

Differential COMT expression and behavioral effects of COMT inhibition in male and female Wistar and alcohol preferring rats

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Highlights

- The effects of COMT inhibition on alcohol seeking and drinking were examined.
- Sex and strain differences in COMT expression were examined.
- Tolcapone reduces reinforcer intake and seeking in male alcohol preferring P rats
- Tolcapone had no effect on consumption or seeking in female rats
- Sex and strain differences in COMT expression were observed in the PFC

Keywords

tolcapone, sex differences, dopamine, prefrontal cortex, alcohol, rodent

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Abstract

Polymorphisms of the catechol-O-methyl transferase (COMT) gene have been associated with alcoholism, suggesting that alterations in the metabolism of catecholamines may be a critical component of the neuropathology of alcoholism. In the current experiments, the COMT inhibitor tolcapone was utilized in an operant behavioral model of reinforcer-seeking and drinking to determine if this compound was capable of remediating the excessive seeking and drinking phenotype of the alcohol-preferring P rat. Tolcapone was administered to male and female alcohol-reinforced P and Wistar rats. Additionally, tolcapone was administered to male sucrose-reinforced P and Wistar rats to determine if its effects also extended to a natural reinforcer. Animals were trained to make an operant response that resulted in 20 min uninterrupted access to the reinforcer solutions. Tolcapone had no effect in female rats on either seeking or consumption of ethanol. However, reductions of both reinforcer seeking and consumption were observed in male P rats, but only of seeking in Wistars. In separate experiments, using reinforcer naïve male and female animals, COMT expression was assessed via Western Blot analysis. Sex differences in COMT expression were also observed, where male P rats exhibited a marked reduction in protein expression relative to females in the PFC. Sex differences were not observed for Wistars or in the striatum and hippocampus. These data complement our previous findings in which tolcapone reduced cue-evoked responses in P rats and further suggest clinical utility of COMT inhibitors in the treatment of addiction disorders, specifically in male high drinkers.

Introduction

Differences in catechol-o-methyl transferase (COMT) genotype have been linked to illicit drug use (Chen et al., 2014) and polysubstance abuse (Vandenbergh et al., 1997). However, with regard to alcohol use, the role of COMT is unclear. Associations between allelic variations in COMT and drinking behaviors are mixed, with some finding associations (Hendershot et al., 2012; Kauhanen et al., 2000; Tammimäki et al., 2008; Tiihonen et al., 1999; Wang et al., 2001), while others do not (Hallikainen et al., 2000; Kweon et al., 2005; Samochowiec et al., 2006; Shibuya et al., 1999). Similarly, the relationship between COMT and the propensity to relapse is complex, and while an association has been observed (Wojnar et al., 2009) other studies have failed to find one (Foroud et al., 2007; Köhnke et al., 2003). COMT is hypothesized to play a role in executive function (Egan et al., 2001; Goldberg et al., 2003; Tammimäki and Mannisto, 2010) and deficits in executive function are associated with risk for alcohol use disorder (AUD; Finn et al., 2009). As such, manipulations of COMT may ameliorate maladaptive drug seeking and taking behaviors.

Alcohol preferring (P) rats are selectively bred for free-choice alcohol preference, exhibit excessive reward consumption (Lankford et al., 1991; McCane et al., 2014), and deficits in

executive function (Beckwith and Czachowski, 2016; Linsenhardt et al., 2016). Additionally, reduced dopamine (DA) levels in the medial prefrontal cortex (PFC) have been observed in P rats, when compared with their progenitor strain, the Wistar rat (Engleman et al., 2006). Given the well documented role of COMT in degrading cortical DA (Käenmäki et al., 2010; Yavich et al., 2007), we hypothesized that differences in COMT may be associated with the excessive alcohol seeking and consuming phenotype of the P rats. Using the COMT inhibitor tolcapone, we have previously observed a tolcapone-mediated suppression of cued ethanol seeking in male P rats but not Wistars, with no effect on free choice ethanol consumption in either strain (McCane et al., 2014). However, it is unclear how differences in COMT might account for differences in tolcapone's efficacy. Strain differences in tolcapone's efficacy may be attributable to differences in COMT, and would suggest a link between COMT and an excessive alcohol seeking/drinking phenotype.

Sex has been shown to mediate the relationship between allelic differences in COMT and behavior (Harrison and Tunbridge, 2008). The COMT promoter region contains 2 estrogen response elements (Xie et al., 1999) that can inhibit the formation of COMT (Jiang et al., 2003). With regard to alcohol drinking, Tammimäki et al. (2008) showed that manipulating COMT affected consumption in male but not female mice. In the absence of COMT manipulations, sex specific effects on alcohol behaviors have also been reported. For instance, female rodents show overall higher ethanol intakes than males (Lancaster and Spiegel, 1992; Morales et al., 2014; Tammimäki et al., 2008). We have previously shown that COMT modulation reduces drinking in male P rats (McCane et al., 2014) but the effects of tolcapone on the drinking behaviors of female P rats is unknown. Therefore, the current study assessed the effects of tolcapone in both male and female P and Wistar rats.

