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Behavioral Markers and Endophenotypes Underlying Individual Differences in Ethanol Use

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Behavioral Markers and Endophenotypes Underlying Individual Differences in Ethanol Use

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Spring 2021

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Abstract

The main concern of this study is to evaluate the neurobiology of ethanol self-administration and cue-induced reinstatement in rats and its implications for Alcohol Use Disorder (AUD) prevention and treatment. The purpose of this study is to use a pre-clinical model of ethanol use to understand the individual effects at the neurobiological level. The study utilized economic demand and negative consequence testing to evaluate the rats' motivation to consume ethanol and their ability to relapse after periods of abstinence. The results showed individual and sex differences in ethanol consumption, with rats who placed a higher value on ethanol being more likely to consume it and relapse. In the future, data will be processed to identify the specific brain regions associated with relapse, which could be targeted with medications to treat AUD. Future research directions include inhibiting specific brain regions in rats with high economic demand and using alcohol long-term drinking rats to better mimic human models.

Introduction

Significance

Alcohol is one of the most used drugs in the world due to its societal and cultural roles. In 2019, 85.6 percent of Americans 18 years and older reported they consumed alcohol in their lifetime (NIH, 2020). Alcohol can be used in three different forms: moderate, binge drinking, and heavy drinking. Moderate drinking is limiting alcohol intake to two drinks or less in a day for men and one drink or less in a day for women. Binge drinking is the most common form of excessive drinking in which women consume four or more drinks in a single occasion and men consume five or more drinks. Heavy drinking in women is defined as eight or more drinks per week and 15 or more drinks per week for men (NIH,2020). The prevalence of alcohol use can influence the risk of people suffering from Alcohol Use Disorder (AUD). Alcohol Use Disorder is a medical condition in which an individual is unable to control alcohol use regardless of adverse social, occupational, or health consequences. According to the NSDUH in 2019, 14.5 million people ages 12 and older had AUD. Of those 14.5 million, 9 million of those individuals were men and 5.5 million were women (NIH, 2020). Excessive drinking can cause various health risks that can lead to the development of chronic diseases or even death. Each year excessive alcohol use in the U.S. is responsible for 140,000 deaths in which the average person dies at age 26 (NIH,2020). This could be caused by injuries, such as motor vehicle crashes, violence, alcohol poisoning, or risky sexual behaviors. Over time, excessive drinking can develop long-term health risks including heart disease, strokes, liver disease, digestive issues, and several types of cancers (CDC, 2022).

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Neurobiology of Ethanol Use in Humans and Rodents

Alcohol can affect the brain's communication pathways making it difficult for individuals to control balance, memory, speech, and judgment. Alcohol inhibits the release of signaling molecules for acetylcholine from the cortex due to its inhibition of voltage-dependent ion channels. N-methyl-D-aspartic acid (NMDA) is a neurotransmitter that is recognized as a central neurotransmitter to alcohol's actions. It inhibits the flow of positively charged ions through the cell membrane. Dopamine is another neurotransmitter that takes prominence in the neurobiology of alcoholism because alcohol activates dopaminergic reward pathways and is linked to being released in the nucleus accumbens (Sullivan et al., 2010).

In humans, alcohol can affect four different reward circuits and neurotransmitter systems including; dopamine systems, opioid systems, y-Aminobutyric Acid System, and glutamate systems. The dopamine system is primarily involved in a circuit called the mesolimbic system which extends from the brain's ventral tegmental area to the nucleus accumbens. The mesolimbic system influences how individuals orient toward incentive changes in the environment which can play a role in rewarding motivation of acute alcohol intoxication. The consumption or anticipation of alcohol can release dopamine in the nucleus accumbens. When individuals that are dependent on alcohol experience withdrawal, there is a decrease in dopamine function which contributes to withdrawal symptoms and alcohol relapse (Ryan et al., 2019)..

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In the opioid system, scientists have hypothesized that positive alcohol reinforcement is mediated by the release of endogenous opioids in the brain (Ryan et al., 2019). This system influences alcohol-drinking behavior both via interaction with the mesolimbic system and the extracellular endorphin content in the nucleus accumbens. This causes opioid receptor

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antagonists to interfere with the alcohol-rewarding effects by acting on sites in the ventral tegmental area, nucleus accumbens, and central nucleus of the amygdala (Ryan et al., 2019).

