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Sex dependent efficacy of taurine as a treatment for cocaine drug use: a study of reward

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Rationale

According to the National Survey on Drug Use and Health (SAMHSA, 2012), 22.2 million Americans were identified as substance dependent. Of those 22.2 million, 1.1 million are dependent on cocaine, making it the third-most widely abused illicit drug. Cocaine is a strong psychomotor stimulant that increases levels of dopamine (DA) within the mesocorticolimbic system, resulting in euphoric effects and sustained feelings of pleasure. Sustained drug use leads to disruptions in reward and motor centers, resulting in increases of locomotor activity, salience of drug-related stimuli, and dysfunction in neurotransmitter regulation (Penberthy et al., 2010; Hyman and Malenka 2001). Recreational use of drugs are attributed to environmental influences, peer-pressure and are used in an attempt to momentarily alleviate negative mood states (Koob 2009). However, acute use fosters the development of compulsive drug use and behaviors due to increased hedonic values or genetic vulnerability (Koob 2000; Dong and Nestler 2014).

Sex differences have been well documented with respect to cocaine addiction. Although males are more likely to use drugs for recreational use, females are at higher risk of developing physiological consequences from drug use and develop a dependency on drugs at a faster rate and lower dose and have a higher risk of relapse (Brady and Randall, 1999; Ignjatova and Raleva, 2009). The difference in vulnerability may stem from dimorphic differences in gonadal hormones with a particular interest in estrogen. Research has shown high levels of estrogen facilitate the rewarding effects of cocaine and a faster preference to the cocaine-paired chamber (Lynch et al., 2001). Upon reaching puberty, females begin to ovulate monthly and synthesize estrogen during the menstrual cycle. Estrogen levels rise and peak during the follicular and steadily decrease in the middle of the luteal phase of the menstrual cycle (Evans and Foltin 2010).

Estrogen receptors are concentrated near midbrain dopamine systems such as the ventral tegmental area (VTA) and serotonergic systems including the raphe nuclei, implicating a critical role for estrogen in modulation of reward value, cognition and motor functions (Nomura et al., 2005; Creutz and Kritzer 2002) by regulating the expression of critical enzymes in the synthesis of neurotransmitters (Gundlah et al., 2005). Estrogen has been implicated in increasing DA firing rate, fibers and striatal DA release (Becker, 1999) and the subjective effects of cocaine are reported as "more pleasurable" during the follicular phase, when estrogen is highest (Lukas et al., 1996). Upregulation of DA processes may result in a more intense vesicle release of DA or prolonged burst firing of DA axons. This contributes to feelings of reward when presented with a stimulus.

Different pharmacological agents have been proposed as effective for cocaine addiction. Some treatments include methamphetamine, levo tetrahydropalmatine, GABAergic medications, and disulfiram. However, increasing concerns regarding efficacy and side-effects have made it difficult to begin implementing treatments into rehabilitation clinics. Pharmacological treatments offer short-term relief but become a liability for abuse and those that are have yet to have their mechanism determined (Shorter and Kosten, 2011). A second obstacle with current treatments is relapse rates for cocaine addicts is disconcerting, with nearly a quarter relapsing to cocaine within the first week of being discharged (DATOS, 2001; Simpson et al., 1999), signaling treatments have limited efficacy on short-term cognitive dysfunctions but do not treat disrupted neural circuitry, which persists for years after abstinence and are problematic for the continuation of improvement in quality of life for recovering addicts (Simpson et al., 2002). Although cocaine addicts often show similar neural dysfunctions and symptoms, many often suffer from comorbidities such as psychiatric disorders, poly substance abuse and cardiovascular problems

(Miller and Ries, 2004; Devlin and Henry, 2008). Research into cocaine treatments must also take this into account, illustrating the complexity and difficulty in developing safe and efficient cocaine treatments.

One of the more promising pharmacological treatments for cocaine addiction is N-acetylcysteine (NAC). NAC is effective in inhibiting reinstatement of cocaine, cue-induced reactivity (Moussawi et al., 2009) and successfully reverses cocaine's plastic effects (McClure et al., 2014; Reichel and See et al., 2012). NAC has been shown to restore glial GLT-1, normalizing extracellular glutamate levels in the nucleus accumbens and increasing 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 levels (Schmaal et al., 2012) and elevated glutathione levels in astrocytes and reverses excitotoxicity (Badisa et al., 2015), suggesting NAC reduces increased glutamate levels associated with impulsivity. In response to viewing slides depicting cocaine and cocaine use, self-reports from hospitalized subjects given NAC for three days showed a decrease in desire to use and a lower response to cocaine slides (LaRowe et al., 2007).

