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THE EFFECT OF VOLUNTARY BINGE CAFFEINE AND ETHANOL CO-EXPOSURE ON NEUROBEHAVIORAL SENSITIVITY TO COCAINE IN MALE C57BL/6J MICE

For the degree of Doctor of Philosophy

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THE EFFECT OF VOLUNTARY BINGE CAFFEINE AND ETHANOL CO-EXPOSURE ON NEUROBEHAVIORAL SENSITIVITY TO COCAINE IN MALE C57BL/6J MICE

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For my family and friends

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Fritz, Brandon M. Ph.D., Purdue University, May 2016. The Effect of Voluntary Binge Caffeine and Ethanol Co-Exposure on Neurobehavioral Sensitivity to Cocaine in Male C57BL/6J Mice. Major Professor: Stephen L. Boehm II.

Recently, the co-consumption of highly caffeinated energy drinks and alcohol has become a public health concern. Consumption of these beverages has been linked to a wide variety negative consequences including alcohol poisoning, driving under the influence, physical harm, and sexual violence. The more protracted consequences of caffeinated alcohol consumption have received very little attention, however. Some evidence suggests that individuals that frequently consume energy drinks mixed with alcohol are more likely to develop an alcohol use disorder. Interestingly, both caffeine and alcohol use alone have been linked to polydrug abuse. It is therefore of interest whether combined caffeine and alcohol consumption may pose an additive risk for substance abuse. Given that both compounds can positively influence dopamine signaling in mesolimbocortical reward circuitry via different mechanisms, this is an important question to address. Psychostimulants, such as cocaine, are of particular interest considering the significant involvement of dopamine in their effects. The current project explored this possibility employing an established mouse model of binge caffeine and alcohol co-consumption. Male C57BL/6J mice underwent 14 days of daily, 2hr limited access to water, alcohol, caffeine, or combined caffeine and alcohol. Water was freely available after these sessions.

In Experiment 1, mice underwent an 11-day locomotor sensitization protocol for cocaine initiating on day 15. Locomotor sensitization has been associated with a greater propensity to self-administer psychostimulants in rodents. Mice were subjected to injections of cocaine (5 or 10 mg/kg; i.p.) or saline every other day, with 15 minute activity monitoring until day 25. In Experiment 2, a separate group of mice underwent an identical drinking procedure. A conditioned place preference (CPP) protocol commenced on day 15. CPP assesses the conditioned rewarding effects of cues associated with drugs of abuse. On day 15, mice received saline injections and were immediately placed onto a neutral floor texture (paper) in the place conditioning box for 15 minutes in order to habituate the animals to the apparatus and injection procedure. Starting on day 16, mice received daily alternating systemic injections of cocaine (1 or 5 mg/kg; i.p.) and saline or saline throughout (naïve controls) and were placed onto one of two particular tactile floor cues: a metal floor with holes punched out or a grid floor made of metal rods. Mice were exposed to the other injection/floor pairing on the alternate days. Mice were placed into these activity monitors for 15 minute conditioning sessions. These sessions alternated drug and vehicle over the course of 8 days so that a total of 4 drug and 4 saline injections were given. The first place preference test occurred on day 24 wherein all mice were injected with saline and offered access to both floor textures. On day 25, mice were returned to the conditioning protocol for another 8 days and a second CPP test on day 33. The results of Experiment 1 suggested that prior caffeine consumption, irrespective of the presence of ethanol, enhanced the initial psychomotor stimulating effect of 10 mg/kg cocaine. However, prior fluid consumption history did not influence the capacity to develop locomotor sensitization. The results of Experiment 2 indicate that prior caffeine and/or ethanol consumption had no influence on

the development or expression of CPP for 1 mg/kg or 5 mg/kg cocaine. Collectively, these results suggest that a history of caffeine consumption may increase the stimulant response to a moderate dose of cocaine, perhaps indicating cross-sensitization. Although the conditioned rewarding effects of cocaine were not altered by prior caffeine and/or ethanol consumption, an enhanced stimulant response may be indicative of enhanced cocaine abuse potential. This study demonstrates that moderate caffeine consumption may influence an individual's early interactions with cocaine which may eventually influence the likelihood of later problematic use.

INTRODUCTION

General Introduction

The public health impact of alcohol (ethanol) abuse is both far-reaching and extraordinarily expensive. In its 2014 global status report, the World Health Organization attributed 3.3 million deaths per year to ethanol use/abuse worldwide and in the United States (U.S.) alone, the economic burden of ethanol consumption has been estimated at 223.5 billion dollars (Bouchery et al., 2011). It is therefore clear that ethanol use/abuse is a serious issue that warrants study to mitigate these costs to the economy and public health.

A specific subtype of ethanol consumption, binge drinking, is quite common with 1 in 6 adults reporting engaging in binge drinking in the U.S. In addition, the Center for Disease Control and Prevention declared that binge drinking accounts for more than half of estimated annual deaths attributed to excessive ethanol drinking in the U.S. (CDC, 2012). This form of excessive drinking has been linked to a variety of acute consequences, such as driving under the influence and physical harm (CDC, 2015), as well as later drinking problems (Chassin et al., 2002). Binge drinking is characterized by consuming a large amount of alcohol in a short period of time, with the ultimate goal of reaching intoxication. The official definition of binge drinking by the National Institute on Alcohol Abuse and

Alcoholism is a pattern of drinking that brings blood ethanol concentration (BEC) levels to 0.08 g/dl within a period of 2hrs, which can typically be achieved by consuming 4 drinks for women and 5 drinks for men. This level of intoxication typically produces impaired reaction time, motor coordination, and attentiveness (Moskowitz and Fiorentino, 2000). Therefore, this BEC level is the nationally-accepted limit for automobile operation for good reason. A troubling trend in binge drinking has recently emerged with the common practice of consuming ethanol and highly caffeinated 'energy drink' mixed beverages.

Caffeinated Alcohol Consumption and its Consequences

Following numerous reports in the media about increases in alcohol poisoning and physical harm associated with the consumption of pre-mixed caffeinated alcoholic beverages (e.g. Joose, Four Loko), the FDA mandated that companies remove the large amount of caffeine from such beverages in 2010. This measure offered little in the way of regulation, however, as these alcohol/energy drink mixers can be readily prepared by individuals as the ingredients (liquor and energy drinks) are widely available.

Consumption of these beverages has been associated with an increased risk for hazardous behavior such as drinking to the point of alcohol poisoning (CDC, 2012), driving under the influence (O'Brien et al., 2013; O'Brien et al., 2008; Thombs et al., 2010; Woolsey et al., 2015), sexual promiscuity (O'Brien et al., 2013), and aggression (Jones et al., 2012; Miller et al., 2016). Particularly concerning is that the analyses in the above studies controlled for overall alcohol intake, thereby suggesting greater concern regarding caffeinated versus alcohol-only intoxication. It has been contested, however, that these

findings may simply be confounded by a generally greater sensation seeking tendency for people who choose to consume these highly caffeinated mixed drinks (Alford et al., 2012; de Haan et al., 2012; Verster et al., 2012a; Verster et al., 2012b). Therefore, individuals who are already prone to engage in risky or hazardous behavior, in general, may more likely to exhibit such behavior when intoxicated by alcohol. Strong evidence against this theory was recently presented, however, in a study by O'Brien and colleagues (2013). Employing a large sample size (N = 3,390), caffeinated ethanol consumption was associated with higher odds of driving under the influence, riding with an intoxicated driver, being taken advantage of sexually, and sustaining physical injury compared to non-caffeinated alcohol consumption; *even after controlling for sensation seeking tendency and the amount of alcohol consumed*. In light of these findings, the consequences of consumption of highly caffeinated alcoholic beverages warrants ample public health concern and further study.

Caffeine as the Primary Psychoactive Ingredient in Energy Drinks

The practice of consuming caffeinated alcoholic beverages is not new and quite common (i.e. rum and cola, Irish coffee, etc.). The introduction of 'energy drinks', however, has influenced how people consume caffeine and ethanol combinations. These beverages contain large quantities of caffeine (~80-300 mg/container) and are also characterized by a blend of other naturally-occurring stimulants (e.g. guarana), sugars (e.g. glucose and fructose), and amino acids (e.g. taurine) (Marczinski et al., 2012). Although manufacturers claim that these are all 'performance-enhancing' ingredients, cognitive assessments have

determined caffeine to be the primary psychoactive ingredient in these beverages (Attwood et al., 2012; Giles et al., 2012; Peacock et al., 2013b). The potential for pharmacological interaction between caffeine and alcohol is enhanced by energy drink co-consumption given that other common caffeinated mixers like standard cola (~30-50 mg) contain far less caffeine. These mixed drinks are also often consumed rapidly in a binge fashion (e.g. 'Jagerbomb' shots) (O'Brien et al., 2008), thus allowing both caffeine and alcohol to accumulate quickly in the individual's system, potentially contributing to the previously described hazards associated with their co-use.

Motives for Caffeinated Alcohol Consumption

The ample evidence for an elevated risk of negative outcomes as a consequence of highly caffeinated ethanol consumption highlights the importance of understanding the motives for its consumption. Self-report data from young adult and college undergraduate populations point to the antagonism of ethanol-induced sedation (Weldy, 2010), increased stimulation (Ferreira et al., 2004a; Marczinski et al., 2011), and the ability to engage in longer drinking sessions (Attwood et al., 2012; Marczinski and Fillmore, 2006) as reasons for consuming these beverages. A recent study by Droste and colleagues (2014) also explored whether reported motives for consumption predicted associated harms. They found that individuals cited 4 core motivation classes for caffeinated alcohol consumption: hedonistic (consuming caffeinated alcoholic beverages is more pleasurable), social (social facilitation), energy/endurance (drink/party longer), and intoxication reduction. Interestingly, hedonistic motives were associated with increased risk for greater

consumption of these beverages, ethanol dependence, and physical harm. Intoxication reduction motives were also associated with an increased risk of harm outcomes as well, suggesting that these individuals' expectations for caffeinated alcohol intoxication may not have been met in reality. Experimental evidence for significant alteration of alcohol intoxication via caffeine co-consumption is mixed, however.

Human Laboratory Studies

Co-consumption of caffeine has been observed to attenuate ethanol-induced impairments in reaction time (Drake et al., 2003; Heinz et al., 2013; Howland et al., 2011), short term memory (Drake et al., 2003), wakefulness (Drake et al., 2003; Marczinski et al., 2012), as well as increase subjective stimulation (Marczinski and Fillmore, 2006; Marczinski et al., 2011; Marczinski et al., 2012; Peacock et al., 2013a). However, these effects on cognitive and psychomotor performance (Marczinski and Fillmore, 2006; Peacock et al., 2013a; Verster et al., 2012b) or subjective intoxication (Alford et al., 2012; de Haan et al., 2012) have not been consistently reported. One reason for this discordance is that these studies differ substantially in ethanol and caffeine dosing (target BECs 0.04-0.12 g/dl; 80-300 mg caffeine). Furthermore, the testing interval following caffeinated alcohol administration varies greatly (15-90 minutes). It is therefore currently unclear the degree to which concurrent caffeine exposure may alter alcohol intoxication.

Importantly, very little is known about the *protracted consequences* of *binge* caffeine and ethanol co-consumption and the practice of mixing ethanol with these highly caffeinated energy drinks is rather new. As such, it is likely that the potential public health

impact of consuming these beverages has not been fully realized and it is critical that we advance our understanding of this drug combination. Individuals that often consume these drinks have been found to be at greater risk for later meeting alcohol use disorder criteria (Droste et al., 2014). Evidence also suggests that ethanol and energy drink/caffeine use alone are significantly predictive of later polydrug abuse (Arria et al., 2010; Arria et al., 2011; Kendler et al., 2006; Kirby and Barry, 2012; McCabe et al., 2006; Reissig et al., 2009). A major potential concern may therefore be that repeated binge caffeine and ethanol co-consumption could additively increase an individual's sensitivity to drugs of abuse. In order to carefully examine the neurobehavioral consequences of binge caffeine and ethanol co-exposure, preclinical work with animal models offers substantial power to understand the cause and effect relationship between caffeine/ethanol consumption and drug sensitivity.

Preclinical Animal Research on Caffeine and Ethanol Combinations

Through precise biological/chemical manipulations and techniques, rodent models of ethanol exposure have greatly advanced the field's understanding of the neurobiology of ethanol use/abuse in ways that human studies ethically cannot. Furthermore, animal models of caffeine and ethanol co-exposure circumvent potential confounds inherent in human studies such as expectancy, personality type, and degree of prior ethanol and/or caffeine experience which may have also contributed to the aforementioned experiment variability.

One of the primary concerns that emerged from the reports on highly caffeinated ethanol consumption in young adults was dangerous ethanol intake. As such, animal studies have directly focused on whether caffeine alters ethanol consumption in existing animal ethanol drinking models. Thus far, findings are mixed with three studies demonstrating an increase in ethanol intake by caffeine co-exposure (Franklin, 2009; Kunin et al., 2000; Rezvani et al., 2013), two studies demonstrating no effect of caffeine (Fritz et al., 2014; Hughes, 2011), and one study showing a caffeine-induced decrease in ethanol intake (Rezvani et al., 2013). These studies employed either a systemic caffeine injection prior to ethanol access (Kunin et al., 2000; Rezvani et al., 2013) or offered access to a caffeinated ethanol solution which was consumed voluntarily (Franklin, 2009; Fritz et al., 2014; Hughes, 2011). Collectively, these results indicate that neither caffeine administration route produced a consistent effect on ethanol intake. Furthermore, caffeine dosing varied widely in these studies (~2-23 mg/kg), adding to the difficulty of interpretation.

Cognition-based tasks have also found mixed evidence for caffeine's effect on ethanol intoxication. Caffeine co-exposure (5-40 mg/kg) was observed to have no effect on ethanol-induced decrements in learning on the plus maze discriminative avoidance task (Gulick and Gould, 2009) or novel object recognition in mice (Fritz et al., 2014). Alternatively, caffeine (5 mg/kg) was found to reduce retrograde memory impairment induced by ethanol in rats (Spinetta et al., 2008). Finally, the anxiolytic effects of alcohol on the elevated plus maze were found to persist following caffeine (~8 mg/kg) co-consumption (Fritz et al., 2014).

The clearest effects thus far in the preclinical literature pertain to caffeine's modulation of ethanol's motor effects. Caffeine has been shown to reduce ethanol-induced ataxia when administered both systemically (~8-15 mg/kg) and centrally (< 25 µg) (Dar, 1988; Fritz et al., 2014). The sedative effects of ethanol can also be mitigated by caffeine as indicated by a reversal of ethanol-induced hypnosis via a systemic injection of 25 mg/kg caffeine (El Yacoubi et al., 2003). Caffeine alone is known to produce locomotor stimulation in mice (Kuribara et al., 1992; Waldeck, 1974) and this has been observed at doses as low as 3 mg/kg (Hilbert et al., 2013). Low to moderate doses of ethanol (0.12-2.0 g/kg) can also produce locomotor stimulation in rodents (Phillips and Shen, 1995). A number of studies have demonstrated that caffeine can have an additive effect on ethanol-induced stimulation over a wide variety of ethanol and caffeine dose ranges (1.0-3.2 g/kg ethanol; 3-50 mg/kg caffeine) (Hilbert et al., 2013; Kuribara et al., 1992; May et al., 2015; Waldeck, 1974). However, this additive stimulant effect was not observed to associate with an increased rewarding value of caffeine/ethanol combinations (3 mg/kg caffeine + 1.75 g/kg ethanol) as assessed by conditioned place preference (Hilbert et al., 2013).

To date, rodent research on concurrent caffeine and ethanol exposure has largely employed forced, non-contingent administration of caffeine and ethanol via systemic injections or intragastric gavages (Dar, 1988; El Yacoubi et al., 2003; Ferreira et al., 2004b; Gulick and Gould, 2009; Hilbert et al., 2013; Kunin et al., 2000; Kuribara et al., 1992; May et al., 2015; May et al., 2012; Rezvani et al., 2013; Spinetta et al., 2008; Waldeck, 1974). It is well known that non-contingent administration of ethanol and other drugs can produce significantly different alterations in behavior and neurobiology relative to self-administration (Jacobs et al., 2003; Mitchell et al., 2012; Moolten and Kornetsky, 1990;

Nurmi et al., 1996). Therefore, self-administration models offer greater translational validity relative to the human experience.

Preclinical Mouse Model of Binge Caffeine and Alcohol Co-Consumption

One of the most widely used and consistently validated binge-like alcohol drinking animal models is the Drinking-in-the-Dark (DID) mouse model. DID offers mice a 20% (v/v) ethanol solution for a discrete period of time (1-4 hours; 2 hours in our lab) so that ethanol consumption occurs during an easily definable time period (Thiele et al., 2014). This is a key strength as the commonly used continuous access, two-bottle choice ethanol consumption paradigm makes it difficult to determine peak ethanol dosing and does not consistently produce sustained, pharmacologically-relevant BECs (Dole and Gentry, 1984); except in mice specifically-bred for high ethanol intake in this paradigm (Matson and Grahame, 2013). The C57BL/6J (B6) inbred mouse strain, the strain with which the DID paradigm was developed, reliably consumes ethanol to the point of behavioral intoxication and reaches BECs in excess of 80 mg/dl (0.08 g/dl) (Rhodes et al., 2005; Rhodes et al., 2007). In addition, mice with a repeated history of binge-like drinking via DID demonstrate motor and metabolic tolerance to ethanol (Fritz et al., 2014; Linsenbardt et al., 2011). This animal model of ethanol consumption therefore appears to mirror what is defined by the National Institute on Alcohol Abuse and Alcoholism as ‘binge drinking’ wherein an individual consumes enough alcohol to reach BECs $\geq$ 80 mg % and intoxication within a limited time frame.

Our lab has previously observed that after repeated binge episodes, B6 mice will consume the 20% ethanol solution to the point of locomotor sedation (Fritz et al., 2014; Linsenbardt and Boehm, 2012, 2013). Given that caffeine has been observed to attenuate ethanol-induced sedation in both humans (Drake et al., 2003; Marczinski et al., 2012) and animals (Dar, 1988; Dar et al., 1987; El Yacoubi et al., 2003) and individuals cite this as a motive for caffeinated ethanol consumption (Ferreira et al., 2004a; Marczinski, 2011), our lab was interested in whether the addition of caffeine to the ethanol solution in DID would increase ethanol intake by way of antagonizing locomotor sedation. Although the addition of caffeine (0.03-0.05% w/v; concentrations similar to common energy drinks) was not observed to have an effect on ethanol intake or attained BEC, caffeine completely reversed the typical ethanol-induced locomotor sedative response and a clear locomotor stimulant effect emerged. Caffeine co-consumption (~8 mg/kg in 2hrs) also reduced ethanol's ataxic effects as measured by the balance beam task. Furthermore, ethanol-induced anxiolysis on the elevated plus maze apparatus as well as memory interference in the novel object recognition task were still apparent when caffeine was co-consumed. This caffeinated ethanol binge co-consumption model therefore appears to model key facets of the "wide awake drunk" state that individuals anecdotally seek via highly caffeinated energy drink-ethanol co-consumption. As this practice is relatively new, the consequences of such consumption have yet to be realized. This mouse model will serve as a useful tool to advance our understanding of the neurobehavioral consequences of voluntary binge co-consumption and the threats they may pose to public health and safety. As previously mentioned, an unanswered and important question is whether a history binge co-exposure of caffeine and ethanol may have the capacity to enhance an individual's sensitivity to

drugs of abuse. Two of the most widely studied phenotypes in the drug abuse research field that could address this question, locomotor sensitization and conditioned place preference, are described and discussed below.

Sensitization

Sensitization in the drug abuse field is essentially defined as an increased response to a fixed dose of drug as a consequence of repeated exposure. Drugs from a wide variety of classes can produce a sensitization response that has been proposed to be reflective of increased incentive salience of drug-paired cues, or a shift from "liking" to "wanting" a drug (Robinson and Berridge, 1993); a key transitional state in chronic drug abusers. In preclinical studies, behavioral sensitization has most often been measured as a progressively increased locomotor stimulant response to a drug as a consequence of repeated exposure. At certain doses, virtually all drugs of abuse produce locomotor stimulation in rodents (Robinson and Berridge, 1993; Wise and Bozarth, 1987) and with repeated exposure, many of these drugs can produce a sensitized locomotor stimulant response. Behavioral observations of sensitization are often tied to substantial changes in the neurochemical/neurophysiological response to the drug in animals (Borgland et al., 2004; Janetsian et al., 2015; Vanderschuren and Kalivas, 2000), such as elevated accumbal dopamine (DA) levels.

Although direct evidence of behavioral sensitization has been observed in chronic drug abusers or non-human primates, the results have not been consistent (Bradberry, 2006). It has been argued that many of these studies, however, failed to consider the

importance of cues that are predictive of either the presence or absence of the drug. Vezina and Leyton (2009) contend that the human laboratory lacks the necessary drug-paired cues to consistently observe sensitization in chronic drug abusers as drug consumption never takes place in such an environment for these individuals, let alone lying in a positron emission tomography (PET) scanner. Indeed, both sensitized behavioral and neurochemical responses are difficult to detect in an environment dissociated from prior drug exposure in animals (Guillory et al., 2006; Stewart, 1992). Studies in animals that have evaluated the response to drug-paired cues alone have observed conditioned responses associated with sensitization such as increased locomotion and striatal DA release (Vezina and Leyton, 2009). These observations have also been extended to humans with drug-associated cues producing elevated mood states and physiological responses such as increased heart rate (Boileau et al., 2007; Foltin and Haney). Furthermore, studies by Bolieu and colleagues observed sensitized striatal DA release when amphetamine was repeatedly given in the context of a PET scan, indicating the importance of drug-paired cues in a human sensitization response (Boileau et al., 2006; Boileau et al., 2007). As Vezina and Leyton note, however, the observation that drug-sensitized DA release can be observed in *in vitro* slice preparations (Castañeda et al., 1988) demonstrates that the sensitization response can occur independent of contextual stimuli. Nevertheless, it is argued that sensitized responses can become entirely regulated by these cues, as outlined above, which may have the capacity to powerfully motivate drug-seeking behavior.

Sensitization has been proposed to reflect significant neuroadaptation underlying a transition to an addictive state, wherein drug-paired cues acquire intense motivational properties, perhaps modeling compulsive drug-seeking (Robinson and Berridge, 1993,

2001). In rodents, self-administration studies have observed that animals sensitized to psychostimulants respond more for these drugs (Piazza et al., 1990; Vezina, 2004). Conversely, self-administration of psychostimulants has also been observed to produce locomotor sensitization (Hooks et al., 1994; Phillips and Di Ciano, 1996). Sensitization can be a highly persistent state, lasting up to 1 year (Paulson et al., 1991), that has been associated with the predictive potential for relapse-like behavior following extinction in an operant self-administration paradigm (De Vries et al., 1998). However, some more recent studies have found that sensitized animals do not always demonstrate a significant reinstatement response (Ahmed and Cador, 2005; Knackstedt and Kalivas, 2007). Although the relationship between sensitization and self-administration is not completely clear, it has been argued that sensitization has significant construct validity given the fact that the neurocircuitry underlying both behaviors is remarkably comparable (Steketee and Kalivas, 2011). Thus, the neurobiological/neurochemical alterations produced by locomotor sensitization are also arguably similar.

Cross-Sensitization

One consequence of exposure to drugs of abuse can be cross-sensitization, whereby a history of drug exposure can result in increased sensitivity to other drugs. Given that ethanol and caffeine share common effector systems (dopamine, adenosine), co-exposure may produce neurobiological adaptations to a greater extent than ethanol or caffeine alone as evidenced by greater behavioral responsivity to another drug. Cross-sensitization has been theorized to be indicative of increased abuse potential for the new drug(s) (Ferreira et

al., 2013; Lessov and Phillips, 2003; Robinson and Berridge, 1993; Yang et al., 2011). Ethanol (Boileau et al., 2003; Ding et al., 2011; Gonzales and Weiss, 1998; Schier et al., 2013; Yoshimoto et al., 1992) and caffeine (Okada et al., 1996; Quarta et al., 2004; Solinas et al., 2002) have both been shown to increase dopamine signaling in the ventral striatum via different mechanisms and experimenter-controlled co-exposure has been demonstrated to produce an additive locomotor stimulant effect (Hilbert et al., 2013; Kuribara et al., 1992; May et al., 2015; Waldeck, 1974). Furthermore, repeated intragastric infusions of a combined caffeine and ethanol solution induced significantly greater locomotor sensitization than caffeine or ethanol alone in mice (May et al., 2015). Therefore, repeated caffeine and ethanol co-consumption may produce more profound or widespread neuroadaptation than exposure to either drug alone; one consequence of which could be enhanced responsivity, or cross-sensitization, to stimulant drugs of abuse such as cocaine.

Conditioned Place Preference

Although cross-sensitization may reflect significant neuroadaptations as a consequence of repeated drug exposure which may be associated with abuse potential, studies suggest that sensitization is separable from the positive motivational effects or rewarding value of a drug. Although the positive effects of drug consumption wane with repeated usage in chronic drug abusers, the rewarding effects of a drug are likely important in the earlier stages of drug use which may eventually influence later, problematic use (Koob et al., 2004). This was nicely demonstrated by King and colleagues (2014) who

showed that individuals that reported stronger positive responses (stimulation, liking) to an acute ethanol challenge were more likely to meet use disorder criteria 6 years later.

The paradigm thought to probe drug 'reward' in rodents is conditioned place preference (CPP). The fairly simple protocol for this assessment alternates administrations of a drug (the unconditioned stimulus; UCS) paired with one context (conditioned stimulus paired with drug; CS+) and administrations of the neutral drug vehicle with another, distinct context (conditioned stimulus paired with the absence of drug; CS-). After a period of repeated exposures to drug/vehicle and their respective contexts, animals are offered a test trial wherein the neutral drug vehicle is administered and they are allowed to choose between both contexts. Historically, drugs of abuse produce a significant preference for the drug-paired context over the neutral context (Tzschentke, 2007). A major advantage of this paradigm is that animals are presented this choice test in a drug-free state. This prevents any interference of the drug's pharmacological effects on performance and the animal therefore must call upon its previous experience to decide whether it wants to approach or avoid the drug-paired cue. As such, CPP is thought to reflect conditioned drug reward. CPP is also sensitive to aversion, as animals can choose to spend significantly more time in the context associated with the absence of the drug. Higher doses of drugs or withdrawal states have been observed to produce place aversion (Risinger and Oakes, 1995; Stinus et al., 2005). The ability of the CPP paradigm to assess behavior along a spectrum from aversion to preference is considered a key strength. Although separate processes, the CPP paradigm allows for easy collection of locomotor activity data during conditioning sessions, allowing for the opportunity to compare locomotor sensitization and CPP locomotor data. Indeed,

previous studies have observed cocaine locomotor sensitization during CPP training (Seymour and Wagner, 2008; Shimosato and Ohkuma, 2000).

Both caffeine and ethanol produce CPP in rodents. Co-exposure may produce stronger effects on mesolimbocortical reward circuitry which may influence sensitivity to the rewarding effects of other drugs of abuse. Indeed, clinical evidence, as well as the pharmacological profiles of ethanol and caffeine, suggest substantial potential for co-exposure to enhance sensitivity to heavily dopamine-involved drugs of abuse such as cocaine.

Caffeine and Ethanol: Adenosine, Dopamine, and Implications for Altered Cocaine Sensitivity

Associations between Caffeine, Ethanol, and Cocaine Use: Clinical Observations

A strong association between cocaine and ethanol abuse has been well-documented, with observations of ~60-84% of cocaine abusers also meeting diagnostic criteria for ethanol use disorder during some point in their lives (Gorelick, 1992; Grant and Harford, 1990; Heil et al., 2001; Helzer and Pryzbeck, 1988). Interestingly, the reverse association (effect of a history of ethanol exposure on cocaine sensitivity) does not appear to be as strong. One report highlighted that only ~20-30% of ethanol-dependent patients also met criteria for cocaine use disorder (Miller, 1991). In humans, a twin study by Kendler and colleagues (2006) found that caffeine intake/dependence was significantly predictive of cocaine abuse/dependence. Both energy drink/caffeine (Arria et al., 2010; Arria et al., 2011;

Reissig et al., 2009) and ethanol (Kirby and Barry, 2012; McCabe et al., 2006; Welte and Barnes, 1985) usage have been linked to polydrug use in humans. As such, co-exposure to both caffeine and alcohol may enhance abuse potential for cocaine in an additive fashion.

Associations between Caffeine, Ethanol, and Cocaine Responsivity: Preclinical Observations

Rodent studies have demonstrated that a history of cocaine exposure enhances the acute locomotor stimulating effect of ethanol (Itzhak and Martin, 1999; Lessov and Phillips, 2003) and vice versa (Itzhak and Martin, 1999). In addition, naive rats selectively bred for high ethanol consumption exhibited increased sensitivity to cocaine (Honkanen et al., 1999), suggesting that this effect may have a genetic component. Rats with a chronic caffeine consumption history have also been found to be more sensitive to the stimulant effect of cocaine (Gasior et al., 2000; Jaszyna et al., 1998; O'Neill et al., 2015). These findings, along with caffeine's previously mentioned effects on the locomotor response of mice to ethanol intoxication, raise questions about the potential for caffeine and ethanol co-exposure to augment cross-sensitization to the locomotor stimulating effect of cocaine.

A history of caffeine consumption during adolescence has been shown to strengthen later CPP for cocaine in adult rats (O'Neill et al., 2015). Furthermore, concurrent caffeine exposure strengthens the development of CPP (Bedingfield et al., 1998). The effect of prior alcohol consumption/exposure is more complex, however, with previous studies showing both similar (Busse et al., 2005; Le Pen et al., 1998) and enhanced (Hutchison and Riley, 2012; Mateos-García et al., 2015; Stromberg and Mackler, 2005) cocaine CPP relative to

rats and mice with no ethanol history. Along with the working hypothesis that binge caffeine and ethanol co-consumption produces unique and potentially more profound changes in the sensitivity to cocaine, a history of co-consumption may enhance the rewarding properties of cocaine as assessed by CPP. Indeed, the pharmacological profiles of caffeine and ethanol offer significant potential for altered sensitivity to the psychomotor stimulating and/or rewarding effects of cocaine.