In the Y-Aminobutyric Acid (GABA) System, alcohol can increase GABA activity in the brain by acting on GABA-release neurons and signal-receiving neurons to increase GABA release and facilitation of the GABA receptors. Chronic alcohol exposure can cause some brain regions to express genes that encode components of the GABA receptor (Ryan et al., 2019).

The Glutamate System is inhibited by alcohol and suppresses glutamate-mediated signal transmission in the central nucleus of the amygdala. This is affected by the altering functions of both NMDA receptors and metabotropic glutamate subtype 5 receptors. NMDA receptors for individuals with AUD play a role in neuroplasticity which contributes to hyperexcitability and craving during alcohol withdrawal (Ryan et al., 2019). Alcohol dependence is hypothesized to involve dysregulation of neural circuits involved in reward but also circuits that mediate behavioral responses to stressors (Ryan et al., 2019). The signaling molecule corticotropin-releasing factor (CRF) is involved in the hypothalamus, the body's major stress system, which contributes to alcohol withdrawal symptoms (Ryan et al., 2019). People with alcohol dependence have an increased extracellular CRF content in the central nucleus of the amygdala. (Ryan et al., 2019).

Studying the neurobiology of addiction in humans can help develop treatment for drug addiction; however, using human subjects can propose ethical and practical limitations. One concern is that the human brain cannot be examined in the same ways as animals' brains due to the ability to manipulate animal brains. However, human brains cannot be manipulated because of ethical reasons. Addiction research using human subjects proposes ethical and practical limitations. A limitation when using human subjects for substance use research is informed

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consent. It is questionable whether drug users can give informed consent due to the nature of addiction which might impede their comprehension or decision making. People are not informed that research protocols include policies for situations that require mandating reporting even if it will decrease the quality of the data. Another limitation that occurs in human studies is confidentiality. Researchers' try to protect the autonomy of participants; however, because of the nature of the research, it is difficult (Ryan et al., 2019). Nevertheless, addiction research can still be performed by using animal models to study the neurobiological effects of drugs. Preclinical models are important to study mechanisms of drug-induced behaviors to provide new knowledge that may reduce or reverse harmful effects that drugs cause to the brain. Animal models are more likely to construct validity when the model mimics the specific sign or symptom of a given disorder. The focus of animal models is to inform on which areas of the brain are affected that are validated across species (Nieto et al., 2021).

Various preclinical models have been used to study the neurobiological effects of ethanol on rodents using self-administration. Self-administration is widely used in preclinical addiction research because it has great face validity in which the neurochemical and neuroanatomical substrates involved in drug intake behavior are similar in rats and humans (Finn et al., 2008). Two types of self-procedures used to study ethanol are non-contingent and contingent. Non-contingent drug self-administration is orally distributed to laboratory animals using two bottles, one containing an aqueous solution of alcohol and the other containing water. (Finn et al., 2008). Contingent drug self-administration is used on a schedule or fixed ratio (Finn et al., 2008). There are different types of schedules for reinforcement such as fixed ratio schedules (FR), variable schedules (VR), progressive ratio schedules (PR), fixed interval schedules, and second-ordered schedules. A fixed ratio reinforcement schedules have a fixed number of responses until the

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individual can get the reinforcement (Killeen et al., 2009). A variable Ratio schedule is when the number of responses can vary widely for a given reinforcer (Killeen et al., 2009). Progressive Ratio Schedules are required by increasing the response for reinforcement delivery after successive sessions (Finn et al., 2008). Fixed Interval Schedules are when reinforcement occurs after a specific number amount of time. (Finn et al., 2008). The second-ordered schedule is defined as when a subject responds according to one schedule for a brief presentation of a stimulus, by responding to the stimulus, reinforcement is given according to another schedule of reinforcement. (Finn et al., 2008). This type of schedule for more complex behavioral sequences reflects the human drug intake situation and is well suited for cue-induced drug-seeking behavioral, neural, and neurochemical basis (Finn et al., 2008). The reinstatement model uses the second-order schedule to assess the properties of addictive behavior to test anti-craving and anti-relapse compounds. Animals are trained to self-administer alcohol by an operant response (on manipulandum), then the response of a non-contingent exposure to the drug or non-drug stimuli is used to reinstate drug seeking (Finn et al., 2008). In our study, we use the fixed ratio (FR) schedule and varied ratio 1-5 (VRF)

