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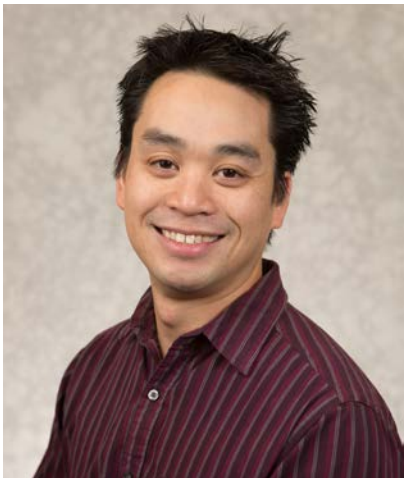

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Alcohol abuse is a substantial public safety and health concern affecting our population at a global level. In the United States alone, alcohol use disorders lead to approximately 75,000 deaths and cost society nearly $200 billion annually (Bouchery, Harwood, Sacks, Simon, & Brewer, 2011). A majority of Americans are affected by alcohol use disorders either by their own personal experience or that of a family member (Alcohol and Drug Abuse Affects Everyone in the Family, 2015). Significant obstructions in the process of recovery from alcoholism are alcohol craving and a chronic relapsing disposition (Seo & Sinha, 2014). Clinical studies have suggested that a common cause for relapse is rooted in the ability of alcohol to reduce negative mood symptoms associated with stress (Hershon, 1977; Cloninger, 1987; De Soto, O'Donnell, & De Soto, 1989; Annis, Sklar, & Moser, 1998). Thus, it is a priority that the neurobiological systems associated with alcohol-related stress are classified in a way that provides a valuable strategy in the long-term prevention of relapse.

Animal models demonstrate that stress produces transient effects on voluntary alcohol consumption (Vangeliene et al., 2003; van Erp & Miczek, 2001). Two stress induction techniques have been previously established as physical and psychological stressors in rodents, namely the forced-swim test and inescapable footshock (Vangeliene et al., 2003; Le et al., 1999). The forced-swim test is a procedure in which rats are placed into cylindrical plastic tanks filled with water. The amount of time spent swimming versus time spent immobile is measured, and increased immobility is thought to be indicative of a heightened stress response. Inescapable footshock is performed by placing rats into chambers connected to a metal rod floor construction that delivers shocks at a varied rate for several seconds, randomized by software. Vengeliene et al. (2003) reported that stress induced by inescapable footshock and forced swim led to significantly increased rates of alcohol consumption. These results demonstrate that stressors are a significant factor in enhanced alcohol self-administration.

The chronic nature of relapse in alcohol use disorders is cause for serious concern in regard to alcoholism, despite recent neurobiological research in pharmacological treatment strategies (Johnson, 2010). With regard to alcohol use disorders and addiction, one of the main neurochemical systems responsible is the endogenous opioid system (Herz, 1997). This opioid system has been connected to pain relief and rewarding properties produced by alcohol consumption (Hay, Jennings, Zitzman, Hodge, & Robinson, 2013). Previous work has shown that alcohol reinforcement may be partially due to activation of opioid mechanisms that are closely linked to increased dopamine transmission in the mesolimbic pathway (Herz, 1997; Johnson, 2010). Due to the reinforcing properties of alcohol and sensitivity to relapse, it is essential to examine behavioral studies involving alcohol self-administration.

The opioid-receptor antagonist naltrexone (NTX) has been shown to decrease alcohol consumption (Herz, 1997; Hay et al., 2013) and is an approved pharmacotherapy for various alcohol use disorders (Johnson, 2010). Previous clinical studies and preclinical models have shown that NTX appears to eliminate rewarding properties previously produced by alcohol consumption (Kreek, LaForge, & Butelman, 2002; Ripley & Stephens, 2011). In clinical studies of alcohol-dependent participants, NTX was reported to decrease relapse (O'Malley, Krishnan-Sarin, Farren, Sinha, & Kreek, 2002). The success of this opioid antagonist is attributed to the ability to decrease cravings of alcohol by interfering with the rewarding properties previously experienced with consumption (Hay et al., 2013). Further, NTX has been reported to reduce alcohol seeking and drinking behavior in preclinical models