Adenosine and Adenosine Receptors

Caffeine is a non-selective competitive antagonist for adenosine receptors (Fredholm et al., 1999) whereas ethanol indirectly increases extracellular adenosine levels indirectly by inhibiting its transporter (Nagy et al., 1990) (Figure 1). Adenosine is a purinergic nucleoside neuromodulator and its release occurs through nucleoside transporters as a result of cell depolarization or elevated intracellular levels of adenosine (Fredholm et al., 2005a). In the brain, this release is not restricted to neurons as microglia and astrocytes can also release adenosine. Adenosine can also be synthesized extracellularly from released adenosine triphosphate or cyclic adenosine monophosphate (cAMP). For an in depth review of the adenosine system and brain function, see Fredholm et al. (2005a).

Adenosine has a primary role as a homeostatic regulator of neural activity as well as central and peripheral blood flow (Cunha, 2001). Throughout wakefulness, adenosine accumulates in the brain as a byproduct of cellular metabolism and these levels are normalized during sleep (Porkka-Heiskanen et al., 1997). Elevated adenosine levels and

receptor activation has been linked to sedation and reduced vigilance in animals (Christie et al., 2008; Mingote et al., 2008; Porkka-Heiskanen et al., 1997). As the main focus of this project was neurobehavioral in nature, only the central effects of adenosine and its receptors will be discussed further.

There are 4 known adenosine receptor subtypes: A_1, A_{2A}, A_{2B}, and A_3. By far, the most common and well-studied subtypes in mammals are A_1 (A1R) and A_{2A} (A2AR). A1Rs are located throughout the brain, with particularly high concentrations in the mammalian cerebellum, hippocampus, and cerebral cortex (Fastbom et al., 1987; Goodman and Snyder, 1982). A2ARs are more localized, with the highest concentrations in the striatum (Rosin et al., 1998), although they are also found at lower levels in the cortex (Svenningsson et al., 1997) and hippocampus (Cunha et al., 1995; Cunha et al., 1994). Both A1Rs and A2ARs are found pre and postsynaptically (Cunha, 2001; Rodrigues et al., 2005). A1Rs inhibit whereas A2ARs stimulate adenylyl cyclase activity through coupling to G_i and G_{olf} G proteins in brain, respectively (Cunha, 2001). Stimulation of A1Rs decreases neuronal excitability through downstream inhibition of calcium channels (MacDonald et al., 1986) and activation of potassium channels (Trussell and Jackson, 1985). Activation of A2ARs primarily acts to facilitate neurotransmitter release through elevated cAMP production (Barraco et al., 1995; Shindou et al., 2002) and increased activity at these receptors has even been linked to excitotoxicity (Popoli et al., 2003; Popoli et al., 2002).

Caffeine Pharmacology and its Behavioral Effects: Adenosine

Caffeine is classified as a competitive non-selective adenosine receptor antagonist. Given the relationship between elevated levels of adenosine and sedation/reduced

cognitive vigilance (Christie et al., 2008; Mingote et al., 2008; Porkka-Heiskanen et al., 1997), caffeine's psychostimulant properties are perhaps not surprisingly mediated through the blockade of these receptors (Fredholm et al., 1999; Fredholm et al., 2005b). Preclinical work has demonstrated specific roles for A1Rs and A2ARs in the neurobehavioral effects of caffeine.

Surprisingly, a literature search yielded no published preclinical studies on the role of A1Rs or A2ARs in caffeine intake. This is likely due to the previously demonstrated difficulty of establishing voluntary, behaviorally-relevant consumption of a simple caffeine and water solution in rodents (Heppner et al., 1986). As such, studies employing a caffeine drinking paradigm typically opt for forced access by adding caffeine to the animal's standard drinking water (Hughes, 2011; Jaszyna et al., 1998; O'Neill et al., 2015) or food deprivation (Heppner et al., 1986) to produce significant caffeine intake. In our mouse model, however, male B6 mice will readily consume 10-15 mg/kg of caffeine in a short period of 2 hours without fluid/food deprivation or sweetening (Fritz et al., 2014). In addition, caffeine consumption is positively related to blood levels of caffeine and its primary psychoactive metabolite, paraxanthine in this model (Figures 2 & 3). Furthermore, this level of caffeine intake produces obvious behavioral motor stimulation (Fritz et al., 2014), confirming that this level of intake is pharmacologically-relevant. This mouse model is therefore likely the best available to address the role of adenosine receptor subtypes in caffeine consumption in future studies.

Nevertheless, repeated caffeine exposure/consumption has been shown to increase cortical A1R expression (Shi et al., 1994) and decrease striatal A2AR expression in rodents (Singh et al., 2009). It has also recently been shown that separate groups of rats that

consumed caffeine in adulthood or adolescence both exhibited increased A1R levels in the nucleus accumbens. However, only rats that consumed caffeine in adolescence displayed a decrease in accumbal A2AR levels (O'Neill et al., 2015). Therefore across key brain regions involved in the response to drugs of abuse, caffeine exposure appears to increase A1R expression and decrease A2AR expression.

The caffeine research field generally agrees that the psychostimulant properties of caffeine are largely due to antagonism of the A2AR. However, it has been hypothesized that A1 and A2AR heteromers may be the primary mediators of these effects (Ferre et al., 2008). CPP studies have found specific roles for these receptor subtypes in the conditioned rewarding effects of caffeine. Blockade of the A2AR, but not A1R appears to be an important mechanism in the development of caffeine CPP (Brockwell and Beninger, 1996; Hsu et al., 2009).

Knockout studies in mice have demonstrated that caffeine produces locomotor stimulation and wakefulness in A1R knockout mice, but not A2AR knockout mice (Huang et al., 2005; Yacoubi et al., 2000). Furthermore, A2AR antagonists (Fritz and Boehm II, 2015; Müller et al., 1998; Nagel et al., 2003), but not A1R antagonists (Brockwell and Beninger, 1996; Griebel et al., 1991), robustly stimulate locomotor activity in rodents. Locomotor sensitization to caffeine is also mediated by blockade of the A2AR (Hsu et al., 2009). A1Rs have been associated with the cognitive-enhancing effects of caffeine as antagonism of these receptors can increase acetylcholine release in the hippocampus (Carter et al., 1995) and cerebral cortex (Kurokawa et al., 1996; Materi et al., 2000). Given the ability of caffeine to improve performance on working memory tasks and maze learning (Angelucci et al., 2002; Castellano, 1976; Prediger et al., 2005), antagonism of the A1R

has been proposed to mediate this effect. In addition, A1R (Karcz-Kubicha et al., 2003), but not A2AR (Halldner et al., 2004), antagonism is an important mechanism for the development of caffeine tolerance. Both A1Rs and A2ARs, however, have been linked to neuroprotective effects in Alzheimer's and Parkinson's disease animal models with chronic treatment decreasing neuronal markers of degeneration and improving performance on cognitive tasks (Dall'lgna et al., 2003; Rodrigues et al., 2015; Rosso et al., 2008).

Collectively, the work reviewed above indicates that the majority of evidence points to the A2AR as the primary target through which caffeine's psychostimulant effects are achieved. However, the A1R is an important mediator of caffeine-enhanced cognition as well as tolerance development. Given that A2ARs, but not A1Rs, are primarily involved in the locomotor and rewarding effects of caffeine, these receptors may also be the likely mechanism through which prior caffeine intake could influence cocaine sensitivity in the present study.

Ethanol Pharmacology and its Behavioral Effects: Adenosine

Ethanol promotes adenosine signaling in an indirect manner by inhibiting its transporter, ENT1 (Allen-Gipson et al., 2009; Nagy et al., 1990), thus allowing a larger amount of adenosine to accumulate extracellularly and interact with receptors. This effect was linked to ethanol-induced sedation and motor incoordination as mice lacking the adenosine transporter ENT1 exhibit significantly reduced sensitivity to these effects (Choi et al., 2004). This is not surprising considering the previously mentioned sedative effects of adenosine signaling.

Voluntary drinking and self-administration studies have primarily found that the A2AR, but not the A1AR is an important mediator of ethanol seeking and consumption. A2AR antagonists have been observed to both increase (Arolfo et al., 2004; Micioni Di Bonaventura et al., 2011) and decrease (Adams et al., 2008; Thorsell et al., 2007) ethanol consumption and seeking whereas agonists have only been observed to decrease these behaviors (Houchi et al., 2013; Houchi et al., 2008; Micioni Di Bonaventura et al., 2011). A recent study also found that a reduction in A2AR activity in the dorsomedial striatum, a region mediating goal-directed behavior, significantly increased ethanol intake in an operant paradigm in mice (Nam et al., 2013). All of these studies either did not assess the effects of A1R-selective drugs or found that they had no effect. However, a recent study in our lab employing the DID model found that A1R antagonism significantly reduced binge-like ethanol consumption whereas the A2AR antagonism had no effect (Fritz and Boehm II, 2015). We proposed that this apparent discordance may have been due to differences in the duration of alcohol access. Our study involved 7 days of ethanol drinking via DID whereas these earlier studies employed drinking paradigms lasting weeks. As the A1R is an important target for the development of tolerance to ethanol (Batista et al., 2005), we proposed that the lack of effect of A1R manipulation in the more protracted drinking studies may have reflected this adaptive mechanism.

Repeated ethanol exposure does not appear to have an effect on the expression or binding capability of A2ARs receptors (Daly et al., 1994; Jarvis and Becker, 1998). However, chronic ethanol consumption significantly increased cortical A1R density in mice (Daly et al., 1994). Repeated ethanol vapor exposure was also observed to increase A1R levels, however, the number of A1R binding sites detected with radioligand labeling

was unaltered (Jarvis and Becker, 1998). An enhancement of A1R binding was observed in cerebellum following chronic ethanol exposure, however, this effect may persist for as little as 3 days (Concas et al., 1996). These findings suggest that any significant adaptation of adenosine receptors produced by ethanol exposure likely involve A1Rs, but not A2ARs. Nevertheless, both receptor subtypes mediate different responses to acute ethanol intoxication.

The A1R appears to be an important target for ethanol-induced ataxia. Rats pretreated with an A1R antagonist exhibit significantly reduced ataxia following an ethanol injection on the rotorod apparatus (Barwick and Dar, 1998; Connole et al., 2004). Furthermore, antagonism of the A1R, but not A2AR, attenuates the development of tolerance to ethanol's ataxic effects (Batista et al., 2005), suggesting that the A1R is an important mediator of ethanol-induced neuroadaptation. Finally, pretreatments with an A1R antagonist significantly blunted the ability of ethanol to increase the time mice spent on the open arms of the elevated plus maze apparatus (Prediger et al., 2004), suggesting an important role for the A1R in ethanol's anxiolytic effects.

A2ARs on the other hand, are important targets for sedation induced by high doses of ethanol ($\geq$ 3 g/kg). Pretreatment with an A2AR antagonist significantly reduces the duration of the ethanol-induced loss of righting reflex (El Yacoubi et al., 2003), a measure of the sedative hypnotic effects of ethanol. Furthermore, A2AR knockout mice also demonstrate a significantly shorter loss of the righting reflex (Naassila et al., 2002) and heightened sensitivity to lower doses of ethanol (0.5-2.0 g/kg) as evidenced by enhanced ethanol-induced locomotor stimulation and anxiolysis (Houchi et al., 2008). Finally, A2AR

knockout mice also exhibit enhanced CPP for these lower doses of ethanol (Houchi et al., 2008).

Collectively, these findings suggest a complicated role for adenosine signaling in ethanol-related behaviors. Ethanol consumption and its conditioned rewarding effects appear to be primarily influenced by the A2AR, however the A1R has been demonstrated to be involved in binge-like consumption in the DID paradigm. The A1R is an important regulator of motor incoordination induced by ethanol whereas the A2AR mediates the sedative hypnotic effects of high doses of ethanol.

Dopamine and Dopamine Receptors

DA is the most well-known neurotransmitter system in substance abuse research, a classical catecholamine neurotransmitter that is initially synthesized from tyrosine in the synaptic terminal by the enzyme tyrosine hydroxylase (Missale et al., 1998). DA cell bodies are located in the ventral midbrain and substantia nigra with projections to the frontal and limbic cortex, hippocampus, amygdala, and striatum. Studies have long demonstrated a strong role for DA in motivated behavior (Nunes et al., 2013; Phillips et al., 2003; Wilson et al., 1995). Furthermore, increased DA signaling has behaviorally-reinforcing effects associated with addiction-like behavior (Pascoli et al., 2015). The DA literature is vast, therefore the introduction below is only a brief overview. The interested reader is referred to a number of reviews on DA and its role in substance abuse (Everitt and Robbins, 2005; Gorwood et al., 2012; Le Foll et al., 2009; Missale et al., 1998; Nutt et al., 2015).

There are 5 DA receptor subtypes (D_1, D_2, D_3, D_4, D_5) with the D_1 and D_2 subtypes being the most pervasive in brain and consequently the most frequently studied (Le Foll et al., 2009). These subtypes are often separated into two classes: D_1-like receptors (D_1 and D_5; D1Rs) and D_2-like receptors (D_2, D_3, D_4; D2Rs). D1Rs are located throughout the mesolimbocortical and nigrostriatal pathways. They are coupled to G_s proteins, thus stimulating cAMP production via adenylyl cyclase, and act to increase neuronal excitability through downstream second messenger signaling effects (e.g. release of internal calcium stores and inhibition of potassium channels) (Missale et al., 1998). D1Rs are located both pre and postsynaptically (Levey et al., 1993). Stimulation of D1Rs within the ventral tegmental area has been demonstrated to increase neurotransmitter release (Cameron and Williams, 1993; Kalivas and Duffy, 1995), however, activation of D1Rs has also been observed to attenuate both excitatory and inhibitory postsynaptic potentials in the nucleus accumbens (Pennartz et al., 1992). D1R signaling therefore does not appear to purely result in an increase in neurotransmitter release.

D2Rs are also found throughout the mesolimbocortical and nigrostriatal pathways, although they are highly concentrated on DA cell bodies in the ventral tegmental area and substantia nigra (Missale et al., 1998). D2Rs are coupled to $G_{i/olf}$ G-proteins and their activation inhibits adenylyl cyclase production of cAMP, and ultimately, inhibition of the cell. D2Rs can be found both pre and postsynaptically and interestingly, presynaptic D_2 receptors serve as autoreceptors, acting to decrease further DA release when activated by higher levels of synaptic DA (Ford, 2014). D2R activation increases outward potassium currents, resulting in hyperpolarization of the membrane potential (Williams et al., 1989). Furthermore, stimulation of D2Rs has been shown to inhibit calcium channels (Lledo et al.,

1992), ultimately decreasing the likelihood of neurotransmitter release. In summary, D1Rs and D2Rs regulate neuronal excitability and neurotransmitter release in an apparently opposing manner in key brain regions associated with substance use and abuse.

Caffeine Pharmacology and its Behavioral Effects: Dopamine

Caffeine consumption/exposure has been observed to enhance D2R, but not D1R, expression or function in the nucleus accumbens of rats (O'Neill et al., 2015; Simola et al., 2008). Chronic caffeine treatment also increases striatal levels of DA transporter (DAT) expression in a limited manner (Singh et al., 2009). However, caffeine consumption throughout adolescence produced a roughly 30% increase in DAT levels in the nucleus accumbens of rats (O'Neill et al., 2015).

Like virtually all classes of drugs of abuse (Di Chiara and Imperato, 1988), caffeine acutely increases DA levels in the nucleus accumbens (Solinas et al., 2002) and this effect has been linked to its psychostimulant properties (Ferré, 2016). However, this DAergic response is likely mediated by the previously mentioned functional interaction between adenosine and DA receptors (Ferré, 2016; Fuxe et al., 2005). Nevertheless, caffeine's psychostimulant effects are influenced by both D1Rs and D2Rs. Caffeine locomotor stimulation in rats is blocked by co-administration of D1R or D2R antagonists (Garrett and Holtzman, 1994). Furthermore, rats exhibiting tolerance to caffeine locomotor stimulation demonstrate cross-tolerance to the stimulant effects D1R and D2R agonists (Garrett and Holtzman, 1994). Repeated caffeine exposure has also been observed to produce cross-sensitization to potent DA-enhancing psychostimulants, such as amphetamine and cocaine (Misra et al., 1986; O'Neill et al., 2015; Schechter, 1977), as well as the D1R agonist SKF

77434 (Cauli and Morelli, 2002) and the D2R agonist quinpirole (Cauli and Morelli, 2002; O'Neill et al., 2015; Pollack et al., 2010).

Collectively, the findings reviewed above indicate that repeated caffeine exposure can induce substantial alterations in DA-signaling-related proteins in key brain areas associated with substance use/abuse. Furthermore, repeated caffeine exposure engages important neuroadaptive processes associated with drug abuse (tolerance and sensitization) that are influenced by DAergic processes.

Ethanol Pharmacology and its Behavioral Effects: Dopamine

Ethanol also induces DA release in the nucleus accumbens (Imperato and Di Chiara, 1986; Weiss et al., 1993; Yoshimoto et al., 1992), although more recent research suggests this observation may be related to ethanol cues, themselves (Doyon et al., 2003). Ethanol consumption/exposure produces complicated and inconsistent changes in D1R and D2R expression in rodent models. Ethanol exposure has been observed to both increase (Lograno et al., 1993; Tajuddin and Druse, 1996) and decrease (Lucchi et al., 1988) the density and binding capacity of striatal D1Rs and D2Rs. However, another study found no effect of 5 weeks of ethanol exposure on D1R or D2R labeling in the striatum or frontal cortex of rats (Hietala et al., 1990). Ethanol consumption has also been associated with increased striatal and accumbal mRNA expression of D2Rs, but not D1Rs, after 5 weeks of 10% ethanol drinking in rats (Kim et al., 1997). Alterations in DA receptor binding following ethanol consumption has also been evaluated in rats selectively bred for high alcohol preference. Previous ethanol intake increased D1R binding in the accumbens core

and amygdala and increased D2R binding in the accumbens core and shell as well as the striatum of alcohol preferring P rats (Sari et al., 2006).

Knockout studies in mice have implicated important roles for both D1Rs (El-Ghundi et al., 1998) and D2Rs (Phillips et al., 1998) in 2-bottle choice ethanol consumption with the knockout mice drinking significantly less ethanol than their wildtype counterparts. Pharmacological studies have also demonstrated significant contributions of both D1Rs and D2Rs in ethanol consumption, although the directions of these findings are not consistent. Both D1R agonists and antagonists have been observed to decrease ethanol intake in rats and mice (Cohen et al., 1999; Dyr et al., 1993; Ng and George, 1994; Silvestre et al., 1996). The same is true for D2R agonists and antagonists (Dyr et al., 1993; Pfeffer and Samson, 1986). These observations suggest that DA receptor activation indeed plays a role in ethanol intake, as antagonists may blunt the receptor level effects of the dopamine response to ethanol consumption whereas agonists may 'substitute' for ethanol's effects. Collectively, these results indicate that DA signaling is highly important in ethanol intake, with an optimal level of DA receptor activation driving consumption.

Ethanol-induced locomotor stimulation (Cohen et al., 1997; Kong et al., 2010; Liljequist et al., 1981) and sensitization (Phillips et al., 1997) have both been tied to enhanced DA signaling in rodents. Viral vector knockdown of D1Rs in the nucleus accumbens significantly reduced ethanol intake as well as ethanol locomotor sensitization and CPP acquisition in mice (Bahi and Dreyer, 2012). D2R knockout mice bred on a B6 background demonstrate enhanced ethanol locomotor stimulation and sensitization (Palmer et al., 2003). Furthermore, these sensitized mice did not display reduced ethanol intake as previously observed in D2R knockouts (Phillips et al., 1998), suggesting

sensitization promoted ethanol consumption. D2R knockout mice also exhibit blunted CPP for ethanol (Cunningham et al., 2000), however, a more recent study from the same lab indicated that pharmacological antagonism of D1Rs, but not D2Rs, in the nucleus accumbens blunted the acquisition of ethanol CPP (Young et al., 2014). DA signaling does not appear to influence tolerance to adverse effects of ethanol intoxication. Rats depleted of brain dopamine via intraventricular infusion of 6-hydroxydopamine displayed similar tolerance to motor-impairing and hypothermic effects of ethanol following ~4 weeks of exposure (Lê et al., 1981).

Although the literature on dopamine's involvement in ethanol-related behavior is expansive and this brief overview is certainly not exhaustive, the above studies clearly demonstrate a significant role for DA in ethanol consumption and reward. A history of ethanol intake can also significantly alter the expression of dopamine receptor subtypes in key brain regions associated with substance abuse. Finally, both classes of DA receptors have the capacity to influence the neurobehavioral adaptive sensitization response.

Adenosine and Dopamine Receptor Functional Interactions

The adenosine and dopamine systems are closely intertwined with A1Rs often co-localizing with D1Rs and A2ARs localizing with D2Rs (see Fredholm, et al. 1999; 2005 for reviews). Furthermore, these adenosine receptors can form functional heteromers with their respective DA receptor (Ferre et al., 2008). Presynaptic A1R activation has been demonstrated to shunt dopamine release and postsynaptic A1R activation antagonistically interacts with coupled D1Rs to decrease binding availability as well as the coupling ability

of G_s proteins. Activation of adenosine A2ARs has also been shown to reduce the binding availability of D2Rs. The functional relationship between adenosine receptor activation and signaling at coupled DA receptors is therefore antagonistic. Concomitant caffeine may suppress the signaling capability of enhanced adenosinergic tone induced by ethanol (Figure 1), potentially relieving inhibition of elevated dopamine signaling induced by both compounds. Therefore, repeated exposure to their combination may produce more pronounced and diverse alterations in neurobiology/neurochemistry than exposure to either drug alone. Indeed, a history of caffeinated ethanol consumption in female alcohol-preferring P rats was associated with increased basal levels of extracellular DA in the medial prefrontal cortex (Franklin, 2009). Therefore, alterations in the mesolimbocortical DA system produced by a history of caffeine and ethanol co-consumption may have significant implications for an individual's sensitivity to the heavily DA-involved effects of psychostimulant drugs of abuse, such as cocaine.

Cocaine Pharmacology and its Behavioral Effects: Adenosine

Although research in the last 20 years suggests the picture is more complex than initially thought (Rocha et al., 1998; Uhl et al., 2001), cocaine's primary mechanism of action is still considered to be dopamine transporter (DAT) blockade (Sulzer, 2011); effectively prolonging the presence of endogenously released dopamine in the synaptic cleft. Given the previously described functional relationship between adenosine and DA signaling, it is not surprising that a role for adenosine receptors has been implicated in cocaine-related behaviors.

Repeated cocaine exposure has been observed to increase A1R expression levels within the nucleus accumbens (Toda et al., 2002). Later work demonstrated discordance between this upregulation and the functional nature of these A1Rs. Even though repeated cocaine did produce an increase in A1R expression level, a reduction in receptor binding was actually observed (Toda et al., 2003), suggesting an internalization of receptors. A history of cocaine self-administration was also shown to increase A2AR levels in the nucleus accumbens (Marcellino et al., 2007), with later work suggesting that repeated cocaine exposure may encourage the dissociation of functional heteromers with D2Rs (Marcellino et al., 2010).

The A1R has been demonstrated to regulate cocaine seeking as pretreatment with an A1R antagonist attenuates extinction (Kuzmin et al., 1999). Both caffeine (non-selective adenosine receptor antagonist) and an A1R/A2AR selective antagonist have been shown to effectively reinstate cocaine seeking after the extinction of responding in non-human primates (Weerts and Griffiths, 2003), and drug discrimination work has suggested that the blockade of these receptors may mimic some of cocaine's discriminative stimulus effects (Justinova et al., 2003). A2AR knockout mice exhibit a significant attenuation of cocaine self-administration acquisition (Soria et al., 2005), suggesting that the A2AR is also an important regulator in the establishment of problematic cocaine consumption. Furthermore, pretreatment with an A2AR agonist effectively attenuates cocaine self-administration and reinstatement in rats and non-human primates (Bachtell and Self, 2009; Knapp et al., 2001; Weerts and Griffiths, 2003). These findings were more recently extended to microinjections within the rat nucleus accumbens (O'Neill et al., 2012), although it was also found that intra-accumbens A2AR antagonism increased cocaine seeking. It should be

noted, however, that adenosine receptor agonists can have potent motor-impairing effects which may interfere with the assessment of these behaviors (Knapp et al., 2001).

A2AR knockout mice demonstrate cocaine locomotor stimulation and CPP equivalent to that of wildtype counterparts (Soria et al., 2005). Activation of the A2AR, however, impairs the development and expression of cocaine sensitization (Filip et al., 2006). Conversely, A2AR antagonism enhances cocaine locomotor sensitization (Filip et al., 2006). Within the nucleus accumbens, pharmacological activation of both A1Rs and A2ARs inhibits the expression of cocaine locomotor sensitization in rats (Hobson et al., 2012). Both receptor subtypes also influence cocaine CPP, with agonists and antagonists both blunting the expression and induction of CPP in rats (Poleszak and Malec, 2002).

As with caffeine and ethanol, adenosine signaling also has a significant capacity to influence cocaine self-administration, stimulation/sensitization, and reward. These effects are likely influenced to some degree by the regulatory role of adenosine signaling on DA transmission. As with all psychostimulant drugs, DA signaling is heavily involved in cocaine's neurobehavioral effects which are briefly reviewed below.

Cocaine Pharmacology and its Behavioral Effects: Dopamine

Cocaine acutely increases DA in the nucleus accumbens (Hernandez and Hoebel, 1988) and repeated administration has been observed to sensitize this effect (Kalivas and Stewart, 1991). Repeated cocaine exposure alters functional D1R and D2R expression in key brain regions. Both D1R and D2R binding sites are significantly reduced following repeated cocaine in rat striatum and nucleus accumbens (Kleven et al., 1990; Tsukada et

al., 1996). Although these changes were persistent in the striatum, they were no longer apparent in the nucleus accumbens 2 weeks following the cessation of cocaine treatment (Kleven et al., 1990). In non-human primates, however, repeated cocaine increased D1R binding and decreased D2R binding in the striatum (Nader et al., 2002). Three weeks of cocaine self-administration has, however, been observed to produce a persistent decrease in D2R levels, but no change in D1R levels, in the medial prefrontal cortex of rats (Briand et al., 2008). Cocaine exposure has also generally been demonstrated to increase DAT expression (Kahlig and Galli, 2003). These findings suggest that repeated cocaine exposure produces significant adaptations in both functional D1Rs and D2Rs as well as DAT, although the persistent nature of these changes may be brain region-specific.

In operant paradigms, D1Rs and D2Rs regulate cocaine self-administration and seeking. Antagonism of D1Rs and D2Rs significantly increases cocaine self-administration in rats and non-human primates (Bergman et al., 1990; Caine et al., 2002; Koob et al., 1987). These findings have been replicated via local administration in the nucleus accumbens of rats (Bari and Pierce, 2005). Interestingly, D1R knockout mice will not reliably self-administer cocaine (Caine et al., 2007) whereas D2R knockout mice self-administer significantly more cocaine than their wildtype counterparts (Caine et al., 2002). For the regulation of cocaine relapse-like behavior, these receptor subtypes have opposite effects with the administration D1R agonists blocking (Self et al., 1996), and D2R agonists enhancing (De Vries et al., 1999; Self et al., 1996; Wise et al.) the reinstatement of responding for cocaine.

DA signaling appears to be crucial for the induction of cocaine locomotor sensitization as co-administration of D1R and D2R antagonists significantly reduces the

development of cocaine sensitization (Fontana et al., 1993). Furthermore, D1R knockout mice exhibit attenuated locomotor sensitization to repeated cocaine (Xu et al., 2000). Activation of D2Rs has also been demonstrated to play an important role in both cocaine locomotor stimulation and sensitization. Microinjections of a D2R agonist, but not a D1R agonist, directly into the medial prefrontal cortex attenuated both the development and expression of cocaine sensitization (Beyer and Steketee, 2002). In addition, this cortical microinjection of a D2R agonist significantly reduced cocaine-sensitized DA release in the nucleus accumbens. Furthermore, the increased locomotor stimulant response to and enhanced CPP for cocaine observed in rats that had previously consumed caffeine as adolescents was associated with an increased stimulant response to an injection of a D2R agonist as well as elevated D2R levels in the nucleus accumbens (O'Neill et al., 2015). D2R knockout mice also exhibit a decreased locomotor stimulant response (Welter et al., 2007).

D1R knockout mice exhibit unaltered cocaine CPP (Miner et al., 1995). However, CPP studies in intact animals have consistently demonstrated that D1R, but not D2R, signaling is crucial for the expression of cocaine CPP (Bardo and Bevins, 2000). These results suggest that although the D1R is a primary target for cocaine CPP, other neurotransmitter systems or receptors can be involved in the development of cocaine CPP.

Collectively, these results demonstrate that cocaine self-administration and seeking, locomotor stimulation/sensitization, and conditioned reward are all influenced by DA receptor signaling. Both D1Rs and D2Rs appear to influence each of these phenotypes in some way. Given the extent of DA and adenosine involvement in cocaine-related behaviors as well as those associated with ethanol and caffeine, the substantial overlap in the systems

involved raises the possibility that co-exposure of caffeine and ethanol may enhance an individual's sensitivity to cocaine.

Pilot Study

To probe for whether caffeine and ethanol co-exposure altered cocaine sensitivity in the established caffeine and ethanol co-consumption model previously described, a pilot study was conducted. Adult male B6 mice were given access to water, caffeine (0.03% w/v), ethanol (20% v/v), or a combined ethanol caffeine solution via 2hr DID for 2 weeks. Mice were then injected with saline (i.p.) each of the following 2 days and baseline locomotor activity was recorded in 15 minute sessions. The next day, mice received a 10 mg/kg (i.p.) cocaine challenge injection or saline. This preliminary study found a statistical trend ($p = 0.07$) towards a particularly robust stimulant response to cocaine in mice that had previously consumed the caffeinated ethanol solution (Figure 4), offering preliminary evidence in the direction of the hypothesis that caffeinated ethanol exposure may increase cocaine sensitivity.

Study Rationale

Heavy caffeine and ethanol use have been associated with polydrug abuse, including cocaine, in the clinical literature (Kendler et al., 2006; Kirby and Barry, 2012). With the rise of energy drinks, individuals are now co-consuming caffeine and ethanol at unprecedented levels. The protracted consequences of such consumption are poorly

understood and given these associative risks, a history of co-consumption may increase the abuse potential for drugs of abuse like cocaine.