Working under the hypothesis that differences in COMT are associated with aberrant ethanol seeking and intake, the current project sought to investigate the influence of alterations in COMT activity and expression on alcohol seeking and drinking. While sex-linked differences in COMT protein expression (Schendzielorz et al., 2011) and effects of tolcapone are documented (Tunbridge et al., 2013), it is currently unknown if COMT expression differs between male and female Wistar and P rats. Thus it is unclear whether pharmacotherapies targeting COMT will be efficacious in both sexes. Therefore, an additional aim of the current project was to quantify sex and strain differences in COMT using western blot analyses to determine whether these differences in COMT expression might be associated with variances in the effects of tolcapone.

Materials and Methods

All procedures were approved by the IUPUI School of Science Institutional Animal Care and Use Committee and were in accordance with the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals (2011).

Subjects: Subjects were alcohol naïve adult male (N=32) and female (N=27) Wistar rats (Harlan, Indianapolis, IN) and male (N=30) and female (N=28) P rats (75th and 76th generations; Indiana University, IN) for all experiments. Animals were single housed in a climate controlled room on a 12-hour light dark cycle. All animals were handled one week prior to testing and were approximately 12 weeks of age at the start of testing.

Intermittent access drinking protocol (IAP): A modified two-bottle free choice drinking protocol as described by Simms et al. (2008) was implemented to expose animals to the liquid solutions later used during conditioning and to initiate solution consumption. Subjects were divided by strain, drinking solution and sex which resulted in six groups: alcohol male P ($n = 9$), alcohol male Wistar ($n = 12$), sucrose male P ($n = 9$), sucrose male Wistar ($n = 8$), alcohol female P ($n=15$) and alcohol female Wistar ($n = 16$). One hour after the start of the dark cycle, all water bottles were replaced with two bottles, one containing water and the other containing either sucrose (1%) or ethanol (20%), counterbalanced by side. After 24 hours of two-bottle access, bottles were removed, fluid intake was measured and the initial water bottle was replaced. Animals had access to their respective solutions 24 hr/d for 3 d/wk (M, W, F) over the course of a four-week period. Animals were weighed immediately before bottle placement to calculate grams of sucrose or ethanol intake per kilogram of body weight.

Procedural separation of seeking and drinking (PSSD) training: All operant conditioning sessions were conducted in modular operant chambers (30 x 30 x 24.5 cm; Med Associates, St. Albans, VT) equipped with a house light, retractable lever and a retractable graduated cylinder tube fitted with stainless steel spouts containing double ball bearings to reduce leakage.

After 4 weeks of IAP drinking, all animals were transitioned to an operant conditioning paradigm. To facilitate lever press training, animals were water restricted in their home cages 24-hours prior to the first conditioning session. Animals were then hand shaped to lever press on a fixed ratio (FR) 1 schedule for 30 seconds of access to water. Animals remained water restricted for approximately one week on an FR1 schedule for water access, which was shifted from 30 seconds to 15 seconds after two consecutive days of 5 or more responses. After successful (≥ 10) lever press responses for water during a 30-minute session, animals were given ad libitum water in their home cages and the operant conditioning reinforcer was changed from water to either sucrose (1%) or ethanol (10%), depending on previous grouping in the IAP. Animals continued lever press training and the FR1 schedule was gradually increased to an FR4 over the course of a week. After successful (≥ 8) responding under an FR4, animals were transitioned to a response requirement (RR) 4 schedule where four responses were reinforced with 20 minutes of access to sucrose or ethanol. Animals were then gradually increased to an RR10, which was maintained for two weeks prior to drug testing. Acquisition of operant responding took approximately, six weeks.

PSSD consummatory test phase: After animals responded for an RR10 for 4/5 days a week, a four week consummatory testing phase was initiated in which animals received intraperitoneal (IP) injections of tolcapone (Valeant Pharmaceuticals; 0; 3.0; 10.0; 30.0 mg/kg) in a balanced design two hours prior to testing on one day (Wednesday) each week. Tolcapone was prepared by dissolving crushed tablets in sterile saline (vehicle; Lapish et al., 2009; McCane et al., 2014).

Tablets were fully dissolved (i.e., not in suspension) prior to drug administration. This method of drug preparation and delivery reliably enhances evoked DA efflux as measured by microdialysis (Lapish et al., 2009). The maximum dose chosen has been consistently observed to inhibit COMT in mice (Tammimäki et al., 2016) and is within the narrow range of drug concentrations which inhibit COMT (Borges et al., 1997). On testing days, the reinforcement schedule was changed to an RR1. The following day (Thursday) animals were moved to an RR5 and an RR10 was implemented all other days. Intakes (g/kg) were determined from change in fluid volume of the sipper tube and daily body weights.