Taurine is an essential amino acid that displays several neuropsychopharmacological roles such as neuromodulator, neurotrophic, and osmomodulatory roles (Wu and Prentice, 2010). Humans mainly obtain taurine through their diet (Laidlaw et al., 1990). Although taurine is found in all foods, taurine is highest in meat and shellfish such as clams and mussels and grains such as beans and nuts. Taurine is also found in many energy drinks, a popular beverage typically consumed by adolescents and has been shown to have adverse effects on the developing brain and increasing the risk of future drug abuse (Reissig et al., 2009), although energy drinks also contain high amounts of caffeine and other stimulants (Aranda and Morlock, 2006). The potential health effects of these other substances have yet to be determined.

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, taurine levels in the brain increase, suggesting taurine may 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 (Banerjee et al., 2013).

The behavioral paradigm employed in the study is conditioned place preference (CPP), a behavioral model commonly used to assess the reinforcing properties of a stimulus in the absence of the stimulus itself (Prus et al., 2009). Using a three chambered apparatus, a drug or stimulus is paired with an outer chamber while the other outer chamber is the vehicle-paired chamber. The middle chamber functions as a walkway between both paired-chambers. Peripheral chambers are distinguished from each other by environmental cues such as rough vs smooth textures, grid patterns, and color schemes. By conditioning the animals to associate a particular context with the rewarding effects of a stimulus, the rewarding effects of the stimulus are extended to the environment, allowing the environment to function as a conditioned stimulus (Pearce and Bouton, 2001). Although both self-administration and CPP measure the rewarding value of a stimulus, CPP allows for testing animals in a drug-free state and testing the motivational effects of drugs (Bardo and Bevins, 2000) .Unlike self-administration, where rats must learn a task, lever-press, and a single behavior is reinforced while other behaviors are weakened (Panlilio and Goldberg 2007), CPP relies on the rewarding properties of a stimulus

and the strength of its associability with environmental cues. A second critical difference between the two paradigms is that distinct neural mechanisms mediate the reinforcing properties (Corbit and Balleine, 2005), demonstrating that addiction is a complex disorder that is not fully explained by any one test. Therefore, by using preference as a measurement of the rewarding strength of the drug, CPP is a reliable indicator of the rewarding value of a stimulus and the animal's ability to associate contextual cues with the rewarding stimulus.

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.

Methods

Thirty-six intact or gonadectomized (GDX) male and female Sprague-Dawley rats are divided into four groups (Table 1). All rats were housed in a climate-controlled facility with a 12-h 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). Pretreatment injections of either taurine (100 mg/kg) or saline were administered for two weeks and were injected intraperitoneal (IP). The volume is based on the animal's weight, volumes ranged from .35 to .52 mL for males and .25 to .36 mL for females.

After two weeks of pre-treatment, a ten-day CPP behavioral paradigm was employed. Day 1 of behavior was for habituation. Animals were placed into the middle chamber and are allowed to freely explore the chamber for 15 minutes. During conditioning (day 2 through day 9), before being placed into the CPP chamber, the animals received two injections of either hydrochloric cocaine (15 mg/kg), saline, or taurine and are placed into the rough texture chamber for 30 minutes. 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. On the tenth day, behavior was assessed. Animals were placed into the middle chamber with dividers in place. The dividers were removed and the animals were allowed to freely explore the chamber for 15 minutes and behavior was recorded. The cohorts of male and female rats were performed at two different times to avoid phenomenal cues.

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

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. Two-way ANOVAs were used to determine how treatment impacted time spent in the paired and unpaired chambers. Planned t-test comparisons were performed to determine significant differences between the paired and unpaired chambers within each treatment. Significance was determined by a $p < 0.05$.

. 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.

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 = $p < 0.01$).

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.