Preclinical work in rodents has demonstrated that caffeine and ethanol co-exposure can produce additive stimulant effects (Ferrario et al., 2005; Hilbert et al., 2013; Kuribara et al., 1992; May et al., 2015) and augment the locomotor sensitization response to repeated administration (May et al., 2015). Furthermore, 14 days of prior binge-like caffeine and ethanol co-consumption produced elevated basal extracellular dopamine levels in the medial prefrontal cortex of female alcohol-preferring P rats (Franklin, 2009). This suggests a potentially enhanced DAergic tone produced by caffeine and ethanol co-consumption that may influence sensitivity to heavily DA-involved psychostimulant drugs of abuse, such as cocaine.

Along these lines, both prior caffeine and ethanol exposure alone can enhance sensitivity to the psychomotor stimulating and conditioned rewarding effects of cocaine in preclinical animal models (Bedingfield et al., 1998; Hutchison and Riley, 2012; Mateos-García et al., 2015; O'Neill et al., 2015). This may be due, in part, to aforementioned alterations in DA signaling-related proteins by caffeine and ethanol and/or alterations of adenosine receptors which regulate DA signaling. Considering the literature reviewed above, combined caffeine and ethanol exposure may have the capacity to additively enhance cocaine sensitivity. Locomotor sensitization and CPP are two of the most widely used and well defined phenotypes in preclinical substance abuse research. As such, both were employed in the current study to gain a multifaceted understanding of how cocaine sensitivity may be altered by repeated binge-like caffeine and ethanol co-consumption.

MATERIALS AND METHODS

Animals

Adult (postnatal day 56 ± 3) male B6 mice were ordered from the Jackson Laboratory (Bar Harbor, ME) and singly housed upon arrival. Lighting was maintained on a 12-hour reverse light-dark cycle with lights off at 0800 for the work conducted in Experiment 1 and lights off at 0600 for the work conducted in Experiment 2. The temperature and humidity of the room were held constant near 20°C and 50%, respectively. Food and water were available ad libitum, except during the 2hr drinking access periods where mice had their water bottles replaced with a sipper tube containing tap water (W), 20% (v/v) ethanol in tap water (E), 0.03% (w/v) caffeine in tap water (C), or a combined 20% ethanol and 0.03% caffeine solution (EC). Mice were allowed 10-14 days to acclimate to the colony room and light cycle prior to the initiation of testing. All experiments were performed under a protocol approved by the IUPUI Institutional Animal Care and Use Committee.

Drugs and Drinking Solutions

The ethanol solution was made by diluting 190 proof ethanol (Pharmco Inc., Brookfield, CT) in tap water. Caffeine was obtained from Sigma Aldrich (St. Louis, MO)

and was dissolved in either tap water or the ethanol solution. Cocaine hydrochloride was also purchased from Sigma Aldrich (St. Louis, MO) and was diluted in 0.9% physiological saline.

Drinking-in-the-Dark

Beginning 3 hours into the animals' dark cycle, mice had their standard glass water bottles removed and replaced with specially-made 10 ml drinking tubes (a picture of the DID setup can be seen in Figure 5). These tubes consisted of a 10 ml plastic serological pipette fitted with a stainless steel double ball bearing sipper. A silicone stopper was fitted to the open end after filling. Tube volumes were read immediately prior to placing the tube on an animal's cage at eye level and the tube was secured to the cage using a metal binder clip. These tubes remained on the cage for the 2hr limited access period and volume readings were again taken immediately after this time elapsed. All volume readings were taken at the 0.01 ml level of measurement. During this 2hr period, the only fluid to which mice had access was their designated test fluid. After the DID session, the standard home cage water bottles were returned. Empty cages were also fitted with tubes containing tap water or the ethanol solution to estimate the relative degree to which each fluid leaked from tubes during the 2hr DID period. Consumption volumes were corrected by the average leak for water (caffeine or water alone) or ethanol (ethanol or ethanol + caffeine) solutions throughout each experiment.

Activity Monitoring

For both experiments, the locomotor activity/position of mice was monitored by 8 AccuScan VersaMax activity monitors (Omnitech Electronics Inc., Columbus, OH) controlled by a Dell computer. These monitors identify the animal's position via the disruption of intersecting photocell beams spaced evenly along the 40 × 40 cm walls of the testing field. In Experiment 1, mice were contained in a Plexiglas box measuring 40×40×31 cm (l×w×h) and fitted with a lid during testing (Figure 6). In Experiment 2, mice were contained in a smaller Plexiglas box centered in the apparatus measuring 25×14×15 cm (Figure 7). All of this testing equipment was housed within a light and sound attenuating chamber measuring 53×58×43 cm, containing a fan providing both ventilation and 'white noise'. At the conclusion of testing, activity data was translated to the position and/or total distance traveled by each mouse (in cm) in each session by the VersaMax software.

Experiment 1: Locomotor Sensitization

The objective of Experiment 1 was to evaluate the effects of various caffeine and ethanol drinking histories on the locomotor stimulation/sensitization response to cocaine (see Figure 8 for a timeline of the procedure used in Experiment 1). Mice (N = 275) underwent a 14-day DID procedure where they received daily 2hr access to W, E, C, or EC (beginning at 1100). As DID is a self-administration paradigm and dosing varies for individual animals, it was anticipated that some mice may not readily consume ethanol, caffeine, or their combination. In order to properly address the hypothesis concerning 'binge' consumption without employing a stressful blood sample analysis, a strategy to extrapolate BEC values on the basis of fluid intake data was adopted. In our lab's

experience as well as others' (Rhodes et al., 2005), ethanol consumption in DID with male B6 mice is very tightly correlated with the attained BEC. Therefore, the BEC achieved via DID ethanol intake can be comfortably predicted. Figure 9 depicts ethanol intake and BEC data following DID on day 14 from a prior study in our lab (Fritz et al., 2014). As can be seen, ethanol intake is highly predictive of BEC. A binge level BEC in this paradigm is generally considered to be ≥ 80 mg/dl and the data in Figure 9 suggest that consumption of ≥ 3 g/kg ethanol will produce BECs of this magnitude. As such, it was planned that mice that drank less than 3 g/kg ethanol per day (19 ml/kg total fluid), on average, would be excluded from the remainder of the experiment. This criterion was applied to both E and EC groups. To also keep consistency with caffeine intake, this fluid volume criterion (19 ml/kg) was also applied to the C group which translates to a minimum average daily DID caffeine intake of 5.7 mg/kg.

The 11-day cocaine sensitization protocol was adopted from Lessov and Phillips (2003) and was initiated on day 15. Each testing day, mice were allowed at least 1hr to habituate to the activity monitor room prior to testing. All locomotor testing occurred under red light. On days 15-16, mice received an injection of 0.9% physiological saline (i.p.) beginning at 1100 and were immediately placed into the Accuscan VersaMax activity monitors for 15 minutes. The purpose of these first 2 days is to habituate the animals to the apparatus and injection procedure. Starting on day 17, mice were subjected to injections of cocaine (5 or 10 mg/kg; i.p.) or saline every other day, with 15 minute activity monitoring until day 25. The baseline activity measures on days 15-16 were used to counterbalance mice for dose and designation to one of three conditions for the remainder of the experiment: cocaine-naïve (CN), acute cocaine (AC), or repeated cocaine (RC). CN mice

received saline throughout the entire experiment, serving as a control for repeated injection stress on basal activity. AC mice received saline until the final day of the sensitization protocol whereupon they received their first and only dose of cocaine. This condition assesses any effect repeated injection stress may have on responsivity to cocaine; an important control considering that stressors have been shown to sensitize responses to drugs of abuse (Kalivas and Stewart, 1991). The RC group received cocaine on each locomotor assessment day for the remainder of the experiment. Sensitization is characterized in two different ways in this design: 1) a significant increase in activity from the first injection of drug (day 17) to the final injection in RC groups (day 25), indicating the classically-defined progressive increase in response to drug and 2) significantly greater activity in RC mice relative to AC mice, which considers the potential for the repeated injection procedure to influence the locomotor response to the drug. Both characterizations offer important information for the interpretation of sensitization data and together, provide the most complete description of the data.

Experiment 2: Conditioned Place Preference

The objective of Experiment 2 was to determine whether various caffeine/ethanol drinking histories differentially influence the conditioned rewarding effects of cocaine as assessed by the CPP paradigm (for a timeline of the procedure used in Experiment 2, see Figure 10). As in Experiment 1, mice (N = 240) first underwent an identical 14-day DID procedure following acclimation to the colony room. The same drinking criterion ($\geq$ 3 g/kg ethanol or 5.7 mg/kg caffeine average daily intake) was implemented for Experiment 2. The CPP protocol commenced on day 15 and mice were moved into the activity monitor

room at least 1hr prior to testing in order to habituate them to the room each day. All testing occurred under red light. On day 15, mice received saline injections starting at 0900 and were immediately placed onto a neutral floor texture (paper) in the place conditioning box for 15 minutes in order to habituate the animals to the apparatus and injection procedure. The activity chambers determined the location of the mouse in the chamber over the course of each session and also recorded general locomotor activity. For conditioning sessions, two different tactile cues were used: a metal floor with punched holes or a floor made of a grid of small metal rods (Figure 11). These exact floor textures have been used extensively in place conditioning with mice since animals consistently show no baseline preference for one texture over the other (Cunningham et al., 2006). Mice were counterbalanced for dose, drug-paired floor, and order of drug exposure (odd or even days) on the basis of their caffeine/ethanol intake and baseline locomotor activity on day 15. Starting on day 16, mice received daily alternating systemic injections of cocaine (1 or 5 mg/kg; i.p.) and saline or saline throughout (naïve controls) and were placed into the monitors for 15 minute conditioning sessions. The low dose was chosen since it has been shown previously to induce mild CPP in male B6 mice and may enhance the ability to detect substantial increases in sensitivity to cocaine reward (Cunningham et al., 1999). The 5 mg/kg dose was chosen because it parallels the low dose in Experiment 1, thereby offering a point of consistency in collective interpretation. These sessions alternated drug and vehicle over the course of 8 days so that a total of 4 drug and 4 saline injections were given. In the conditioning literature, each pair of saline and drug exposures is referred to as a ‘trial’ (i.e. days 16-17 constitute ‘trial 1’). The first place preference test occurred on day 24 wherein all mice were injected with saline and offered access to both floor textures (Figure 7

illustrates an example of a CPP test floor setup). Each animal's drug-paired floor was placed on the non-preferred side of the chamber during the test to ensure that side preference did not artificially inflate CPP expression. On day 25, mice were returned to the conditioning protocol for another 8 days for 4 more trials and a second CPP test on day 33. This design offers the sensitivity to not only determine whether the fluid groups exhibit stronger/weaker CPP for cocaine, but also whether CPP may develop after fewer/more drug-cue pairings across 2 doses of drug. The durations of these sessions and drug doses were chosen due to previously demonstrated success with cocaine CPP in B6 mice (Cunningham et al., 1999).

Statistical Analysis

Experiment 1

Fluid intake was analyzed by repeated measures analyses of variance (ANOVAs) with caffeine (yes or no) and ethanol (yes or no) consumption history as the between subjects factors and day as the repeated within subjects factor. Ethanol and caffeine intake were also specifically analyzed with fluid (E/EC or C/EC) as the between subjects factor and day as the repeated within subjects factor.

Habituation activity during the first 2 days of the sensitization protocol was also analyzed via repeated measures ANOVA with caffeine (yes or no) and ethanol (yes or no) consumption history as the between subjects factors and day as the repeated within subjects factor. The activity of CN mice was analyzed independently because this group does not

contain the same 2 levels of the dose factor as in AC and RC groups and therefore cannot be appropriately included in the overall analysis. Furthermore, the main experimental question addressed was whether caffeine/ethanol consumption histories influence the locomotor response to cocaine and CN mice never received cocaine in the experiment. A repeated measures ANOVA with caffeine and ethanol consumption history as the between subjects factors and day as the repeated within subjects factor for the activity of CN mice on Days 3-11 of the sensitization protocol therefore evaluated whether baseline differences existed, an important consideration in the interpretation of the response to cocaine in the other groups. To determine fluid group and dose effects on cocaine locomotor sensitivity, activity was specifically analyzed on Day 3 and Day 11 of the sensitization protocol with caffeine consumption history, ethanol consumption history, dose (1,5 mg/kg), and condition (AC,RC) as the between subjects factors. This approach was chosen because the important experimental manipulations occur on these two days and thus determine whether group differences exist. Furthermore, a repeated measures ANOVA across all 5 test days is arguably unnecessarily complicated considering the inconsistent treatment history of the AC group (saline on days 3-9 and cocaine on day 11). In order to evaluate whether repeated cocaine exposure induced a progressive increase in locomotor activity in RC mice, a sensitization score (SENZ) was computed for each mouse (Day 11 activity – Day 3 activity). SENZ scores for each fluid group significantly greater than '0' as determined by t-test indicate the existence of a sensitized locomotor response. In addition, direct comparisons were planned between AC and RC groups within each dose for day 11 activity in each fluid consumption group (W, C, E, EC). This planned analysis helped determine whether the AC versus RC manipulation was successful in inducing sensitization within

each fluid group, an important consideration obscured by the overall analysis of Day 11 activity. Bonferroni-corrected t-tests were employed for these planned comparisons. Finally, regression analyses were conducted to assess whether prior ethanol or caffeine intake via DID influenced any observed differences in cocaine sensitivity. Newman-Keuls post-hoc statistics were run where applicable and the significance level was set at $p < 0.05$. A total of 20 mice were removed from the analyses in Experiment 1 due to insufficient caffeine intake as determined by the previously mentioned criteria (11 C), injection error (1 EC) extremely anxious behavior (1 E, 1 W, 1 EC), escape from cage for a prolonged period of time (2 W & 1EC), or death (1 W & 1 EC).

Experiment 2

Fluid intake was analyzed by repeated measures ANOVAs with caffeine and ethanol consumption history as the between subjects factors and day as the repeated within subjects factor. Ethanol and caffeine intake were also specifically analyzed with fluid (E/EC or C/EC) as the between subjects factor and day as the repeated within subjects factor.

Habituation activity was analyzed by a two-way ANOVA with caffeine and ethanol consumption history as the between subjects factors. Activity data across CS+ and CS- trials were separately analyzed by 4-way repeated measures ANOVAs with dose, caffeine history, and ethanol history as the between subjects factors and conditioning trial as the within subjects factor. The activity analysis of CS+ trials allows for comparison to the data from Experiment 1 to assess whether locomotor sensitization differences are also apparent in the context of place conditioning at the 5 mg/kg dose.

The typical way in which CPP is analyzed is by comparing the mean seconds/minute spent on the drug-paired floor relative to the saline paired floor during the CPP test. Mice were counterbalanced for which floor texture was paired with cocaine within each dose assignment and mean seconds/minute on the grid floor was compared between mice that had the grid floor paired with cocaine (G+) and those that had it paired with saline (G-). This allows for the most careful determination of whether CPP was indeed achieved as the groups are compared solely on their different learning experience about one common cue (the grid floor in this case) and this analysis is conventional in the place conditioning literature (Cunningham et al., 1999; Cunningham et al., 2006). To determine whether previous fluid consumption history influenced baseline floor preference, mice in the '0' dose group that never received cocaine were first compared for the time they spent on the grid floor during the CPP tests. This was assessed via a 3-way repeated measures ANOVA with caffeine and ethanol consumption history as the between subjects factors and test as the repeated within subjects factor. For groups receiving cocaine, a repeated measures ANOVA was employed with caffeine consumption history, ethanol consumption history, dose (1 or 5 mg/kg), and grid floor assignment (G+ or G-) as the between subjects factors and test as the repeated within subjects factor. CPP is also assessed by comparing groups simply based on the time spent on the drug-paired floor. A repeated measures ANOVA was also run to compare groups on this measure of CPP with caffeine consumption history, ethanol consumption history, and dose (1 or 5 mg/kg), as the between subjects factors and test as the repeated within subjects factor. Although not as stringent as the analysis described earlier, this approach may allow for the detection of CPP that may have been differentially influenced by which texture was assigned as the CS+. In addition,

the G+/G- comparison does not provide a continuous measure with which to correlate prior caffeine and ethanol consumption via DID. Regression analyses were conducted to assess whether prior ethanol or caffeine intake via DID influenced any observed differences in time spent on the drug-paired floor. Newman-Keuls post-hoc statistics were run where applicable and the significance level was set at $p < 0.05$. A total of 5 mice were excluded from the analyses of Experiment 2 due to insufficient caffeine intake (2 C), insufficient ethanol intake (1 E), extremely anxious behavior (1 E), or death (1 C).

RESULTS

Experiment 1

Fluid Consumption during DID

Across the 14 days of DID drinking, mice with a caffeine consumption history (C,EC) consumed more total fluid than those that did not consume caffeine [$F(1,250) = 7.295$, $p < 0.05$] (Figure 12). Ethanol history significantly interacted with the repeated factor, day, and indicated that mice with an ethanol consumption history (E,EC) significantly increased their fluid intake over days [$F(13,3250) = 10.312$, $p < 0.001$]. The potential for differences in caffeine or ethanol intake was also evaluated between groups. Although C and EC mice did not differ in overall caffeine intake ($p > 0.05$), a significant fluid × day interaction indicated that EC mice initially consumed slightly less caffeine than C mice on certain days during the first half of DID [$F(13,1625) = 9.176$, $p < 0.001$] (Figure 13). Ethanol consumption over the course of DID was not different between E and EC mice ($p > 0.05$), however intake increased over days as previously reported [$F(13,1742) = 35.650$, $p < 0.001$] (Figure 14).

Habituation

To assess the influence of fluid consumption history on baseline activity in the sensitization paradigm, habituation activity was analyzed across the first two days when mice received saline injections prior to activity monitoring. A significant caffeine × day interaction indicated that mice that had a caffeine consumption history were more active than those that did not on Day 1 [$F(1,250) = 4.478$, $p < 0.05$], however groups were equivalent by Day 2 (Figure 15). In addition, a caffeine × ethanol × day interaction indicated that E mice demonstrated the least change in activity from Day 1 to Day 2 [$F(1,250) = 3.992$, $p < 0.05$].

Sensitization

Prior to the initiation of the sensitization protocol mice were counterbalanced for caffeine/ethanol intake and baseline activity for dose and condition assignments (all p's > 0.1). The activity data for all mice across the entire sensitization phase of the experiment can be seen in Figures 16-19. For Days 3-11, the activity of only the CN mice in each fluid group was first analyzed. This was done to determine whether or not basal activity was generally influenced by fluid consumption history. As CN mice do not have 5 mg/kg or 10 mg/kg dose groups, it is not possible to include them in an overall analysis of condition and dose on activity throughout Days 3-11. Neither caffeine ($p > 0.05$) nor ethanol ($p > 0.05$) consumption history influenced basal activity throughout Days 3-11. As the primary objective of the study was to determine whether fluid consumption histories altered locomotor responsivity to cocaine and these fluid consumption histories did not influence basal activity, CN mice were excluded from the rest of the analyses.

As the major differences between groups are determined on two key days of the sensitization protocol, Day 3 and Day 11, locomotor activity was specifically analyzed on these days in lieu of an unnecessarily complicated repeated measures analysis. The analysis of Day 3 activity found main effects of caffeine history [$F(1,195) = 13.083$, $p < 0.001$], dose [$F(1,195) = 41.673$, $p < 0.001$], and condition [$F(1,195) = 232.868$, $p < 0.001$] (Figure 20). Caffeine history positive mice were generally more active than those that were not exposed to caffeine ($p < 0.001$) and RC mice (which received cocaine) were significantly more active than AC mice (which received saline) ($p < 0.001$). The main effect of dose is best explained by higher order interactions. A significant dose × condition interaction [$F(1,195) = 55.197$, $p < 0.001$] indicated that within the RC groups, the 10 mg/kg dose produced greater locomotor activity than the 5 mg/kg dose ($p < 0.001$) as expected. In addition, a significant caffeine history × dose × condition interaction [$F(1, 95) = 8.605$, $p < 0.01$] indicated that among the RC mice that received the higher 10 mg/kg dose, those that had a positive caffeine history (C,EC) were significantly more stimulated than those that did not ($p < 0.01$). Therefore, prior caffeine consumption produced the greatest initial locomotor stimulant response to the 10 mg/kg dose of cocaine.

The analysis of Day 11 activity, when mice in the AC condition received their first dose of cocaine and RC mice received their fifth dose, found main effects of dose [$F(1,195) = 65.674$, $p < 0.001$] and condition [$F(1,195) = 22.229$, $p < 0.001$] with mice receiving the 10 mg/kg dose and mice in the RC group demonstrating significantly greater stimulant responses to cocaine than 5 mg/kg and AC mice, respectively (Figure 21). In addition, a caffeine history × dose interaction neared significance [$F(1, 195) = 3.559$, $p = 0.06$], indicating a trend for mice with a positive caffeine history being activated to a greater

extent by the 10 mg/kg dose on Day 11 than those that had no prior experience with caffeine. This characterization of sensitization accounts for the possibility that repeated injection stress influences the development of locomotor sensitization. In this case, sensitization is defined by significantly greater activity in the RC group compared to the AC group in response to a common dose of drug and this was observed in the current study. Although RC groups were indeed generally more activated by cocaine than AC mice on Day 11, it is also of interest how the various injection conditions influenced locomotor behavior on Day 11 in each fluid group. This information assists in the interpretation of the overall Day 11 activity analysis because it more carefully addresses whether the injection protocol did indeed produce sensitization in each fluid group. This question is obscured by the complexity of the overall ANOVA.

In order to determine whether this indeed occurred in the current study, Bonferroni corrected T-tests compared the Day 11 activity of RC and AC mice in each dose group, within each fluid group. Among the C mice, repeated 5 mg/kg [$t(23) = 2.509$, $p < 0.01$; one-tailed] and 10 mg/kg cocaine [$t(23) = 2.234$, $p < 0.05$; one-tailed] induced significant sensitization relative to the acute injection (Figure 16). Within E mice, neither the 5 mg/kg ($p > 0.05$) nor the 10 mg/kg dose ($p > 0.05$) produced sensitization as determined by this analysis (Figure 17). In EC mice, repeated 5 mg/kg cocaine did not increase the locomotor response relative to the acute injection ($p > 0.05$). However, repeated doses of 10 mg/kg cocaine was effective in increasing this response [$t(27) = 1.975$, $p < 0.05$; one-tailed] (Figure 18). W mice that received repeated doses of 5 mg/kg mice were stimulated similarly to those receiving an acute injection ($p > 0.05$). However, repeated 10 mg/kg cocaine

robustly augmented the stimulant response relative to an acute injection [$t(20) = 4.004$, $p < 0.001$; one-tailed] (Figure 19).

As sensitization is also classically defined as a successive increase in response to a fixed dose of drug with repeated administration, this was also evaluated within the RC mice. A 'SENZ' score was calculated for each RC mouse as follows: *SENZ = (Day 11 activity – Day 3 activity)*. This difference score therefore reflects the change in locomotor activity from the first cocaine exposure to the last, with positive values reflecting sensitization. In order to determine whether mice expressed sensitization, SENZ score means were compared to '0' for each fluid/dose group via t-test. Among the 5 mg/kg RC mice, only W [$t(12) = 3.94$, $p < 0.01$)] and EC [$t(13) = 3.94$, $p < 0.01$)] mice had mean SENZ scores significantly greater than 0 (Figure 22A). Among the RC mice receiving 10 mg/kg, only the W mice demonstrated a significant SENZ score [$t(10) = 3.694$, $p < 0.01$)] which was particularly robust (Figure 22B). Overall ANOVAs were not deemed appropriate for these analyses of SENZ as relative comparisons of SENZ are not very meaningful if not all groups express significant SENZ in the first place.

Relationship between Prior Caffeine/Ethanol Intake and Cocaine Stimulation

In order to determine how caffeine intake was related to the observed group difference in acute cocaine stimulation on Day 3 in RC 10 mg/kg mice, a linear regression analysis was performed to determine the predictive value of mean caffeine intake throughout DID for Day 3 activity of these C and EC mice. This analysis found no relationship between the relative amount of caffeine consumed and locomotor activity on day 3 ($p > 0.05$; $R^2 < 0.001$; Figure 23A). The same analysis was conducted for prior

ethanol intake and Day 2 activity and again, no relationship was found ($p > 0.05$; $R^2 = 0.03$; Figure 23B). Therefore prior caffeine consumption, regardless of relative levels of consumption, heightened sensitivity to the acute stimulant response to the 10 mg/kg cocaine dose.

Experiment 2

Fluid Consumption during DID

Across the 14 days of DID drinking, mice with an ethanol consumption history consumed more total fluid than those that did not consume ethanol [$F(1,231) = 5.917$, $p < 0.05$] (Figure 24). Ethanol history significantly interacted with the repeated factor, day, and indicated that mice with an ethanol consumption history significantly increased their fluid intake over days [$F(13,3003) = 7.229$, $p < 0.001$]. The potential for differences in caffeine or ethanol intake was also evaluated within each group. EC mice were found to consume slightly more caffeine, overall, than C mice [$F(1,115) = 4.258$, $p < 0.05$] (Figure 25). A significant fluid × day interaction indicated that EC mice generally increased their intake over days and consumed significantly more caffeine than C mice specifically on days 11-14 of DID [$F(13,1495) = 6.721$, $p < 0.001$]. Ethanol consumption over the course of DID was not different between E and EC mice ($p > 0.05$), although ethanol intake generally increased over days as previously reported [$F(13,1508) = 47.34$, $p < 0.001$] (Figure 26).

Habituation

On day 1 of the CPP protocol, basal activity was measured during the habituation session. Neither caffeine ($p > 0.05$) nor ethanol ($p > 0.05$) consumption history influenced basal locomotor activity on Day 1 (Figure 27). Mice were counterbalanced for dose (0, 1, or 5 mg/kg cocaine), drug-paired floor (GRID+ or GRID-), and drug exposure day (odd or even) on the basis of mean ethanol/caffeine intake during DID and day 1 locomotor activity (p's > 0.1).

Locomotor Activity during Conditioning

Activity during CS- (saline only) trials (Figure 28) and CS+ (cocaine) trials (Figure 29) were analyzed separately. For control mice in the '0' dose group, roughly half of the mice have data represented from odd days and the other half have data represented from even days. This approach was taken because although these mice never receive cocaine, data from each day of each trial is represented. During drug-free CS- trials, mice in the 5 mg/kg dose group were significantly more active than mice in the 0 and 1 mg/kg groups [$F(2,223) = 21.636$, $p < 0.001$], perhaps indicating some degree of conditioned locomotion (Figure 28). In addition, a significant trial × dose interaction [$F(14,1561) = 2.067$, $p < 0.05$] indicated that the 5 mg/kg group was particularly active during trials 6-8 (p's < 0.05). Finally, a significant trial × dose × ethanol history interaction [$F(14,1561) = 1.943$, $p < 0.05$] determined that mice that had previously consumed ethanol in the 5 mg/kg group were particularly active during trials 6-8 (p's < 0.05).

During CS+ trials, where the 1 and 5 mg/kg dose groups received cocaine, a main effect of dose [$F(2,223 = 83.158$, $p < 0.001$] determined that the 5 mg/kg group was

significantly more active than the other groups (p's < 0.001; Figure 29). In addition, a main effect of trial [$F(7,1561) = 6.391$, $p < 0.001$] indicated that activity was significantly lower on trials 5-8 relative to trials 1-4 (p's < 0.05). Finally, a significant trial × ethanol history interaction [$F(7,1561) = 2.078$, $p < 0.05$] indicated that mice that had consumed ethanol (E,EC) were generally less active on trials 5-8 relative to trials 1-4 (p's < 0.05). All other main effects and interactions did not reach statistical significance (p's > 0.05).

Conditioned Place Preference

Prior to CPP analysis, all 0 dose group mice were compared for their time spent on the grid floor for across the CPP tests to determine whether prior fluid history may have influenced time spent on the grid floor in a neutral condition. Neither caffeine ($p > 0.05$) nor ethanol ($p > 0.05$) history was found to influence time spent on the grid floor among mice in the 0 group during CPP tests (Figure 30). Test was also not a factor ($p > 0.05$) and none of the potential interactions reached statistical significance (p's > 0.05). Activity during the CPP tests was also analyzed within the 0 dose group to determine if prior fluid consumption history influenced activity specifically during testing conditions. Neither fluid history (p's > 0.05) nor test number ($p > 0.05$) influenced activity during CPP tests (Figure 31). As this group of mice did not receive cocaine at any point and therefore did not have a G+ and G- designation, it was excluded from further analyses.

Time spent on the grid floor for Test 1 and Test 2 are represented in Figures 32 and 33, respectively. For the CPP test analysis, time spent on the grid floor was compared between mice that had the grid floor paired with cocaine (G+) and mice that had the grid floor paired with saline (G-). A main effect of grid floor assignment [$F(1,180) = 87.16$, $p <$

0.001] indicated that G+ mice spent significantly more time on the grid floor than G- mice, suggesting that the CPP protocol was effective in producing CPP for cocaine. In addition, a significant dose × grid floor assignment interaction [$F(1,180) = 37.02$, $p < 0.001$] demonstrated that although G+ mice spent more time on the grid floor than G- in both the 1 and 5 mg/kg groups ($p < 0.05$), this difference was far more pronounced in the 5 mg/kg group ($p < 0.001$). Finally, a significant test × grid floor assignment interaction [$F(1,180) = 4.75$, $p < 0.05$] revealed that the difference between G+ and G- groups increased in Test 2 (p's < 0.05), suggesting a strengthening of conditioning. All other main effects and interactions did not reach statistical significance (p's > 0.05). Therefore, prior fluid consumption in DID had no bearing on cocaine CPP development or expression.

Although this is viewed as the most rigorous statistical approach to assess CPP because it is strictly comparing the learning that took place for a consistent cue (the grid floor), another common analysis is to compare the amount of time animals spent on the drug-paired floor. This other analysis offers increased power and simplicity to detect potential differences in CPP as it removes the G+ and G- distinction. This analysis only found a main effect of dose [$F(1,188) = 34.15$, $p < 0.001$] with the 5 mg/kg dose producing greater times on the drug-paired floor (Figure 34). In line with the analysis above, the main effect of test approached significance [$F(1,188) = 3.16$, $p = 0.07$] suggesting conditioning may have been slightly stronger in Test 2 relative to Test 1. All other main effects and interactions did not reach statistical significance (p's > 0.05).

The analysis of activity during drug-free CPP tests revealed that mice that had been receiving the 5 mg/kg dose were significantly more active than the 1 mg/kg group [$F(1,188) = 21.698$, $p < 0.001$], suggesting conditioned locomotion evident during CS- trials in the

higher dose group may have also carried over to the CPP tests (Figure 35). Caffeine history, ethanol history, and test number all had no bearing on test activity and none of the potential interactions reached statistical significance (p's > 0.05).

