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Effects of a Synthetic Cannabinoid on the Reinforcing Efficacy of Ethanol in Rats

Ericka M. Bailey
Utah State University

EFFECTS OF A SYNTHETIC CANNABINOID ON THE REINFORCING EFFICACY OF SELF-ADMINISTERED ETHANOL IN RATS

by

Ericka M. Bailey

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ABSTRACT

Effects of a Synthetic Cannabinoid on the Reinforcing Efficacy of Ethanol in Rats

by

Ericka M. Bailey, Master of Science

Utah State University, 2007

Major Professor: Dr. Amy L. Odum
Department: Psychology

The co-abuse of alcohol and marijuana is widespread, although the mechanisms underlying this behavior are unclear. There is some evidence of a relationship between the neural processes that mediate the effects of ethanol and marijuana. For example, research has shown that exposure to marijuana increases responding for, and intake of, ethanol. The alcohol deprivation effect is an animal model of alcoholism that suggests that the reinforcing efficacy of ethanol, as measured by intake, increases following a period of deprivation. Recent research indicates that rats chronically exposed to marijuana during periods of alcohol deprivation consume ethanol above and beyond deprivation alone. It is unclear, however, whether the marijuana exposure or the repeated deprivations increased motivation to consume ethanol. In the present experiment, rats were trained to self-administer ethanol on a progressive ratio schedule and subjected to

two separate periods of deprivation during which either drug or saline was chronically administered for 7 days. Breakpoint (i.e., last ratio completed) was recorded as a measure of the reinforcing efficacy of ethanol. Following deprivations, breakpoint was initially lower than baseline, regardless of whether the drug or saline was administered. Breakpoint recovered to, but did not exceed, baseline levels following both deprivations, indicating a lack of increased reinforcing efficacy of ethanol after repeated deprivation or chronic exposure to marijuana. The lack of an expression of an alcohol deprivation effect following deprivation may have been due to the length and number of deprivations employed. Furthermore, lowered breakpoint recorded following chronic drug administration during deprivation may have been due to the dose administered or stress generated by chronic injections. Further investigation is necessary to separate and clarify the variables responsible for the present results.

(62 pages)

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Ericka M. Bailey

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INTRODUCTION

Individuals who abuse one drug often abuse a second drug at or near the same time. The abuse of both marijuana and alcohol is especially common. Alcohol is easily obtained, particularly if one is at or over the legal age of 21. Although marijuana is illegal, it is easily obtained relative to other illicit drugs such as cocaine or heroin. The number of persons admitted to inpatient treatment for drug addiction who report the use of marijuana with alcohol is sufficiently high to warrant investigation into the processes underlying the co-abuse of the two drugs.

In experimental settings, marijuana exposure increases ethanol consumption in nonhumans. Exposure to marijuana has also been shown to instigate relapse to drinking in subjects who have been alcohol deprived for a period of time. The process mediating the increase in motivation to seek or consume alcohol, however, is unclear.

The behavioral and neural changes resulting from chronic exposure to ethanol have been investigated, implicating the involvement of neurotransmitter receptor systems, including the glutamatergic and GABAergic systems. Chronic ethanol exposure results in neuronal changes instigated by the brain in an attempt to maintain homeostasis. It is not known, however, if or how these neuronal changes may affect marijuana's ability to increase motivation to consume alcohol.

Animal models of drug abuse suggest the participation of neural systems mediating drug abuse. Observations of behavior indicate an increase in the sensitivity to the effects of self-administered drugs following marijuana use. Subjects exposed to

marijuana prior to or in conjunction with self-administration of a drug respond for the drug sooner as well as consume more of the drug than subjects not exposed to marijuana.

The endocannabinoid system in the brain is responsible for mediating the effects of marijuana. The active component in marijuana binds to the numerous cannabinoid receptors found mainly in the central nervous system. An internally produced cannabinoid receptor agonist normally activates these receptors and is responsible for mediating appetite, perception, and memory. The cannabinoid agonist has also been shown to increase levels of dopamine, a neurotransmitter involved in the rewarding properties of drugs of abuse. Marijuana, or its psychoactive components, mimics the effects of the internal cannabinoid and can substantially alter behavior.

Exposure to marijuana can result in behavioral sensitization. After repeated exposure, smaller doses of the drug produce readily observable effects on behavior. Marijuana and ethanol have both been shown to induce behavioral sensitization in humans and nonhumans. Behavioral effects include slowed locomotor activity and repetitive or nonpurposeful movement. Long-term exposure to marijuana can also result in sensitization of the endocannabinoid system that mediates the effects of the drug. Specifically, relapse and/or increased intake of alcohol may result from changes in neuronal activity due to prior exposure to marijuana.