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 1

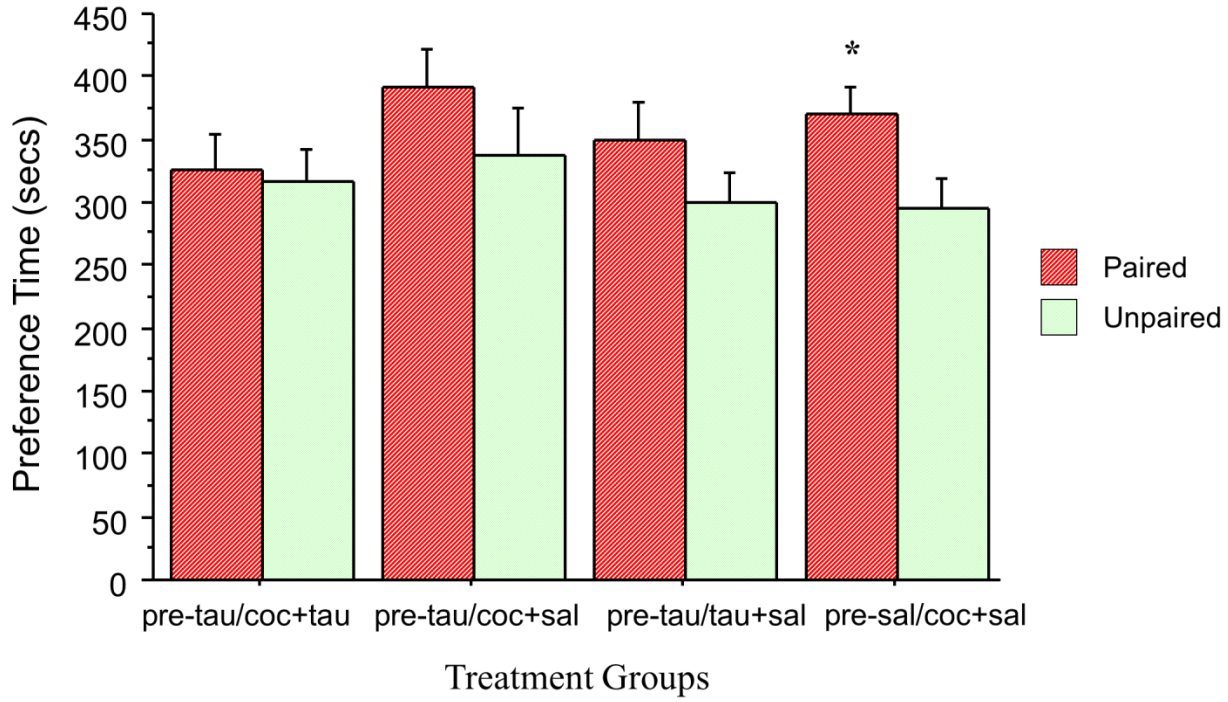

Figure 1: Taurine attenuates cocaine preference in intact male rats. Males conditioned to cocaine-only show a significant preference (*) towards the cocaine paired chamber ($p<0.036$). Taurine pre-treatment as well as taurine co-administration diminishes cocaine preference as observed when there are no significant differences in the time spent in the paired and unpaired chambers. Pre-tau/tau+sal group do not form a preference towards the taurine-paired chamber.

Figure 2

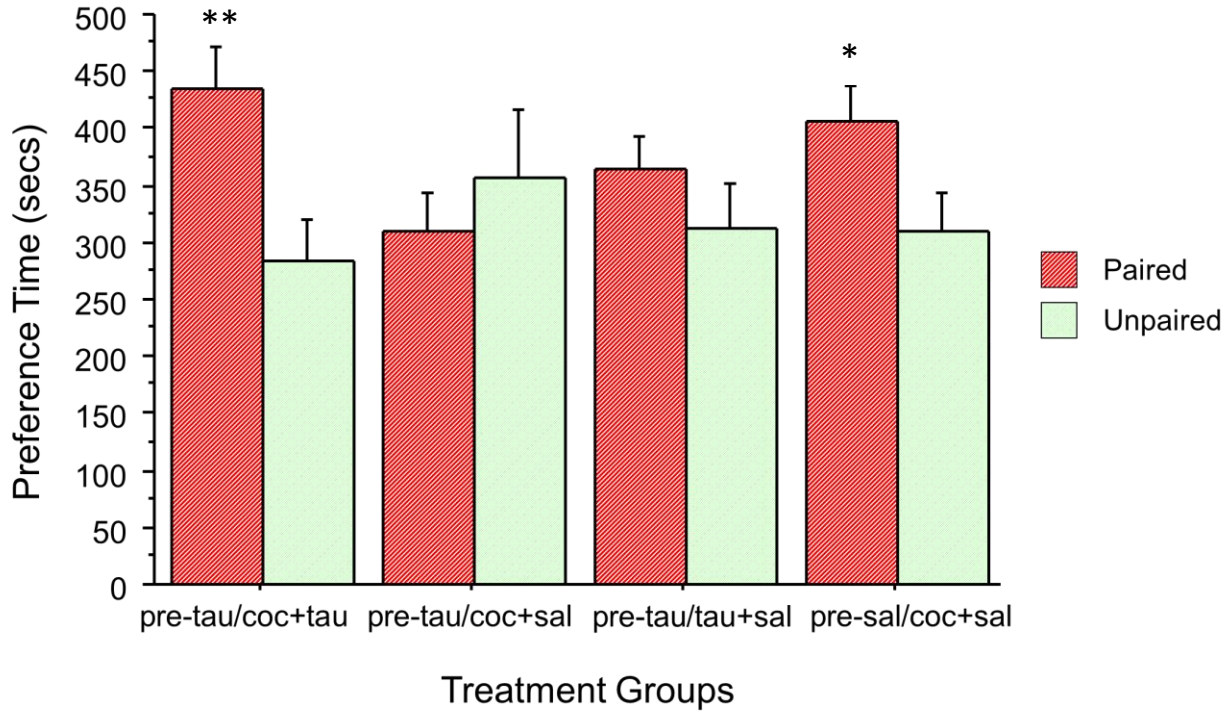

Figure 2: Co-administration with taurine enhances cocaine preference in intact females. Pre-treatment with taurine decreases cocaine preference; however, co-administration of taurine enhances preference to the drug-paired chamber in female rats (**; $p<0.01$). Females conditioned to pre-sal/coc+sal show a significant preference (*) towards the cocaine-paired chamber. Taurine pre-treatment attenuates cocaine-preference. Pre-tau/tau+sal group do not form a preference towards the taurine-paired chamber.

