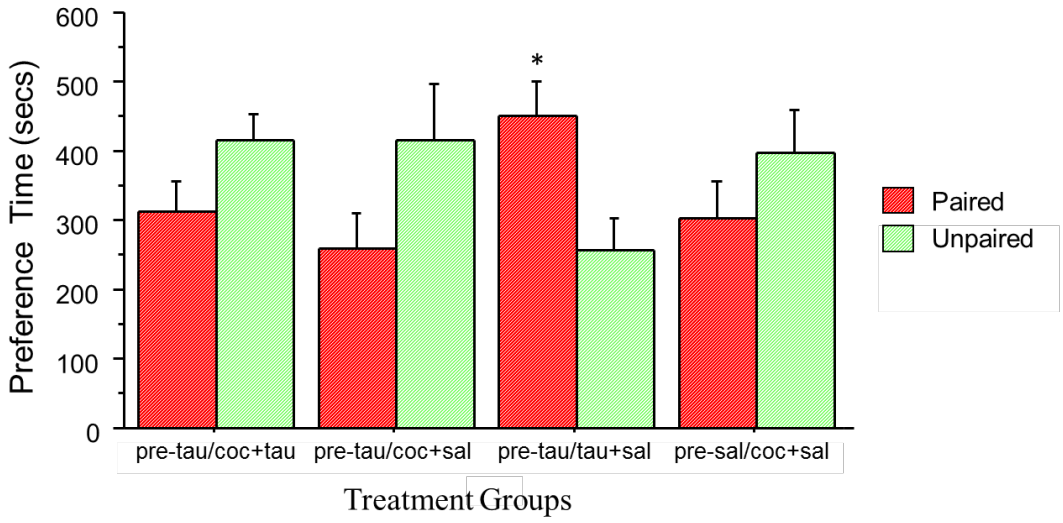

Figure 4: Lack of ovarian hormones results in taurine preference while cocaine preference is not acquired. OVX females do not form a preference to the cocaine-paired chamber. However, a preference to the taurine-paired chamber is formed (p <0.0064).

Figure 4 depicts the time spent in the paired and unpaired chambers of OVX males across experimental conditions. The statistical analyses revealed a significant interaction between treatment and preference ($F_{(3,62)}$=4.11, p=0.10). Post-hoc analyses revealed OVX females did not show a significant preference to either the cocaine-paired or unpaired chambers under any conditions involving conditioning with cocaine. Interestingly, OVX females pre-treated and conditioned with taurine display a significant preference to the taurine-paired chamber ($p < 0.05$).

Discussion

Preclinical studies have demonstrated dysregulated excitatory transmission contributes to the acquisition and maintenance of cocaine addiction. Successful interventions such as NAC and Acamprosate have been shown to reduce cocaine-induced behaviors and relapse by restoring dysfunctional glutamatergic neurotransmission in the mesocorticolimbic pathway (Moussawi et al., 2011). Our studies contribute to the existing literature by showing that a naturally-occurring amino acid, taurine, blocks the rewarding effects of cocaine in both female and male rats. Pretreatment with taurine inhibits the formation of cocaine preference in females and males regardless of hormonal status. Co-administration of taurine and cocaine inhibits cocaine preference in males but potentiates drug reward in intact females. Taurine is not a rewarding stimulus in intact females, males, and GDX males but is rewarding in OVX females.