Using human participants to investigate the underlying relation between sensitization and drug abuse is difficult and costly, as well as potentially ethically undesirable. The alcohol deprivation model is an animal model of drug abuse in which animal subjects engage in drug-related behavior similar to that exhibited by humans,

including acquisition of stable drug self-administration as well as relapse. Experiments conducted with animal subjects in a controlled laboratory setting enable precise measurement of certain behavioral aspects of drug use.

One behavioral measure is the reinforcing efficacy of a drug. Reinforcing efficacy is measured by how much effort an organism puts forth to receive the drug. A progressive ratio (PR) schedule of reinforcement measures the effort expended by requiring an increasing number of responses to obtain the drug. Progressive ratio schedules can also be used to study the effects of one drug on the reinforcing efficacy of another drug.

Few studies have investigated how exposure to marijuana affects subsequent alcohol-related behavior. This experiment investigated the role of chronic exposure to marijuana on the reinforcing efficacy of self-administered ethanol. Previous research has indicated that administration of a cannabinoid during alcohol deprivation results in an increase in responding for alcohol as well as an increase in alcohol consumption. It is unclear, however, whether the increased reinforcing efficacy of alcohol was due to exposure to the cannabinoid or to the repeated alcohol deprivation. In the present experiment, rats were trained to self-administer ethanol under a PR schedule of reinforcement. The reinforcing efficacy of ethanol was measured following baseline responding, a period of ethanol deprivation paired with chronic administration of a cannabinoid receptor agonist, and a period of ethanol deprivation paired with chronic administration of the drug vehicle. Subjects were exposed to the deprivation conditions in different orders. Within-subject behavior following each period of deprivation was measured and compared with the previous baseline. It was expected that the reinforcing

efficacy of ethanol, as measured by breakpoint, would increase following deprivation and furthermore that chronic exposure to a cannabinoid receptor agonist would increase the reinforcing efficacy of ethanol above deprivation alone.

REVIEW OF THE LITERATURE

Polydrug Abuse

According to the 2003 National Survey on Drug Use and Health (Substance Abuse and Mental Health Services Administration, 2005), 21.6 million Americans were categorized as clinically dependent on, or abusing, drugs. Of these persons, 4.2 million abused marijuana, and 3.1 million reported being dependent on both alcohol and some illicit drug. The Community Epidemiology Work Group (CEWG, 2005) has reported widespread polydrug abuse in the United States. Polydrug abuse is gaining prevalence partly due to the availability and low cost of some illegal drugs. Marijuana continues to be the most used illicit drug and a large number of persons who abuse it report simultaneous abuse of alcohol. In 2000, state drug treatment facilities reported that patients who abused marijuana were more likely to also abuse alcohol than any other drug (CEWG). According to the Substance Abuse and Mental Health Services Administration (SAMHSA), among persons admitted into drug treatment facilities for polydrug abuse in 2002, alcohol (76%) and marijuana (55%) were the most commonly abused drugs.

Marijuana is often easier to obtain than other drugs of abuse partly due to its reputation as being less harmful than drugs such as cocaine, heroin, or even alcohol (Raphael, Wooding, Stevens & Connor, 2005). It is still an illegal substance, however, with the possibility of negative consequences attached to its use. The factors underlying

the polydrug abuse of marijuana and alcohol appear to be more complex than solely availability. Therefore, the increase in the abuse of marijuana, particularly with alcohol, has led researchers to investigate the possibility that neurological mechanisms, among other processes, play a role in polydrug abuse of this nature.

Humans who use marijuana and alcohol have reported that taking the drugs at or near the same time results in an additive drug effect that is more pleasurable than when either drug is taken alone (Lukas & Orozco, 2001). This effect could in part be accounted for by alcohol's ability to reduce or eliminate the negative subjective effects of marijuana, like paranoia. Marijuana may also alleviate nausea caused by increased alcohol consumption (Lukas & Orozco).

Experiments using animal subjects in a controlled setting have shown similar additive effects of marijuana and alcohol. Dar (2000) administered median doses of ethanol (1.0 g/kg) and the psychoactive component in marijuana, delta-9-tetrahydrocannabinol (THC, 15 μg/kg), to rats to investigate the effects on motor coordination. Co-administration of ethanol and THC severely impaired coordination while the same dose of either THC or ethanol alone had no effect. While concurrent administration of marijuana and alcohol is of interest, it is also important to understand how the consumption of one drug affects the consumption of another drug taken later.

The Effects of Cannabinoid Exposure

Several studies have shown that animals exposed to synthetic cannabinoid compounds (e.g., WIN 55,212-2 and CP 55,940) respond at an increased rate for alcohol as well as consume more alcohol. For example, Colombo et al. (2002) showed an increase in ethanol self-administration by selectively bred alcohol-preferring rats following acute administration of CP 55,940. Gallate, Saharov, Mallet, and McGregor (1999) also showed that the motivation for beer in rats increased following acute administration of CP 55,940.