PSSD appetitive test phase: Following the consummatory phase of testing, the response requirement was gradually increased to an RR20, which was maintained for two weeks prior to drug testing. After consistent responding on an RR20, a four week appetitive testing phase was initiated in which animals received IP injections of tolcapone (0; 3.0; 10.0; 30.0 mg/kg) in a balanced design two hours prior to testing on one day each week (Wednesday). On testing days, all animals were given an extinction session. During extinction, retractable sippers containing reinforcers to control for scent cues were present but lever pressing was not reinforced. The following day (Thursday) animals were moved up to an RR10 and an RR20 was implemented all other days. Once a week, animals would receive a vehicle injection two hours prior to a reinforced session to control for the possibility that animals may associate an injection with an extinction session.

Western Blot: Following behavioral testing, separate cohorts of rats were used for western blot analyses. We first assessed COMT protein levels in male P and Wistar rats to determine whether strain differences in COMT were present. Following behavioral testing, we subsequently assessed sex differences to better understand how differences in COMT protein level may be associated with behavioral differences observed. Alcohol naïve male and female P (n = 11-12 male, n=13 female) and Wistar (n = 12 male, n=11 female) rats were euthanized and brains were extracted. Coordinates were based on the rat brain atlas (Paxinos and Watson, 2007). Tissue from the medial (m)PFC (AP, 3.2; ML, 0.6; DV, -2.5), hippocampus (AP, -4.16; ML, 2.8; DV, -3.2), and ventral striatum (vST; AP, 1.6; ML, 1.5; DV, -6.7) were dissected for Western Blot analyses. These regions were chosen because of their known involvement in drug seeking behavior and previous evidence of COMT activity. Tissue was placed in a Potter-Elvehjem tissue grinder and homogenized in 2% Sodium Dodecyl Sulfate (SDS). Protein concentration was determined by bicinchoninic acid assay kit (Pierce Biotechnology, Inc., Rockford, IL., USA). Proteins were separated on 12% SDS-Page gels (Bio-Rad) then transferred using a Transblot Turbo System (Bio-Rad Laboratories, USA) to nitrocellulose paper via 1.3A 25V for 7 minutes (COMT ~24 Kd). The blots were then stained with Ponceau S (BP10-10, Thermo Fisher Scientific, Waltham, MA., USA) and scanned as a TIFF image on Epson V700 photo scanner for loading control analysis. For immunostaining, the blot was blocked for 1 hour in TBS-T (TBS with 0.1% Tween 20) and 5% nonfat dry milk. The blot was then incubated in anti-COMT

(BDB611970, BD Transduction Laboratory) diluted 1/1000, for 18 hours at 4°. Then the blot was rinsed in TBS-T with 5% nonfat dry milk. The second antibody (Goat anti-Mouse Alexa 790) was diluted 1/10,000 in TBS-T with 5% nonfat dry milk. The blot was incubated in the secondary at room temperature for 1 hour on a rocker and then rinsed in TBS. As a control, gels were run in duplicate with one gel receiving no primary antibody. Bands were visualized with the LI-Cor CLx Odyssey Infrared Imaging System (Imaging system Li-Cor Inc, Lincoln, NE).

Data analysis and statistics: All statistical analyses were performed in R (<http://www.r-project.org>). All group effects were first assessed by analysis of variance (ANOVA) and Dunnett's post-hoc was used for all multiple comparison procedures when appropriate. For Western blots, to control for loading differences, Ponceau signal intensity was assessed between male versus female Wistars and P rats via unpaired t-tests in the mPFC, hippocampus, and vST, separately. To compare COMT protein levels between sexes for each brain region for each treatment, COMT, the following formula was used: Total COMT/Ponceau signal intensity. To compare COMT protein levels between strains, COMT was normalized by total protein content and then compared across strains. To get a normalized value for each animal, the following formula was used: (total COMT/total protein) / (averaged total COMT/total protein of all Wistar rats). Data are presented as normalized to Wistar (%).

Results

IAP

A three-way repeated measures ANOVA (RMANOVA) of ethanol intake by strain, sex, and day indicated that female rats consumed significantly more ethanol than males overall all [main effect of sex: $F(1,46)=22.89$, $p<0.001$]. Data were therefore stratified by sex.

In females, P rats consumed more ethanol than Wistars [two-way RMANOVA intake by strain and day, main effect of strain: $F(1, 28) = 32.52$, $p<0.001$] while both strains increased their ethanol intake over days [main effect of day: $F(1,28)=9.92$, $p=0.0039$, Fig 1].

Similarly, in male rats, P rats drank more ethanol than Wistars [two-way RMANOVA, intake by strain and day, main effect of strain: $F(1,18) = 30.9$, $p < 0.0001$] and both P rats and Wistars increased their ethanol [main effect of day: $F(11,208) = 3.2$, $p=0.0005$; Fig 1] intake over days.