(Hill & Kiefer, 1997; Le et al., 1999; Hay et al., 2013). A study by Hay et al. (2013) utilized NTX to reduce alcohol-seeking behavior and reported a reduction in self-administration in rats conditioned in goal-directed (immediately rewarded) drinking behavior. Rats trained to self-administer alcohol showed a decrease in lever presses and alcohol consumption after receiving NTX. Thus there was a decreased preference for the previously rewarding effects produced by alcohol consumption. Due to the success of previous work, it is evident that stress is a significant factor in alcohol consumption and rate of relapse. Additionally, NTX is successful in blocking the rewarding properties experienced with alcohol drinking that leads to continued consumption.

As a result of multiple factors contributing to relapse, these studies can be interpreted to suggest that NTX, as a pharmacological therapy, may be an effective tool in relapse prevention. Due to alcohol consumption relieving negative symptoms associated with stress, it is of crucial importance to discover a technique in order to eliminate this association. Due to previous work supporting both stress as a significant factor in relapse and NTX effects on blocking rewarding properties, there is a need for a therapy that promotes a decrease in alcohol consumption enhanced by stress. We hypothesize that the use of the opioid antagonist NTX will block stress enhanced alcohol self-administration. Using these results, we intend to aid in the development of improved medications in the treatment of alcohol abuse and relapse prevention.

METHOD

Animals and Housing

Male Wistar rats (n=11, Charles River, Kinsgston, NY) weighing 150-200g upon arrival were used in the current study. Rodents were housed (2-3 per cage) with food and water available *ad libitum* except at the initiation of self-administration training as described below. The colony was sustained by a 12-hour reverse light/dark cycle with lights coming on at 10:00 p.m. Prior to conducting experimental procedures, animals were habituated to colony housing for 7-14 days as well as handled and weighed daily. A statistical power analysis was utilized for the configuration of an appropriate number of rodents required for the current procedure in conjunction with previous reported studies with similar methods. All procedures were approved by the Grand Valley State University Institutional Animal Care and Use Committee (Protocol #13-01-A).

Ethanol Self-Administration Training

Rats were trained to lever press for ethanol solutions by utilizing an adapted sweetened solution fading procedure (Samson, 1986) that ultimately resulted in animals consuming unsweetened 10% ethanol solution that produced pharmacologically sufficient blood alcohol levels (Roberts et al., 1999). At training initiation, rats were water restricted in home cages for three days. During this time, rats began self-administration training in operant boxes. Response on one lever resulted in the delivery of a 10% sucrose solution and response on another lever resulted in the delivery of water. Following the initial three-day training period, water restriction was discontinued. Ethanol concentrations gradually increased from 3% to 10% in conjunction with a 10% sucrose solution as sessions advanced. When stable levels of responding for 10% sucrose/10% ethanol solution were established, sucrose concentration was gradually decreased until rats consumed strictly 10% ethanol solution. It was maintained throughout the training procedure that response on one lever produced ethanol/sucrose solution and the other resulted in water. In order to counterbalance solution delivery, half of the operant cages dispensed ethanol/sucrose solution following left lever response and the other delivered solution following right lever response. Water was concomitantly available on an opposing lever, respectively, in order to control for general fluid intake. Training sessions were conducted for 30 minutes Monday-Friday.

Footshock Stress

Operant conditioning chambers equipped with ethanol self-administration units utilized metal grid floors connected to electric shock generators to administer mild stress. A constant-current of intermittent, inescapable electric footshock was delivered for 5 minutes prior to the ethanol self-administration test sessions. Each footshock (0.8 mA) lasted for 0.5 seconds. Shock exposure was delivered under a variable time schedule at irregular intervals ranging from 10-70 seconds between shocks.