Cannabinoids have also been shown to affect other aspects of drug self-administration. Acute exposure to cannabinoids (e.g., THC, WIN 55,212-2 and CP 55,940) has been shown to increase levels of responding for drugs of abuse as well as the amount of the drug consumed following periods of extinction. In one study (Fattore, Spano, Cossu, Deiana, & Fratta, 2003) rats were trained to self-administer heroin. This behavior was then put on extinction for 21 days. Priming injections of median doses of WIN 55,212-2 (0.3 mg/kg) or CP 55,940 (0.05 mg/kg) increased heroin-seeking behavior and consumption to levels substantially above pre-extinction levels. Acute administration of THC has also been shown to restore alcohol-seeking behavior following extinction (McGregor, Dam, Mallet, & Gallate, 2005). Rats were trained to self-administer beer and this behavior was subsequently put on extinction. A median dose of THC (1.0 mg/kg) reinstated responding for the alcohol to significantly higher levels compared to responding during extinction.

Early research showed that periods of alcohol deprivation increased responding for ethanol when it was available again (Sinclair & Senter, 1967). Cannabinoid pre-exposure further increases responding for ethanol following periods of alcohol deprivation. Lopez-Moreno and colleagues (Lopez-Moreno, Gonzalez-Cuevas, Rodriguez de Fonseca, & Navarro, 2004) showed that rats chronically exposed to WIN 55,212-2 during 5 days of ethanol deprivation significantly increased their responding for ethanol above and beyond that after deprivation alone. Additionally, following

administration of 2.0 mg/kg or 10.0 mg/kg, responding at the end of the second week after deprivation was greater than after the first week suggesting that chronic administration of moderate or higher doses of cannabinoids extends the effects of alcohol deprivation. Together, these experiments suggest that cannabinoids increase the motivation to consume other drugs, including alcohol.

Some conflicting results have also been found with regard to exposure to cannabinoids on responding for alcohol. In one experiment, a high dose of THC (10.0 mg/kg) administered acutely to rats prior to ethanol self-administration sessions decreased the amount of ethanol consumed compared to control (McMillan & Snodgrass, 1991). The authors also reported that chronic administration of a range of doses (3 mg/kg to 30 mg/kg) decreased ethanol intake. If subjects had not received THC within 24 hours of a test session, however, ethanol intake was significantly higher than baseline. These results may reflect the well-documented biphasic effects of cannabinoids on motoric functioning or consummatory behavior. THC has been shown to increase activity at

lower doses (0 mg/kg to 3.0 mg/kg), while higher doses (5.0 mg/kg and higher) have been shown to initially retard functioning only to have it return some time after administration (e.g., Stark & Dews, 1980). Evidence of the biphasic effects of cannabinoids indicates that a high dose of THC administered soon before a session could decrease ethanol intake as well as motoric function. That is, motoric effects could be confounded with measures of consumption. Overall, the effects of marijuana on alcohol consumption highlight the need for an overarching explanation of the process underlying the effects of marijuana on later drug use, including alcohol use. Therefore, the effects of chronic ethanol consumption are initially discussed, followed by a review of neurological and behavioral effects of the psychoactive agent in marijuana. Then, the relationship between ethanol and cannabinoids at the neural level is examined.

Neurological Effects of Chronic Ethanol Exposure

Research suggests that the glutamate and gamma-aminobutyric acid (GABA) neurotransmitter and receptor systems play the most important roles in the neuronal and behavioral changes following chronic alcohol consumption (see Fadda & Rossetti, 1998, for review). Glutamate is the most widespread excitatory neurotransmitter in the brain and is responsible for rapid neurotransmission. Gamma-aminobutyric acid is the most widespread inhibitory neurotransmitter in the brain and effectively slows the "pace" of brain activity (e.g., Tsai, Gastfriend, & Coyle, 1995). Alcohol acts as an indirect antagonist on glutamatergic receptors and an indirect agonist on GABAergic receptors (e.g., "Alcohol, the Brain, and Behavior: Mechanisms of Addiction," 2000). Therefore,

when alcohol is chronically administered, the brain's effort to maintain homeostasis results in the alteration of receptor expression to balance the rate of neurotransmission (Kelly, 1995). Chronic alcohol consumption results in a decrease in activated GABA receptors (i.e., downregulation) and an increase in activated glutamate receptors (i.e., upregulation; Heinz, Schafer, Higley, Krystal, & Goldman, 2003).