Additionally, in sucrose drinking male rats, P rats drank more than Wistars [RMANOVA, intake by strain and day, main effect of strain: $F(1,12) = 131.6$, $p < 0.001$] but Wistars and P rats showed different patterns of intake over days [strain \times day interaction: $F(11,181) = 9.4$, $p < 0.001$; Fig 2], where only P rats exhibited a change in intake over days [main effect of day: $F(1,8) = 30.76$, $p = 0.0005$].

PSSD and Tolcapone

In the consummatory test phase, there were significant three way [three-way RMANOVA, group \times strain \times treatment: $F(1,32) = 4.7, p = 0.03$] and two way [strain \times treatment: $F(1,32)=8.8, p=0.006$] interactions in male rats. Data were next stratified by strain which yielded a significant group by treatment interaction in P rats [$F(1,16)=5.9, p=0.03$] but not Wistars [$F(1,16)=0.07,p=0.8$]. In P rats, there was main effect of treatment on both ethanol [$F(1,8)=7.1, p=0.03$; Fig 3A1] and sucrose [$F(1,8)=9.8,p=0.01$; Fig 3B] intake. There was no main effect of treatment in the Wistars [$F(1,16)=1.4,p=0.3$].

To test the a priori hypothesis that tolcapone's effects may differ by sex, tolcapone was administered to a separate group of female rats during the consummatory test phase using ethanol as the reinforcer. A null effect of tolcapone on ethanol intake in females was observed [two-way RMANOVA, intake by strain and treatment, main effect of treatment: $F(3,82)=0.23, p=0.9$, Fig 3A2].

In the appetitive test phase, male rats were stratified by strain which was justified by a significant main effect of strain [$F(1,29)=24.1, p<0.001$]. Significant main effects of treatment were observed in both Wistars [$F(1,15)=10.9, p=0.005$] and P rats [$F(1,15)=29.7, p<0.001$] on lever-press responding. For both strains, Dunnett's post hoc test indicated that the effect of tolcapone to decrease seeking was driven by the 30.0 mg/kg dose relative to vehicle [$p<0.05$, Fig 4]. There was no effect of tolcapone on latency to first response [$F(1,31)=0.33, p=0.57$] or latency to first lick [$F(1,31)=0.06,p=0.81$], suggesting that tolcapone treatment did not alter locomotor activity.

In females, there was a significant main effect of strain [$F(1,29)=23.63, p<0.001$] with P rats responding more than Wistars overall, however, tolcapone had no effect on seeking in either P [main effect of treatment: $p=.64$] or Wistar rats [$p=.99$].

COMT protein expression

For western blot analyses, we tested two separate hypotheses yielding two different blots. In the first western blot, strain differences in protein expression were assessed in male rats. There were significant decreases in COMT in the P rats compared to Wistars in the PFC [$t(12)=3.5, p=0.004$], in the hippocampus [$t(12)=3.4, p=0.006$] and in the vST [$t(12)=2.5, p=0.03$;Fig 5A]. Sex differences were next evaluated in separate blots. There were no differences between sexes in Ponceau signal intensity in each brain region for each treatment (independent samples t-tests, $p>0.05$; data not shown). Furthermore, there were no significant differences in the PFC [$t(21)=0.687, p=0.4996$], hippocampus [$t(21)=0.0481, p=0.9621$], or vST [$t(21)=0.4131,$

$p=0.6837$] when examining sex differences in Wistars for Total COMT/Ponceau signal intensity (Fig. 5B). Interestingly, female P rats had higher Total COMT/Ponceau signal intensity in the PFC compared to male P rats [$t(23)=3.784$, $p=0.0010$] (Fig 5C). These differences were not observed in the hippocampus [$t(22)=0.09352$, $p=0.9263$] or vST [$t(23)=0.1294$, $p=0.8981$].

Discussion

In our previous study, we observed that tolcapone treatment resulted in a reduction in cued reward consumption in male P but not Wistar rats (McCane et al., 2014). Additionally, we observed null effects of tolcapone on free choice drinking for both P and Wistar rats (McCane et al., 2014). Importantly, both these findings were observed in male rats and females were not tested. Here we report that tolcapone reduces “earned” reinforcer consumption in male P but not Wistar rats with no effect on females of either strain. In other words, consummatory behavior was consistently modulated by tolcapone under conditions of cued, limited access, but not in a free choice condition (IAP; McCane et al., 2014). Therefore, we hypothesize that the behavioral effects of this compound are not mediated by a general suppression of consummatory behavior but rather an attenuation of the salience of reward-paired cues. Moreover, since both sex and strain differences in COMT expression were detected in the PFC, we further hypothesize that COMT-mediated differences in cortical catecholamine levels contribute to the efficacy of tolcapone.