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Preclinical models the molecular and biological mechanisms of drug-related behavior and identify strategies that are useful for understanding human drug consumption. These methods avoid the risk of ethical issues, accidents, medical issues, and psychological issues that would be present in human studies (Woodward, J. J., & Ahmadi, S, 2017). However, there are some disadvantages in preclinical models. The main disadvantage is that animals that are non-human primates do not provide good predictive validity inhibiting treatment options (Woodward, J. J., & Ahmadi, S, 2017). Another disadvantage is that there is limited pre-clinical data that reports randomization, binding, or sample size calculation which are critical for designing clinical trials

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for human studies (Woodward, J. J., & Ahmadi, S, 2017) Rat self-administration is valuable when studying ethanol because they readily learn complex operant training schedules and are social animals (Woodward, J. J., & Ahmadi, S, 2017)

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Behavioral Economics of Substance Use

Commented [AK17]: Assessment of ethanol and nicotine interactions using a reinforcer demand modeling with grouped and individual levels of analyses in a long-access self-administration model
Christopher L. Robison, Nicole Cova, Victoria Madore, Tyler Allen, Scott Barrett, Sergios Charntikov
doi: https://doi.org/10.1101/2022.10.17.512519

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Behavioral economics applies psychology, economics, and pharmacology and its purpose is to distinguish decision-making mechanisms through psychology and economics to understand the rationale of individuals' decisions (Strickland and Lacy, 2020). The relationship of behavioral economics can be explained through economic demand, which measures the rate of consumption over the rand of prices (Robison et al., 2022). For example, in addiction studies, economic demand can be measured by the amount of money individuals are willing to spend on a drug. This model can demonstrate the individual levels of demand for certain drugs, therefore, individuals that spend more on drugs have a higher demand. In addiction research, behavioral economics can generate mathematical models to assess drug demand in animal trials (Strickland and Lacy, 2020). For example, some experimental models will evaluate the economic demand by assessing the quantity of lever presses for a given drug. The amount of lever presses indicates the rat's motivation to work/spend to access the drug; comparably to humans' motivation to spend money on certain drugs. Behavioral economics allows researchers to align their animal addiction research with humans. Previous research has shown that the economic demand model is strongly associated with addiction-like-behaviors in human and animal experiments (Bentley et al., 2014).

Self-Administration Procedure in Substance Use Research

In ethanol research, the self-administration procedure is typically used. During self-administration, rats are trained to make a response, via nose poke or press a lever, under a schedule of reinforcement. Once the specified reinforcement is met, the reinforcer (ethanol) is presented to the rat to consume (Lopez & Becker, 2014). In the training phase, rats are presented with a liquid reinforcer, such as sucrose. Once rats learn how to receive the reinforcement, ethanol would slowly fade in with a sucrose mixture until desired ethanol percentage is reached. Then sucrose percentage would fade until only ethanol is given to rats to consume (June & Gilpin, 2010). Self-administration procedure allows for appetitive and consummatory components that are essential for studying the motivational effects of substance use. In addition, drug concentration and schedule reinforcement enable the analysis of the reinforcing efficacy of a drug. In relapse models, the self-administration model is essential in measuring the behavioral response when the expected drug is delivered which demonstrates drug-seeking behavior (Lopez & Becker, 2014).

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Purpose of the Study

The purpose of this study is to use a pre-clinical model of ethanol use to understand the individual effects at the neurobiological level. In this study, we investigate the behavior and neurobiology of cue-induced ethanol reinstatement. Due to the high prevalence of people who consume ethanol and experience AUD, this study is necessary to help understand alcohol addiction and provide information to later help the development of treatment. The study consists of seven phases; sucrose fading, economic demand testing, negative consequence testing, elevated plus maze withdrawal testing, extinction, reinstatement, and c-Fos processing microscopy. Ethanol use is measured and analyzed using these tests to understand the factors that

contribute to the vulnerability of ethanol relapse: motivation, craving, and withdrawal of ethanol consumption. The c-Fos immunoreactivity testing is to help determine which areas of the brain are activated during reinstatement testing. This study hypothesizes that rats who obtain higher demand of ethanol will be more vulnerable to reinstatement. The results of this study will help us understand how the consumption of ethanol affects individuals differently based on responses during reinforcer demand testing, negative consequence testing, withdrawal testing, reinstatement, and expression of brain activity.