The Endocannabinoid System

A large body of neuropharmacological research has shown that the psychoactive agent in marijuana, THC, is activated when it binds to cannabinoid (CB_1) receptors concentrated in the central nervous system. Tetrahydocannabinol, as well as synthetic cannabinoid agonists like CP 55,940, is a receptor agonist. It mimics the effects of the endogenous cannabinoid, anandamide, when it binds to cannabinoid receptors (Felder & Glass, 1998; Hungund & Basavarajappa, 2004). Anandamide is responsible for the regulation of several brain functions including appetite, memory, and motor control (Felder & Glass) as well as the process of dopamine transmission. Dopamine is a neurotransmitter that has been linked to the rewarding and motivational properties of reinforcers such as food and sexual activity (e.g., Di Chiara, 1995) as well as drugs of abuse (e.g., Chaperon & Thiebot, 1999). When cannabinoid receptor agonists come into contact with CB_1 receptors, the level of dopamine transmitted is increased above the level normally transmitted following anadamide binding (Chen et al., 1990). Additionally, the effects of cannabinoid receptor agonists on memory, appetite, perception, and so forth, are increased above anandamide-induced effects.

Interaction of the Endocannabinoid System and Ethanol

Studies have suggested that the endocannabinoid system mediates the effects of ethanol. Hungund, Szakall, Adam, Basavarajappa, and Vadasz (2003) showed that mice genetically altered such that they lacked CB_1 receptors (CB_1 knockout mice) consumed substantially less alcohol than their wild-type littermates. CB_1 knockout mice provide a model of drug-related behavior without the influence of the endocannabinoid system. Additionally, microdialysis measures showed no increase in dopamine release in the nucleus accumbens (NAcc; an area of the brain implicated in a major dopamine pathway) that generally follows ethanol administration indicating that the reinforcing efficacy of ethanol may be mediated by the endocannabinoid system. Thanos and colleagues also investigated ethanol consumption using CB_1 knockout mice (Thanos, Dimitrakakis, Rice, Gifford, & Volkow, 2005). Two groups of mice (knockout mice and wild-type mice) were given access to both water and an ethanol solution in their home cages for 4 weeks. Once baseline intake of both fluids was determined, the CB_1 receptor antagonist SR141716A (Rimonabant) was administered. Receptor antagonists function by binding to cannabinoid receptors and blocking cannabinoid receptor agonists (including anandamide) from binding to the receptor sites. Results showed that wild-type mice consumed more ethanol during baseline training phase than CB_1 knockout mice and that the administration of Rimonabant significantly decreased ethanol consumption in wild-type mice but not CB_1 knockout mice. These results indicate that the deactivation of CB_1 receptors by receptor antagonism decreases the reinforcing efficacy of self-administered ethanol. Additional studies have examined the effects of Rimonabant on ethanol self-

administration and found that the antagonist substantially decreases ethanol intake in alcohol-preferring rats (Colombo et al., 1998).

Neural Sensitization

The ability of a cannabinoid receptor antagonist to decrease alcohol consumption indicates an important relationship between alcohol and the cannabinoid system. Newman, Lutz, Gould, and Domino (1972) demonstrated that rats made tolerant to the behavior attenuating effects of THC during a shock-avoidance procedure also rapidly became tolerant to the sedative effects of alcohol during the same procedure. Basavarajappa and Hungund (1999) showed that human neuroblastoma cells chronically exposed to ethanol increased production of anandamide. Furthermore, administration of Rimonabant to the same cells ceased all production of anandamide. These results suggest that the activity of endogenous cannabinoids and the receptors that they bind to is facilitated by the presence of ethanol.

When cannabinoids are administered, they mimic the effects of anandamide, but also alter the level of dopamine normally activated by anandamide. Changes in behavior have been associated with changes in the level of dopamine release (see Chaperon & Thiebot, 1999, for a review). The abuse of marijuana has also been linked with changes in CB_1 receptor activation.

Repeated exposure to a cannabinoid can cause a decrease in the number of receptors to which the cannabinoid can effectively bind, resulting in tolerance to the effects of cannabinoids (Romero et al., 1998). Early research with THC demonstrated

that doses as large as 36 mg/kg had no effect on food-reinforced responding in pigeons following repeated administration of increasing drug doses (McMillan, Harris, Frankenheim, & Kennedy, 1970). Additionally, research has shown that chronic ethanol exposure in laboratory mice can result in the downregulation of CB_1 receptors (Basavarajappa, Cooper, & Hungund, 1998). Following ethanol exposure by inhalation for 4 days, administration of CP 55,940 showed substantially decreased drug/receptor binding activity although the binding affinity of the remaining receptors was not affected. Downregulation of CB_1 receptors could affect consumption of alcohol following cannabinoid administration. As the neural processes mediating marijuana and alcohol abuse are at least peripherally connected, then decreasing the number of binding sites for cannabinoids could lead to an increase in motivation for alcohol.