Our previous findings showed that tolcapone attenuated cued ethanol consumption in Wistars, but only in high drinkers (McCane et al., 2014). However, in the current study, tolcapone had no effect in female rats of either strain, both of which drank more than their male counterparts. It is not likely that alcohol history alone can explain the differences in tolcapone’s efficacy. In our previous study (McCane et al., 2014) we observed that during the IAP, male P rats drank levels of ethanol comparable to what females in the same paradigm consume in the current experiments. Additionally, we have previously observed that tolcapone was more efficacious in high drinkers, compared to low drinkers (McCane et al, 2014). As such, one might hypothesize that in females tolcapone would be more efficacious, which was not the case, indicating sex-mediated differences in COMT, independent of level of intake. Our findings are consistent with results obtained from Tammimäki et al. (2008), where COMT gene disruption resulted in sex-mediated differences in ethanol intake in mice. Specifically, male mice with COMT gene disruptions consumed more ethanol than wild type mice and, while females consumed more ethanol than males, there was no effect of COMT gene disruption on their ethanol intake (Tammimäki et al. 2008). Interactions between sex and COMT genotype have also been observed in behavioral responses to pharmacological challenges. Male and female mice showed different responses to amphetamine-induced locomotor activity where male mice homozygous for COMT deletion showed enhanced activity, an effect not observed in female homozygotes or wild type animals (Huotari et al., 2004). Moreover, sex differences in

pharmacological therapies for AUD have been reported where naltrexone in combination with topiramate reduced responding for ethanol in male rats to a greater extent than in female rats (Moore and Lynch, 2015). Consistent with these findings, in the current experiments, female behavior was not sensitive to pharmacological manipulations of COMT. However, it is possible that a higher dose is required in females. Additionally, in mice, sex/COMT genotype interactions were observed in a behavioral measure of impulsivity (Papaleo et al., 2012), a phenotype associated with addiction disorders (Perry and Carroll, 2008). Lastly, sex differences in COMT inhibition on catecholamine metabolism have been observed in rats (Laatikainen et al., 2013). Estrogen decreases COMT activity as well as gene and protein expression (Cohn and Axelrod, 1971; Jiang et al., 2003). Sex specific compensatory mechanisms may therefore account for interactions between sex, COMT, and behavior (Harrison and Tunbridge, 2008). The present experiments add to a growing body of literature which suggest that COMT genotype has sexually dimorphic effects on behavior (Harrison and Tunbridge, 2008).

COMT protein expression was investigated as a potential mechanism by which tolcapone may differentially affect male and female P and Wistar rats. COMT protein levels in the PFC were lower in P rats compared to Wistars but female P rats expressed greater levels of COMT in the PFC relative to males. Engleman et al. (2006) reported that P rats exhibited lower extracellular DA levels relative to Wistars which may result in a down regulation of COMT expression. Importantly, strain differences in PFC DA tone were only previously assessed in male rats (Engleman et al., 2006), making it unclear whether or not this strain difference is present in females. The current studies assessed levels of COMT protein but not enzymatic activity of COMT. While allelic variations in COMT have been modeled in mice (Papaleo et al., 2008; Risbrough et al., 2014), to date no such rat models exist. However, mice with increased COMT activity show deficits in attentional processes (Papaleo et al., 2008) and response inhibition (Simpson et al., 2014) similar to individuals with AUD (Noel et al., 2007; Vollstadt-Klein et al., 2012) and P rats (Beckwith and Czachowski, 2014; Beckwith and Czachowski, 2016; Linsenhardt et al., 2016). Additionally, polymorphisms of the COMT gene are characterized by differences in enzyme activity (Chen et al., 2004) and these differences are associated with alterations in executive functioning (Egan et al., 2001; Farrell et al., 2012). Thus, greater enzymatic activity of COMT in P rats may explain reduced cortical DA tone and COMT protein levels relative to Wistars. Similar to observations in clinical populations (Farrell et al., 2012; Mattay et al., 2003), animals with greater enzymatic activity may show increased sensitivity to pharmacological compounds which target PFC DA and PFC-mediated behaviors. Differences in COMT metabolism of DA may underlie the behavioral differences reported here, but future studies are needed to fully explore these possibilities.

The efficacy of tolcapone in male rats for each of the reinforcers tested here may be associated with differences in PFC function. It has been hypothesized that the PFC encodes, among other things, subjective value (Kable and Glimcher, 2007). Therefore, the efficacy of tolcapone in the PSSD task may be mediated by each animal's appraisal of its respective reinforcer. This is consistent with the non-ethanol specific effects of tolcapone on consummatory

behavior in P rats which may be due to the perceived value of reward in these animals which show excessive reward consumption for both ethanol and sucrose (McCane et al., 2014). In fact, Shnitko and Robinson (2014) recently reported statistically indistinguishable cue-evoked striatal DA activity in ethanol- and sucrose-reinforced rats. The authors posited that these results may indicate that both solutions were equally reinforcing, evident by similar operant responding in both groups (Shnitko and Robinson, 2014). Cue-evoked DA release is consistently reported (Brown et al., 2011; Fotros et al., 2013) and is hypothesized to play a role in encoding of salient stimuli (Berridge and Robinson, 1998). Enhancement of DA tone may shift the signal to noise ratio (Servan-Schreiber et al., 1990), dampening the effects of stimulus presentation on subsequent behavior. Under this hypothesis, in the current experiments, a tolcapone-mediated suppression of cue salience may lead to a reduction in reward seeking and consumption. Subjective value assigned to a reinforcing substance would influence the salience that one attributes to cues predictive of said substance. Importantly, the nonspecific nature of tolcapone's effects on reinforcers in general suggests that COMT inhibition may possess clinical utility in treatment of addiction disorders broadly.