Figure 3

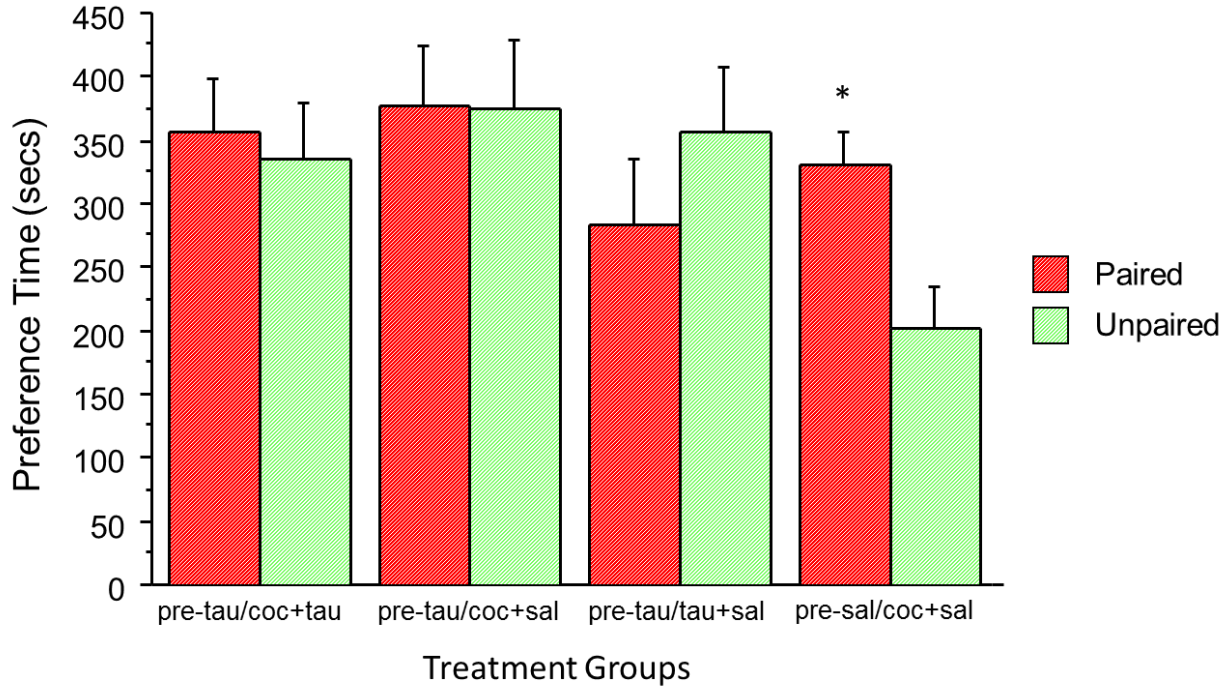

Figure 3: Cocaine preference and taurine efficacy is not testosterone-dependent. GDX males conditioned to cocaine-only show a significant preference (*) towards the cocaine paired chamber ($p<0.025$). GDX males do not form a preference to the taurine-paired chamber. Pre-treatment and co-treatment is sufficient to reduce cocaine preference to non-significant levels.