Evidence of neural sensitization has also been found following high dose chronic exposure to THC (5 mg/kg to 40 mg/kg). Rubino, Vigano, Massi, and Parolaro (2003) demonstrated through autoradiographic brain imaging that cannabinoid receptors in certain areas of the brain were functionally altered even 3 weeks after the last dose of THC. Brain imaging showed an increase in CB_1 receptor binding of the administered cannabinoid (CP 55,940) in two areas known to have very high CB_1 receptor densities (i.e., the cerebellum and caudate putamen). As the two areas are known to be involved in motor functioning, it is clear that changes occurring in these areas could result in observable changes in behavior due to sensitization.

Behavioral Sensitization

It has been suggested that the mechanisms involved in the expression of the effects of a drug become "plastic" with repeated administration (e.g., Stewart & Badiani, 1993). That is, biological and neurobiological changes occurring as a result of chronic drug administration most likely play a role in the effects of the drug expressed behaviorally. When chronic administration of a drug ceases for a period of time, behavior following a smaller dose will often resemble previous behavior following a larger dose. Diana, Melis, Muntoni, and Gessa (1998) demonstrated the plastic nature of neural systems involved in the expression of the effects of cannabinoid agonists and antagonists. They showed that the administration of Rimonabant to rats that had been chronically exposed to THC resulted in behavior indicative of "withdrawal" (i.e., facial rubbing, wet dog shakes, licking, etc.). Additionally, electrophysiological recording revealed a decrease in dopamine activity in the NAcc. Subsequent administration of THC ceased withdrawal-induced behavior and increased dopamine activity. When Rimonabant was administered to control rats (no THC exposure), they showed no behavioral signs of withdrawal and dopamine activity was not affected. These results suggest that chronic exposure to a cannabinoid sensitizes the cannabinoid receptors so that they are more susceptible to the effects of a cannabinoid antagonist.

Much of the research on behavioral sensitization has focused on measuring increases in locomotor activity and/or stereotypy following the administration of a psychomotor stimulant. Rubino, Vigano, Massi, and Parolaro (2001) chronically administered THC (5.0 to 40.0 mg/kg) to rats. Following a period during which no drugs

were administered, a challenge dose of 5.0 mg/kg was administered to all subjects. Rats exposed to THC spent substantially more time engaged in stereotyped behaviors (e.g., licking, gnawing, purposeless confined area sniffing) than those that initially received vehicle. Similar results were found with rats using smaller doses of THC (2.0 to 8.0 mg/kg; Cadoni, Pisanu, Solinas, Acquas, & Di Chiara, 2001). The indication that behavioral changes can occur following the administration of a relatively small amount of a cannabinoid is important, especially with regard to human marijuana use. For example, it may be possible for a short exposure to, or a small amount of, a cannabinoid agonist to substantially affect immediate as well as long-term behavior.

Animal Models of Relapse

Animal models of relapse have been widely used for some time (Carroll & Comer, 1996). Based on the data obtained during animal studies, inferences can be made regarding human drug abuse. For example, the alcohol deprivation model provides evidence for the restoration of previously maintained alcohol self-administration following a period of alcohol deprivation (Sinclair & Senter, 1967). Self-administration is recovered by allowing access to alcohol. Further, animals that have been deprived of alcohol following reliable alcohol self-administration consume substantially greater amounts of alcohol soon after it has been made available again than animals that have had continuous access to alcohol.

The reinstatement model has also been shown to parallel aspects of human relapse, including the ability of a relatively small drug dose to instigate a return to drug-

related responding (Carroll, 1998). Reinstatement refers to behavior initially maintained by drug self-administration that is then extinguished and restored later by administering the same drug or a drug from a different pharmacological class. The ability of a drug to reinstate responding following extinction was first demonstrated by Stretch and Gerber (1973). Self-administration of *d*-amphetamine by monkeys was extinguished and subsequently reinstated with priming doses of *d*-amphetamine immediately prior to test sessions. In a later experiment, Gerber and Stretch (1975) showed that different drugs from the same pharmacological class could reinstate self-administration. They trained squirrel monkeys to self-administer cocaine, followed by extinction sessions in which cocaine was replaced with saline. During reinstatement testing, *d*-amphetamine was administered immediately before the beginning of a session in which only saline was available. *d*-Amphetamine dose-dependently increased response rate and number of saline infusions per session for cocaine well above extinction rates.