Given innate differences in PFC DA tone between P and Wistar rats (Engleman et al., 2006), coupled with our findings that COMT expression differs by strain and sex in the PFC, we hypothesize that tolcapone exerts its effects primarily on cortical catecholamines. However, high COMT expression in the hippocampus has also been reported (Matsumoto et al., 2003) and tolcapone affects hippocampal DA and behavior in hippocampus-mediated tasks (Laatikainen et al., 2012; Laatikainen et al., 2013). Furthermore, hippocampal DA has been hypothesized to contribute to attribution of incentive salience (Fotros et al., 2013). Thus in the present experiments, it is unclear to what extent hippocampal DA levels may contribute to tolcapone-mediated changes in drinking behaviors. Experiments that seek to tease apart the involvement of DA transmission in the hippocampus versus PFC in ethanol-motivated responding are thus warranted.

Collectively, these data suggest the potential clinical utility of COMT inhibitors for the treatment of addiction disorders. Similar to current pharmacotherapies such as naltrexone, tolcapone's effects were not ethanol specific, therefore tolcapone may be effective in reducing other drug reinforced behaviors and should be investigated accordingly. Importantly, the degree to which tolcapone may suppress natural reinforcers in clinical populations is unknown, and as such, future clinical studies should exercise caution when working with this compound. It should be noted that the differential effects in P rats and Wistars suggest that this compound may be more efficacious for "at risk" populations susceptible to drug abuse. Preclinical findings support this hypothesis where allelic variation of the COMT gene was associated with differences in tolcapone's efficacy in ameliorating cognitive deficits (Farrell et al., 2012; Grant et al., 2013). Tolcapone's sex specific effects are consistent with observations made in COMT deficient mice (Tammimäki et al., 2008) and suggest that sex differences in COMT activity may be associated with differences in the neurophysiological mechanisms underlying drinking behaviors (Harrison and Tunbridge, 2008). Lastly, it should be noted that sex and strain differences in

pharmacokinetics may underlie the results attained here, and should be considered as a caveat to be investigated in future studies. Overall however, the behavior-specific effects observed suggest this compound may hold promise as a means to remediate reward seeking behaviors, indicating that COMT inhibitors should be investigated as a tool to mitigate reward craving and reduce propensity to relapse.

ACCEPTED MANUSCRIPT

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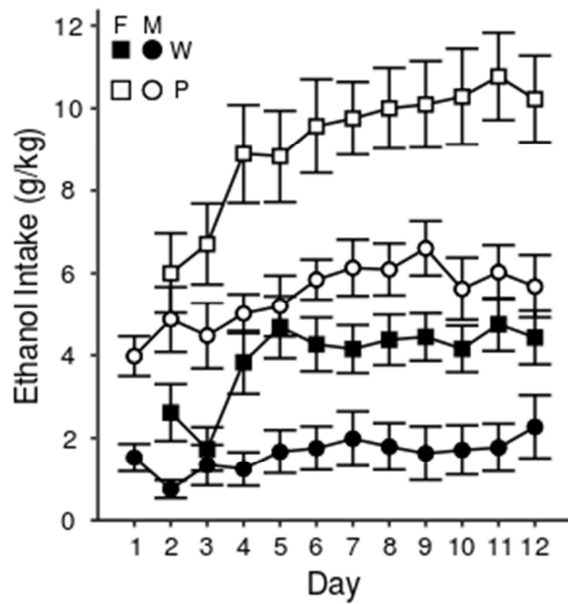


Figure 1. Ethanol intake in the intermittent access protocol for female (square) and male (circle) P (open) and Wistar (closed) rats. P rats consume more ethanol compared to their Wistar counterparts. Female rats of both strains consume more EtOH than males.