Figure 4

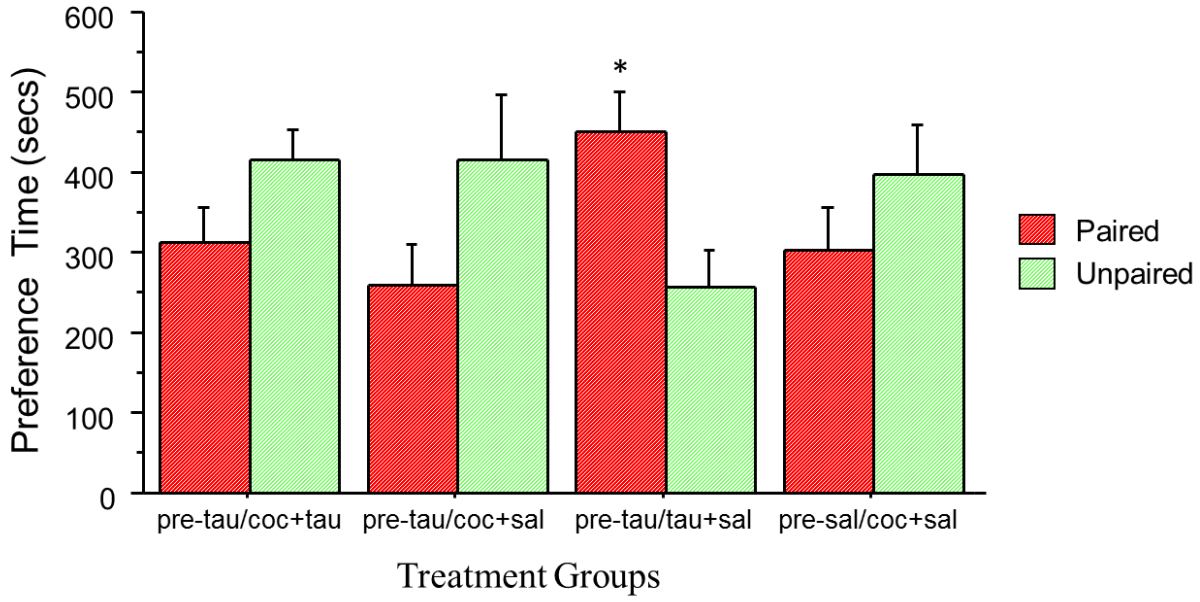

Figure 4: Lack of ovarian hormones results in taurine preference while cocaine preference is not acquired. A two-way ANOVA revealed a treatment by preference interaction ($F(3,62) = 4.11$; $p < 0.01$). OVX females do not form a preference to the cocaine-paired chamber. However, a preference to the taurine-paired chamber is formed ($p < 0.0064$).

Discussion

Intact males and females form a preference to the cocaine-paired chamber. Pre-treatment of taurine is sufficient to attenuate cocaine preference in both intact males and females. Neither intact male or intact female groups show a preference to the taurine-paired chamber. Taurine pre-treatment and co-administration of taurine+cocaine further decreases cocaine preference in intact males but exacerbates cocaine preference in intact females. OVX females do not show a significant preference towards the cocaine-paired chamber but, interestingly, do show a preference to the taurine-paired chamber. There is an interaction in the OVX-females with treatment and preference . GDX males form a preference to the cocaine-paired chamber but pre-treatment and co-administration of taurine reduces cocaine preference to non-significant levels.

Experiment 1: Taurine pre-treatment reduces cocaine preference in intact males

Taurine pre-treatment is sufficient to reduce cocaine preference in males. Co-administration of taurine during conditioning further reduces cocaine preference. Similar results have been seen in treatments for relapse. NAC, in addition to its effect in glutamatergic systems, also has been implicated in neurotrophic, antioxidant, and inflammatory pathways (Dean et al., 2011), in virtue by acting as a glutathione precursor (Asevedo et al., 2014). Rats chronically-fed ethanol are found to have reduced glutathione in their plasma and tissue, resulting in oxidative stress and damage to cellular structure and function (Pushpakiran et al., 2004). Simultaneous taurine administration with ethanol was found to restore glutathione levels, suggesting that taurine and NAC may work through similar, but different pathways as neuroprotective molecules in drug-induced toxicity.

Previous biochemical studies done by Banerjee at al. 2013demonstrated taurine injected directly into the striatum reduced spontaneous locomotor activity and CPP for cocaine. Findings from this experiment validate these findings. Pre-treatment of taurine is sufficient to reduce cocaine-preference to non-significant levels and co-administration of taurine reduces it even further. Studies in the same lab (Chan et al., 2014), found taurine modulates NMDA receptors through multiple mechanisms including reducing affinity of NMDA for glycine and through TAG receptors. Yablonsky-Alter et al. found that after chronically-withdrawn rats are given a cocaine challenge, there is an increase of glutamate release in the striatum but, concurrently, there is an increase of taurine. Taurine's inhibition of NMDA may be a mechanism through which the effect of glutamate-induced neurotoxicity is blunted, providing a self-protection mechanism against cocaine addiction and excitotoxicity.

Experiment 2: GDX males show the same response to taurine as intact males

GDX males show the same development of preferences as intact males. GDX males form a preference towards the cocaine-paired chamber ($p<.03$) but pre-treatment and co-treatment of taurine reduces cocaine's rewarding effect. Acute or chronic exogenous administration of testosterone did not differ in time spent in the cocaine-paired chamber when compared to GDX males. The finding that GDX males show similar results as intact males (Minerly et al., 2008), suggests testosterone offers minimum contribution to the rewarding effects of cocaine or taurine. In terms of locomotion, GDX males did show a transient increase in locomotion, but did not show a progressive increase of locomotion in response to cocaine sensitization (Menendez-Delmestre and Segarra 2011) when compared to intact. However, the GDX+ testosterone replacement group did show sensitization to cocaine but this effect has not been consistently

found as other studies found testosterone treatment in castrated males does not cause behavioral sensitization (Forgie and Stewart 1994). In addition, testosterone does not regulate the dopaminergic release in either the striatum or nucleus accumbens (Becker 2009; Triemstra et al., 2008), suggesting that unlike estrogen, testosterone does not confer any biochemical facilitatory effects.