Drugs from one pharmacological class have also been shown to reinstate behavior originally maintained by drugs from a different pharmacological class. de Wit and Stewart were the first researchers to demonstrate this phenomenon with drug self-administration (de Wit & Stewart, 1981). They showed that acute injections of amphetamine, morphine and apomorphine produced dose-dependent increases in responding during extinction sessions following cocaine self-administration. Reinstatement of responding following injections of morphine and apomorphine was not as high as following amphetamine. The fact that drugs known for their depressant behavioral effects were able to reinstate behavior initially maintained by a stimulant

suggested that neural systems mediating drug effects might be interacting (see Corchero, Manzanares, & Fuentes, 2004, for a review). de Wit and Stewart proposed that the mechanism underlying some aspect of reinstatement might be that the drugs shared neural sites of action. Therefore, administration of one drug could affect the value of a second drug. One way to measure the value of a drug as a reinforcer is by using a PR schedule.

Progressive Ratio Schedule of Reinforcement

A PR schedule of reinforcement is similar to a fixed ratio (FR) schedule in that a reinforcer is presented following a set number of responses. The PR schedule differs from the FR by requiring an increasing response output within the session to receive reinforcement (Hodos, 1961). The PR schedule was designed to distinguish the reinforcing strength or efficacy of a reinforcer from the rate of responding for the reinforcer (see Richardson & Roberts, 1996; Stafford, LeSage, & Glowa, 1998, for reviews). For example, if the concentration of a self-administered drug is increased, the rate of drug intake (directly related to responding) decreases even though the overall amount of the drug consumed may be high. A drug may have value for an organism, but pharmacological effects can be such that high rates of responding are difficult or impossible. Therefore, response rate does not necessarily reflect reinforcing efficacy.

In a PR schedule, reinforcer efficacy is determined by the measure of "breakpoint." Breakpoint is defined by the last ratio completed before the subject stops responding (under specified criteria) or the session time is up. Breakpoint is sensitive to

both reinforcer magnitude and the level of motivation related to obtaining the reinforcer (Hodos & Kalman, 1963; Solinas & Goldberg, 2005). Therefore, the reinforcing efficacy of the drug is measured as opposed to direct pharmacological effects on behavior.

Progressive ratio schedules used in experiments investigating ethanol self-administration often employ an arithmetic increase in ratio size (Gomez & Meisch, 2003; Rodd et al., 2003, for example). This type of an increase may be desirable over the exponential increase proposed by Roberts and Bennett (1993) as such marked increases in work requirement from one ratio to the next might result in the inability of the subjects to respond fast enough to consume pharmacologically effective doses of ethanol in an appropriate amount of time (i.e., before it is metabolized). Therefore, a small increase in step size facilitates self-administration of an effective amount of ethanol during a session. Additionally, work requirement continues to increase and breakpoint can be measured.

Progressive ratio schedules are widely used as a way to determine the reinforcing efficacy of a self-administered drug (see Stafford et al., 1998, for review). The measure of reinforcing efficacy often follows some kind of pre-exposure to a drug and can be used to test the effectiveness of a drug therapy for drug abuse or assess different variables involved in polydrug abuse. For example, a drug designed to attenuate responding for ethanol could be administered prior to a session in which ethanol is available under a PR schedule. A decrease in breakpoint compared with baseline measures would suggest that the drug decreased the reinforcing efficacy of ethanol.

Rationale for Present Experiment

Cannabinoid receptor agonists have been shown to alter subsequent drug-related behavior (e.g., Colombo et al., 2002). Both chronic and acute exposure to cannabinoid agonists increase responding for ethanol. Gallate et al. (1999) showed that acute administration of CP 55,940 increased the reinforcing efficacy of alcohol and a nonalcoholic fluid as measured by a PR schedule. Drug administrations, however, were separated by a single day in which no drug was administered. While the order of drug dose and vehicle was randomized, previous literature suggests that the effects of cannabinoid exposure may last longer than a day (Huestis, 2005). Thus, behavior measured following the second dose of CP 55,940 may have been influenced by the first dose administered.

The biphasic effects of cannabinoids have been well documented (e.g., Stark & Dews, 1980). Further, cannabinoids can produce a general increase in appetite soon after exposure. For example, Williams and Kirkham (2005) showed that the administration of a cannabinoid agonist increased the motivation for food in presatiated rats. Similarly, cannabinoid administration increases the motivation to eat sweet foods in humans (Mattes, Engelman, Shaw, & Elsohly, 1994). Therefore, in the present experiment, behavioral measurements did not occur during the acute effects of the cannabinoid agonist.

Chronic administration of a cannabinoid agonist during a period of alcohol deprivation has been shown to increase the reinforcing efficacy of ethanol when it is made available again. Lopez-Moreno and colleagues (2004) showed that when subjects

were allowed access to ethanol under an FR 1 schedule following an initial period of deprivation, the rate of responding increased substantially above baseline. A second period of deprivation was paired with chronic administration of a cannabinoid that resulted in an increase in responding that exceeded the response increase following deprivation only. Further, the increase in responding measured after cannabinoid exposure was longer lasting than deprivation only. The results, however, may have been due to an artifact of the experimental design rather than due to exposure to the cannabinoid agonist. That is, all subjects trained to self-administer ethanol were first exposed to a period of deprivation only. This period was followed by re-exposure to the ethanol self-administration procedure. Finally, all subjects were deprived of ethanol for a second time while receiving chronic injections of a cannabinoid. The authors concluded that changes in the CB_1 receptors due to chronic exposure to the cannabinoid were most likely responsible for the more robust and longer-lasting increase in responding for ethanol.