Although DID fluid consumption history did not influence either of these measures of CPP, group differences may have emerged at certain time points within the CPP tests that this analysis was not able to detect. Time spent on the drug floor was compared between test groups across the 3, 5-minute bins of each 15-minute CPP test. The results of the repeated measures analyses of both Test 1 [$F(1,188) = 17.946$, $p < 0.001$] and Test 2 [$F(1,188) = 30.64$, $p < 0.001$] yielded identical results to the earlier analysis, with only a significant effect of dose indicating that the 5 mg/kg group spent significantly more time on the drug-paired floor, overall, in each test (Figures 36 & 37). All other main effects and interactions did not reach statistical significance (p's > 0.05). Therefore, no fluid group differences emerged early or late in the CPP tests.

With the evidence of conditioned locomotor activity in the CPP tests, it is important to determine whether this increased activity may have interfered with CPP expression. If mice are sufficiently active, their ability to remain on one side and demonstrate preference or aversion could be hampered and this has been seen in previous CPP work with cocaine (Cunningham et al., 1999). To address this, regression analyses were run for both tests to address whether activity was negatively predictive of time spent on the drug-paired floor. For test 1, activity was actually *positively* predictive of time spent on the drug-paired floor [$F(1,194) = 16.25$, $p < 0.001$; $R^2 = 0.077$] (Figure 37A). However, no effect was found for test 2 ($p > 0.05$; $R^2 = 0.011$] (Figure 37B). Therefore, any conditioned effect on locomotion does not appear to have interfered with CPP expression.

Relationship between Prior Caffeine/Ethanol Intake and Cocaine CPP

In light of the small caffeine intake difference between C and EC mice, regression analysis evaluated the predictive ability of mean caffeine intake during DID on the time C and EC animals spend on the drug-paired floor for both tests. Caffeine intake during DID was not a significant factor in either test (p's > 0.05; Figure 38A,C). For comparative purposes, the same analysis was carried out for ethanol groups and again, previous intake was not predictive of time spent on the drug-paired floor in either test (p's > 0.05; Figure 38B,D).

DISCUSSION

The objective of Experiment 1 was to determine whether a history of binge consumption of caffeine, ethanol, or their combination influenced the locomotor stimulant response or the capacity to develop locomotor sensitization to the psychostimulant drug, cocaine. The objective of Experiment 2 was to determine whether these prior drinking histories influenced cocaine conditioned 'reward' as assessed by the CPP paradigm. It was found that caffeine consumption, either with or without ethanol, increased the initial locomotor stimulant response to the highest dose of cocaine used in the study (10 mg/kg). However, these prior fluid consumption histories were not found to influence the expression of cocaine CPP. These results suggest that prior caffeine, but not ethanol, consumption increases sensitivity to the stimulant response of cocaine, however the conditioned rewarding effects of lower doses were not altered.

Experiment 1: Locomotor Sensitization

In Experiment 1, no differences were found in ethanol or caffeine intake between groups across the 14-day DID pre-exposure period (Figures 13 & 14). This is in line with our previously published work with this model (Fritz et al., 2014). The onlyknown published animal study that observed an effect of voluntary caffeine co-consumption on ethanol intake was in female selectively-bred alcohol preferring rats (Franklin et al., 2013).

The drinking paradigm was similar with 1hr limited daily access for 14 days, using the same caffeine concentration (0.03% w/v) and a slightly lower ethanol concentration (15% v/v). Animals were found to consume significantly more of the caffeinated ethanol solution than either ethanol or caffeine alone. Recently, we also observed increased ethanol intake via caffeine co-consumption in adolescent and adult male B6 mice via DID when the access period was extended from to 2hr to 4hr (Fritz et al., in press). It is not entirely clear why caffeine does not influence binge ethanol intake in 2hr DID in adult male B6 mice, however genotype, sex, and ethanol concentration may be important factors. Perhaps the genetic predisposition for high alcohol intake in P rats enhances the capacity for caffeine to influence ethanol consumption. Female rats and mice have also been consistently observed to consume more ethanol than male animals in voluntary access paradigms (Li et al., 1993; Matson and Grahame, 2013; Rhodes et al., 2005), perhaps increasing the likelihood that caffeine may alter the interaction with ethanol. In addition, caffeine may exert a stronger influence over the consumption of lower concentrations of ethanol. Caffeine has been observed to enhance the stimulant properties of low/moderate doses of ethanol (Hilbert et al., 2013; Kuribara et al., 1992; May et al., 2015; Waldeck, 1974) and may therefore potentially increase sensitivity to lower concentrations of ethanol. Although a primary concern in the human literature is that the high amount of caffeine in energy drinks may encourage dangerously high levels of ethanol consumption, it could be argued that the lack of caffeine or ethanol consumption differences between groups in the current experiment is an interpretational strength. Because these intakes are equivalent, any differences observed in cocaine-induced locomotor stimulation and/or sensitization are likely due to

the caffeine/ethanol consumption history and not relative differences in the amounts caffeine or ethanol consumed.

At the initiation of the locomotor sensitization protocol, mice were habituated to the activity testing boxes and received saline injections on days 1-2. This allowed for the determination of any baseline differences in activity or the capacity to habituate to the testing chamber, both of which were important interpretational considerations for the remainder of the experiment. Mice that had previously consumed caffeine (C,EC) were found to be significantly more active than those that had not on Day 1 (Figure 15). However, the activity of all fluid groups was equivalent by Day 2. Prior caffeine exposure may therefore have interacted with the novelty of the activity testing chamber and these mice may have been somewhat more exploratory. Acute caffeine treatment has been shown to increase the response to novelty in both animals (Hughes and Greig, 1976) and humans (Davidson and Smith, 1991). Furthermore, a heavy caffeine consumption history has been associated with a greater propensity for novelty-seeking in humans (Gurpegui et al., 2007). Surprisingly, a literature search yielded no published studies directly addressing the effect of prior caffeine on novelty responses or seeking in animals. This seems to be an important question to address given the demonstrated overlapping relationships between novelty seeking (Bardo et al., 1996; Palmer et al., 2013; Wills et al., 1994) and caffeine use (Kendler et al., 2006) with drug seeking and use/abuse. Nevertheless, the activity of all groups was equivalent by Day 2, suggesting that no baseline differences in activity existed which could have influenced subsequent observations on Days 3-11. This was further confirmed by the analysis of activity among CN mice on Days 3-11 which revealed no

influence of prior caffeine or ethanol consumption histories on baseline activity (Figures 16-19).

On Day 3, mice in the RC groups experienced their first dose of cocaine. Not surprisingly, cocaine stimulated locomotor activity with the 10 mg/kg dose producing stimulation greater than the 5 mg/kg dose (Figure 20). Interestingly, mice that had previously consumed caffeine irrespective of ethanol (C,EC) were significantly more stimulated by the 10 mg/kg cocaine dose than those not previously exposed to caffeine (E,W). Although this may be evidence for cross-sensitization in mice that had previously consumed caffeine, the data collected on Day 11 determine whether group differences emerged in sensitization development over the course of the experiment. On Day 11, RC groups received their fifth dose of cocaine and AC received their first dose. Significantly greater activity in RC groups relative to AC groups indicates that repeated cocaine administration induced a sensitized response greater than a single acute injection. Not surprisingly, mice that had received the 10 mg/kg dose (collapsed on AC and RC) were also more stimulated than those that were given the 5 mg/kg dose (Figure 21). The observed statistical trend for mice that did not consume caffeine to have a lower response to the 10 mg/kg dose (collapsed on RC and AC) appears to be driven by a dampened response in W AC mice in the 10 mg/kg group. Therefore, prior caffeine and ethanol intake (alone or in combination) may have also positively influenced the acute response to 10 mg/kg cocaine 11 days after the cessation of DID.

Sensitization was also assessed within each fluid group by directly comparing the Day 11 activity of AC and RC groups for each dose. Although the overall ANOVA on Day 11 technically made this comparison, its complexity obscures the question of whether the

various injection conditions produced the expected pattern of results in each fluid group. In other words, it is still important to address whether repeated cocaine in each fluid group indeed produced greater stimulation than an acute injection in order to more carefully interpret the results of the overall analysis of Day 11 activity. It was found that only mice consuming caffeine alone expressed this characterization of sensitization to the 5 mg/kg dose of cocaine (Figure 16). In addition, all fluid groups, except for mice that consumed ethanol alone, demonstrated this characterization of sensitization to the 10 mg/kg dose of cocaine (Figures 16-19).

The above findings are best interpreted when also considering the SENZ score (change in activity from Day 3 to Day 11) for RC mice. Analysis of the SENZ score indicated that the cocaine-induced stimulation response was significantly increased only in W and EC groups receiving the 5 mg/kg dose (Figure 22A). In addition, only W mice exhibited a significant increase in activity via repeated exposure to the 10 mg/kg dose (Figure 22B). Given the observation that relative stimulation by cocaine did not increase with repeated exposures in 10 mg/kg C and EC mice, prior caffeine consumption may have cross-sensitized mice to the 10 mg/kg dose to the fullest extent possible on Day 3. It is also important to note that 10 mg/kg AC groups did demonstrate lower levels of stimulation than RC groups on Day 11 (except for mice that consumed ethanol, only). Therefore, the time between the cessation of DID and first cocaine exposure appears to be an important factor in this response. Indeed, previous work has demonstrated that significant neurobiological changes induced by chronic caffeine intake in mice can be normalized within 7 days following the cessation of caffeine consumption (Shi et al., 1994). Perhaps by Day 11, any changes in neurobiology/neurochemistry induced by prior caffeine

consumption were diminishing and as such, the acute response to cocaine was dampened. This may also explain the observation of significantly greater activity in RC 5 mg/kg mice relative to the AC group in mice consuming caffeine, only (Figure 16). This possibility is also supported by the fact that that RC 5 mg/kg mice in the C group did not achieve a significant SENZ score (Figure 22A). Therefore, continued cocaine exposure may maintain these observed higher levels of initial response in mice that had consumed caffeine in DID. Interestingly, the relative level of caffeine intake was not related to the observed increased stimulant response to 10 mg/kg cocaine on Day 3 in mice that had previously consumed caffeine (Figure 23A). The same was true for prior ethanol consumption (Figure 23B). Therefore, a caffeine consumption history of at least 5.7 mg/kg/day in DID, elevated the initial stimulant response to 10 mg/kg cocaine soon after the cessation of caffeine consumption.

Alternatively, repeated injection stress could have potentially dampened the response to cocaine in mice that previously consumed caffeine. If injection stress was indeed interfering with cocaine stimulation/sensitization, it could be acting as an opponent process, resulting in no change in activity in RC 10 mg/kg groups. The acute response of AC mice on Day 11 could also be dampened as a result. This could explain the null SENZ score in their respective RC 10 mg/kg groups as well as significantly greater Day 11 activity relative to AC 10 mg/kg groups. This may also offer an explanation for similar results in C mice that had received 5 mg/kg of cocaine. A simple follow up study could address whether repeated injection stress may have been a factor in these observations. Following 2 weeks of DID drinking, mice could be assigned to a group that receives saline injections until Day 11 where they receive their first injection of cocaine (AC condition) or another group that

receives its saline injections on Days 9-10 and its first cocaine injection on Day 11. The standard AC condition could be slightly altered to also receive saline on Day 10 to keep the chamber exposure consistent. This design keeps the duration following the cessation of DID before receiving cocaine consistent and compares the effect of 2 prior saline injections versus 7 prior saline injections on acute cocaine responsiveness, thus answering the question of whether repeated injection stress may have differentially influenced the locomotor stimulant response to cocaine as a result of various fluid consumption histories. These findings would clarify whether repeated injection stress indeed contributed to the results of the initial study.

Nevertheless, these findings suggest that prior caffeine consumption cross-sensitized mice to the psychomotor stimulant effect of cocaine soon after the cessation of caffeine consumption. This observation is in line with previous findings in rats (Gasior et al., 2000; Jaszyna et al., 1998; O'Neill et al., 2015). However, there are key differences between these studies and the current one that are worth noting. In the study by Jaszyna and colleagues (1998), caffeine consumption was forced by adding an extremely high concentration of caffeine (0.3 % w/v; 10 times the concentration used in the current study) to the rats' drinking water, resulting in daily intakes approaching 175 mg/kg/day. For a frame a reference, a 70 kg individual would need to consume an astounding 122.5 cups of coffee (assuming 100 mg per 8 oz serving) per day to reach that level of dosing. Of course, the pharmacokinetics of a human are very different than that of a rat, but even so, a standard 'cup of coffee' equivalent in rodents has been estimated at a dose of around 5 mg/kg when accounting for pharmacokinetic factors (Fredholm et al., 1999). Even after this consideration, the level of caffeine intake in these rats would be roughly equivalent to a

still extremely high level of 35 standard cups of coffee. This extreme caffeine intake does not appear to have much translational relevance. In addition, the rats in this study were responding for food in an operant paradigm and the observation of a sensitized motor response was an increased response rate induced by cocaine in rats that had been consuming caffeine. Interestingly, the largest differences observed between groups was at a cocaine dose of 10 mg/kg, consistent with the current study.

The study by O'Neil and colleagues (2015) also forced caffeine consumption in their experimental animals, although the concentration was identical to the current study (0.03% w/v). This resulted in daily intakes of ~25-35 mg/kg for a period of 4 weeks, a much more translationally-relevant dose range (equivalent of ~5-7 cups of coffee). One major difference was that they were testing both adolescent and adult rats that had previously consumed caffeine. They observed that prior caffeine consumption as an adolescent, but not as an adult, increased the locomotor stimulant response to 15 mg/kg of cocaine (a higher dose than that used in the current study; 10 mg/kg). The locomotor activity assessment, however, was over a period of 4 hours with rats receiving increasing doses of cocaine each hour (0, 2.5, 7.5, 15 mg/kg). This finding is not very clear, however. Although animals previously exposed to caffeine as adolescents and adults were not compared statistically, the age-specific effect appears to be driven by a slightly decreased response to 15 mg/kg of cocaine in adolescent water controls and a high degree of variability in both the caffeine and water adult groups. Furthermore, this progressive dosing design, requiring 4 successive injections within the same session, may be more prone to the influence of repeated stress. As previously mentioned, stressors can act to cross-

sensitize animals to the effects of stimulant drugs of abuse (Robinson, 1988). Therefore, these previous findings are not entirely clear.

Finally, Gasoir and colleagues (2000) also employed a forced access model of caffeine intake in adult rats. The caffeine concentration was also similar to that employed in the current study (0.025% w/v) and produced translationally-relevant daily caffeine intakes ~20-25 mg/kg. It was found that a high 30 mg/kg dose of cocaine produced particularly robust locomotor stimulation in rats consuming caffeine. One major difference relative to the current study, however, is that caffeine remained in the drinking water *throughout* behavioral testing. Therefore, it cannot be ruled out that this observation is due to an additive stimulant effect of cocaine and caffeine as has been previously reported (Bedingfield et al., 1998).

Previous studies have demonstrated that rats and mice sensitized to cocaine exhibit cross-sensitization to ethanol (Itzhak and Martin, 1999; Lessov and Phillips, 2003) and vice versa (Itzhak and Martin, 1999), although this was observed at a dose double of that used in the current study (20 mg/kg). These prior studies employed experimenter-administered injections of ethanol. A main concern with this approach is that non-contingent drug/alcohol exposure can produce very different effects and experimenter administration lacks translational validity (Jacobs et al., 2003; Moolten and Kornetsky, 1990). As such, the primary goal of the current study was to address whether *voluntary* ethanol, caffeine, or co-consumption influenced cocaine sensitivity. Of course, the mice in the current studies were receiving experimenter-administered injections of cocaine, which may seem incongruent with this concern. However, the experimental design allowed for any alterations in the observed locomotor response to cocaine to be attributable to fluid

consumption history. Therefore, any neurobiological/neurochemical adaptations that may have occurred as a result of prior caffeine/ethanol exposure would account for any differential response cocaine. Self-administration paradigms produce differing levels and patterns of drug intake that makes interpretations of drug sensitivity difficult. So although mice were not self-administering cocaine in the current study, employing fixed doses of cocaine allows for clearer interpretation of cocaine sensitivity which may have implications for later assessments of self-administration behavior. Nevertheless, mice that consumed ethanol only in DID exhibited no evidence of either characterization of sensitization in the current study. Although cocaine clearly stimulated activity in these mice, there is no evidence that repeated cocaine exposure positively influenced locomotor stimulation at either dose. The other three fluid consumption groups expressed at least some evidence of cocaine sensitization.

In genetically heterogeneous mice, a history of prior ethanol injections (2.0-2.5 g/kg) did not influence the acute locomotor responses to a range of cocaine doses (5-20 mg/kg) in females (Lessov and Phillips, 2003; Wise et al., 1996), however cross-sensitization to 20 mg/kg cocaine was observed in male animals (Itzhak and Martin, 1999). In addition, three weeks of forced heavy ethanol consumption (23-28 g/kg/day) was not observed to influence the initial response to 10 mg/kg cocaine in male mice, although repeated cocaine exposure produced an increase in cocaine-stimulated locomotion only in mice that previously consumed ethanol (Manley and Little, 1997). Finally, a study evaluating the effect of repeated ethanol injections during adolescence on later cocaine-induced stimulation found that females previously exposed to ethanol demonstrated enhanced locomotor stimulation by 1 mg/kg cocaine relative to females that had received

saline during adolescence (Mateos-García et al., 2015). However, this effect was not apparent at higher doses (5-20 mg/kg). Ethanol-exposed male mice exhibited blunted sensitivity to 20 mg/kg cocaine, but responded similarly to saline-treated mice at lower doses (1-10 mg/kg). In light of these findings, it is possible that the 10 mg/kg dose was not sufficient to detect an enhanced stimulant response to cocaine as a consequence of prior DID ethanol consumption. Alternatively, ethanol intake in the current study may not have produced BECs high enough to influence locomotor sensitivity to cocaine. Injections at the doses of ethanol used in these prior studies (2.0-2.5 g/kg) as well as the extreme ethanol intake in the study by Manley and Little (23-28 g/kg/day) would be expected to produce BECs around 200-250 mg/dl by injection and potentially similar, or even higher, levels produced by consumption. The ethanol intake in the current study would be expected to produce average BECs in the range of 120-160 mg/dl (Figure 9). Nevertheless, mice in the other 3 fluid groups demonstrated some evidence of sensitization. Therefore, binge-like ethanol intake, alone, via DID may have somehow blunted the potential for cocaine to induce significant neuroadaptation resulting in sensitization.

This is the first known investigation of the effect of prior voluntary binge caffeine, ethanol, or co-consumption on the locomotor sensitization capacity for cocaine. The results demonstrated that a history of binge caffeine, but not ethanol, consumption in adulthood can increase sensitivity to the locomotor stimulating effect of a moderate dose of cocaine, perhaps indicating cross-sensitization. Although this has been previously observed as a consequence of forced, continuous adolescent caffeine consumption in rats (O'Neill et al., 2015), this is the first observation of this effect in adult animals that had previously consumed caffeine. Therefore, the age of caffeine pre-exposure does not appear to be a

critical factor for this effect. In addition, mice in the current study consumed approximately 8-11 mg/kg of caffeine in each 2hr DID session, less than half of the daily caffeine intake of rats in the study by O'Neil and colleagues (25-35 mg/kg). Therefore, substantially lower total dosing of caffeine can also augment the cocaine locomotor stimulant response. It may be that binge-like caffeine consumption in a short period of 2hrs via DID produces a substantial accumulation of caffeine in the brain, thus eliciting this effect with lower total caffeine dosing. Furthermore, this observation occurred at a lower dose of cocaine than the previous study (10 mg/kg vs 15 mg/kg), suggesting that this level of limited-access caffeine intake may further enhance cocaine sensitivity. Finally, a significant SENZ score for the moderate dose of cocaine was only observed in W mice, suggesting that this elevated stimulant response may possibly be indicative of maximal sensitization on the very first cocaine exposure.

The findings of this experiment suggest that a modest level of caffeine exposure (equivalent of ~2 cups of coffee a day) has the capacity to enhance the stimulant effects of cocaine. This is of potential concern for public health as this elevated response may be indicative of increased abuse liability. Rodent studies suggest that a sensitized locomotor response is highly persistent and may be associated with a greater propensity to self-administer psychostimulants and elevate drug-seeking behavior during abstinence (to be discussed in further detail below). Indeed, a carefully controlled twin study found that caffeine use was associated with cocaine abuse/dependence in humans (Kendler et al., 2006). Together, these findings suggest that even modest caffeine intake may have harmful implications for individuals' early interactions with cocaine.

Experiment 2: Conditioned Place Preference

In Experiment 2, EC mice were found to consume slightly more caffeine than C mice. However, ethanol intake between E and EC mice was equivalent. Although small, the difference in caffeine intake between EC and C mice was considered in the interpretation of subsequent CPP analysis. However, it is important to note that the amount of caffeine consumed during DID was very similar to that in the Experiment 1 (~8-11 mg/kg).

On Day 1 of the CPP protocol, mice were habituated to the CPP chamber and exposed to a neutral paper floor cue. Activity on this day determined whether group differences existed in baseline activity. This baseline activity measure was not influenced by DID fluid consumption history (Figure 27). This appears to be inconsistent with Day 1 activity data in Experiment 1 where mice that had previously consumed caffeine were significantly more active than those that had not (Figure 15). One reason for this could be that the Plexiglas box used in CPP was significantly smaller than that used for locomotor sensitization. Perhaps the larger space was more reflective of group differences in novelty-seeking or potentially anxiety-like behavior as these are behaviors commonly measured in the classic open field task (Britton et al., 1982; Prut and Belzung, 2003).

Baseline activity was also not found to be different in saline control mice throughout the 8 training trials (Figures 28 & 29). Therefore any differences between groups could be attributed to dose and/or fluid consumption history. Across the CS+ trials where mice received cocaine with their assigned paired floor cue, the 5 mg/kg dose effectively stimulated locomotion whereas the 1 mg/kg dose did not (Figure 29). Furthermore, a progressive increase in the locomotion was not apparent in any fluid group,

indicating that cocaine locomotor sensitization was not observed during CPP training. In fact, activity was actually significantly lower during later CS+ trials (Figure 29). The reason for this is not clear, however this decrease took place after the first CPP test. Perhaps the CPP test was somehow anxiogenic or even produced modest extinction of conditioned locomotion. This is possible because psychostimulants, including cocaine, have been shown to produce strong conditioned locomotor stimulation (Vezina and Leyton, 2009). Indeed, mice that had received 5 mg/kg cocaine during training were significantly more active than the 1 mg/kg groups during testing, suggesting conditioned locomotion carried over to the drug-free test. Perhaps exposure to the drug-paired cue during the CPP test, in the absence of cocaine, may have extinguished conditioned locomotion to some degree.

For drug free CS- trials, mice that were receiving 5 mg/kg of cocaine on their drug-paired floors were again significantly more active than the other groups, perhaps indicating conditioned locomotion also occurred to the CPP process, in general (Figure 28). Interestingly, activity was found to be *increased* in the 5 mg/kg mice during the last 4 trials following Test 1, an effect opposite of that observed in CS+ trials. The fact that these observations are opposite of those in CS+ trials is somewhat puzzling. Repeated exposure to psychostimulants has been observed to produce sensitization to the point of stereotypic behavior, such as head bobbing and incessant grooming (Janetsian et al., 2015; Kuczenski et al., 1991), behaviors which have previously interfered with locomotor activity assessments in rodents. This seems unlikely in the current study, however, as neither the 5 mg/kg nor 10 mg/kg RC groups in Experiment 1 exhibited any indication of stereotypic behavior. Another possibility is that although conditioned locomotion was still intact, mice developed tolerance to the stimulant effects of 5 mg/kg cocaine. Again, this seems unlikely

as no evidence of tolerance to cocaine locomotor stimulation (RC group significantly less activated than AC group) was observed in Experiment 1. In addition, tolerance to this effect of cocaine has been observed after a long period of abstinence (60 days) but not after a shorter period of abstinence (14 days) (Ben-Shahar et al., 2005). It is therefore unclear why locomotor activity during CS+ trials diminished over time in the current study.

These findings differ from the observations of locomotor activity in Experiment 1 where there was some evidence of sensitization to repeated dosing of 5 mg/kg cocaine in EC and W mice. Furthermore, the only influence of fluid history on cocaine-stimulated activity was a very slight decrease in latter half of the Experiment 2 in mice that previously consumed ethanol (E,EC). There are a couple of distinct differences between these experiments that may explain these apparent inconsistencies. Firstly, mice were injected and placed in the CPP boxes daily, whereas mice undergoing the sensitization protocol were exposed to the box and injected every other day during the induction phase of the experiment (Days 3-11). This equates to far more exposure to the box and injection procedure in mice that underwent CPP. In addition, mice that were receiving cocaine during CPP were also receiving saline injections during CS- trials. Therefore, mice in the sensitization experiment that were receiving repeated cocaine *only* had the activity box and injection procedure associated with a cocaine dose during the induction phase, whereas mice in the CPP experiment were receiving *both* cocaine and saline associated with injections and the box. Therefore, any effect of conditioned locomotion to the procedure could have more strongly influenced the observation of sensitization in Experiment 1. It also cannot be ruled out that the far larger space the mice had in the activity chamber in Experiment 1 allowed group differences to more easily emerge. Upon examination of the

total distance traveled for mice receiving 5 mg/kg in Experiment 1 (~5,000-7,500 cm; Figures 16-19) versus Experiment 2 (~1,500-2000 cm; Figure 29), this larger space clearly resulted in substantially more locomotion which may have ultimately contributed to the expression of these fluid group differences in Experiment 1.

The CPP analyses revealed that the protocol was effective in producing cocaine CPP and CPP was strengthened with 4 additional conditioning trials (Figures 32 & 33). However, prior fluid consumption history via DID had no influence on CPP expression or the number of trials required to produce CPP. Prior work with male B6 mice suggests that CPP should have been observed with both 1 and 5 mg/kg cocaine (Cunningham et al., 1999). Although 1 mg/kg cocaine technically produced CPP as indicated by a significant dose × grid floor assignment interaction (thus collapsing on all fluid groups for 1 mg/kg mice), examination of Figures 32 and 33 suggests this was not a particularly strong CPP, at least not as strong as previously observed in male B6 mice (Cunningham et al., 1999). One reason may be that the dosage reported in the earlier study could have been the weight of the cocaine free base rather than the weight of the cocaine hydrochloride salt. As it was not reported in the prior study, if the dose was indeed calculated as weight of the free base, mice could have received approximately 12% more cocaine, thus increasing the likelihood of stronger CPP. Another possibility is that the mice in the current study were tested during their dark cycle whereas the vast majority of CPP tests occur during the animals' light cycle. Perhaps conditioning for this low dose occurs with greater efficacy if training takes place during the light cycle. Finally, the duration of the CPP tests in the current study was 15 minutes as opposed to 30 minutes in the earlier study. Although the 15 minute duration was chosen for the sake of consistency across both experiments in the current study, it is

possible that this shorter testing period may not have detected group differences that may emerge with a longer test. Indeed, some studies have shown that CPP effects may not emerge until later in a test (Cunningham et al., 2006). Therefore, CPP was also analyzed across each 5 minute bin of the 15 minute CPP tests. No evidence was found for strengthened CPP towards the end of the tests (Figures 36 & 37). As such, longer CPP test sessions may not have necessarily revealed any group differences that the 15 minute test was unable to detect.

Caffeine has been previously shown to enhance cocaine CPP when administered concomitantly in mice when both drugs are administered at a low doses (0.32 mg/kg caffeine; 1.0 mg/kg cocaine) (Bedingfield et al., 1998). The only known study, however, to directly address the effect of prior voluntary caffeine consumption on cocaine CPP by O'Neil and colleagues (2015) found that adolescent, but not adult, consumption of caffeine produced CPP for a 7.5 mg/kg dose of cocaine in rats. This dose is higher than either dose of cocaine used in the current study. Therefore, neither caffeine nor ethanol consumption history via DID appears to enhance sensitivity to the conditioned rewarding effects of lower doses of cocaine in male B6 mice. It is possible, however that higher doses of cocaine may, elicit group differences produced by these various DID fluid consumption histories as evidenced by an observed effect of caffeine consumption history in Experiment 1 at the 10 mg/kg dose.

Collective Interpretation of Experiments 1 and 2

In the current study, the primary objective was to explore whether a history of binge-like caffeine and/or ethanol consumption influenced two different domains of

cocaine responsiveness: locomotor sensitization and conditioned reward. Only the highest dose of cocaine (10 mg/kg) produced cross-sensitization in mice that had previously consumed caffeine via DID and fluid consumption history had no influence on conditioned reward. Although cross-sensitization and CPP are argued to be models reflective of abuse liability, it is important to discuss the degree to which these phenotypes are related to each other, as well as their associations with abuse potential.

Firstly, the complete lack of an ethanol consumption history to influence either sensitization or CPP should be acknowledged. This was rather surprising as the existing data reviewed above suggests that, if anything, ethanol pre-exposure would have increased cocaine sensitivity. A history of binge-like drinking via DID has been shown to induce substantial neurobiological and neurophysiological changes. Therefore, the model does indeed produce levels of ethanol intake that are sufficient to produce neuroadaptation. However, much of this previous work employed DID protocols lasting up to 6 weeks (Fritz and Boehm II, 2016; Sparrow et al., 2012). It may therefore be that 14 days of ethanol access in the current work was not sufficient to induce neurobiological/chemical changes influencing cocaine sensitivity.

Nevertheless, a caffeine consumption history via DID was sufficient to produce cross-sensitization to the locomotor stimulant, but not the conditioned rewarding effects of cocaine. The lack of an effect on CPP, does not mean that there is little concern for the abuse potential for cocaine, however. The rewarding components of drug consumption have long been dissociated from drug-seeking and drug-taking in chronic drug abusers as their motivation to obtain drugs increases over time while they report the positive effects of consumption wane (Camí and Farré, 2003). Furthermore, animal models also

demonstrate that self-administration and CPP are measuring fundamentally different processes (Bardo and Bevins, 2000). A sensitized state, however, has been associated with increased drug-seeking behavior in operant studies. For example rodents sensitized to a variety of psychostimulants have demonstrated an enhanced propensity to self-administer those drugs (Piazza et al., 1990; Vezina, 2004). In addition, self-administration of psychostimulants, including cocaine, can produce locomotor sensitization (Hooks et al., 1994; Phillips and Di Ciano, 1996). Finally, sensitization has been demonstrated to be predictive of relapse-like behavior potential (De Vries et al., 1998; Ferrario et al., 2005), however this has not been consistently observed (Ahmed and Cador, 2005). Nevertheless, it has been argued that the close overlap in neurocircuitry between sensitization and reinstatement holds strong construct validity for the sensitization model (Steketee and Kalivas, 2011). Furthermore, sensitization of motivation circuitry may be related, but not entirely associated with motor effects, perhaps contributing to the previously mentioned discordance between reinstatement and locomotor sensitization (Steketee and Kalivas, 2011). Sensitization may be reflective of a compulsory drug-seeking state due to the increased incentive salience of drug cues (Robinson and Berridge, 2001).