Drug Administration Procedure

All subjects were initially exposed to shocks following a saline injection in order to associate alcohol consumption with stress relief without pharmacological manipulation. Treatment solution NTX was then rotated between animals each day. Rats received either NTX (10 mg/kg s.c.) or saline 10 minutes prior to shock exposure. Rats were counterbalanced by a condition of shock or no shock in conjunction with a NTX treatment or saline solution injection every other day. Subjects were placed in operant chambers following shock/no shock exposure and provided with ethanol access for 30 minutes. Data were collected and compared against saline consumption levels in order to measure for any difference in self-administration following shock.

Data Analysis

Statistical reliability of results were analyzed using a repeated measures ANOVA, with NTX and shock exposure as within subjects factors.

Results

There was no significant interaction between NTX and shock condition on ethanol response [$F(1, 10) = 0.17$; $P = 0.68$]. Further there was no main effect of shock [$F(1, 10) = 0.06$; $P = 0.81$] on ethanol response (see Figure 1). Although not statistically significant, a main effect of NTX on ethanol responding appeared to approach significance [$F(1, 10) = 3.39$; $P = .096$] evidencing suppression in ethanol self-administration. Additionally, there was no significant difference between the pre-shock and the post-shock exposure on ethanol responding (see Figure 2).

Discussion

There were no interactions or significant main effects between shock condition and NTX treatment on ethanol responses (see Figure 1). However, there was a trend towards decreased responding for ethanol following treatment with NTX regardless of shock condition. Further, no significant differences were observed between baseline ethanol responding during training and responding following shock exposure (see Figure 2). Contrary to what we predicted, there was no main effect of footshock on ethanol self-administration. Work from previous studies has revealed mixed results in regard to shock increasing ethanol responses, such that there is greater success in repeated shock exposure prior to testing. For instance, studies reviewed reported using repeated footshock stress used over three consecutive days in ethanol dependent rats. The results showed a significant increase in alcohol consumption among rats. This supports the notion that an increase in ethanol response can be effected by duration of stress exposure and has a large inter-individual variability (Vangeliene et al., 2003.) Acute shock exposure, as was conducted in the current study, has a greater likelihood of being confounded by rats displaying a fear conditioning response, also known as a freezing response. Because it is a natural response for rodents to initially freeze when experiencing a stressful situation, had we used repeated exposure to shocks in order to familiarize the rats, we may have extrapolated significant results. Although significant levels were not met, those in the treatment condition with shock exposure were approaching significance. Therefore, NTX may reduce ethanol self-administration, consistent with previous literature (Hay et al., 2013). Overall, NTX led to a suppression of behavior approaching significance regardless of stress condition. Therefore, it is possible that NTX may reduce ethanol self-administration, which is consistent with work that has been done before (Hay et al., 2013; Hill and Kiefer, 1997; Le et al., 1999).

Limitations within the current study were a result of time constraints, small sample size, acute stress exposure and use of a non ethanol-dependent sample. Due to time constraints, a repeated stress exposure of footshock was not plausible, defaulting to an acute exposure. Previous research reports that repeated exposure to stress is significant in yielding an increase in ethanol self-administration. Further due to time constraints, rats were not made ethanol dependent prior to stress exposure. Previous studies show that ethanol dependent rodents consume significantly more alcohol when exposed to stress (Vangeliene et al., 2003). Pilot data collected previously in the lab observed acute shock exposure yielding an increase in self-administration following long-term withdrawal in ethanol dependent rats. The pilot procedure delivered a liquid diet over four weeks, known to be sufficient in inducing physical dependence in animals. Unreported observations revealed that rats consuming an ethanol liquid diet compared to a control diet had significantly higher ethanol responses following acute stress exposure after protracted withdrawal. As a result, the current study intended to induce ethanol dependence as well as a protracted withdrawal period, but we were unable due to time. Finally, not all rodents were successful in operant training therefore limiting the initial sample size from 18 to 11, resulting in an insignificant number of subjects to observe effects of treatment conditions. The lack of significant results in the current study may be attributed to these shortcomings.

Moving forward, future work should look to address the effects of repeated exposure in self-administration as previous work reveals as more reliable. Additionally, the differences in self-administration should be investigated between rodent samples that are ethanol dependent compared to samples that are non dependent. Previous work has shown that rats with a history of physical ethanol dependence are more consistent in establishing an association between alcohol consumption and an alleviation of stress symptoms. Though the current study imposed intoxication evidenced by sufficient blood alcohol levels, the level of intoxication was not significant enough to witness withdrawal symptoms identifying alcoholism. Looking forward, ethanol dependence and repeated exposure of mild stressors should be established when measuring alcohol self-administration.