This explanation does not take into account, however, possible procedural sequence effects when conducting more than one experimental condition. One possible effect is that subjects have already experienced one condition prior to being exposed to the second, which could influence outcomes following exposure to the second condition (see Kazdin, 1982). Previous research has shown that ethanol self-administering rats subjected to multiple periods of alcohol deprivation increase responding for ethanol across successive deprivation periods (e.g., Rodd et al., 2003). Therefore, sequence

effects need to be controlled to provide an accurate assessment of the increase in the reinforcing efficacy of ethanol following chronic administration of a cannabinoid agonist.

Lopez-Moreno and colleagues (2004) used an FR 1 schedule to measure response rates following alcohol deprivation conditions and found increased responding for ethanol following alcohol deprivation paired with administration of a cannabinoid. Fixed ratios measure rates of responding, which do not necessarily reflect the reinforcing efficacy of a drug. That is, an FR 1 may measure ethanol consumption, but not the motivation to consume ethanol. For example, high rates of responding for a drug on an FR 1 schedule can occur even when the reinforcing efficacy of a drug is relatively weak. Progressive ratio schedules are a widely accepted measure of the reinforcing efficacy of a drug during self-administration procedures (see Richardson & Roberts, 1996, for review). Progressive ratios incorporate increasing work requirements for drug reinforcement, and the direct pharmacological effects of a drug on behavior are minimized during sessions. Therefore, the present experiment used a PR schedule to measure the reinforcing efficacy of ethanol following each deprivation condition.

The present experiment investigated the effects of the synthetic cannabinoid receptor agonist, CP 55,940 on the reinforcing efficacy of ethanol during a self-administration procedure. Synthetic cannabinoid agonists have been shown to affect behavior in the same or similar ways as the natural cannabinoid agonist, THC, and are more readily available for research purposes. Administration of the synthetic agonist, CP 55,940 has been shown to dose-dependently substitute for THC in discriminative stimulus tests (Wiley, Barrett, Lowe, Balster, & Martin, 1995). Additionally, rates of

responding under the effects of CP 55,940 did not differ from rates under the effects of THC. Behavior on a simple schedule of reinforcement was similarly affected following administration of four cannabinoid agonists, including THC and CP 55,940 (Carriero et al., 1998).

At this time, the effects of cannabinoids on the reinforcing efficacy of self-administered ethanol are unclear. Despite recent research the effect of cannabinoid exposure per se, above and beyond the effects associated with repeated alcohol deprivation, are not clear. Therefore, the present experiment examined the effects of chronic administration of the synthetic cannabinoid agonist CP 55,940 during a period of alcohol deprivation on the reinforcing efficacy of subsequently available ethanol.

STATEMENT OF THE PROBLEM

Alcohol and marijuana are frequently used in combination. An increasing number of studies have been addressing the hypothesis that the psychoactive component in marijuana increases consumption of many different drugs of abuse, including alcohol. The processes underlying this behavior are still unclear.

The endocannabinoid system has been thought to mediate the effects of alcohol for some time. Promotion or disruption of receptor activity in this system can alter the motivation to consume alcohol and change the amount consumed. Specifically, cannabinoid receptor agonists (e.g., THC, CP 55,940) have been shown to increase ethanol consumption (Colombo et al., 2002; Gallate et al., 1999). Further, the cannabinoid receptor antagonist Rimonabant substantially decreases ethanol intake in rats previously trained to self-administer ethanol (Freedland, Sharpe, Samson, & Porrino, 2001; Thanos et al., 2005).

Few studies have addressed the effects of chronic administration of a cannabinoid receptor agonist on the reinforcing efficacy of ethanol. Recent research, however, has shown that chronic exposure to a cannabinoid receptor agonist during alcohol deprivation can restore responding for alcohol above baseline performance (Lopez-Moreno et al., 2004). The process mediating the increase in motivation to consume ethanol following cannabinoid exposure is still unclear. The present experiment attempted to separate the effects of chronic administration of a cannabinoid agonist from the effects of multiple periods of alcohol deprivation on the reinforcing efficacy of ethanol. The alcohol deprivation model (an accepted animal model of drug relapse) and the PR schedule (a

widely used measure of the reinforcing efficacy of drugs of abuse) were used to examine whether the endocannabinoid system mediates the reinforcing efficacy of self-administered ethanol.