Our experiments revealed taurine is effective in decreasing cocaine reward in both intact females and males. Studies have found that pretreatment with Acamprosate, a structural analog to taurine, inhibits the formation of cocaine CPP in male mice (McGeehan and Olive, 2003). Furthermore, mice conditioned with Acamprosate do not form a preference to the Acamprosate-paired chamber, suggesting Acamprosate is not rewarding and is consistent with our results. Acamprosate has also been shown inhibit the reinstatement of CPP (Mcgeehan and Olive, 2006) and cocaine self-administration (Bowers et al., 2007) despite having no significant effect on reducing cocaine use and craving in clinical settings (Kampman et al., 2011). However, there were limitations in the study such as subject enrolled in the study continued to use cocaine and a high drop-out rate that limit the validity of the findings. Studies have found the neuromodulatory effects of Acamprosate and taurine are mediated through the glutamatergic system (al Qatari et al., 1998; Boothby and Doering 2005; Witkiewitz et al., 2012; Kalk and Lingford-Hughes, 2014). Taurine has several proposed mechanisms for its neuroprotective effect to drugs of abuse, one of which may involve interactions with the ionotropic NMDA receptor and the metabotropic glutamate receptor 5 (mGluR5). Activation of NMDA and mGluR5 have been implicated in the compulsive behaviors associated with alcohol (Blednov and Harris 2008) and cocaine addiction (Reissner et al., 2014). Chan et al. (2013; 2015) has found taurine interacts directly with NMDA via the polyamine and glycine binding sites, selectively inhibiting the N2 evoked field potential and inhibiting responses mediated by NMDA. Suarez and Solis (2006) found that in the rat hippocampus, presynaptic NMDA receptors contain a specific binding site for taurine, providing further evidence taurine directly interacts with NMDA receptors. Following abstinence from chronic cocaine, a cocaine challenge not only increases glutamate but also taurine concentration in the striatum (Yablonsky-Alter et al., 2009), suggesting the increase in taurine is an endogenous neuroprotective mechanism to counteract excitotoxicity due to the increased glutamate. In addition to causing an influx of positive ions to enter into cells, NMDA also excites neurons by increasing intracellular calcium levels and release (Hardingham et al., 2001). Spanagel et al., (2014) suggests the anti-relapse effects of acamprosate are mediated through the reduction of intracellular Ca2+ release. Taurine may inhibit the activation of mGluR5 by decreasing Ca2+-evoked currents and inhibiting glutamate-induced elevation of intracellular Ca2+, suggesting taurine alters axon excitability by regulating the opening of Ca2+ channels (Chen et al. 2001; Albinana et al., 2010).

Co-administration of taurine and cocaine produces a sexually dimorphic response, where males display a reduction in cocaine reward but females display a potentiation of drug reward. The increase in cocaine preference seen in females may be due to the presence of ovarian hormones, specifically estradiol. Estradiol has been shown to facilitate the sensitization, locomotion and rewarding effects of psychostimulants (Lynch et al., 2002; Anker and Carroll, 2011) and enhances NMDA excitatory post synaptic potentials (EPSP) (Foy et al., 1999) and LTP (Smith and McMahon 2006). Interactions between mGluR5 and estrogen have been shown to facilitate of cocaine's rewarding effects (Bobzean et al., 2014; Martinez et al., 2014). Cocaine has also been shown to induce increases of estradiol and testosterone in intact females but not intact males, suggesting a self-renewing effect that contributes to the abuse of cocaine (Mello et al., 2004). Estrogen not only modulates excitation through NMDA but also through

modulation of GABA neurotransmission. Estradiol-dosed hippocampal interneurons express decreases in glutamate-decarboxylase, the GABA-synthesizing enzyme, and GABAergic miniature IPSC's (Murphy et al, 1998), suggesting estrogen facilitates contextual-cue retrieval by reducing inhibitory signals. Estrogen-mediated NMDA activation increases nigral dopamine release despite tonic GABA release (Cobb and Abercrombie, 2002), suggesting estrogen may induce opposite effects, dependent on which receptor it binds to. Estrogen-binding to NMDA potentiates Ca2+ (Nilsen et al 2002), increasing excitatory output, while binding to GABA decreases inhibitory potentials, resulting in facilitation of axon excitability. Although, there is limited research on the interaction between taurine and the neuroendocrine system, estrogen has direct implications on taurine concentrations as it increases the uptake of taurine into cells via the taurine transporter, TauT (Shennan and Thomson et al., 2007). With the uptake of taurine from the extracellular space, estrogen may limit taurine's actions on NMDA and mGluR5 and disinhibit glutamatergic transmission. Taken together, the potentiation of cocaine reward seen in intact females may be due to the and estrogen's biochemical effects in reducing GABAergic inhibition and increasing glutamatergic activity.