Experiment 3: Taurine pre-treatment and co-administration potentiates cocaine preference in intact females

In intact females, pre-treatment of taurine is sufficient in reducing cocaine preference. In the brain, taurine functions as a neurotransmitter and may modulate cocaine's rewarding effect. Taurine supplementation has been shown to be effective in reversing and treating the deleterious physiological effects of chronic ethanol consumption (Chen et al., 2009; Park et al., 2009). Acamprosate, a glutamatergic neuromodulator, has been recently investigated as a potential treatment for cocaine addiction. Similar to taurine, acamprosate was first touted as an intervention treatment for reducing ethanol consumption and ethanol-drinking behavior (Oka et al., 2013). Recently, acamprosate has been investigated as a potential treatment for cocaine dependence and reinstatement. Acamprosate inhibited reinstatement of both cocaine-preference (Mcgeehan and Olive 2006) and self-administration (Bowers et al., 2007). Although its exact mechanisms remain unknown, similar mechanisms that mediate the efficacy of acamprosate mediate the effects of taurine through the glutamatergic system (Boothby and Doering 2005). mGluR5 has been implicated in the compulsive behaviors associated with alcohol (Blednov and Harris 2008) and cocaine dependence (Reissner et al., 2014), making it an ideal candidate for drug treatments. Interactions between mGluR5 and estrogen have been shown to facilitate of cocaine's rewarding (Peterson et al., 2014) and motor sensitization (Grove-Strawser et al., 2010), suggesting the receptor may be the driving force between the dimorphic difference in drug escalation and addiction rates between males and females. Taurine pre-treatment may be inhibiting the activation of mGluR5 by decreasing calcium-evoked currents (Albinana et al., 2010) or the binding of estrogen to the receptor.

However, co-administration of taurine exacerbates cocaine preference in intact females. The intensification of preference may be due to the presence of gonadal hormones, specifically estradiol. Estradiol has been shown to facilitate the rewarding effects of drugs of abuse such as cocaine and methamphetamine and may be responsible for the increased vulnerability female's display to drugs of abuse. Bobzean et al., (2014) showed that acute estradiol injections in OVX female rats prior to test for CPP produced a significant preference for the cocaine-paired chamber, demonstrating a prominent role for estradiol in enhancing cocaine preference. 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).

Experiment 4: OVX females form a preference to the taurine-paired chamber

OVX females do not show a preference to the cocaine-paired chamber. This may be due to the lack of estrogen present in the body. Van Swearingen et al. (2013) showed that OVX female rats given a 30mg/kg challenge dose of cocaine do not develop a preference to the cocaine-paired chamber but when given estradiol replacement injection and then a challenge dose, a preference is formed. This emergence of preference after estrogen treatment shows that

that the presence of estrogen is required for the formation of cocaine-preference. Bobzean et al. (2014) has shown OVX females develop preference for the cocaine-paired chamber at 5mg/kg and 10 mg/kg cocaine doses but not at 2.5 mg/kg and 15 mg/kg despite no difference in locomotor activity in the treatment groups. This demonstrates a dose-response curve in OVX-females to cocaine. Moderate doses create a preference but low or high doses may produce aversive properties to the chamber or may too low a dose to create a substantial rewarding effect or satiates any cravings in the rats.

The most interesting result is that OVX females form a preference to the taurine-paired chamber since intact females did not form a preference to the taurine-paired chamber. Loss of estrogen in females may occur due to surgery or natural cause. Limited studies have shown pre-mature loss of estrogen is accompanied by cognitive defect, primarily in the verbal episodic memory but there has not been enough studies to substantiate these conclusions (Henderson and Sherwin, 2007; Henderson 2007). What is true for both conditions is loss of estrogen results in an increase in anxiety, cognitive defects, and increases in dementia (Brann et al., 2007). The natural loss of estrogen occurs when women reach menopause, where estrogen levels begin to decline, resulting in cognitive defects in memory and learning. Taurine may play a role in reducing the anxiety associated with the decline in estrogen. Furuta et al., (2013) found that 3 week postpartum primiparous rats spent the least time in the open arms of the elevated plus maze and highest immobility time during the forced swim test. When given estradiol (E2) injections daily for 6 days, E2 significantly reduced anxiety and depressant actions in the elevated plus maze and forced swim tests. In addition, administration of E2 directly to the hippocampus and amygdala decreased anxious and depressive behaviors (Walf and Frye, 2006). This may suggest that while the presence of estrogen potentiates cocaine's rewarding effect, its absence creates negative mood states (Pandaranandaka et al., 2009).