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Methods

Animals

Six male Wistar rats that ranged from 250-300g and six female Wistar rats ranged from 200-250g were purchased from Envigo (Indianapolis, IN, USA). Each rat was housed in a single-housed temperature vivarium on an inverse 12-hour light/dark cycle (lights turned on at 1900). All experimental procedures were conducted in the dark cycle. Rats had one week to acclimate in the colony in which they received free feed and water. Throughout the study, rats were food restricted to maintain 90% of their free-feeding weights with water ad libitum. All procedures and training were done following the Guide for the Care and Use of Laboratory Animals (National Research Council et al., 2010) under review and approval of the University of New Hampshire Institutional Animal Care and Use Committee.

Apparatus

Behavioral tests were conducted in Med Associates conditioning chambers measuring 30.5 x 24.1 x 21.0 cm (l x w x h). Each chamber was enclosed in a sound and light attenuating cubical

and equipped with an exhaust fan (ENV-018MD; Med Associates, Inc.; St. Albans, VT, USA). The chambers had aluminum sidewalls, metal rod floors, and all other surfaces were made of polycarbonate. Two nose pokes on the right side of the chamber that would operate the retractable sipper equipped with a licktometer. The nose poke hole (2.5 cm in diameter) contained yellow LED lights mounted inside, and the infrared beam monitored entry. Cue lights were above each of the nose pokes (ENV-252M; Med Associated, Inc; St. Albans, VT, USA). A house light (two white 28 V, 100 mA lamps) was located 10 cm above the conditioning chamber ceiling. The metal grid floor delivered foot shocks to the rats by using a Med Associated current aversion stimulation module. Med Associates network and software (Med-PC for Windows, version IV) were used to collect data and present programmed events.

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Preliminary Nose Poke Training

For preliminary nose poke training, rats were trained to drink 12% sucrose from a retractable sipper. The training sessions lasted 10 hours for two weeks on a fixed ratio (FR1) reinforcement schedule. At the start of each session, cue lights would turn on to indicate that there is access to sucrose/ethanol and the house lights would turn off. Once rats responded, the house light would turn on and cue lights would turn off.

Sucrose Fading Self Administration

Rats were trained daily through 10-hour sessions to self-administer ethanol by the sucrose fading procedure on a variable ratio (VR3) reinforcement schedule. Prior to the start of the session, all the lights were off and once the session started the cue lights turned on. Once sucrose ethanol concentration was earned, the house light turned on and cue lights turned off. Training began with 12% of sucrose solution for one week and then ethanol faded every three days by 2%, 4%,

8%, and 12%. For six days 12% ethanol and 12% sucrose are administered and then sucrose concentration is decreased every three days by 12%, 8%, 4%, and 2%. Once the fading procedure was finished, rats were administered 12% ethanol for the remainder of the study.

Economic Demand Testing

The cue lights worked in a similar procedure above. Subjects underwent demand testing in which the FR increased Each day by 1,5,8,12,18,26,38,58,86,130,195,292,438, and 657. Rats progressed through this daily schedule escalation until failing to earn at least one reinforcer. Once all rats finished demand testing, they could self-administer ethanol on a VR3 schedule for 10 days for 10-hour sessions.

Negative Consequence Testing

Rats were placed in the chamber for the 10-hour session where negative consequence testing occurred for the first two hours and the 8 hours followed normal ethanol self-administration on a VR3 reinforcement schedule. During this phase, 50% of the rewarded nose pokes were accompanied by concurrent foot shock lasting 0.5-s foot shock through the metal grid floor. For each test day, the shock intensity increased by 0.18, 0.24,0.30, 0.36, and 0.42 mA over 11 days with one rest day between each test.