GDX males form a preference towards the cocaine-paired chamber ($p<.05$) but taurine pre-treatment and co-administration prevents cocaine preference, suggesting taurine efficacy is not testosterone-dependent. Previous findings have shown testosterone offers minimum contribution to the rewarding effects (Minerly et al., 2008) and the sensitizing effects of chronic cocaine (Forgie and Stewart 1994; Chin et al., 2002). Testosterone also does not regulate dopaminergic release in either the striatum or NAc (Becker 2009; Triemstra et al., 2008), suggesting that unlike estrogen, testosterone does not confer any biochemical facilitatory effects. Ovariectomy in females abolishes cocaine's rewarding effect and no preference is formed to the cocaine-paired chamber. There is existing literature showing estrogen promotes cocaine's rewarding (Calipari et al., 2017; Van Swearingen et al., 2013) and reinforcing effects (Martinez et al., 2016) in part by altering dopaminergic transmission. An interesting result is that OVX females form a preference to the taurine-paired chamber. We believe OVX rats form a preference because of taurine's anxiolytic properties. A decline of ovarian hormones results in an increase in negative affective states such as anxiety, cognitive defects, and depressive-like symptoms (Walf and Frye, 2006; Brann et al., 2007; Pandaranandaka et al., 2009; Furuta et al., 2013). Whirley and Einat (2008) found that taurine administration was not effective in reducing anxiety and depressive behaviors in male mice. However, the authors suggest taurine's anxiolytic effects are subtle and may only be observed in an animal that displays a high anxiety phenotype. McCool and Chappell (2007) initially found strychnine, a glycine antagonist, reduced anxiety in the elevated plus maze and light/dark box but taurine's anxiolytic effect was negligible. However, when the same rats were separated into two populations of "high" and "low" anxiety, taurine showed a significant anxiolytic effect in the "high" anxiety rat. This illustrates taurine is effective in reducing anxiety in high anxiety but not a low anxiety phenotype. We suggest OVX females constitute a high anxiety phenotype as replacement of physiological estradiol and/or progesterone has antianxiety effects in OVX females (Frye and Walf 2004; Walf and Frye, 2005; 2007). Our results suggest taurine has no rewarding properties in intact females and studies have found taurine by itself does not cause an increase in anxiety (Whirley and Einat, 2008). Therefore, the removal of ovarian hormones produces a negative affective state taurine administration alleviates, functioning as a neuroprotective substance for OVX females.

Sexual Dimorphisms in Mechanisms Regulating Drug Reward

Many drugs of abuse elicit their rewarding effects through the mesocorticolimbic pathway, modulating the release of amino acids (glutamate and GABA) and catecholamines (dopamine, serotonin, and norepinephrine). Sexually dimorphic mechanisms differentially regulate the mesocorticolimbic pathway in females and males. Within the NAc, GABAergic medium spiny interneurons modulate the hedonic value of drugs of abuse (Balfour, 2009; Berridge and Krigelbach, 2013). Female rats display greater expression of estrogen receptor-β (ER-β), located on the terminals of GABAergic medium spiny neurons (Yoest et al., 2016), and these interneurons have recurrent collaterals on VTA dopaminergic

terminals (Grove-Strawser et al., 1996; Mermelstein et al., 1996), suggesting ovarian hormones promotes the rewarding effects of cocaine (Becker, 1999; 2012). Estradiol attenuates GABAergic medium spiny neuron activity in the NAc via inhibition of L-type Ca2+ channels (Hu et al., 2006), reducing GABAergic activity, inhibition of dopamine release and increasing the salience of drug-associated cues (Smith et al., 2017). Activation of ER-β by estradiol inhibits the activity of these GABAergic interneurons, promoting increased dopamine release in the NAc (Becker and Hu, 2008; Hu et al., 2006). Due to the higher ER-β expression on the GABAergic interneurons, females display greater inhibition of GABA release and increased dopamine release as compared to males, suggesting an ovarian-hormone based mechanism that increases the drug reward.