Whirley and Einat (2008) found that taurine administration was not effective in reducing anxiety and depressive behaviors in mice. However, this disparity may be explained because of the anxiety phenotypes the mice showcased. McCool and Chappell (2007) initially found strychnine, a glycine antagonist, reduced anxiety in the elevated plus maze and light/dark box while taurine's anxiolytic effect was negligible in intact rats. However, when separated into "low" anxiety and "high" anxiety groups, taurine showed a significant effect in increasing time spent in the open arms in "high" anxiety animals, representing a reduction in anxiety. This effect illustrates that taurine is effective in reducing anxiety in highly anxious animals but not as effective in low anxiety animals. In accordance with our findings, pre-treatment and co-administration of taurine reduces preference to cocaine, a psychostimulant known to induce high levels of anxiety (Paine et al., 2002). OVX females, a high anxiety phenotype, do form a preference to the taurine-paired chamber but intact females do not. Furthermore, taurine has been shown to be effective as an anti-depressant in diabetic rats, a population known to be at higher risk for depression (Caletti et al., 2012; Toyoda and Lio, 2013). Studies performed in our lab have shown taurine reduces freezing in forced swim in intact and OVX females (unpublished data)

Limitations

The subjective feelings associated with psychostimulants changes with the phases of the menstrual cycle but with other classes of drugs, this effect is moderate. Taurine may be an effective prevention model for cocaine but may not be as effective in preventing addiction from sedatives, ethanol, prescription drugs and benzodiazepines because estrogen has been found to

not alter the physiological and subjective effects of other classes of drugs except for psychostimulants (Terner and Wit, 2006). Taurine's preventive effect may only be effective in drugs that work through DA and its mechanisms such as blocking its reuptake or reversing the flow through the dopamine transporter (DAT). Terner and Wit also believe estrogen modulates the subjective effect of psychostimulants because this class of drug has direct actions on DA processes, while other classes of drugs indirectly alter the DA mechanism. Although many studies inject exogenous estrogen to portray the rise in estradiol seen in women entering the follicular phase of the menstrual cycle or in rats entering the diestrus cycle (Caligioni 2009; Owen Jr., 1975), gonadectomy also excludes other important hormones such as follicle stimulating hormone, luteinizing hormone, progesterone, and testosterone. There is limited literature investigating the role of other gonadal hormones which are important in regulating the normal human physiology and may impact cocaine pharmakinetics and treatment options.

While CPP is a useful tool in measuring reward value, the model does have several limitations. A concern with CPP is that it involves a cognitive component. 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. CPP is a behavioral paradigm designed to assess the reward value of a drug by time spent in the paired-chamber and does not tell us about the reinforcement of a drug.

The behavioral paradigm, self-administration, more closely mimics the human drug addiction with drugs being immediately administered and better models the compulsive nature of cocaine addiction (Panlilio and Goldberg) than CPP. Self-administration is a model designed to reinforce responses through the effects of drugs, leading to behavioral sensitization, a crucial aspect of addiction seen in the natural world. Self-administration can also be better used to test drug treatments and produces a more valid predictor of clinical efficacy (O'Connor et al., 2011).

Possible Mechanisms by which Taurine can inhibit cocaine preference

Previous research on taurine has elucidated taurine's role in physiological and cellular functions (Schaffer et al., 2010; Hussy et al., 2001) but never in the context of a reward. This study is the first to test if taurine can act as a rewarding stimulus. Therefore, a pre-tau/tau+sal group is included to investigate if reductions of cocaine preference are due to the neuroprotective role of taurine and not because of a competing stimulus. In both intact males and females and GDX-males, there is no significant preference to the taurine-paired chamber, showing taurine is not a rewarding stimulus and reductions of cocaine-preference is due to taurine's neuromodulatory effects. Therefore, decline of preference in the pre-tau/coc+sal suggests taurine reduces cocaine's rewarding effect.

One mechanism through which taurine may reduce cocaine preference is reduction of stress. Females have also been found to higher levels of corticotrophin-releasing factor receptors, the main stress hormone, suggesting a higher risk for the effects of stress such as cardiovascular disease, anxiety, depression, and substance abuse (Bangasser et al., 2010; Valentino et al., 2013). Deviations from normal corticosteroid levels may produce anxiolytic-like symptoms and discomfort (McEwen 2007), pushing individuals to self-medicate. Taurine has been implicated in combining with antioxidants to create a more stable antioxidant to suppress the inflammatory effects of cytokines (Wojcik et al., 2010). Activation of the hypothalamic-pituitary-adrenal axis (HPA) axis during bouts of stress release glucocorticoids and catecholamines that suppress the

action of cytokines (Elenkov and Chrousos 2002). During self-administration, plasma corticosterone levels follow a dose-related curve (Goeders et al., 1998). Cocaine induces anxiety and panic in humans and animals (Goeders, 2002). It may be taurine reduces the strength of the negative reinforcement motivation (Koob et al., 2014) or suppress the impact of cytokines (Park et al., 2009), decreasing the need to alleviate the distress and craving to self-medicate. This implicates taurine as being a potential treatment for preventing cue-induced drug seeking behavior, a major cause for recovering addicts to relapse.