Elevated Plus Maze

Elevated plus-maze tests were conducted using the elevated plus-shaped platform (Shoelting Co; Wood Dale, IL, USA; lane width =10 cm, arm length = 50cm, wall height = 40 cm, leg height = 40cm). Rats were acclimated in the test room for 60 minutes before the test was performed. One rat was placed inside the middle of the maze for 10 minutes and placed back in the vivarium after

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testing. ANY-maze software was used to collect data on the total time in open arms, total time in closed arms, and total freezing time. Data was collected for the last five minutes to allow the rats to acclimate to the maze. This test was conducted to test withdrawal after ethanol self-administration.

Extinction

After ethanol self-administration, the extinction procedure began with 10-hour sessions for 14 days. At the beginning of the session, cue lights were turned on and no ethanol was present. Response to nose pokes did not induce cues.

Cue-Induced Reinstatement

After extinction, cue-induced reinstatement occurred in a four-day phase. The cues were the same as self-administration. Ethanol was present for olfactory cues, but the response on the nose pokes did not retract the lixits. Rats were placed in the chambers at thirty-minute intervals.

Histological Assessment

After reinstatement testing, rats were euthanized with Euthasol (Virbac, Fort Worth, TX, USA) perfused with 150 mL 0.9% saline (NaCl, pH 7.4) followed by 150 mL 4% paraformaldehyde solution was dissolved in 0.3M PBS (pH 7.4). Subsequently, brains were collected and post-fixed in 4% paraformaldehyde for 24 hours at 4°C, followed by cryoprotection in 30% sucrose solution diluted in 0.02M PB stored at 4°C until brains sunk to the bottom. Brains were then dried and placed at –80 °C for preservation for future tissue collection.

Results

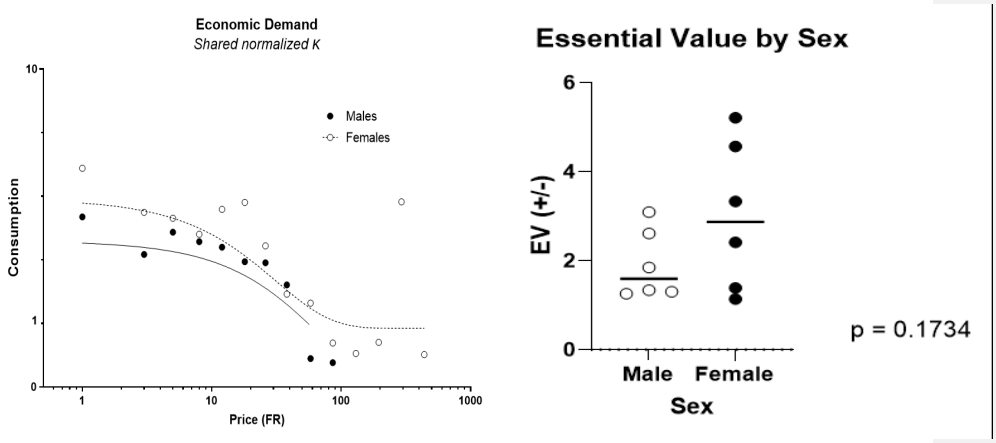

Figure 1: This graph plots the economic demand and essential value of ethanol consumption between sex. An unpaired t-test is performed on the group data (p- value=0.1734).

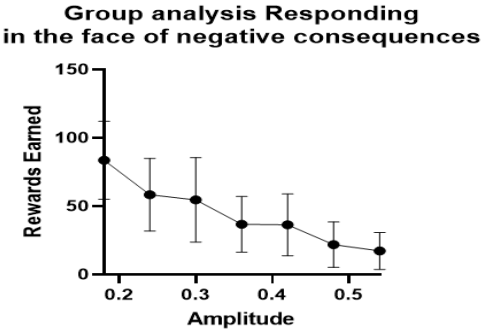

Figure 2: This graph presents testing the results of negative consequence testing between sexes. The y-axis is rewards earned (# of ethanol presentations retrieved) during the two-hour test and the x-axis is measured in the amplitude shocks administered. An unpaired t-test was done on the group data (p-value=0.001).