Antagonizing estrogen receptors in intact females or the removal of ovaries in female rats (OVX) disrupts the formation of sensitization and CPP to cocaine administration (Bobzean et al., 2014; Cummings et al., 2014; Segarra et al., 2014). Estradiol supplementation restores stereotypical and drug-seeking behaviors (Becker et al., 2001; Hu and Becker, 2003; Quinones-Jenab et al., 2000; Segarra et al., 2010) and cocaine self-administration (Zhao and Becker, 2010; Larson et al., 2007) in females but not males. This may possibly be due to the restoration of cocaine-induced dopamine release (Peris et al., 1991; Cummings et al., 2014) and facilitate effects of ovarian hormones. An important sexually dimorphic structure that integrates neuroendocrine signals and information about rewarding stimuli is the medial preoptic area (mPOA). Situated in the rostral hypothalamus, the mPOA has widespread projections to the brain (Simerly and Swanson, 1988; Swanson, 2004) and is subdivided into several nuclei and specific regions, each of which regulates maternal, sociosexual, appetitive and consummatory behaviors including drug addiction (Stack et al., 2002; Dominguez, 2009; McHenry et al., 2017). The mPOA expresses the highest density of estrogenic receptors (Petrulis, 2013; Pfaff and Keiner, 1973), suggesting, the mPOA modulates a variety of neural systems by integrating hormonal signals and altering the motivational and rewarding properties of drug-linked cues through its widespread projections to the VTA. Indeed, lesions of the mPOA has been shown to directly augment cocaine-induced locomotion and cocaine conditioned preference. Upon lesioning the mPOA, female and male rats display greater cocaine-induced locomotion (Tobiansky et al, 2013; Will et al., 2016) and female rats display greater conditioned preference to the cocaine-paired chamber (Tobiansky et al, 2013). One possible mechanism through which mPOA alters cocaine-induced behavioral effects is through its GABAergic projections to the VTA. Lesioning the mPOA removes an inhibitory source to VTA dopaminergic neurons and indirectly, increases dopamine release in the NAc. In addition, Tobiansky et al., (2016) showed these mPOA-VTA projections are directly modulated by estradiol and microinjection of estradiol into the mPOA increases cocaine-induced dopamine release in the NAc. Estradiol microinjections into the mPOA of OVX female rats facilitates the development of cocaine preference and increases c-fos expression in the NAc (Robison et al., 2017). These studies suggest estradiol in the mPOA facilitates a strong and durable association between cocaine and the contextual environment where it was administered. Taken together, the evidence strongly suggests the sexually dimorphic mPOA may be a neuroanatomical locus for sex differences by integrating ovarian hormonal signals and directly modulates activity of the mesocorticolimbic pathway and subsequently, cocaine's rewarding effects.

Two treatments used for cocaine addiction, NAC and acamprosate, share similar mechanisms of action with taurine. Pretreatment of acamprosate inhibits the formation of cocaine CPP (Mcgeehan and Olive, 2003), in agreement with our results. Similar to acamprosate, taurine binds to NMDA receptors and decreases excitatory postsynaptic potentials, suggesting taurine's neuroprotective is mediated through direct modulation of the glutamatergic system. Additionally, similar to NAC, taurine may also function through the cystine-glutamate exchange. Chronic cocaine administration impairs the cystine-glutamate exchange in the NAc and may be a key mechanism that underlies cocaine-induced meta-plasticity and the emergence of maladaptive drug-seeking behaviors (Kau et al., 2008). Dysfunctional cystine-glutamate exchange blunts extrasynaptic glutamate levels, reducing the activation of mGlu2/3, metabotropic glutamatergic autoreceptors. When NAC is administered, it is oxidized into cystine, increasing the availability of free cystine and promoting the exchange of glutamate into the extrasynaptic space and restoring mGlu2/3 function. Effectively, activation of mGlu2/3 will impair the increase in glutamatergic