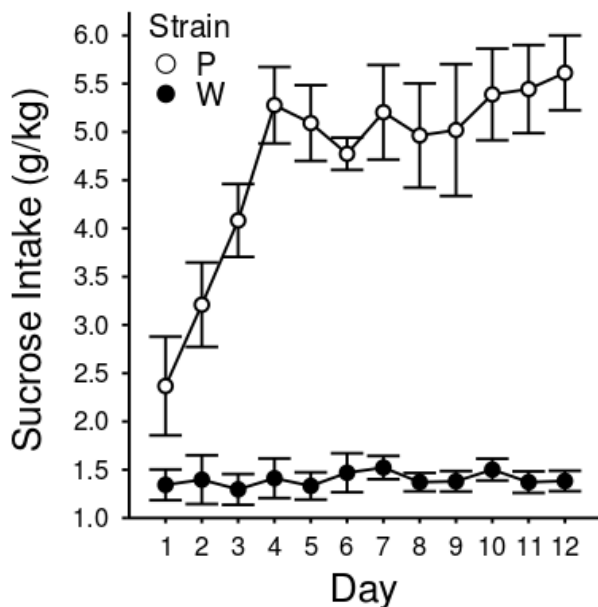


Figure 2. Sucrose intake in the intermittent access protocol for male P (open) and Wistar (closed) rats. P rats consume more sucrose than Wistars and show an escalation in intake over days.

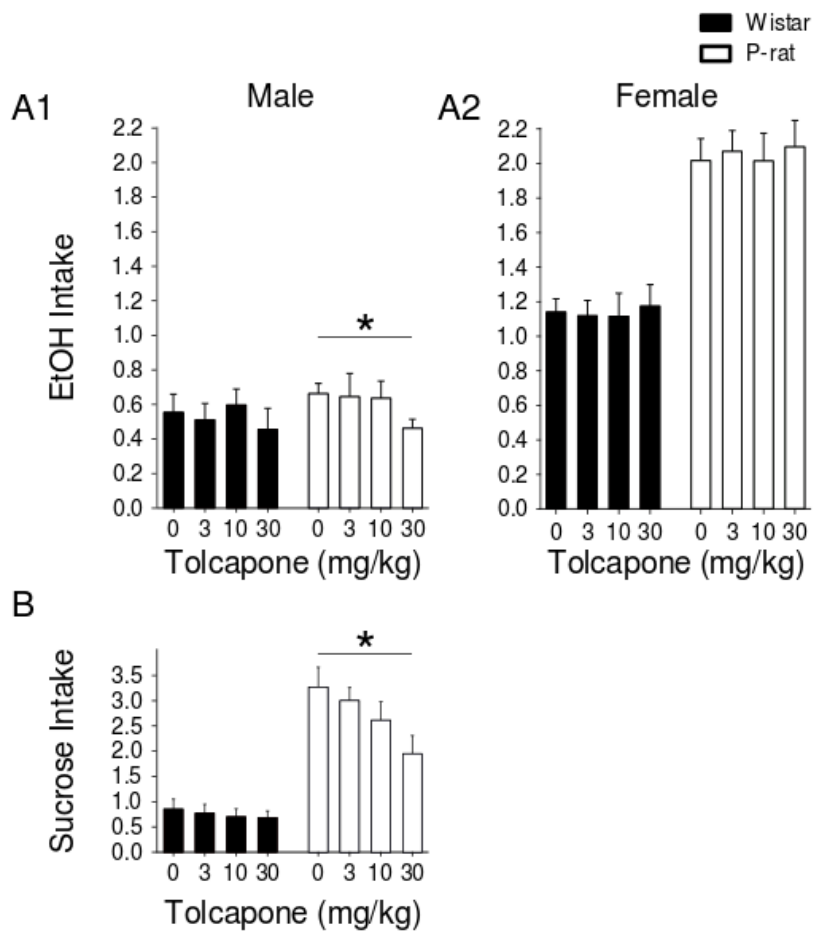


Figure 3. EtOH (A) and Sucrose (B) intake in male (A1,B) and female (A2) rats during the consummatory test phase. Tolcapone reduces both ethanol and sucrose consumption in male P rats. *main effect of treatment, $p < 0.05$