Taurine is also a structural analog of GABA and glycine, suggesting that its neuroprotective role may stem from its activation of GABA and glycine receptors (Horikoshi et al., 1988). Chronic high-dose of cocaine induces long term potentiation (LTP) in ventral tegmental area (VTA) neurons and disinhibits VTA DA neurons through inhibition of GABA-voltage-sensitive sodium channels (Liu et al., 2005; Steffensen et al., 2008), enhancing mesocorticolimbic DA transmission. It is possible midbrain GABA neurons modulate the hedonic value and salience of cocaine and other drugs of abuse. In disregulated systems, drug-seeking behavior and reward value are reinforced, resulting in drug abuse and addiction. Albinana et al., found acute application of taurine induces a dose-dependent chloride conductance through $GABA_A$ channels and regulates calcium conductance. Medium and high concentrations of taurine bind to glycine and GABA receptors, respectively, in the basolateral amygdala, hippocampus, and nucleus accumbens (Jia et al., 2008) as well as inhibit thalamic relay neurons. Taurine is also transported by the GABA transporter at the blood brain barrier as well as by its own transporter, (Takanaga et al., 2001), illustrating the importance of taurine in the CNS. Albinana also found intracellular dialysis of GDP-β-σ inhibited the reduction of calcium conductance seen by taurine binding, demonstrating taurine modulation on voltage-gated calcium channels is mediated by G-protein coupled receptors (GPCR). This mechanism is poorly understood and future research may be directed to investigate if similar GPCR's are found in the rat and human brain.

Current research has been investigating the role of taurine and NMDA receptors and their role in cocaine addiction. Chan et al., (2014) showed that taurine does interact with NMDA receptors through multiple mechanisms. Continuing down this avenue, Chan et al., (2015) elucidated that taurine modulates NMDA activation through interactions with the GluN1/GluN2B subunit. Blocking of GluN2B NMDA receptors rescued dysfunctional long-term depression in the oval bed nucleus of the stria terminalis, suggesting an important role of NMDA receptors in the metaplasticity evoked by cocaine and relapse (deBacker et al., 2015). Since, taurine inhibits NMDA through GluN2B and GluN2B has been shown to be critical for addiction and relapse (Yuan et al., 2013) and glutamate has been implicated as the mediator of cocaine-induced reinstatement and relapsing (Cornish and Kalivas, 2000), taurine is a potential candidate for treatment for substance abuse disorders.

Implications

Taken together, the data suggests taurine is an effective intervention for substance use disorders but its efficacy is dependent on hormonal status. Taurine's efficacy is not contingent on the presence of testosterone as intact and castrated males show similar results. Dimorphic differences to cocaine and taurine may be due to differing levels of estrogen. Estrogen enhances NMDA excitatory post synaptic potentials (EPSPs) (Foy et al., 1999) and LTP (Smith and McMahon 2006). Acute cocaine has been found to increases mesocorticolimbic spine density and excitatory synapses (Luscher and Malenka 2011), an attribute shared with estrogen

(Srivastava et al., 2008). Cocaine produces hyperactivation of PFC-NAc efferents, leading to increased salience of drug-associated cues and occurrence of compulsive behaviors and cocaine use fosters ΔFosB accumulation, resulting in the potentiation of synaptic NMDA insertion in NAc neurons (Dong and Nestler), a critical reward structure. Taurine pre-treatment inhibits GluN1/GluN2B subunit, reducing NMDA excitation and decreasing cocaine reward and preference. However, crossing a certain threshold of taurine levels may reverse its protective effect and facilitate cocaine's rewarding effect.

Addiction is a disorder of cognition and behavior and involves the interplay of several disrupted brain structures and functions. When cocaine-preference is brought to extinction and a challenge dose is administered, rats still show a significant preference towards cocaine (Brenhouse and Andersen 2008), demonstrating the presence of disrupted neurocircuitry even after prolonged, suggesting treatment for drug addicts is a complex process. Progesterone and exercise have been shown to be effective in reducing the reinstatement of cocaine preference but this effect is greater in females than males (Zlebnik et al., 2014), indicating the importance of taking into account and investigating the impact sex differences have on the effectiveness of drug abuse treatments. Rehabilitation clinics should implement multimodal treatment programs to address the global disruptions caused by drug abuse. By implementing multimodal programs, treatments are able to treat drug abuse from multiple facets, treating the myriad of familial, biological and maladjusted social behaviors associated with drug abuse and tailor specific treatments for patients. These studies support that taurine is a viable candidate for cocaine addiction, with potentially few side effects, and, in addition, could be neuroprotective. Further studies need to be performed to assess how taurine is acting on the mesocorticolimbic circuit to inhibit cocaine reward.

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