Cocaine locomotor sensitization has been dissociated from reward in rodents. Riday and colleagues demonstrated that although drugs of abuse acutely increase the rewarding value of electrical medial forebrain bundle stimulation, mice sensitized to cocaine did not exhibit a potentiation of stimulation reward (Riday et al., 2012). Furthermore, cocaine CPP and sensitization responses were found to be uncorrelated within individual rats with doses similar to those used in the current study (Seymour and Wagner, 2008) and a study with inbred strains of mice found no genetic correlation between cocaine

sensitization and CPP (Eisener-Dorman et al., 2011). Thus, although these phenotypes may be unrelated, they can still nevertheless be observed concurrently (Shimosato and Ohkuma, 2000).

Cocaine CPP and self-administration also appear to be separate processes. D2R antagonists are highly effective at reducing cocaine self-administration, however they are ineffective at influencing cocaine CPP which is instead strongly blunted by the administration of D1R antagonists (Bardo and Bevins, 2000). CPP offers unique utility, however, to gain important insight on the response to drug-associated environmental cues (Tzschentke, 1998). An environment associated with a drug is likely to facilitate or allow the opportunity to take the drug in humans. Therefore, the ability for a drug-associated context to elicit approach behavior is an important assessment, modeling one facet of use/abuse potential. CPP tests also occur in a drug-free state. This has construct validity for the rewarding value of the drug as the animal makes an appetitive choice to spend time in the context in which the drug was received without the interference of the drug's pharmacological effects.

Together, these findings suggest that the psychomotor stimulant response to cocaine can be enhanced by a history of caffeine consumption. However, this may not be a particularly persistent effect. Furthermore, neither caffeine nor ethanol consumption histories influenced the development or expression of cocaine CPP, indicating that the appetitive properties of cocaine-associated environmental cues were not affected by these drinking histories. Given the association between sensitization and self-administration, it is possible that caffeine pre-exposure may influence early interactions with cocaine which may, in turn, increase its use/abuse potential.

Mechanisms of Findings

The fact that cross-sensitization, but not conditioned reward, for cocaine was altered provides some possibilities for the potential mechanisms of this observation. D2Rs do not appear to influence cocaine CPP, however, D1Rs are highly influential (Bardo and Bevins, 2000). However, both D1Rs and D2Rs have the capacity to influence cocaine stimulation and sensitization (Fontana et al., 1993). The study by O'Neil and colleagues which observed enhanced cocaine CPP and locomotor stimulation as a consequence of adolescent caffeine consumption observed alterations in adenosine and DA receptor levels in the nucleus accumbens which may assist in the interpretation of the observations in the current study. They found that adolescent caffeine consumption increased D2R levels and decreased A2AR levels in the nucleus accumbens, whereas D1R levels were unchanged and A1R levels were increased (O'Neill et al., 2015). Furthermore rats that had a history of adolescent caffeine consumption exhibited heightened sensitivity to the locomotor stimulant effect of the D2R agonist quinpirole at doses that suggested an increase in postsynaptic D2Rs. The authors attributed their observed increased locomotor response to and CPP for cocaine to a decrease in A2AR regulation of D2Rs in the accumbens, thus resulting in enhanced D2R signaling via cocaine-induced increases in DA. This hypothesis is supported for their observed increase in cocaine-stimulated locomotor activity. The A2AR antagonist MSX-3 potentiates the locomotor stimulant response to cocaine (Filip et al., 2006), although this has yet to be assessed as a consequence of direct infusion into the nucleus accumbens. As for decreased A2AR inhibition of the D2R as their proposed mechanism of enhanced cocaine CPP, the aforementioned lack of D2R antagonist effects

within the nucleus accumbens on cocaine CPP (Baker et al., 1996) is in disagreement with this contention.

Although the current study corroborates the effect of prior caffeine consumption on cocaine locomotor sensitivity, this was observed at a lower dose in the current study (10 mg/kg versus 15 mg/kg). Furthermore, O'Neil and colleagues (2015) observed enhanced CPP as a result of prior caffeine consumption at a higher dose of cocaine than either used in the current study (7.5 mg/kg versus 1 and 5 mg/kg). One factor that makes comparisons between these two studies difficult is that the 10 mg/kg dose that elicited caffeine group differences in cocaine stimulation was not used in the CPP experiment in the current study. It is therefore not clear whether enhanced cocaine CPP may have also been observed at this dose. Nevertheless, decreased A2AR inhibition of D2Rs within the nucleus accumbens via prior caffeine consumption is a plausible mechanism of the observation in Experiment 1 that would not necessarily influence the results in Experiment 2. In fact, it is the most reasonable mechanism to propose as no other study has addressed these scientific questions in this manner. Future work will therefore evaluate DA and adenosine receptor levels in the accumbens of cocaine-naïve and cocaine-treated mice in the current study to determine whether the observations in Experiment 1 may be attributable to a similar mechanism. The consequences of enhanced D2R signaling in the nucleus accumbens could be inhibition of GABAergic projections to the VTA, ventral pallidum, and ventromedial prefrontal cortex, thus relieving inhibition of this circuitry. Enhancing activity within the mesolimbocortical circuit may therefore have problematic implications for individuals' early interactions with cocaine.

Future Directions

In agreement with previous research, prior caffeine exposure enhances sensitivity to the psychomotor stimulant properties of moderate, but not low doses of cocaine. Additional data that would clarify the interpretation of the existing data would be the use of a higher, consistent dose of cocaine in CPP as was used in the locomotor sensitization experiment. This will allow for direct comparisons to be made between observations in each paradigm. The current study unfortunately observed an effect of fluid consumption history in Experiment 1 at the 10 mg/kg dose that was not utilized in Experiment 2. As prior research has observed a caffeine consumption history to enhance cocaine CPP employing a higher dose than was used in the current study (O'Neill et al., 2015), this seems to be an essential point of clarification.

Furthermore, the effect of adolescent caffeine and ethanol co-exposure on cocaine sensitivity is also highly relevant. Caffeinated ethanol consumption is highly popular with adolescents and young adults (Arria et al., 2010; Marczinski, 2011). The adolescent brain is optimally-suited for drugs to induce neuroplastic changes (Spear, 2000) and thus, may be uniquely sensitive to the combined effects of caffeine and ethanol co-exposure. Indeed, our lab recently observed that adolescent, but not adult B6 mice that co-consumed caffeine and ethanol via DID exhibited additive locomotor stimulation (Fritz et al., in press). Along with previous observations of adolescent caffeine consumption enhancing cocaine sensitivity (O'Neill et al., 2015), this potential line of research holds promise for answering key questions about the protracted consequences of caffeine and ethanol co-consumption.

Finally, caffeine and ethanol co-consumption also has the potential to alter sensitivity to another highly addictive psychostimulant, methamphetamine. Excessive

ethanol consumption has been observed in individuals abusing methamphetamine (Parsons et al., 2007; Semple et al., 2003). Up to 60% of urban methamphetamine users have reported frequent co-use with ethanol (Green and Mourgues, 2005) and frequency of ethanol intoxication has been observed to be positively associated with methamphetamine use (Furr et al., 2000). Animal studies have found that a history of ethanol exposure sensitized striatal dopamine release in response to an acute challenge of methamphetamine (Nishiguchi et al., 2002) and a genetic predisposition for high ethanol intake enhances the acute locomotor stimulant response to combined methamphetamine and ethanol in rats (Yamauchi et al., 2000). Caffeine use statistics among methamphetamine abusers was not readily available, however it is known that methamphetamine is often 'cut' with caffeine (Kuribara, 1994) and has even been intentionally combined with caffeine in a tablet referred to as 'Ya-Ba' in Thailand (Sinchai et al., 2011). While it may seem that the primary reason for this practice is to reduce the cost of production and/or consumption, findings from animal studies suggest that caffeine may greatly amplify the stimulant effect of low, to moderate doses of methamphetamine (Fujii et al., 1989; Kuribara, 1994). Methamphetamine has been theorized to work through reversal of both the vesicular monoamine transporter and the DAT, releasing vesicular stores of dopamine inside the presynaptic terminal which then flood the synapse (Fleckenstein et al., 2007). In addition, the activation of D2Rs has been demonstrated to play an important role in methamphetamine locomotor stimulation and sensitization (Kelly et al., 2008). Repeated cycles of binge caffeine and alcohol co-exposure may therefore have the capacity to alter neural sensitivity to methamphetamine.

In conclusion, caffeine may not be as harmless as previously thought. For most individuals, caffeine is likely the first drug intentionally consumed with the goal of altering one's psychological state. In addition, it is the most used psychoactive substance in the world (Fredholm et al., 1999). Individuals experimenting with potent psychostimulants (such as cocaine), who readily consume caffeine, may respond more strongly to their stimulant properties. This enhanced response may ultimately influence their later interactions with these substances. As such, the potential risks associated with significant caffeine use should be noted as energy drink consumption is increasingly popular and has been linked to polydrug abuse (Arria et al., 2010).

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APPENDIX

APPENDIX: FIGURES

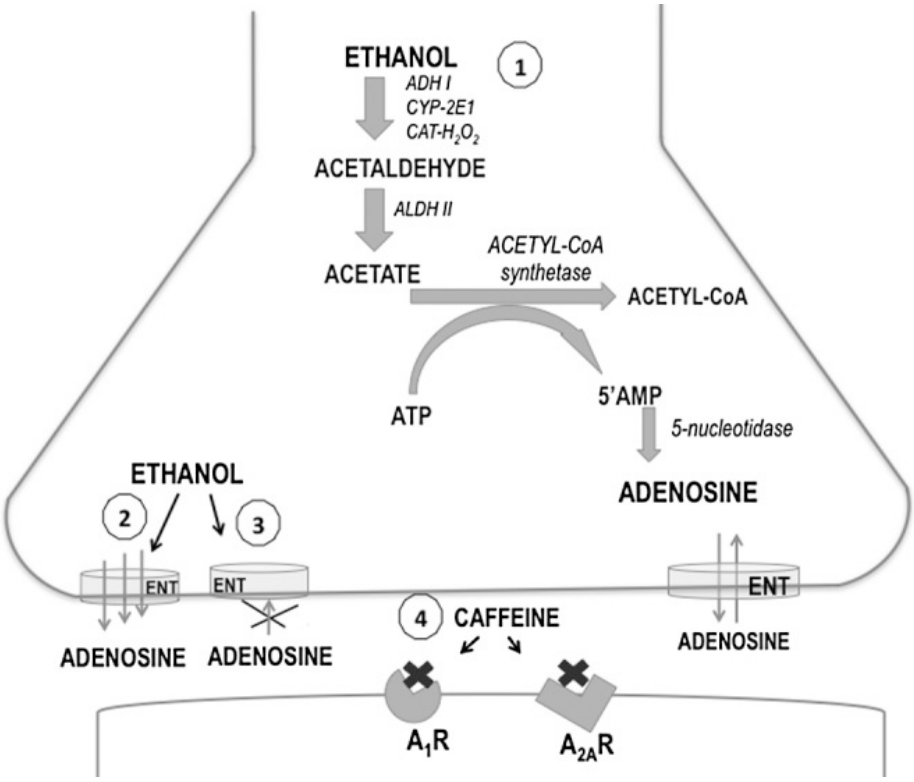

Figure 1. Schematic of ethanol/caffeine adenosine pharmacology. Figure from from López-Cruz et al. (2013). Ethanol increases adenosine signaling by 1) increasing adenosine as a product of ethanol metabolism 2,3) inhibiting transport of extracellular adenosine into the cell and instead, releasing adenosine through its transporter. 4) Caffeine is a nonselective adenosine receptor antagonist. Collectively, the positive dopamine signaling effects of both caffeine and ethanol, and the inhibition of ethanol-induced adenosine receptor regulation of dopamine receptors by caffeine, may allow for an additive effect of caffeine and ethanol on dopamine signaling.

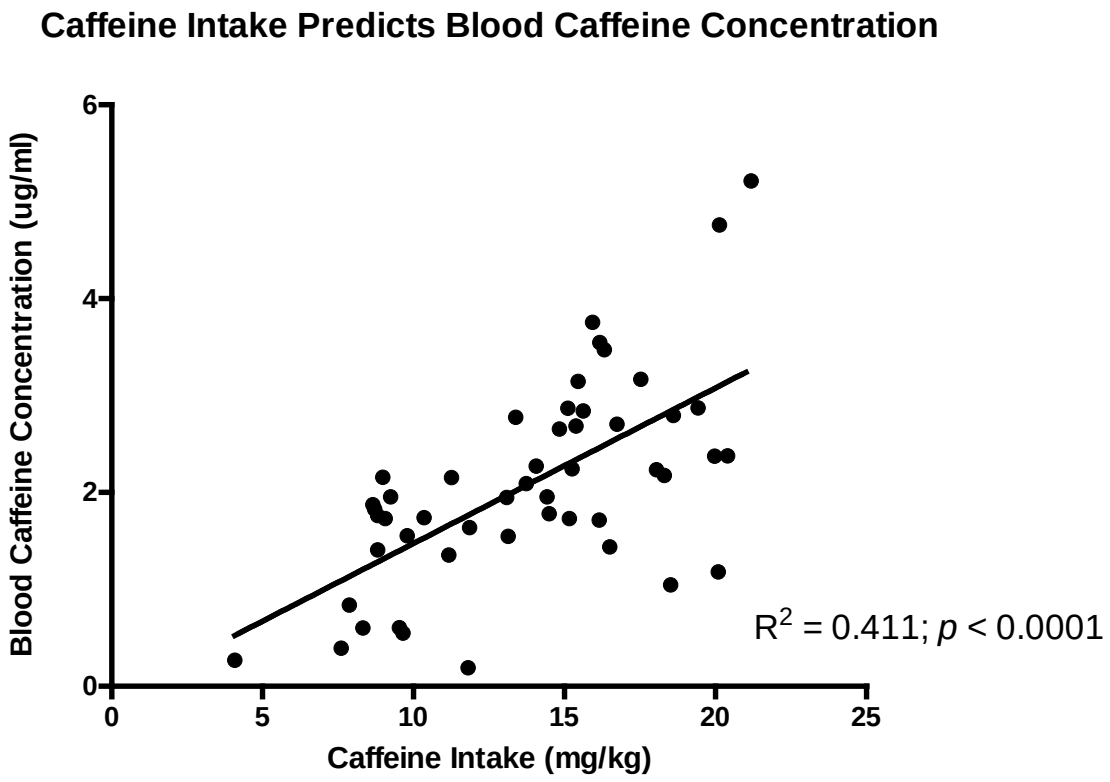

Figure 2. Relationship between caffeine intake/blood content. Following day 14 of DID, periorbital blood samples were taken from C and EC mice in the study by Fritz et al. (2014). Blood caffeine content was later analyzed by high performance liquid chromatography. Caffeine intake was significantly predictive of blood caffeine concentration (N = 50).

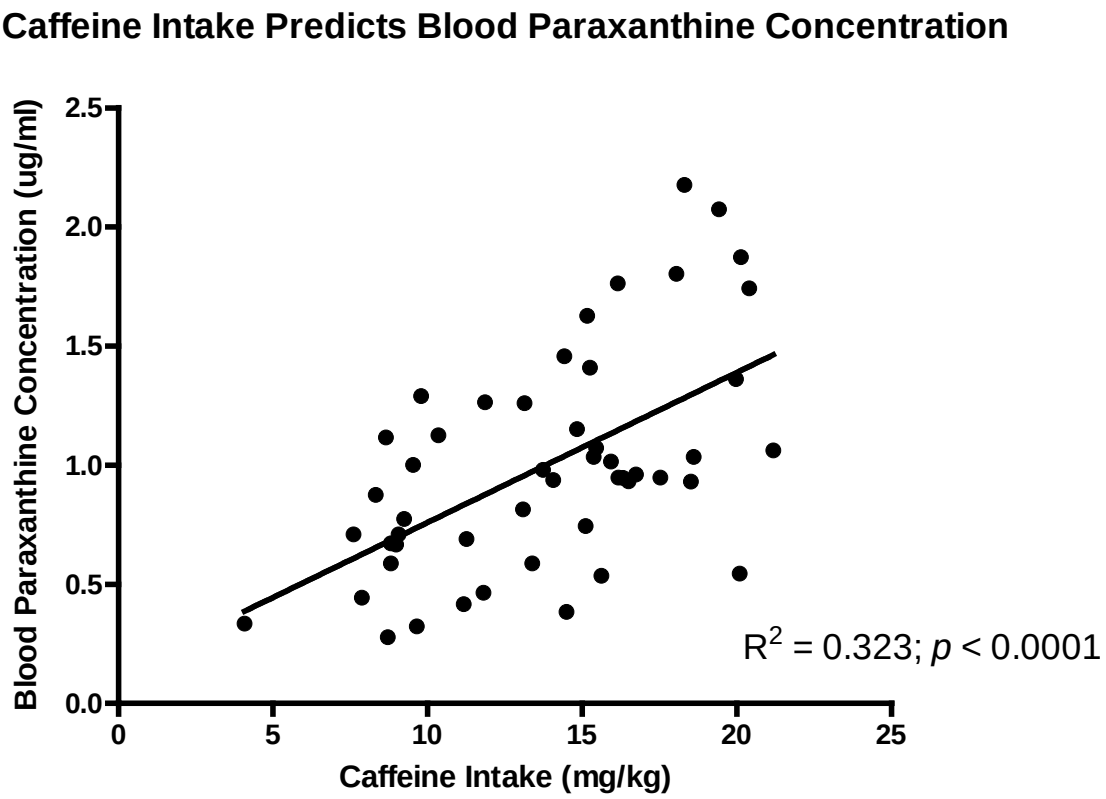

Figure 3. Relationship between caffeine intake and blood paraxanthine content. Following day 14 of DID, periorbital blood samples were taken from C and EC mice in the study by Fritz et al. (2014). Blood paraxanthine content analysis was later conducted by high performance liquid chromatography. Caffeine intake was significantly predictive of blood paraxanthine concentration (N = 50).

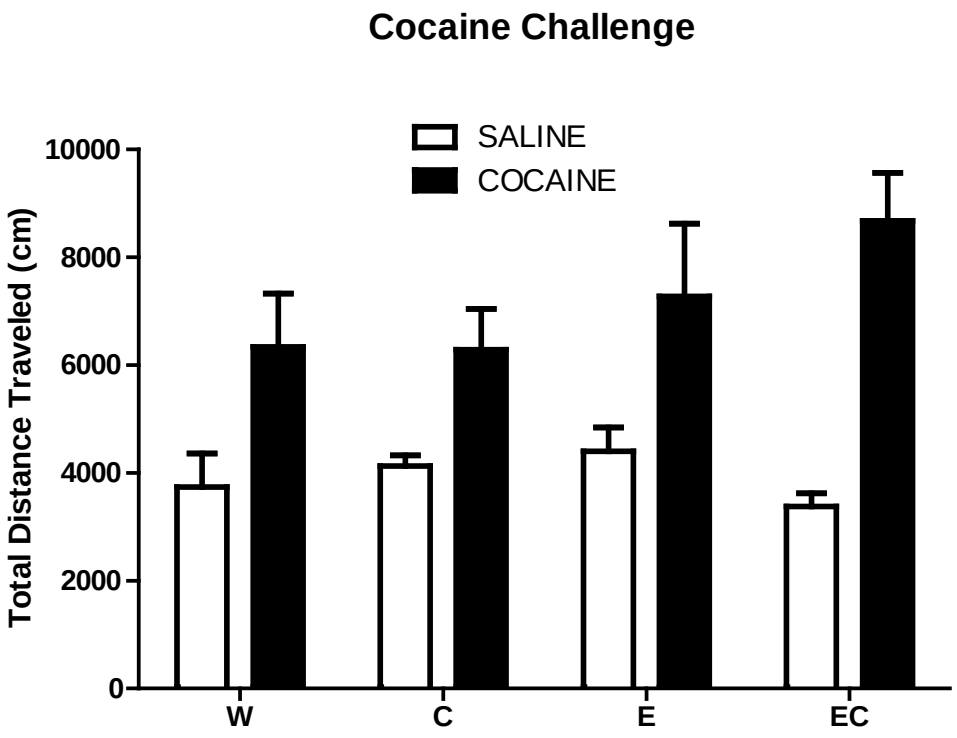

Figure 4. Pilot study data. Following 14 days of DID, mice received an injection of saline in order to habituate the animals to the handling/injection procedure as well as the apparatus for 2 days. The following day, mice received either saline or cocaine (10 mg/kg; i.p.) and activity was recorded for 15 minutes. There was statistical trend ($p = 0.07$) for particularly robust cocaine induced locomotor stimulation in EC mice ($n = 5$-12).

Figure 5. Standard DID setup. Figure from Thiele et al. (2014) depicting a typical cage setup for DID drinking sessions.

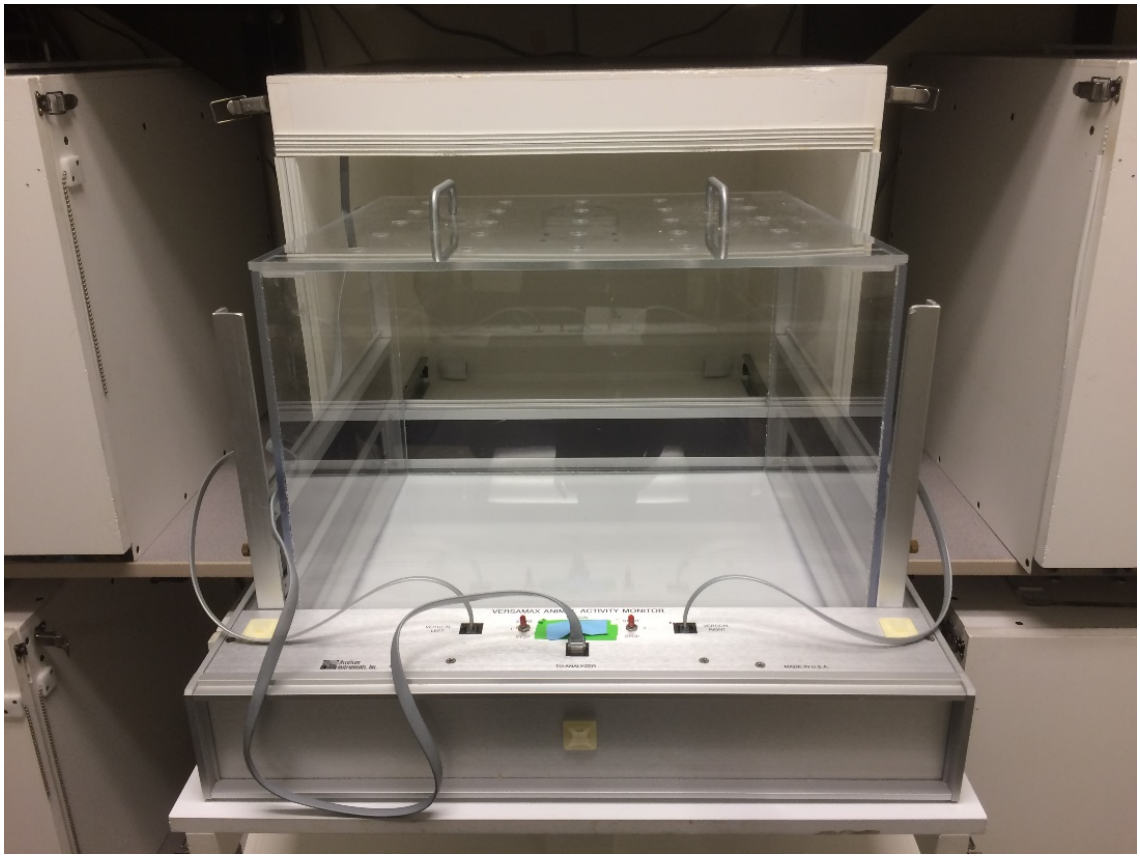

Figure 6. AccuScan activity monitor.

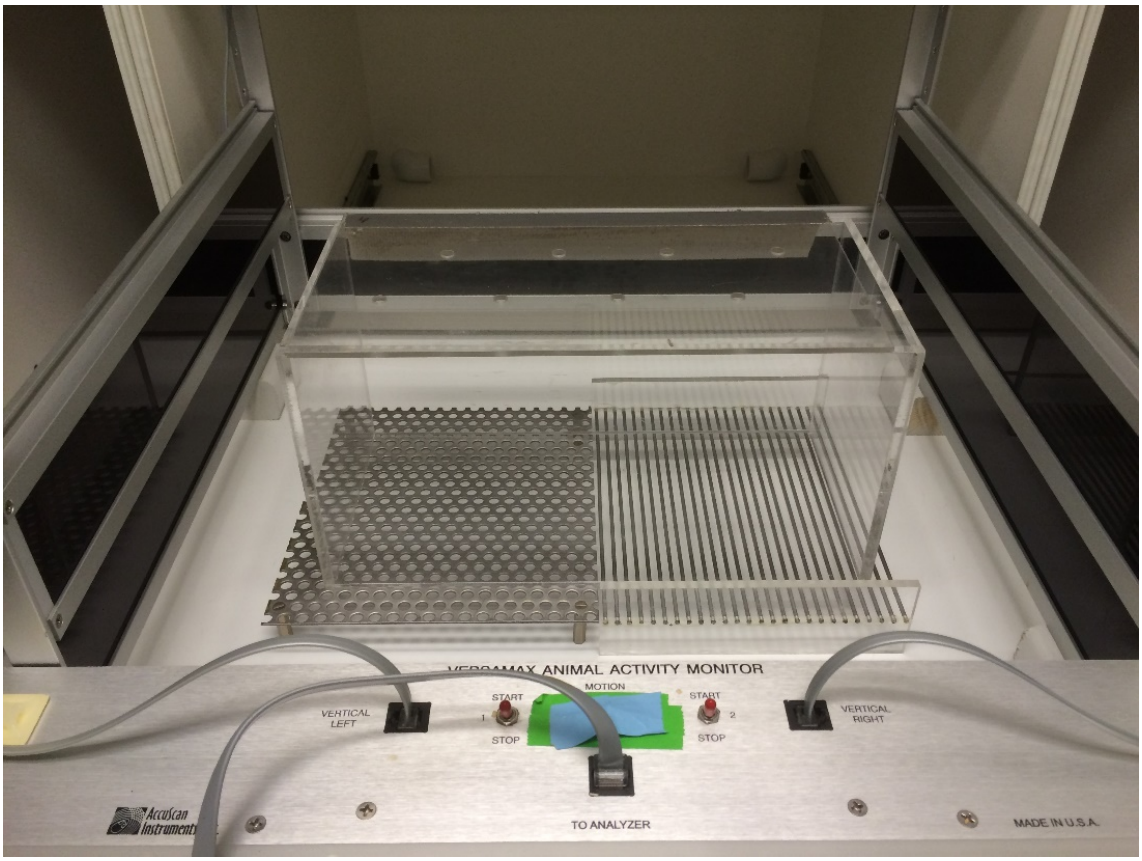

Figure 7. CPP setup for AccuScan activity monitors. Example of a floor cue orientation setup for a CPP test in Experiment 2.

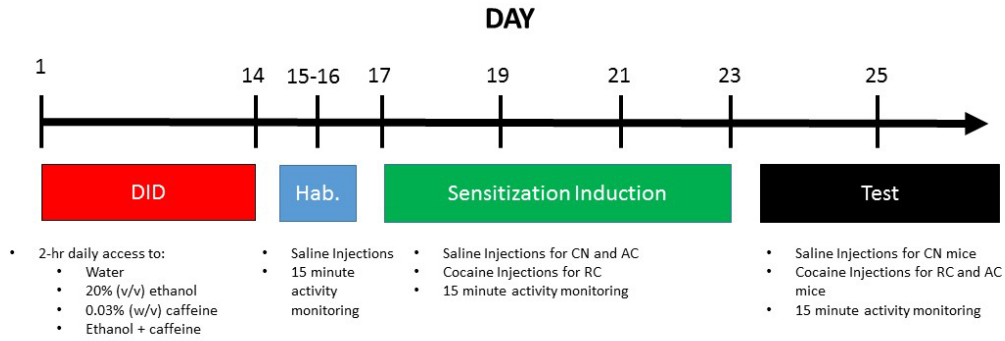

Figure 8. Timeline for Experiment 1.

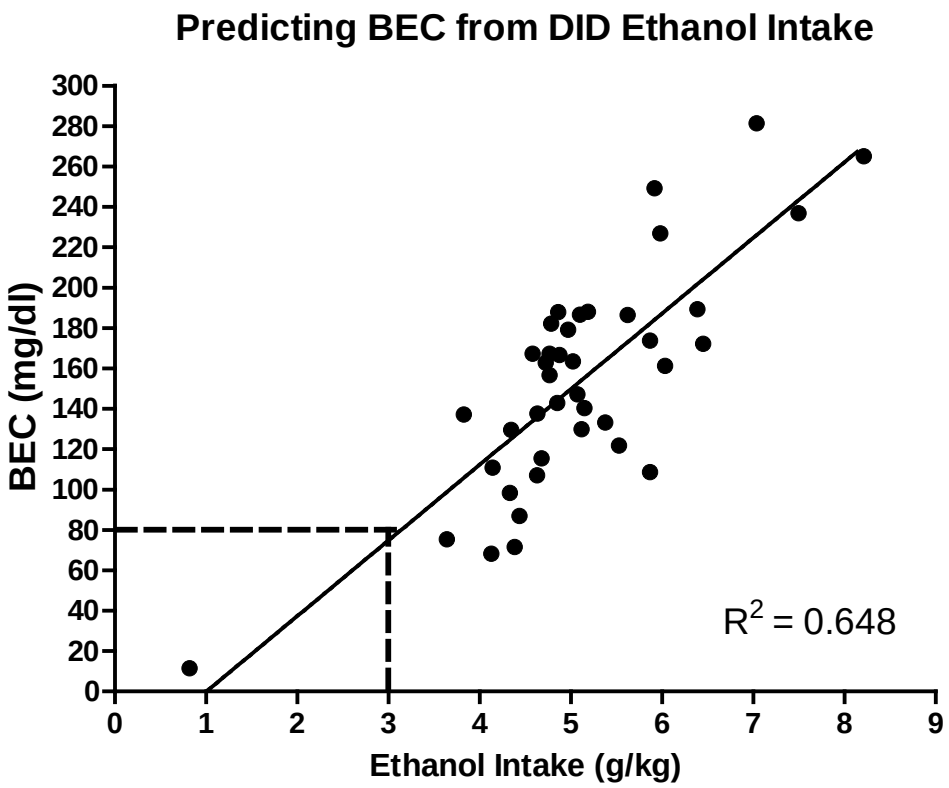

Figure 9. Relationship between ethanol intake/blood levels. Following day 14 of DID, periorbital blood samples were taken from E and EC mice for ethanol content analysis in the study by Fritz et al. (2014). Ethanol intake was highly predictive of BEC, $p < 0.001$ ($N = 40$).