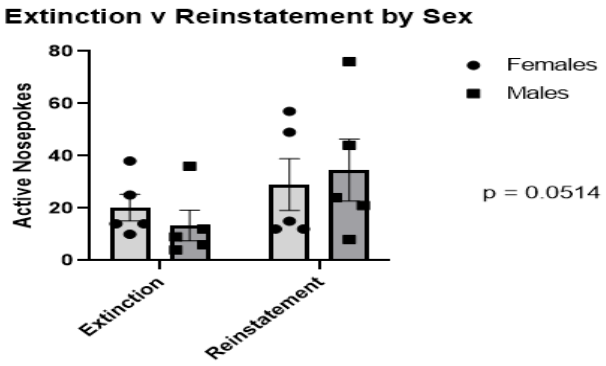

Figure 3: The bar graph shows the average number of nose pokes during the extinction phase verses during the reinstatement phase by sex. An unpaired t-test is performed on the group data (p- value=0.00514.

In this study, the goal is to identify the individual differences in ethanol consumption between males and females. During economic demand testing both male and female rats consumed more ethanol when the price was low; however, once the price increased, consumption decreased (Figure 1). As we look at the essential value by sex, there is no statistical significance due to having fewer subjects; however, a replication of the study is being performed where the data will be combined predicting the essential value to be significant. Looking at the essential value, there is a wide variation between each individual and by sex. Females had a wider variation and consumed more ethanol than males. During negative consequence testing, rats' consumption of ethanol decreased as the amplitude increased (Figure 2). There was no effect on sex, therefore the data was grouped. This data is statistically significant with a p-value being less than 0.001 (Figure 2). The extinction data was collected by taking the last 30 minutes of the last two sessions and averaging them to compare them to reinstatement (Figure 3). Comparing extinction and reinstatement testing, rats responded more after reinstatement (Figure 3). Overall, there were

no sex differences between extinction versus reinstatement rewards (Figure 3). However, there is wide variation among individuals for both males and females during reinstatement (Figure 3). This data is not statistically significant with a p-value of 0.0514; but a replication of the study is being performed where the data will be combined predicting the data to be significant (Figure 3). Currently, the results for c-fos expression are still being processed; but it is predicted that rats that had higher economic demand would have higher c-fos in certain brain regions associated with ethanol relapse and vice versa. The elevated plus maize data is currently being processed, it is predicted that rats with higher economic demand will show higher levels of anxiety when exposed to the elevated plus maize.

Discussion

Interpretation of results

There were many key findings when analyzing the results for economic demand, negative consequence testing, extinction, and reinstatement. A key finding from the economic demand data was that as the price increased, consumption decreased for both male and female rats. There was also a wide variation of the essential value of ethanol among individuals and sex. For negative consequence testing, there was a key finding in which as the amplitude increased, the rewards earned decreased. A key finding for extinction and reinstatement testing was rats had slightly more active nose pokes after reinstatement compared to extinction. There was also a wide variation between individual rats' responses after reinstatement compared to extinction. The anticipated results for the elevated plus maze are that rats that have a higher economic demand will have more anxiety when placed in the elevated plus maize. The anticipated results of the c-fos expression are that rats with a higher economic demand will have an increase of c-

fos expression in certain brain regions associated with relapse. From analyzing this data, the overall implications are that there are individuals' differences as well as sex differences in ethanol consumption. Individuals who place more value on ethanol are going to consume more even in the face of negative consequences; this in turn will influence their ability to relapse after a period of extinction due to their motivation towards ethanol.

Furthermore, the above results demonstrate the studies' strengths and limitations when comparing preclinical models to human models of ethanol use. One strength this study contains is its practical application to ethanol research and cue-induced reinstatement. As our results show, individuals who are motivated to consume more ethanol have a strong likelihood to relapse. This data can support the idea of recovering alcoholics who enter a bar in which they are more likely to relapse due to a history of high ethanol consumption and the cue of the bar environment. However, comparing pre-clinical models to humans is a limitation because in a lab environment, animals' brains can be manipulated and controlled whereas in human models they cannot. Another limitation of this study is that the data for economic demand, extinction, and reinstatement is not statistically significant which affects the validity of the data. Although, a replication of the study is being performed where more subjects will be combined with this data to make the data statistically significant.