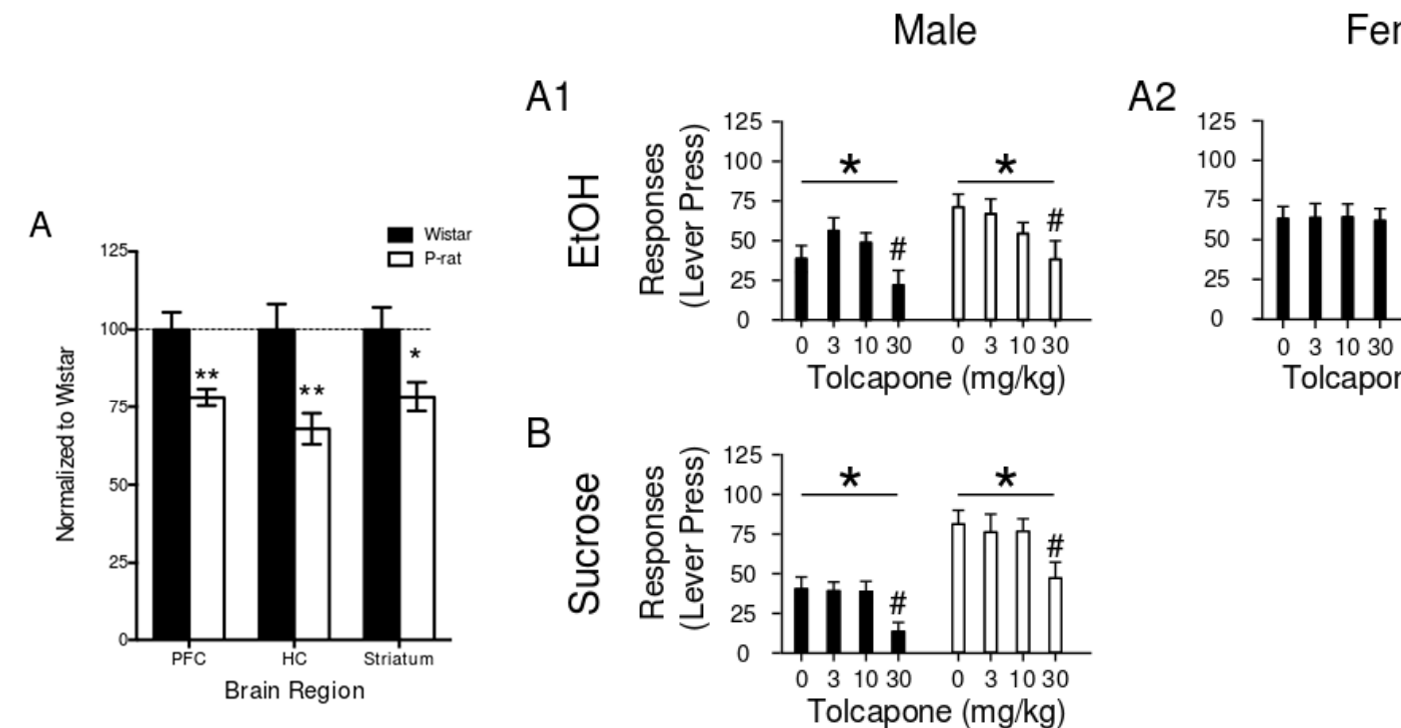


Figure 4. Lever press responding in EtOH (A) and Sucrose (B) reinforced male (A1,B) and female (A2) rats during the appetitive testing phase. Tolcapone reduced responding in male P and Wistar rats, regardless of reinforcer group. This effect was driven by the 30 mg/kg dose of Tolcapone. *main effect of treatment, $p < 0.05$. # Dunnett's post hoc, dose different from vehicle, $p < 0.05$.

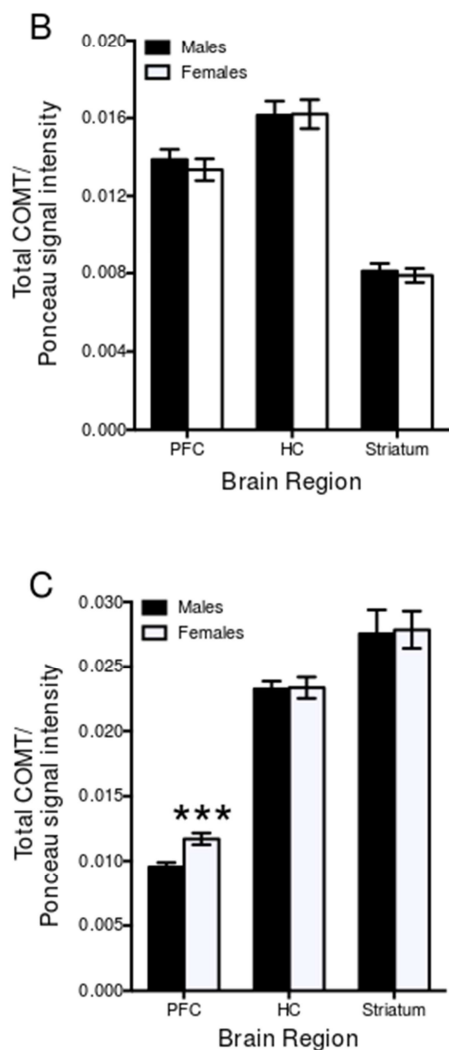


Figure 5. Protein expression in the PFC, hippocampus (HC) and striatum of male and female P and Wistar rats. COMT levels in male P and Wistar rats, show that P rats exhibit reduced COMT expression in all brain regions, relative to Wistars (A). COMT levels compared between male and female Wistars show no sex differences in any region assessed (B). However, in P rats, male P rats exhibit reduced COMT expression compared to females (C). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

Highlights

- The effects of COMT inhibition on alcohol seeking and drinking were examined.
- Sex and strain differences in COMT expression were examined.
- Tolcapone reduces reinforcer intake and seeking in male alcohol preferring P rats
- Tolcapone had no effect on consumption or seeking in female rats
- Sex and strain differences in COMT expression were observed in the PFC