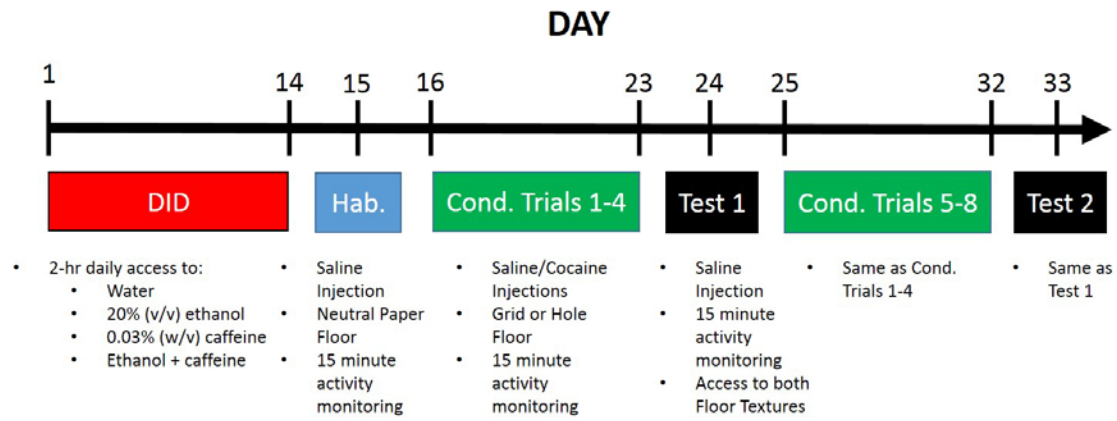

Figure 10. Timeline for Experiment 2.

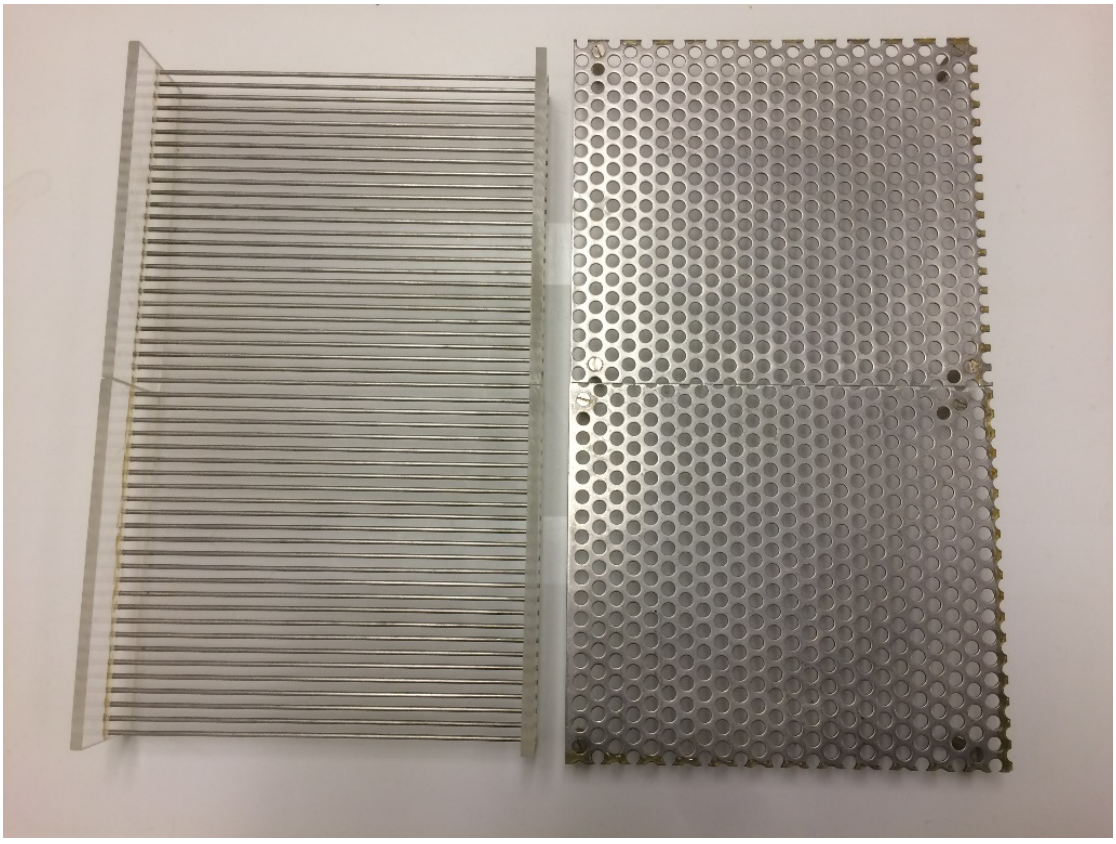

Figure 11. CPP Floor textures. Grid (left) and hole (right) floor textures used in the CPP protocol in Experiment 2.

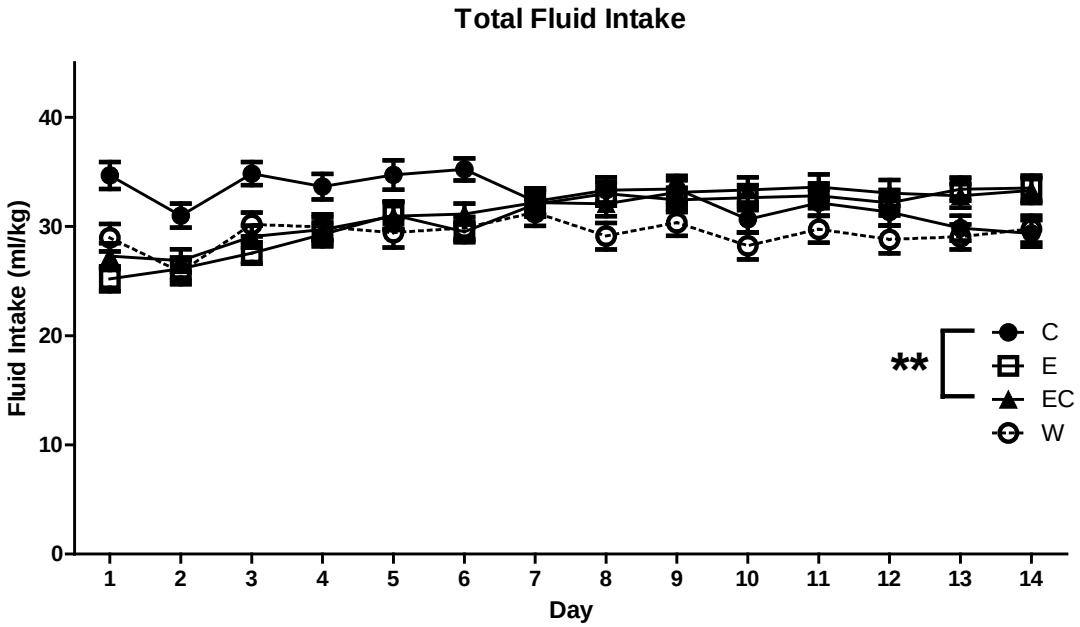

Figure 12. Experiment 1: Fluid intake. Total fluid intake (ml/kg) over the course of the 14-day DID procedure Experiment 1. Mice that consumed caffeine (C,EC) consumed significantly more fluid than mice that did not over the course of the DID procedure. $^{**}p < 0.01$ versus mice that did not have access to caffeine (E,W) (n = 58-69).

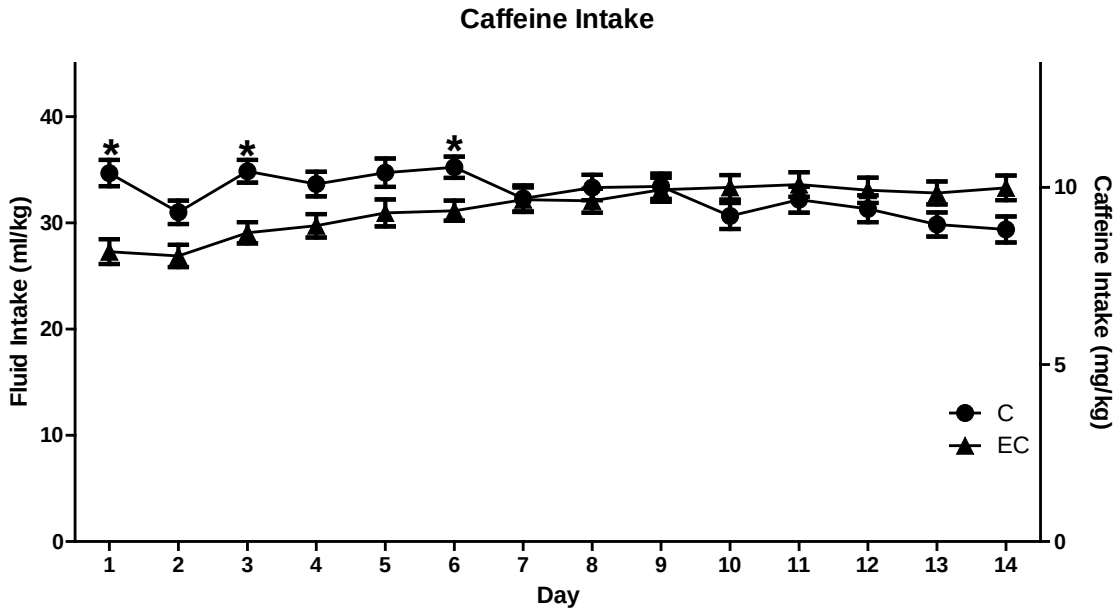

Figure 13. Experiment 1: Caffeine intake. Total caffeine intake (mg/kg) over the course of the 14-day DID procedure. Mice in the C group consumed significantly more caffeine early on in the DID phase than EC mice. *$p < 0.05$ versus EC (n = 60-67).

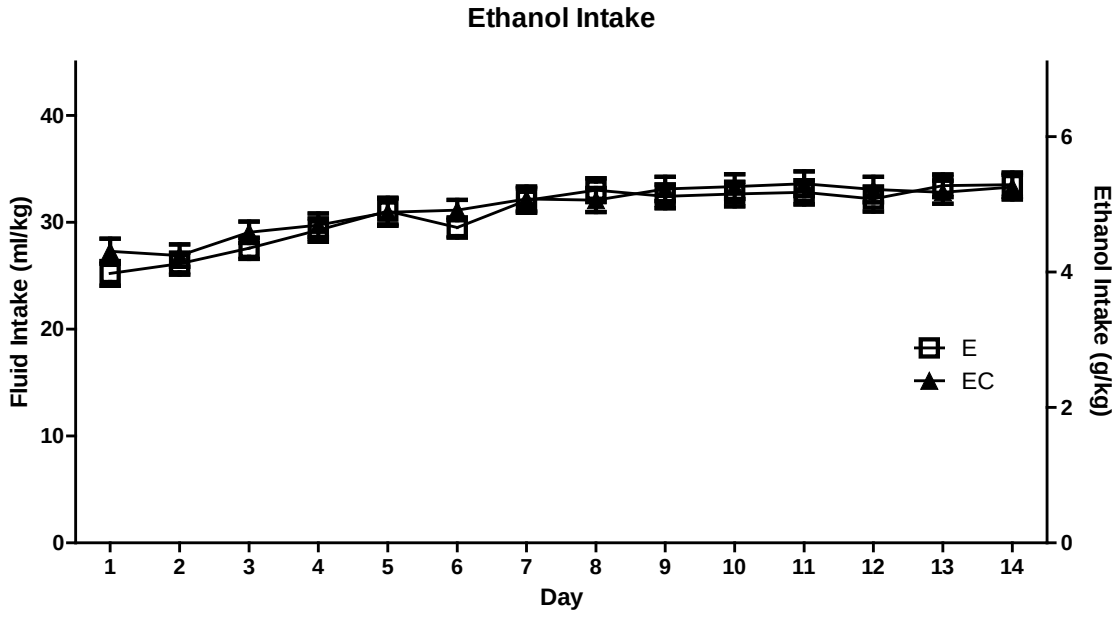

Figure 14. Experiment 1: Ethanol intake. Total ethanol intake (g/kg) over the course of the 14-day DID procedure. Mice in the E and EC groups consumed equivalent amounts of ethanol throughout the DID phase of the experiment (n = 67-69).

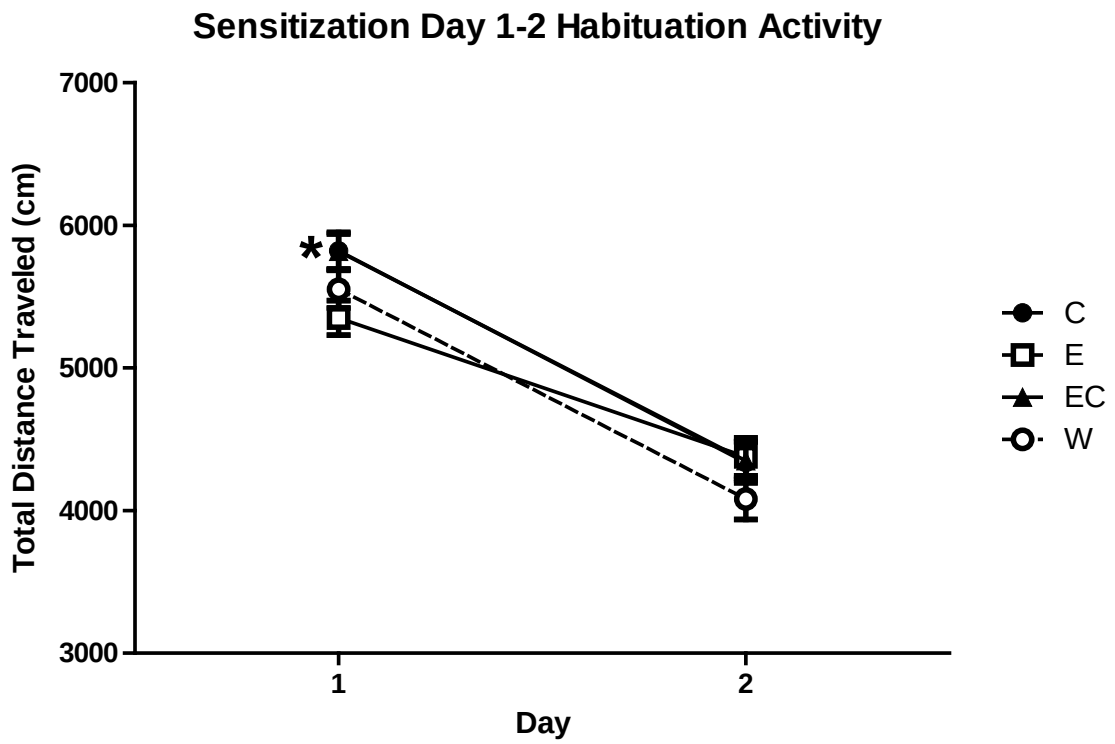

Figure 15. Experiment 1: Habituation Activity. Habituation activity during the first two days of the sensitization protocol. Mice that had consumed caffeine (C,EC) in DID were significantly more active than those that had not on Day 1. *$p < 0.05$ versus mice that did not have access to caffeine in DID (W, E) (n = 58-69).

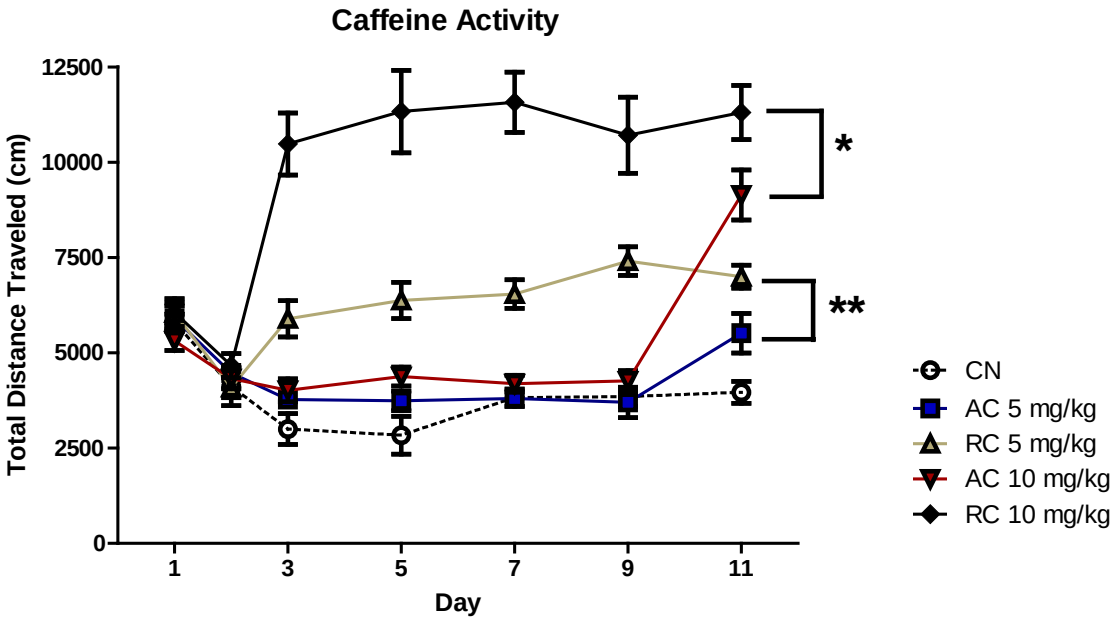

Figure 16. Experiment 1: Caffeine group activity. Activity throughout the 11-day sensitization protocol in mice that consumed caffeine only in the DID phase of the experiment. Mice that received repeated cocaine were significantly more stimulated than those that received acute cocaine on the final day of the sensitization protocol. *$p < 0.05$, **$p < 0.01$ RC versus AC of the same dose group (n = 11-13).

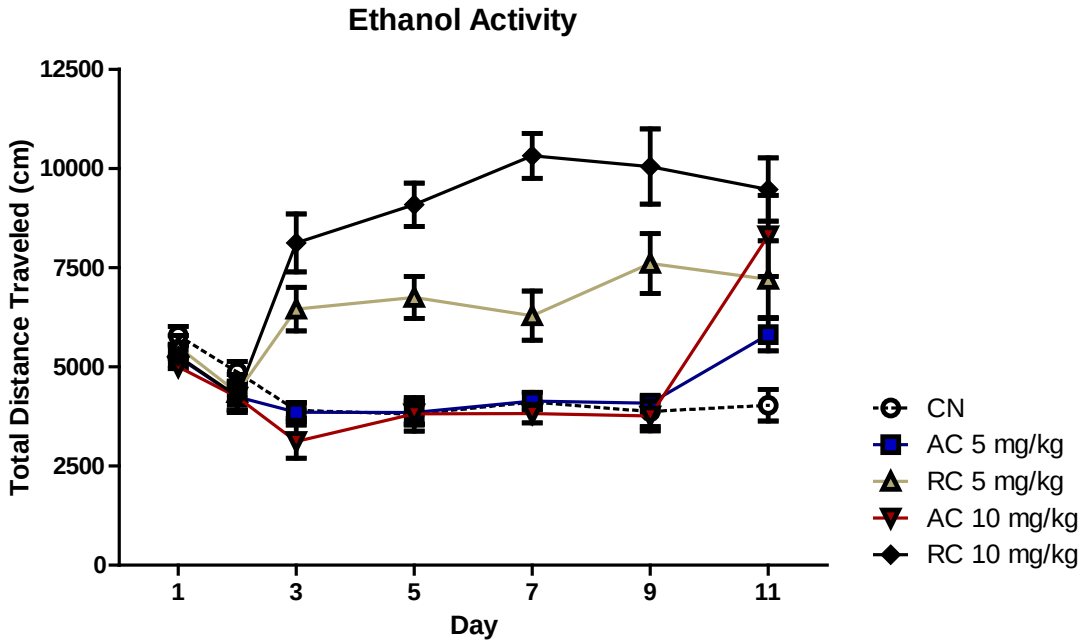

Figure 17. Experiment 1: Ethanol group activity. Activity throughout the 11-day sensitization protocol in mice that consumed ethanol only in the DID phase of the experiment. Repeated cocaine did not differentially influence cocaine-induced locomotor stimulation relative to an acute injection (*n* = 12-15).

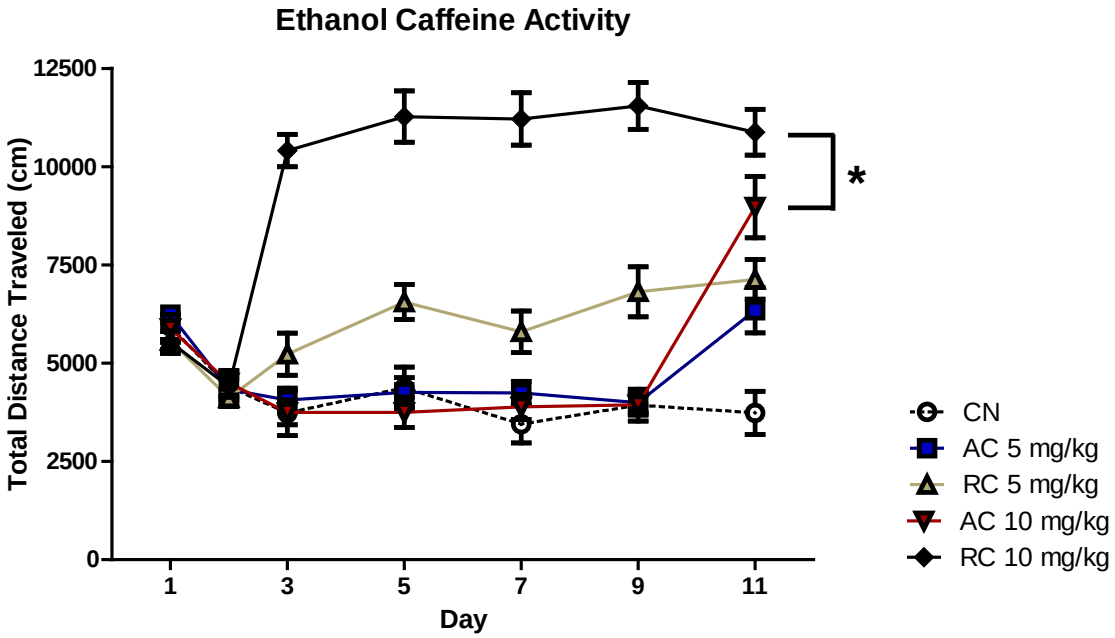

Figure 18. Experiment 1: Ethanol + caffeine group activity. Activity throughout the 11-day sensitization protocol in mice that consumed combined caffeine and ethanol in the DID phase of the experiment. Mice that received repeated 10 mg/kg cocaine were significantly more stimulated than those that received acute 10 mg/kg cocaine on the final day of the sensitization protocol. *$p < 0.05$ RC versus AC (n = 11-15).

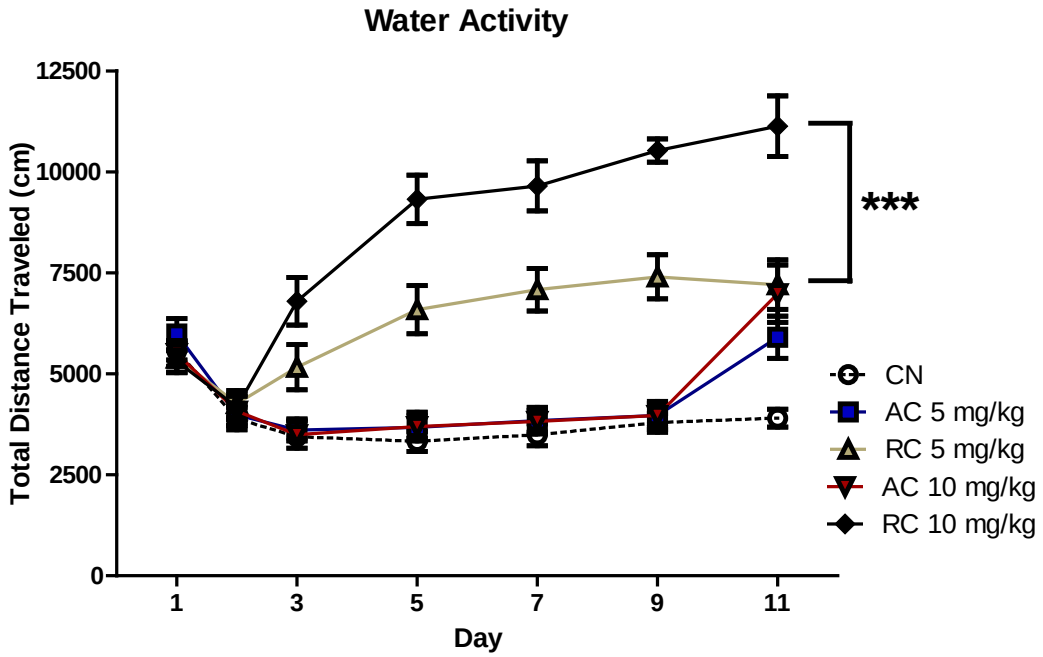

Figure 19. Experiment 1: Water group activity. Activity throughout the 11-day sensitization protocol in mice that consumed water only in the DID phase of the experiment. Mice that received repeated 10 mg/kg cocaine were significantly more stimulated than those that received acute 10 mg/kg cocaine on the final day of the sensitization protocol. ***$p < 0.001$ RC versus AC (n = 11-13).

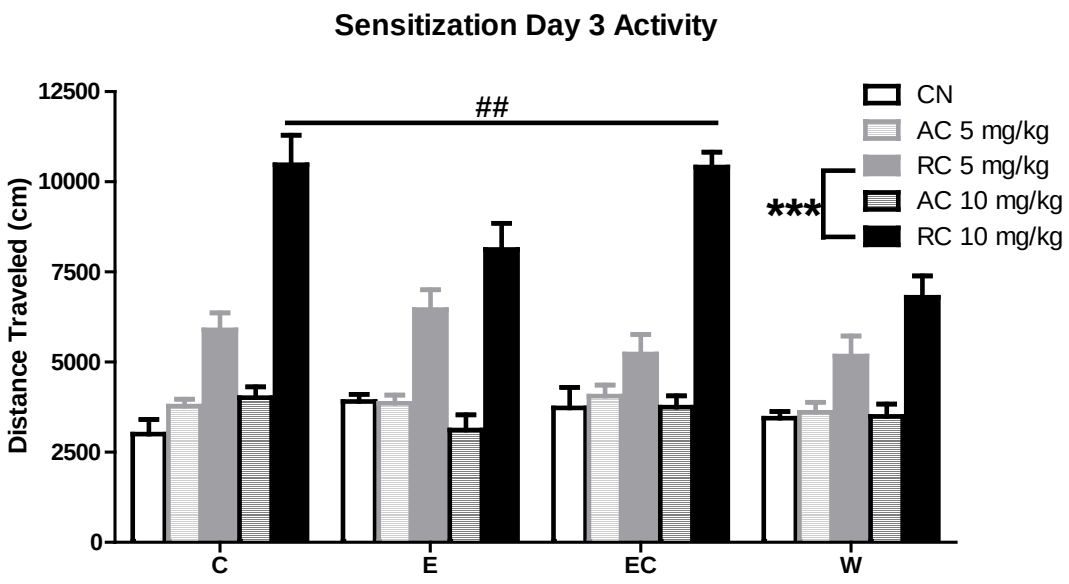

Figure 20. Experiment 1: Day 3 activity. Mice in the RC groups received their first dose of cocaine on Day 3 whereas all other groups received saline. Mice that received cocaine (RC) were significantly more active than those that did not. In addition, mice that received 10 mg/kg cocaine were also significantly more stimulated than mice that had received 5 mg/kg of cocaine ($p < 0.001$). Finally, 10 mg/kg cocaine stimulated mice that had consumed caffeine (C,EC) to a significantly greater degree than those that did not previously consume caffeine in DID (E,W). Although CN mice were not included in the analysis, their activity is presented here as a contextual baseline measure. $^{***}p < 0.001$ RC 10 mg/kg versus RC 5 mg/kg; $^{\#\#}p < 0.01$ versus RC 10 mg/kg mice that had not previously consumed caffeine (E,W) (n = 11-15).

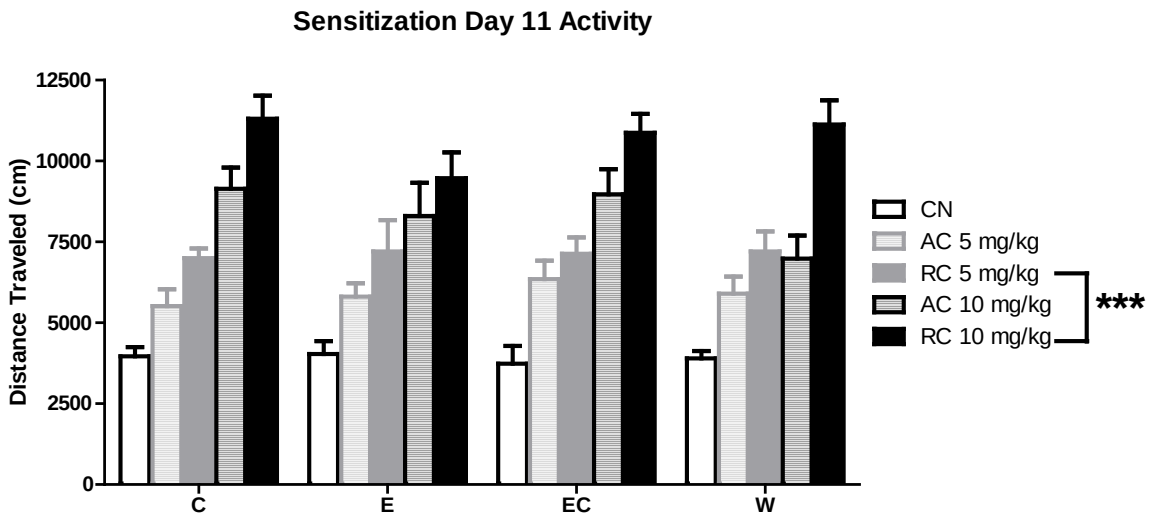

Figure 21. Experiment 1: Day 11 activity. Mice in the RC groups received their fifth dose of cocaine on Day11 whereas AC groups received their first dose. Mice that received repeated cocaine (RC) were significantly more active than AC mice. In addition, mice that received 10 mg/kg cocaine were also significantly more stimulated than mice that had received 5 mg/kg of cocaine ($p < 0.001$). Although CN mice were not included in the analysis, their activity is presented here as a contextual baseline measure. ***$p <$ 0.001 RC versus AC mice; (n = 11-15).