Comparison with Previous Research

Behavioral economics is used widely for addiction of drug seeking in animal studies to understand human behaviors. In this study, economic demand and negative consequence testing tested rats' behavior and their motivation on how willing they are to consume alcohol. Other

studies have demonstrated the use of behavioral economics and the use of negative consequence testing for ethanol self-administration in which results were similar to our study. Studies have shown that as animals alcohol intake would decrease after the reinforcement schedule was changed from FR-1 to an intermittent schedule (Marchant et al., 2013). As for negative reinforcement testing, a study found that groups that were punished had decreased active lever presses over time compared to groups that were not punished. However, punishment with mild shock intensity at the beginning of the study had constant ethanol intake. (Marchant et al., 2013).

Other studies have shown similar results to understanding the neurobiological behavior and targeted brain regions of cue-induced reinstatement. Prior research has found that groups that undergo the extinction phase are more likely to relapse during cue-induced alcohol memory, whereas groups that were being treated for relapse of cue-induced memory present no alcohol seeking or ethanol consumption (Vena et al., 2020). Certain brain regions have been determined to affect cue-induced relapse and in our study were looking at the following regions: lateral hypothalamus, nucleus accumbens, basal lateral amygdala, prefrontal cortex, hippocampus, orbitofrontal cortex, ventral pallidum, ventral tegmental area. The lateral hypothalamus had been a target region for reinstatement in which it has been shown that inactivating the lateral hypothalamus via B/M infusions has prevented reinstatement after long periods of extinction (Marchant et al., 2009). Another study found that there was an increase in c-fos expression in the nucleus accumbens during the reinstatement of alcoholic beer-seeking (Marchant et al., 2015). This same study found that there was an increase expression in the basal lateral amygdala and lateral hypothalamus (Marchant et al., 2015).

Implications for Treatment and Prevention

The results of this study and other ethanol self-administration studies provide more understanding of ways to develop prevention and treatment for AUD. Understanding behavioral economics of alcohol use can help understand why certain individuals are more likely to relapse; therefore, it will help create preventative treatment. Studying ethanol self-administration in the face of negative consequence testing can help us understand how to prevent ethanol use. The results of our study for cue-induced reinstatement can help create treatment for individuals with AUD. Being able to look at specific brain regions that affect reinstatement is beneficial in the development of treatment that can be manipulated using medications.

Future directions

In addition to exploring treatment and prevention options for alcohol use disorder (AUD), future studies could focus on inhibiting specific brain regions such as the nucleus accumbens shell, prefrontal cortex, basal amygdala, hippocampus, lateral hypothalamus, orbitofrontal cortex, ventral pallidum, and ventral tegmental area to observe whether rats voluntarily seek ethanol and reinstate ethanol-seeking behavior. Based on the previous study, rats with high economic demand could be used to mimic people with AUD, where the specific brain regions could be inhibited to observe whether the rats seek out ethanol. Using preclinical models for ethanol cue-induced reinstatement help mimic a human model, but using rats with long-term drinking patterns would be better for mimicking people with AUD. It is important to note that not everyone who has AUD will have the same effects, as individual differences exist. Therefore, testing the individual differences of ethanol-seeking rats can help us better understand which parts of the brain are activated in rats with high or low economic demand. Understanding individual differences can also help us develop different treatments for people with AUD

because one treatment may not work for everyone, and understanding these differences can help tailor the treatment to everyone's needs.

Conclusion

The key findings of this study underscore the importance of understanding the neurobiological effects of ethanol use at the individual level for the development of effective treatments and prevention strategies for addiction and relapse. The study highlights individual and sex differences in ethanol consumption and motivation, as well as the limited effectiveness of negative consequences in deterring consumption in individuals with high demand. The economic demand paradigm was found to be a useful tool for understanding the reinforcing effects of ethanol, and the study suggests that rats with higher economic demand may have increased anxiety and increased c-fos expression in brain regions associated with relapse. However, the study also acknowledges the limitations of using pre-clinical models and emphasizes the need for further research to better understand the complex mechanisms underlying addiction and relapse. Future studies could inhibit specific brain regions to better understand ethanol-seeking and reinstatement behavior in rats with high economic demand or explore other individual-level factors. Overall, the study highlights the importance of individualized approaches to addiction treatment and prevention, recognizing that one-size-fits-all interventions may not be effective for all individuals. By understanding the neurobiological effects of ethanol use at the individual level, researchers can develop more targeted and effective treatments that consider the complex and individualized nature of addiction.

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