Acknowledgments

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Figure 1. Ethanol Responding to Shock/No Shock Following Administration of Naltrexone or Vehicle Solution. Rats (n=11) were trained to self-administer ethanol. Once stable consumption levels were achieved, rats were then injected with saline vehicle or naltrexone (10 mg/kg, s.c.). Following, rats were exposed to a shock/no shock condition and then measured for responses for ethanol self-administration. Data are expressed as mean+SEM

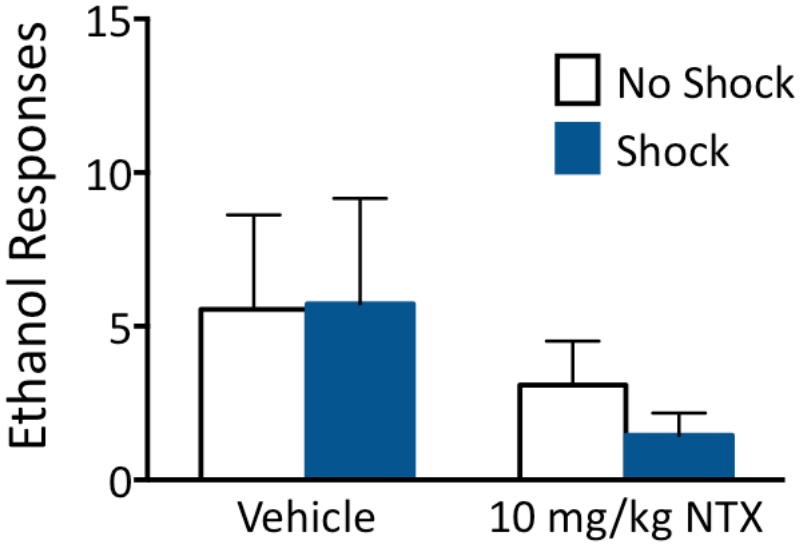
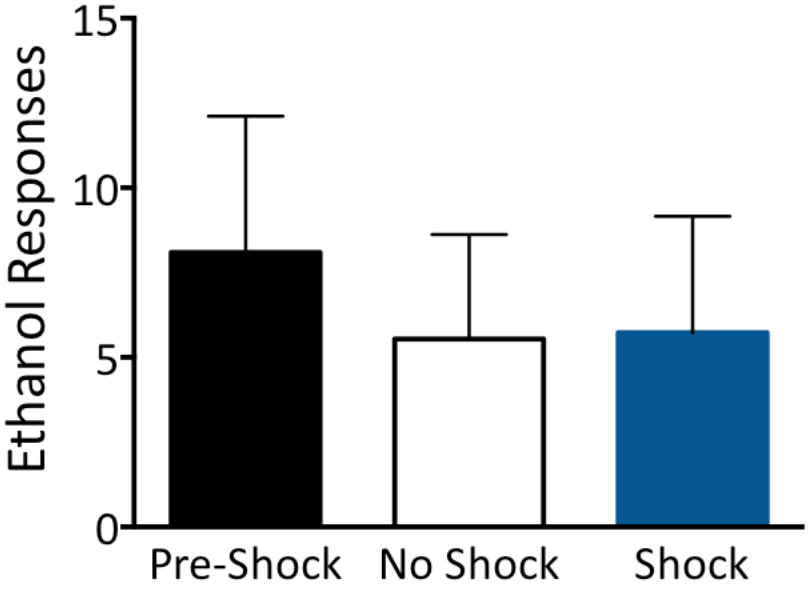


Figure 2. Ethanol Responding to Pre-Shock, Shock and No Shock Exposure. The pre-shock condition represents ethanol responding on the final day of training before the initial shock exposure. The no shock and shock conditions represent responses following saline injections after stable self-administration levels were achieved. Data are expressed as mean+SEM.



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