transmission associated with cue-induced reinstatement and cocaine relapse (Moran et al., 2005). Like cysteine, taurine is a sulfur-containing compound and is synthesized from cysteine through the action of two rate-limiting enzymes, cysteine sulfinic acid decarboxylase (CSAD) and cysteine dioxygenase (CDO) in the liver (Ripps and Shen, 2012), although studies have found evidence of CDO and CSAD expression in the rat mammary gland (Ueki and Stipanuk, 2007) and nonhepatic tissues (Hirschberger et al., 2001; Ide et al., 2002). Chronic pretreatment of NAC reduced cocaine self-administration and was effective in preventing cocaine-evoked metaplastic changes in the cystine-glutamate transporter and glutamatergic transmission (Madayag et al., 2007). In comparison to NAC reversing cocaine metaplasticity (Moussawi et al., 2009), taurine may reverse cocaine-induced changes as blockade of NMDA receptors rescues dysfunctional long-term depression in the oval bed nucleus of the stria terminalis (Cornish and Kalivas, 2000; deBacker et al., 2015) by reducing the apparent affinity for glycine (Chan et al., 2013). Consistent with our results, taurine pretreatment inhibited the formation of cocaine CPP in intact females and males. Co-administration of taurine further disrupts cocaine preference in intact males but potentiates cocaine preference in intact females. The conflicting results may be reconciled as taurine is effective within a range of total taurine concentration in the body. Pretreatment with 50 mg/kg chloral hydrate for six days blocked morphine-induced preference but pretreatment with 100 mg/kg had no effect on morphine CPP (Sun et al., 2015), resulting in a U-shape of treatment efficacy. Additionally, the same U-shape was also seen with a low dose of NAC reducing EPSC while a high dose of NAC potentiated EPSC amplitude (Kupchik et a., 2011). These studies suggest there is a therapeutic range where the intervention is effective in reducing drug reward and in interrupting the evoked metaplastic changes associated with drug use. Outside of the therapeutic range, these same interventions either have no effect or potentiate the rewarding effects of the drug of abuse. Several studies have shown medium and high doses of taurine bind to different receptors, eliciting distinct neuromodulating effects (Jia et al., 2008; Albinana et al., 2010) and provide a mechanistic basis for the therapeutic range. With regards to our results, it seems taurine's therapeutic range is more constrained in intact females when compared to intact males. Castration does not affect taurine's therapeutic range but ovariectomy does, illustrating the important role ovarian hormones play in modulating the efficacy of treatments. Altogether, this suggests taurine is a potential candidate for treatment for cocaine abuse disorders and implicates taurine as being a potential treatment for preventing cue-induced drug-seeking behavior, a major cause for relapse.

Limitations and Implications

While CPP is a useful tool in measuring reward value, the model does have several limitations. CPP evaluates preferences based of associations animals make between context and the reward (Dixon et al., 2013). Therefore, it measures an animal's overall experience with an environment and does not address an animal's specific behavioral and physiological response to a drug. The behavioral paradigm, self-administration, more closely mimics the compulsive nature of cocaine addiction (Panlilio and Goldberg) and reinforcing effects. However, NAC has been shown to be effective in reducing cocaine self-administration, suggesting taurine may also be effective.

Previous research on taurine has elucidated taurine's role in physiological and cellular functions (Schaffer et al., 2010; Hussy et al., 2001). The current study brings taurine's electrophysiological and neurochemical properties (Yablonsky-Alter et al., 2009; Chan et al., 2013; 2014) into a behavioral model of cocaine reward. Even after prolonged extinction, rats and non-human primates still show a significant preference towards cocaine (Brenhouse and Andersen 2008), demonstrating the presence of disrupted neurocircuitry and the difficulty in treatment. Current treatments are applied in a unilateral manner in the clinical setting and have not been effective, with females showing lower cessation and higher relapse rates, suggesting there needs to be fundamental reform in how we conceptualize treatments directed towards females. Furthermore, in a recent World Health Organization (WHO) survey (Degenhardt et al., 2017), over 60% of participants had a perceived need of treatment among those with substance use disorders but only 19% received minimally adequate treatment for substance use disorders. The study illustrates the importance of treatment for substance use disorders, the lack of effective pharmacotherapies being implemented in therapies, the low number of people with substance use disorder receiving adequate

treatment. The current study shows that taurine is a viable candidate for cocaine addiction, with potentially few side effects, and, in addition, could be neuroprotective for cocaine use disorder. Further studies need to be performed to assess if taurine acts directly on the mesocorticolimbic circuit to inhibit the rewarding effects of cocaine.