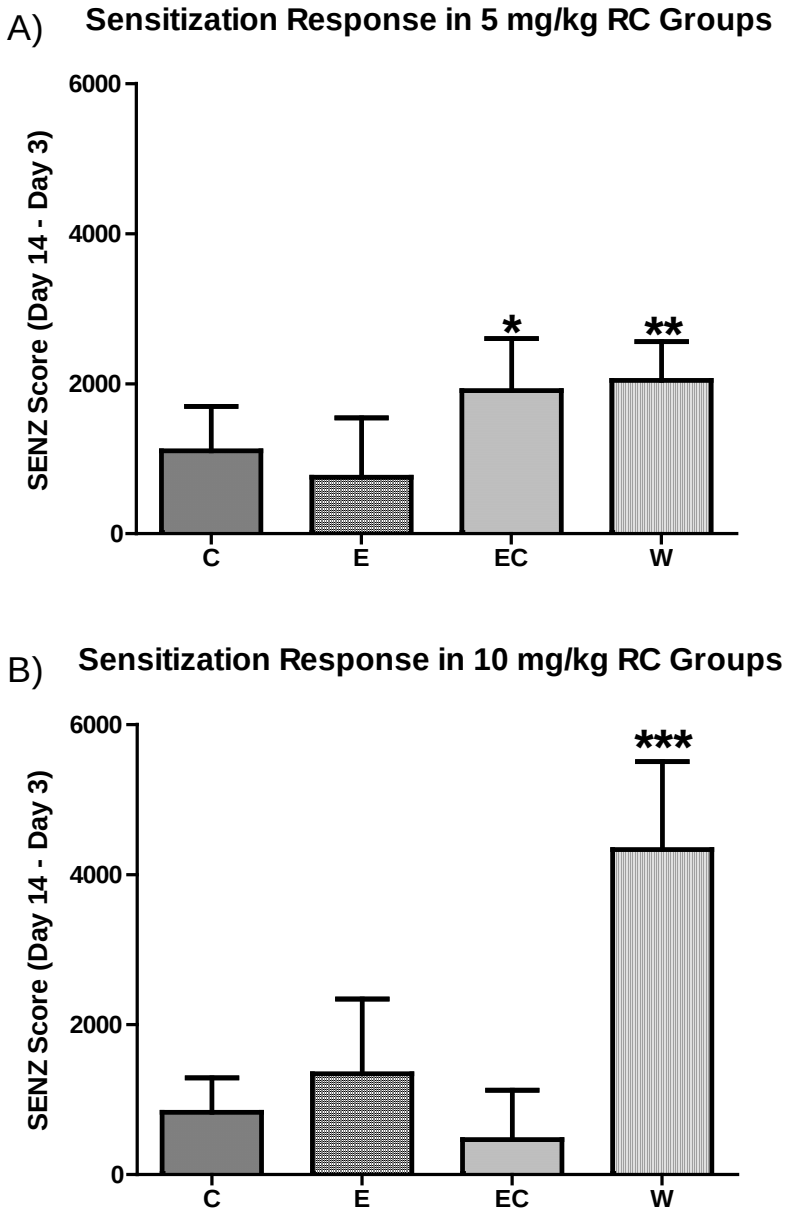

Figure 22. Experiment 1: Sensitization scores. Sensitization response in mice that received repeated cocaine. SENZ scores were computed (Day 11 activity – Day 3 activity) for mice that had repeatedly received cocaine (RC) in Experiment 1. A) Among mice receiving 5 mg/kg cocaine, only EC and W mice achieved significant SENZ scores. B) Among mice receiving 10 mg/kg cocaine, only W mice achieved a significant SENZ score. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ versus '0' (n = 11-15).

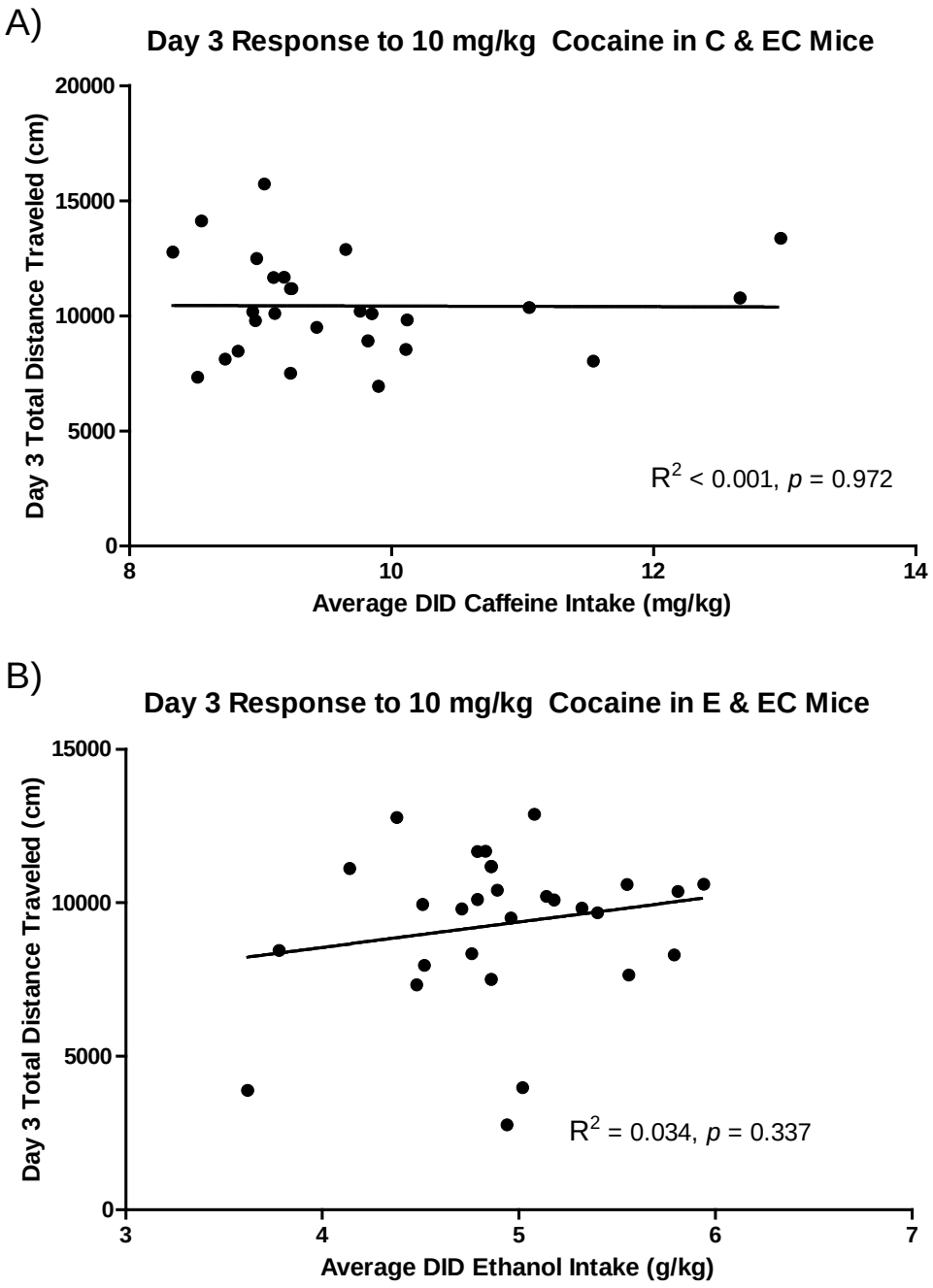

Figure 23. Experiment 1: Caffeine/ethanol intake and Day 3 activity regressions. Neither prior DID caffeine or ethanol intake is predictive of 10 mg/kg cocaine response on Day 3. Regression analysis determined that the observed enhanced stimulant response to 10 mg/kg cocaine in mice that had previously consumed A) caffeine (C,EC) was not influenced by the relative amounts of caffeine consumed. B) Prior ethanol intake (E,EC) in DID also had no effect on the Day 3 response to 10 mg/kg cocaine (*N*'s = 27-29).

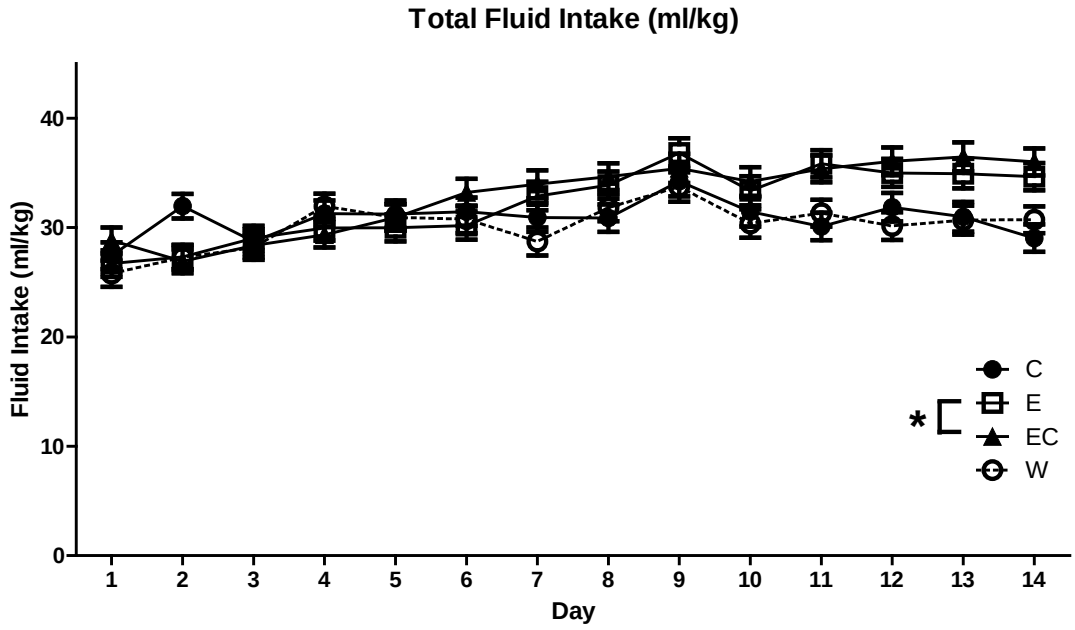

Figure 24. Experiment 2: Total fluid intake. Total fluid intake (ml/kg) over the course of the 14-day DID procedure in Experiment 2. Mice that consumed ethanol (E,EC) consumed significantly more fluid than mice that did not over the course of the DID procedure. *$p < 0.05$ versus mice that did not have access to ethanol (C,W) (n = 57-60).

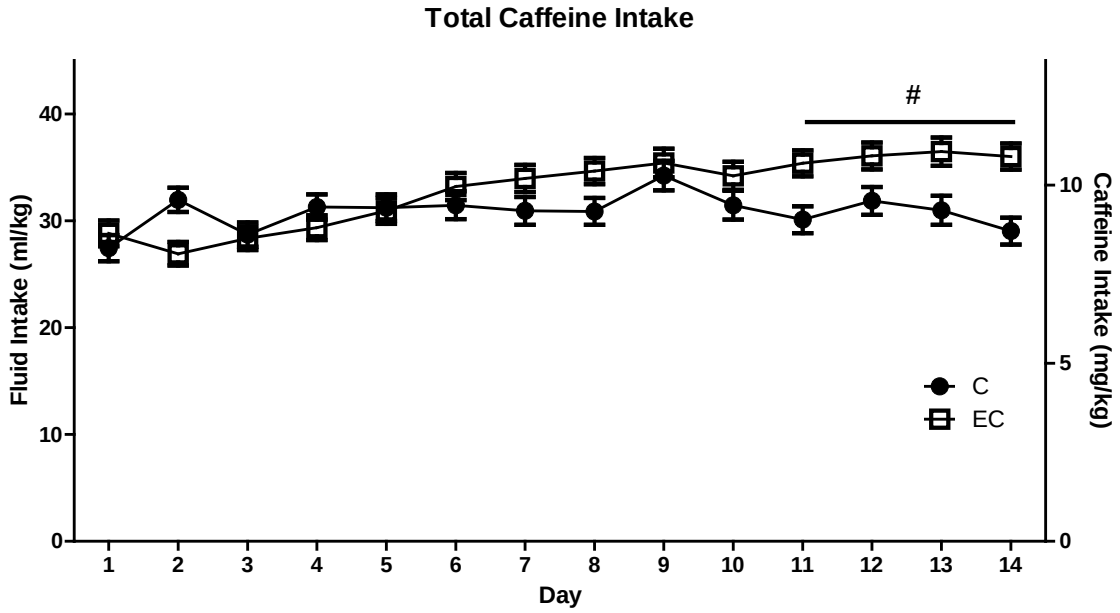

Figure 25. Experiment 2: Caffeine intake. Total caffeine (mg/kg) intake over the course of the 14-day DID procedure in Experiment 2. EC mice consumed significantly more caffeine than C mice towards the end of the 14-day DID procedure. $^{\#}p < 0.05$ versus C (n = 57-60).

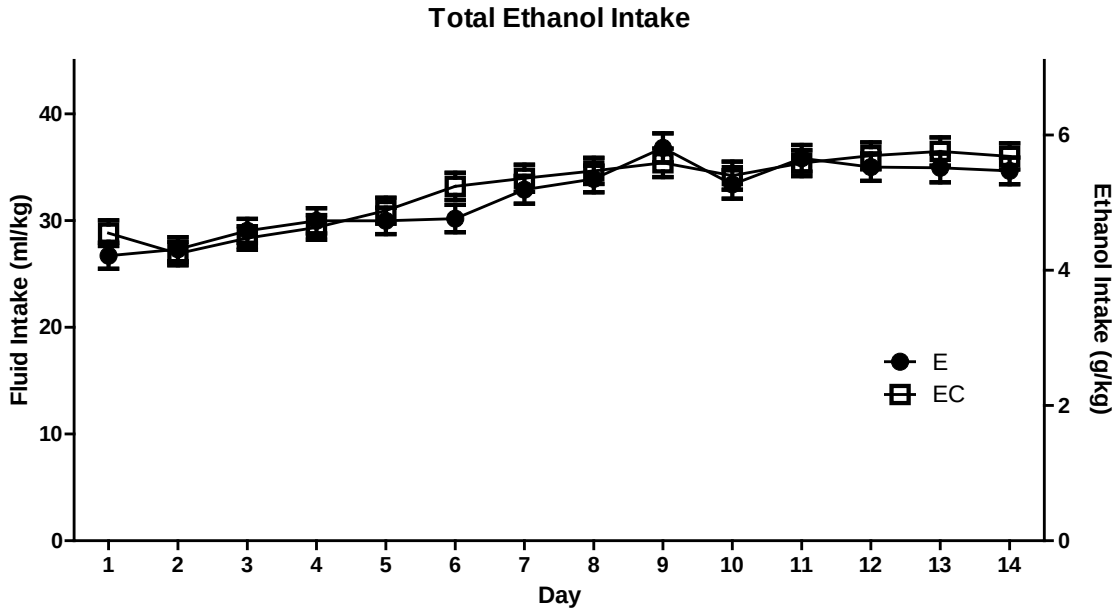

Figure 26. Experiment 2: Ethanol intake. Total ethanol intake (g/kg) over the course of the 14-day DID procedure in Experiment 2. Mice in the E and EC groups consumed equivalent amounts of ethanol throughout the DID phase of the experiment (n = 58-60).

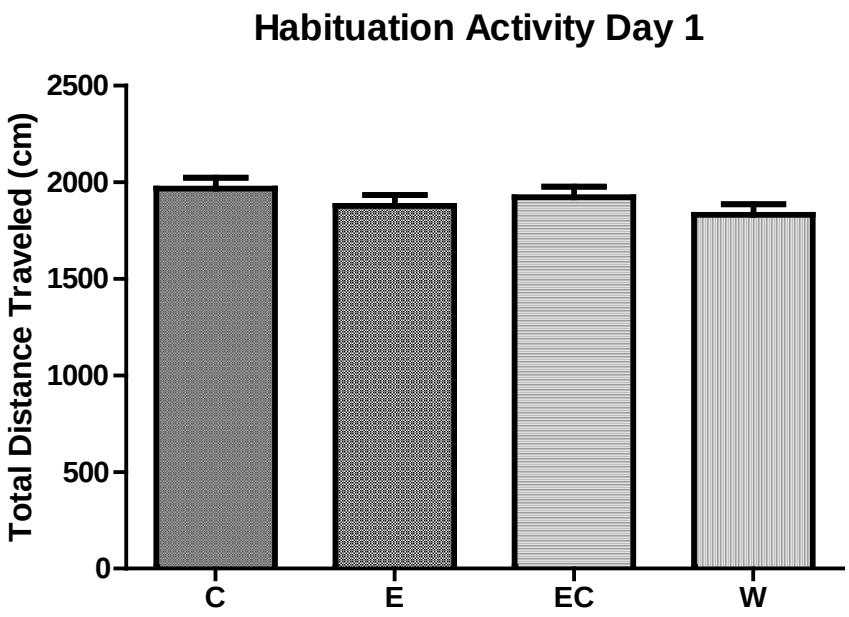

Figure 27. Experiment 2: Habituation activity. Habituation activity during the first day of the CPP protocol. Prior fluid consumption in DID produced no differences in baseline activity on Day 1 of the CPP protocol (*n* = 58-69).

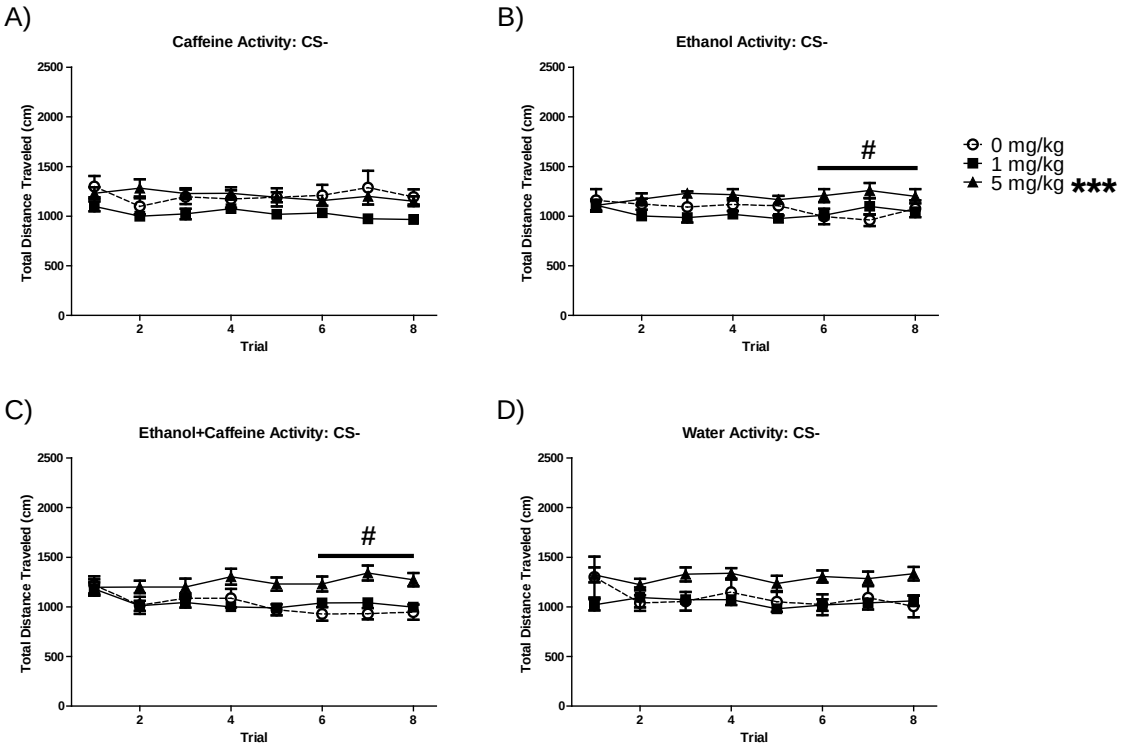

Figure 28. Experiment 2: CS- trial activity. Activity during cocaine-free CS- conditioning trials. Animals receiving 5 mg/kg cocaine on CS+ trials were significantly more active during drug-free CS- trials than mice that had received 1 mg/kg or saline. Furthermore this effect was particularly apparent in mice that previously consumed ethanol via DID (E,EC) over the last 3 trials. ***$p < 0.001$ versus 0 mg/kg and 1 mg/kg; $^{\#}p < 0.05$ for significant ethanol × dose × trial interaction with mice that had consumed ethanol and received 5 mg/kg cocaine being significantly more active than 0 mg/kg and 1 mg/kg mice over the last 3 trials relative to mice that had not consumed ethanol (C,W) (n = 9-25).

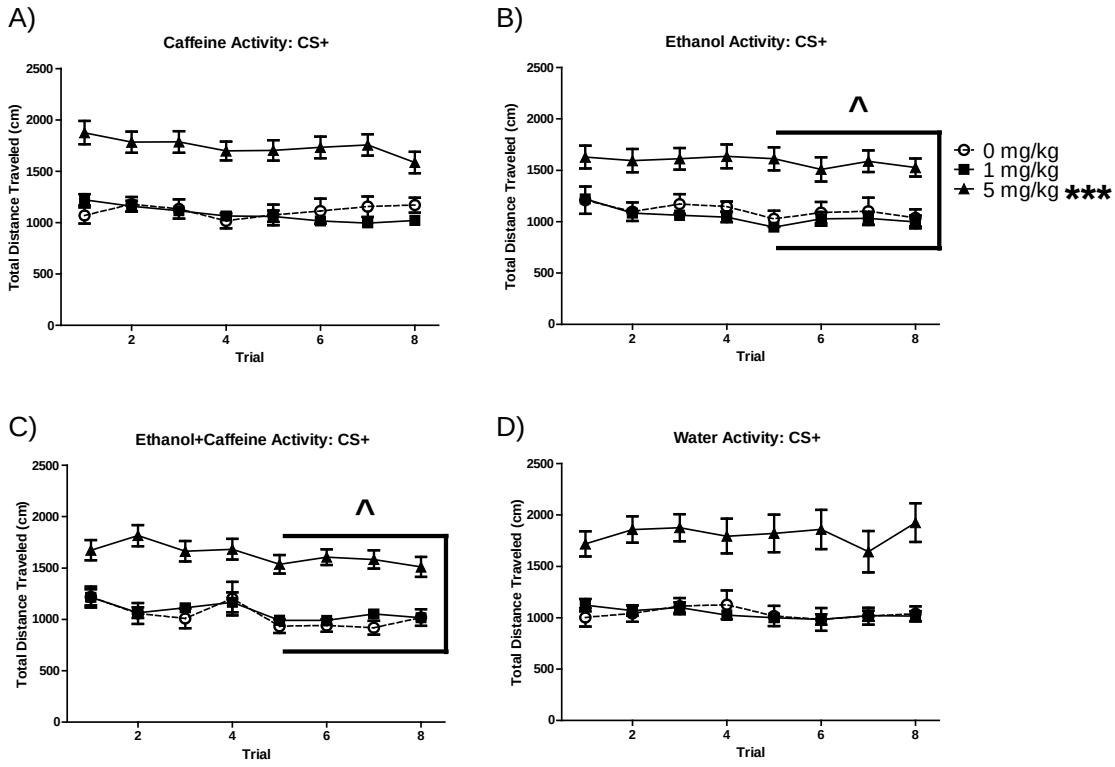

Figure 29. Experiment 2: CS+ trial activity. Activity during cocaine CS+ conditioning trials. Animals receiving 5 mg/kg cocaine on CS+ trials were significantly more active than mice that had received 1 mg/kg or saline. Furthermore, mice that previously consumed ethanol via DID (E,EC) were significantly less active over the last 4 trials than mice that had not (C,W). ***$p < 0.001$ versus 0 mg/kg and 1 mg/kg; ^$p < 0.05$ for significant ethanol × trial interaction with mice that had consumed ethanol being significantly less active over the last 4 trials relative to mice that had not consumed ethanol (C,W) (n = 9-25).

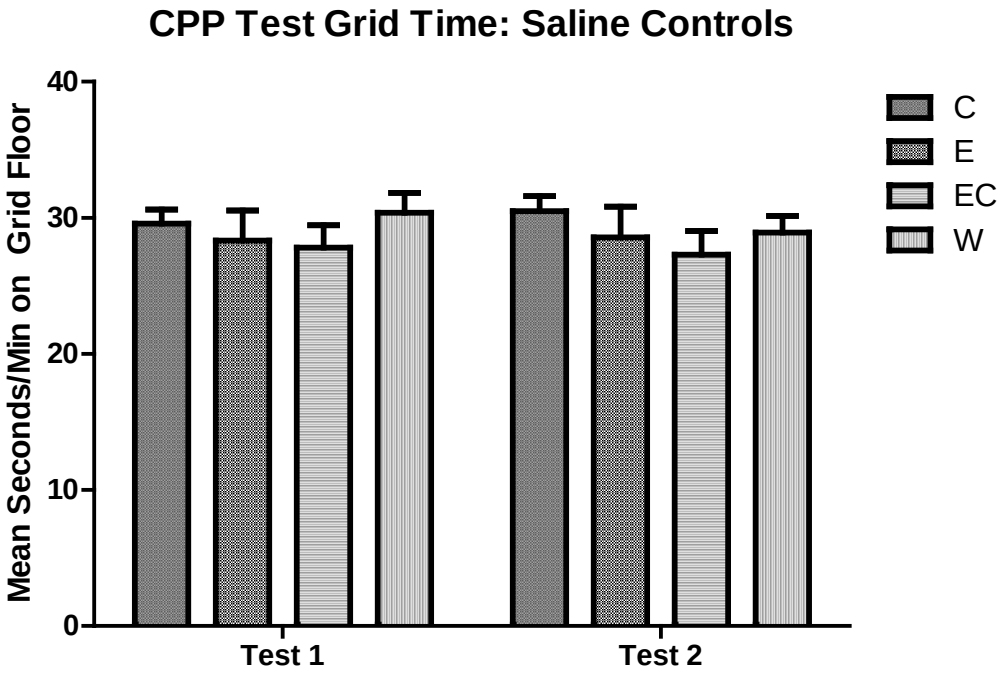

Figure 30. Experiment 2: Control group test grid floor time. Time spent on the grid floor during the CPP tests in control 0 mg/kg mice. Previous DID fluid consumption did not influence time spent on the grid floor during the CPP tests in saline control CN mice (*n* = 9-11).

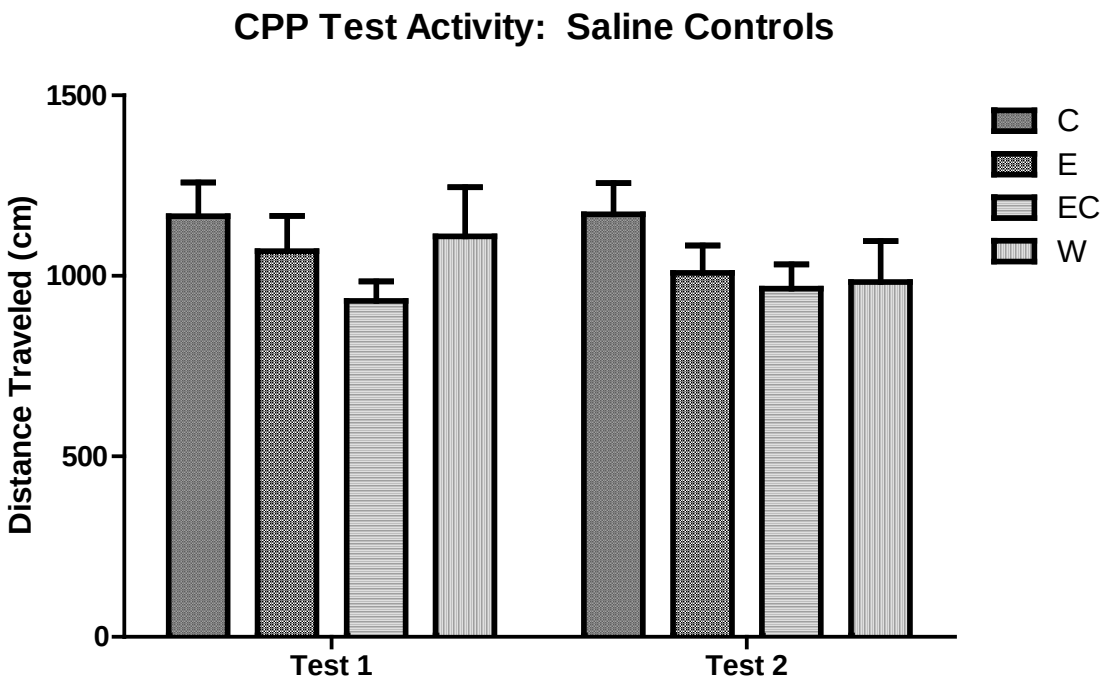

Figure 31. Experiment 2: Control group test activity. Activity during the CPP tests in control CN mice. Previous DID fluid consumption did not influence basal activity during the CPP tests in saline control CN mice (*n* = 9-11).

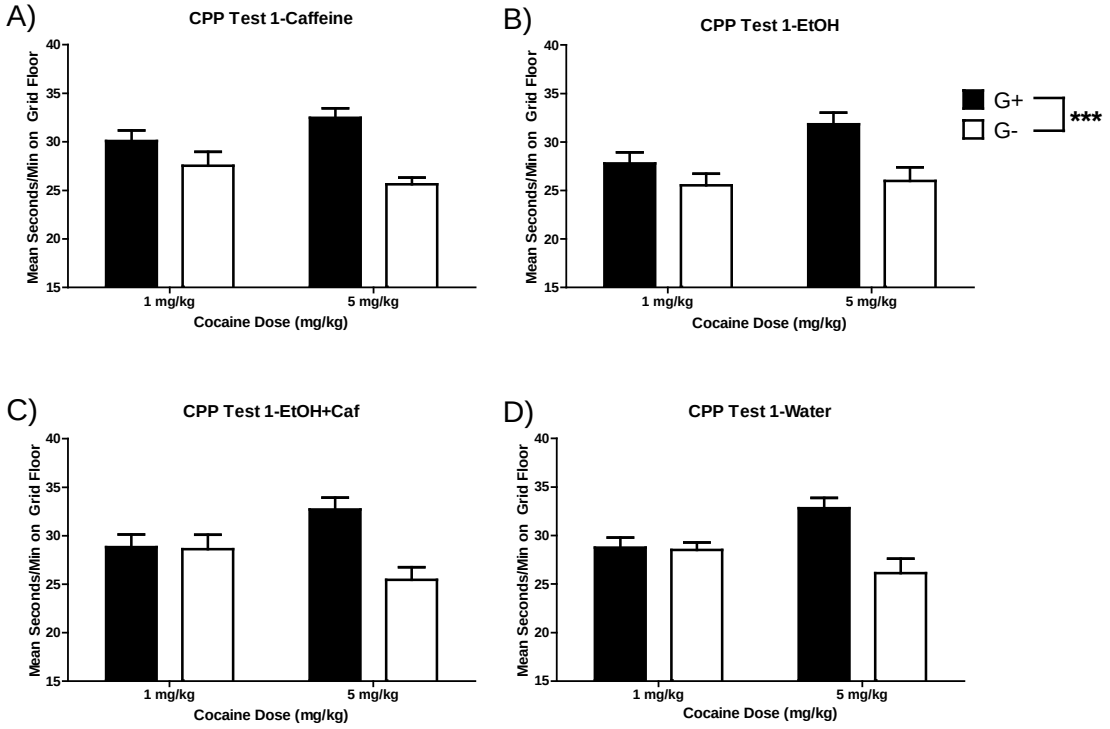

Figure 32. Experiment 2: Test 1 grid floor time. Time spent on the grid floor for mice that had cocaine previously paired with the grid (G+) or hole (G-) floor in Test 1. G+ mice generally spent more time on the grid floor than G- mice. In addition, this difference was far more pronounced in mice that had received 5 mg/kg rather than 1 mg/kg cocaine (dose × grid floor assignment interaction, $p < 0.001$). ***$p < 0.001$ versus G- (n = 11-15 per dose/floor assignment combination).

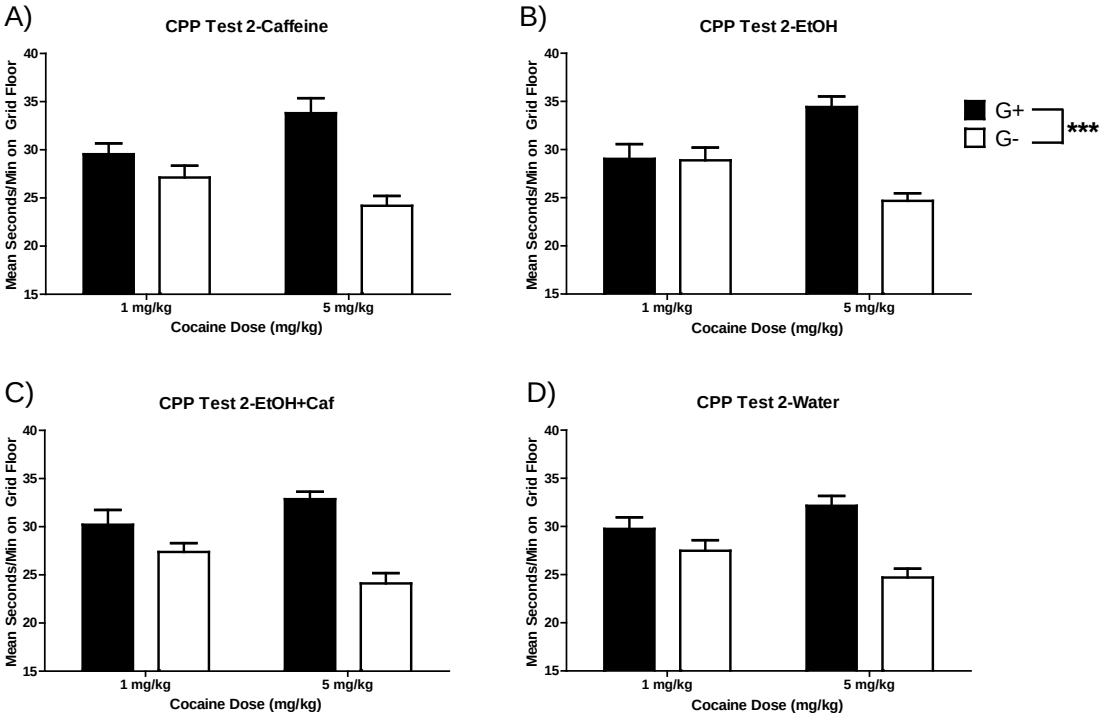

Figure 33. Experiment 2: Test 2 grid floor time. Time spent on the grid floor for mice that had cocaine previously paired with the grid (G+) or hole (G-) floor in Test 2. G+ mice generally spent more time on the grid floor than G- mice. In addition, this difference was far more pronounced in mice that had received 5 mg/kg rather than 1 mg/kg cocaine (dose × grid floor assignment interaction, $p < 0.001$). ***$p < 0.001$ versus G- (n = 11-15 per dose/floor assignment combination).

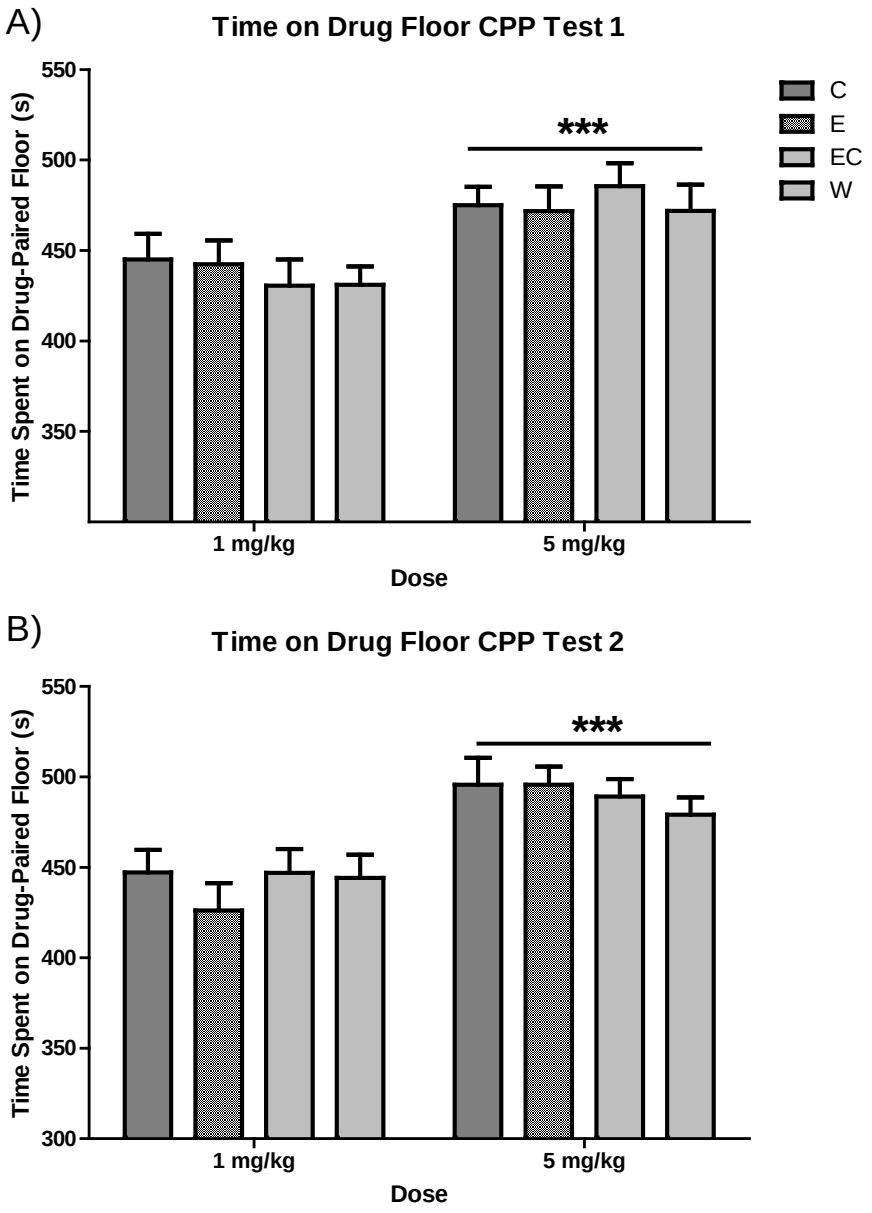

Figure 34. Experiment 2: Time on drug paired floor. Total time spent on the drug-paired floor (either grid or hole) during the CPP tests. Mice that received 5 mg/kg cocaine spent significantly more time on the drug-paired floor than mice that received 1 mg/kg cocaine. ***$p < 0.001$ for main effect of dose versus 1 mg/kg (n = 23-25).

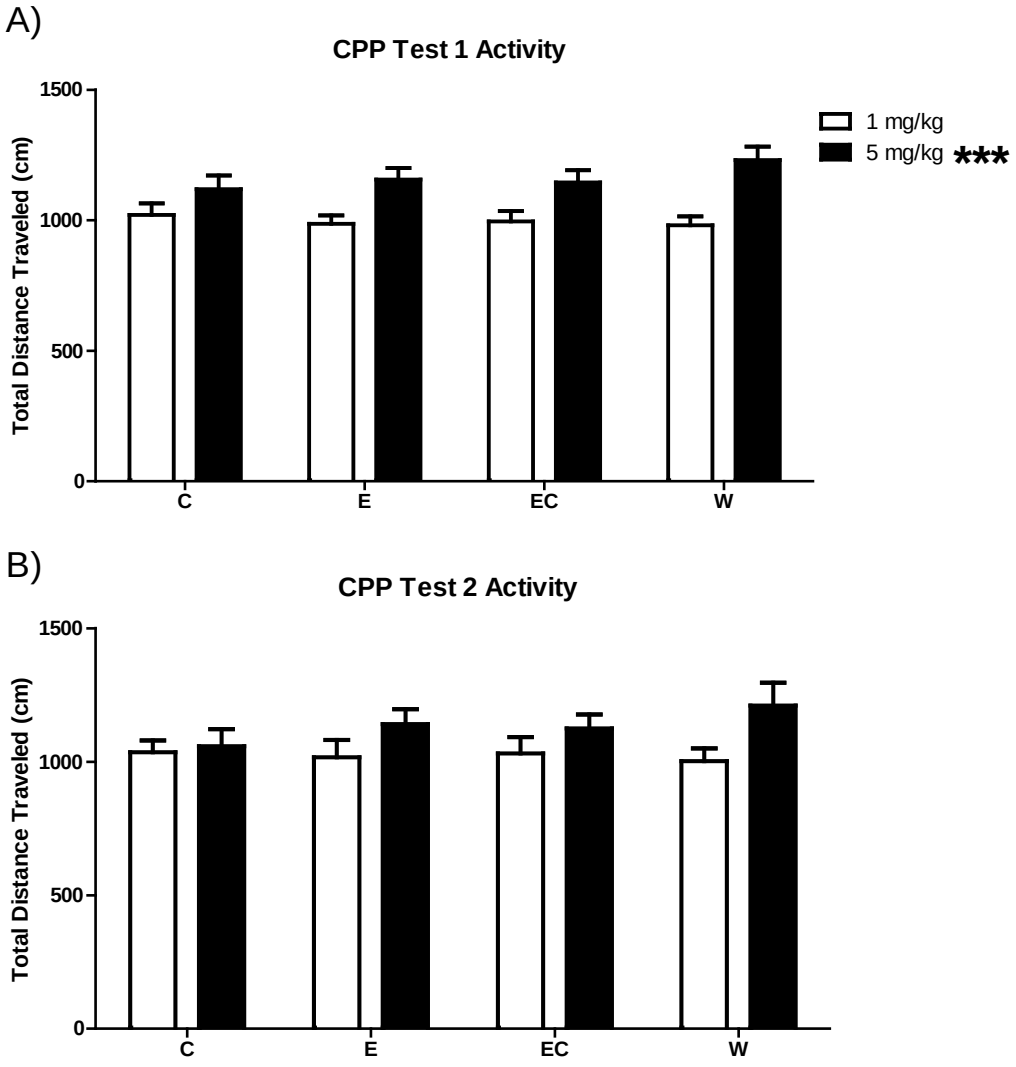

Figure 35. Experiment 2: CPP test activity. Activity during the drug-free CPP tests in mice that received cocaine during conditioning. Mice that had received 5 mg/kg cocaine during CS+ conditioning trials were significantly more active during the drug-free CPP tests than those that had received 1 mg/kg cocaine. ****p* < 0.001 versus 1 mg/kg (*n* = 23-25).

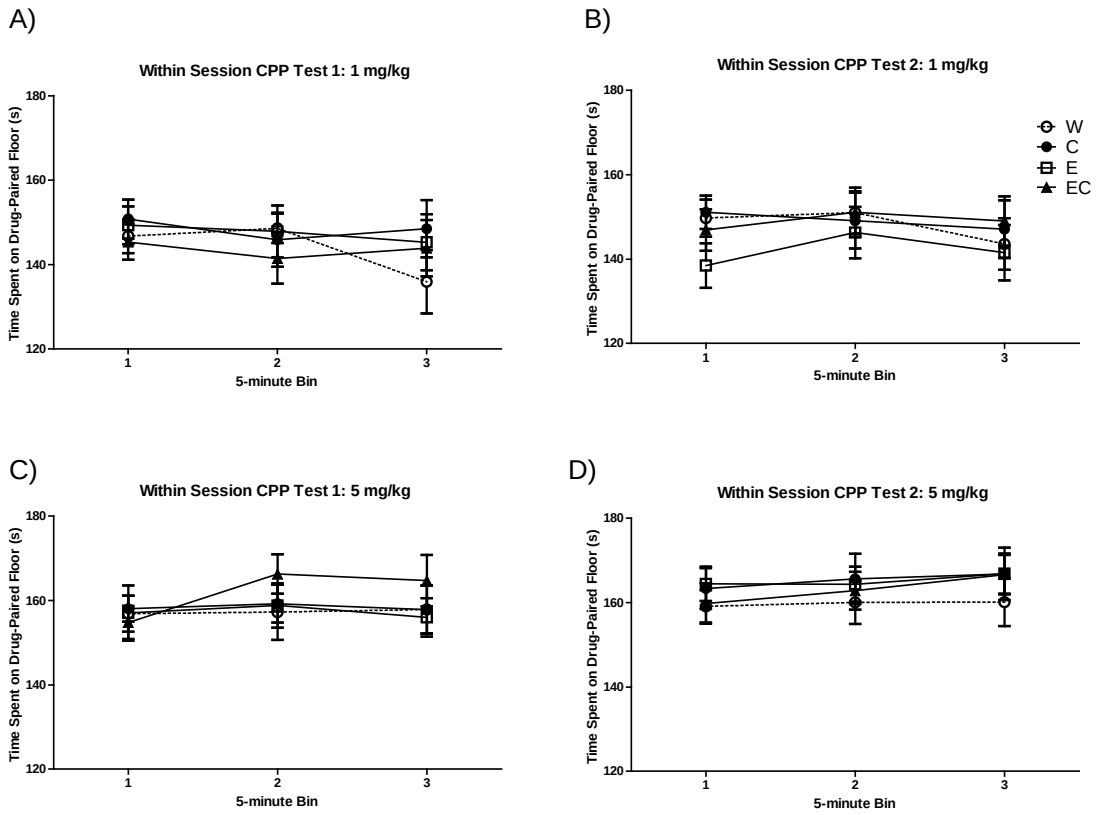

Figure 36. Experiment 2: Within session CPP. Time spent on the drug floor during CPP Tests. The time that mice spent on the drug-paired floor was not differently expressed across each of the 5-minute bins of in the CPP tests (n = 23-25).

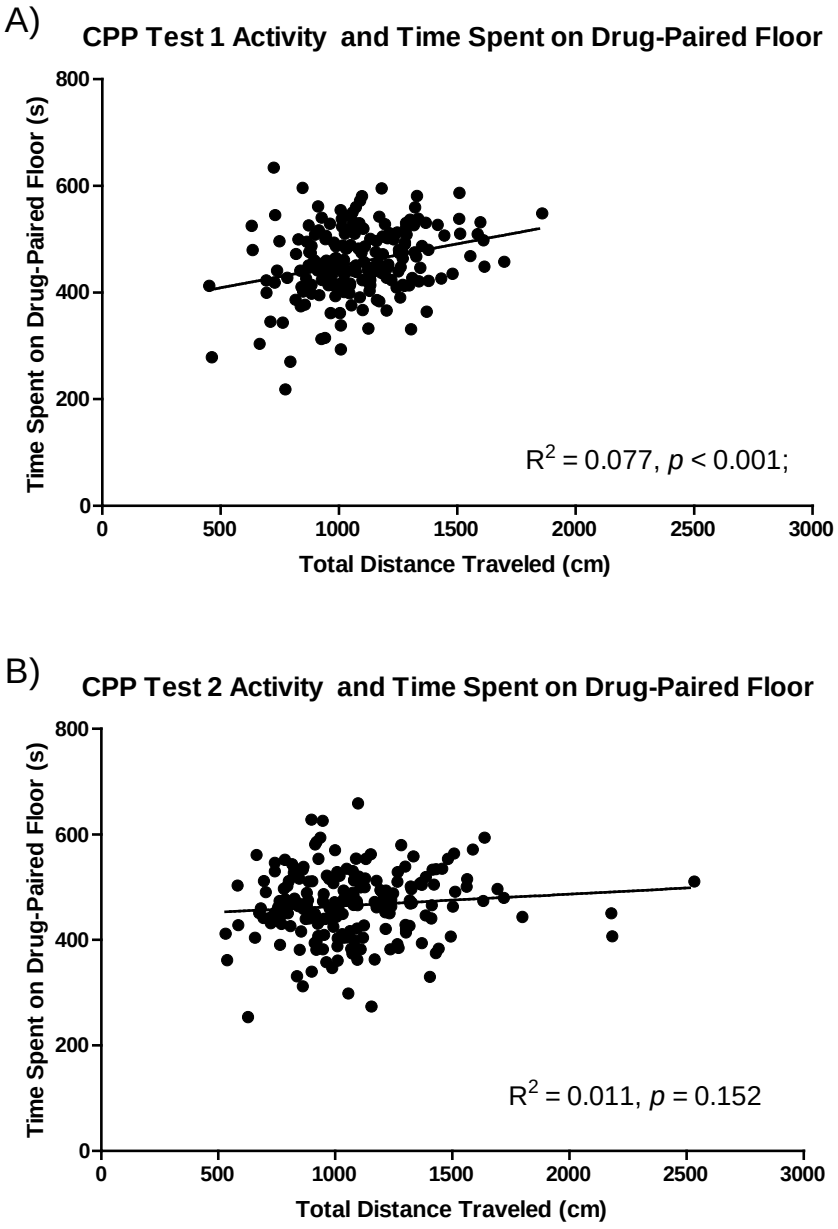

Figure 37. Experiment 2: Test activity/CPP regression. Relationship between activity and time spent on the drug-paired floor during CPP tests. Activity was positively predictive of time spent on the drug-paired floor in A) Test 1, but not B) Test 2 (N = 196).

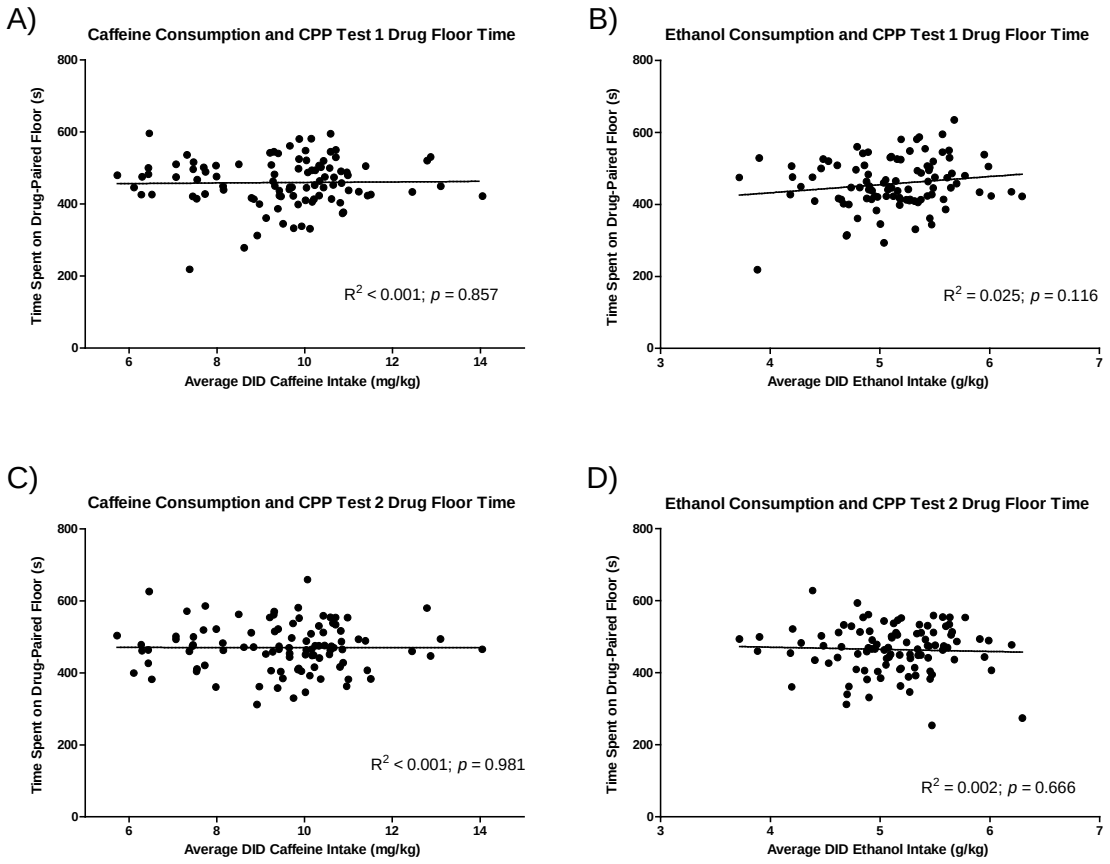

Figure 38. Experiment 2: Prior caffeine/ethanol intake and CPP regression. Relationship between prior caffeine and ethanol intake with time spent on the drug floor during CPP tests. Neither prior A,C) caffeine or B,D) ethanol consumption via DID influenced the time spent on the drug paired floor in either CPP test (*n* = 98-99).

VITA

VITA

BRANDON M. FRITZ

CURRICULUM VITAE

EDUCATION

St. Olaf College
B.A. in Psychology, Cum Laude **2010**
Foci: Biopsychology, Addiction
Concentration: Neuroscience
Mentor: Dr. Shelly Dickinson

Indiana University-Purdue University Indianapolis (IUPUI) **2013**
M.S. in Psychobiology of Addictions
Thesis Title: Investigating the role of extrasynaptic $GABA_A$ receptors located in the infralimbic cortex in the binge-like alcohol intake of male C57BL/6J mice
Mentor: Dr. Stephen L. Boehm II

Indiana University-Purdue University Indianapolis (IUPUI) **2016**
Ph.D in Addiction Neuroscience
Thesis Title: The effect of voluntary binge caffeine and ethanol co-exposure on neurobehavioral responsivity to cocaine in male C57BL/6J mice
Mentor: Dr. Stephen L. Boehm II

Cumulative Graduate GPA: 3.958

RELEVANT COURSEWORK

Behavioral neuroscience, behavioral genetics, animal learning, psychopathology, psychopharmacology, statistical inference, experimental design, drugs of abuse, neuroanatomy, methods in cell and neurobiology

HONORS/AWARDS

Buntrock Academic Scholarship, St. Olaf College	**2006-2010**
Cassler Music Scholarship, St. Olaf College	**2006-2010**
Psychology Vision Fund Travel Award, St. Olaf College	**2010/11**
Student Merit Award, Research Society on Alcoholism	**2011-2016**
Young Investigator Travel Award, IBANGS	**2012-2014**
IUPUI School of Science Graduate Student Council Travel Award	**2013**
American Psychological Foundation COGDOP Dissertation Award	**2015**
Paul J. McKinley Graduate Research Excellence Award	**2016**

FELLOWSHIPS/GRANTS

NIAAA T32 IUPUI Institutional Training Grant Appointee	**2013-2016**

RESREACH EXPERIENCE

St. Olaf College

Undergraduate Research Assistant

Supervisor: Shelly Dickinson PhD **2009–2010**

- Assessed the effect of the opiod receptor antagonist Naloxone on the expression of conditioned place preference for ethanol in outbred Swiss Webster mice
- Phenotyped adult and adolescent DBA/2J mice for the development of locomotor sensitization to ethanol
- Determined whether or not pre-exposure to ethanol differentially affected the expression of conditioned place avoidance in adolescent versus adult DBA/2J mice

Indiana University-Purdue University Indianapolis
Graduate Research Assistant
Advisor: Stephen L. Boehm II PhD **2010-2016**

- Studying sensitivity and tolerance to the motor impairing actions of ethanol in mice selectively bred for high and low alcohol preference drinking (HAP & LAP)
- Lentiviral knockdown of alpha-2 $GABA_A$ receptor subunit expression in the ventral tegmental area of C57BL/6J mice and its effect on ethanol consumption
- Microinjection of the $GABA_A$ receptor partial agonist gaboxadol into the infralimbic cortex to evaluate the role of δ subunit-containing $GABA_A$ receptors in binge-like alcohol consumption
- Investigating caffeine and alcohol interactions in C57BL/6J mice following binge-like drinking and later responsiveness to cocaine
- Assessment of adolescent and adult THC exposure alone or in combination with ethanol on future motor and cognitive performance measures

LABORATORY SKILLS

Voluntary drinking studies in mice, intraperitoneal mouse injection, transcardial mouse perfusion, mouse stereotaxic surgery, intracranial microinjection, lentiviral microinjection, immunohistochemistry, laser-capture microdissection, wet tissue collection, periorbital sinus blood collection, Analox blood ethanol concentration analysis, locomotor sensitization paradigm, conditioned place preference paradigm, novel object recognition assay, locomotor activity assays, motor coordination assays (e.g. rotarod, static dowel, balance beam), elevated plus maze assay, forced swim assay, data analysis and graphing (Statistica; SPSS; GraphPad Prism)

TEACHING EXPERIENCE

General Psychology (2010: Lab Instructor, St. Olaf College)
Psychology as a Biological Science (2010-2012: Teaching Assistant, IUPUI)
Learning (2011: Teaching Assistant, IUPUI)

PEER-REVIEWED PUBLICATIONS

Brandon M. Fritz, Caroline Quolin, Chelsea R. Kasten, Michael Smoker, Stephen L. Boehm II (2016). Concomitant caffeine increases binge consumption of ethanol in adolescent and adult mice, but produces additive motor stimulation only in adolescent animals. *Alcoholism: Clinical and Experimental Research, in press*.

Brandon M. Fritz & Stephen L. Boehm II (2015). Adenosinergic regulation of binge-like alcohol consumption and associated locomotor effects in male C57BL/6J mice. *Pharmacology, Biochemistry, and Behavior 135*, 83-89.

Brandon M. Fritz & Stephen L. Boehm II (2014). The effect of prior alcohol consumption on ataxic responses to alcohol in high-alcohol preferring mice. *Alcohol 48*(8), 765-772.

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INVITED REVIEW

Brandon M. Fritz & Stephen L. Boehm II (2016). Rodent models and mechanisms of voluntary binge-like ethanol consumption: Examples, opportunities, and strategies for preclinical research. *Progress in Neuro-Psychopharmacology & Biological Psychiatry 65*(4), 297-308.

MANUSCRIPTS IN PREPARATION

Brandon M. Fritz, Michel A. Companion, Stephen L. Boehm II. Exploring the relationship between alcohol and cocaine locomotor sensitization: A behavioral genetic approach in mice.

Brandon M. Fritz, Benjamin A. Dowden, Stephen L. Boehm II. Assessment of Δ9-THC and ethanol exposure alone or in combination on later sensitivity and tolerance in adolescent and adult mice.

Brandon M. Fritz & Stephen L. Boehm II. The effect of voluntary binge caffeine and ethanol co-exposure on neurobehavioral responsivity to cocaine in mice.

INVITED RESEARCH TALKS

"Role of adenosine A1 and A2A receptors in the modulation of binge alcohol drinking". To be presented at the Research Society on Alcoholism meeting, New Orleans, LA, June 2016.

POSTER ABSTRACTS

Fritz, B.M., Dowden, B.A., Boehm, S.L. II. Evaluation of Δ9-THC withdrawal and tolerance in adolescent and adult mice following ethanol and Δ9-THC exposure alone and in combination. To be presented at the Research Society on Alcoholism meeting in New Orleans, LA, June 2016.

Fritz, B.M. & Boehm, S.L. II (2015). Adenosinergic regulation of binge like ethanol drinking and associated locomotor behavior in male C57BL/6J mice. *Alcoholism: Clinical and Experimental Research*, *39*(s1), 62A.

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Fritz, B.M., Companion, M.A., Boehm, S.L. II (2014). Cocaine locomotor sensitivity and sensitization in mice selectively bred for high and low alcohol preference drinking. Presented at International Behavioural and Neural Genetics Society Meeting, Chicago, Illinois, USA.

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Dickinson, S. D., **Fritz, B. M.**, Crumly, A., Deeg, A. K., Flesness, K. G., Jeong A. (2009). Sensitization to ethanol's activating effects in adolescent mice. Program No. 445.14. *2009 Neuroscience Meeting Planner*. Chicago, IL: Society for Neuroscience. Online.

MANUSCRIPT REVIEWER FOR:

Alcoholism: Clinical and Experimental Research

PROFESSIONAL AFFILIATIONS

2010 – 2014 *Society for Neuroscience*
2011 – Present *Research Society on Alcoholism*
2012 – 2014 *International Behavioural and Neural Genetics Society*