METHOD

Subjects

Eight experimentally naïve Long Evans rats served as subjects. Three subjects were removed from the study during ethanol self-administration training when they stopped responding for the ethanol solution. Rats were individually housed in a temperature- and humidity-controlled room on a 12-h light/dark cycle. Upon arrival, subjects were handled daily and were given free access to food in the home cages for 2 weeks followed by food restriction. Subjects were maintained at approximately 80% of their ad libitum weight (± 15 g), which was achieved by assessing weight daily and postsession supplemental feedings. Water was freely available in home cages throughout the experiment.

Design

The present experiment was conducted using counterbalancing procedures in which all subjects experienced two conditions and the order of exposure to each condition varied across subjects. This type of within-subject design was chosen to allow for assessment of the possible influence of sequence effects with regard to conditions. That is, each subject experienced all conditions in the experiment and therefore served as its own control (Keppel, 1991). Following baseline training, subjects were assigned to Group 1 or Group 2.

Apparatus

Eight Med Associates® operant conditioning chambers were used. Dimensions of chambers 1 through 4 were as follows: Each chamber was approximately 30 cm long, 24 cm wide, 29 cm high, and housed in a sound-attenuating cubicle. The front panel of each chamber was equipped with two response levers centered 13 cm apart. Each chamber contained a 28-V houselight at the top center of the front panel, a sonalert (2900 ± 500 Hz, 75-85 dB), a solenoid-operated dipper located between the two levers that delivered the liquid solutions, and light emitting diodes (LEDs) in a horizontal array of red, yellow, and green lights located above each lever. During dipper presentations, lever lights and houselights were darkened. A light inside the opening for the dipper activated during dipper presentations. Extraneous noise was masked by a chamber ventilation fan and white noise. Dimensions of chambers 5 through 8 were the same as 1 through 4, except that the height was 21 cm. Control of experimental events and data recording were conducted in an adjacent room with Med Associates® interfacing and programming.

Procedural Overview

Subjects were trained to respond for ethanol under a PR schedule. When responding was deemed reliable, subjects were matched into pairs based on their rank for breakpoint. Pairs were separated and assigned to Group 1 or Group 2 and the fifth unmatched subject was randomly assigned to Group 2. Matching was used to control the influence of the reinforcing efficacy of ethanol (measured by the last ratio completed)

between subjects prior to alcohol deprivation conditions. Group 1 was exposed to ethanol deprivation plus vehicle administration (condition A) first and ethanol deprivation plus cannabinoid administration (condition B) second. Group 2 was exposed to ethanol deprivation plus cannabinoid administration (condition B) first and ethanol deprivation plus vehicle administration (condition A) second. Both conditions involved twice daily injections of either vehicle or cannabinoid agonist for 7 consecutive days (i.e., chronic administration). The reinforcing efficacy of ethanol was measured after each deprivation period. On the third day following the last injection (cf. Lopez-Moreno et al., 2004), access to ethanol was made available again under a PR schedule.

Procedure

Ethanol Self-administration Training

Subjects were trained to self-administer ethanol using a fading procedure in which increasing amounts of ethanol are gradually added to a sucrose solution, while the sucrose is gradually faded out (Samson, 1986). All subjects were initially exposed to a variable time (VT) 60 s schedule to train rats to drink a 10% sucrose solution available during dipper presentation. Then, rats were trained to respond on a lever for the solution under a random-ratio 2 (RR 2) schedule of reinforcement in which the probability of each response resulting in reinforcement was .50 (see Lattal, 1991). At this time, only one lever in each chamber was programmed as "active." That is, responses on the active lever resulted in the presentation of the dipper containing 0.1 ml of a 10% sucrose solution, while responses on the inactive lever were recorded but had no programmed

consequences. The active lever was signaled by illumination of the LEDs above the lever. Responses on active and inactive levers were recorded for the entire experiment. Active levers were counterbalanced across subjects to minimize the possibility of the influence of a side bias. Immediately following rapid

responding under the RR 2, the ratio value was increased, usually during sessions until rapid, reliable responding occured on a RR 20 in which the probability of reinforcement following each response was .05. At this point, ethanol was slowly added to the sucrose solution, while the concentration of sucrose was decreased. The order of sucrose/ethanol concentrations was as follows: 10% sucrose in 0% ethanol v/v, 10% sucrose in 2% ethanol v/v, 10% sucrose in 5% ethanol v/v, 10% sucrose in 10% ethanol v/v, 8% sucrose in 10% ethanol v/v, 5% sucrose in 10% ethanol v/v, 3 % sucrose in 10% ethanol v/v, 1% sucrose in 10% ethanol v/v, 0% sucrose in 10% ethanol v/v. Sessions were 30 min in length and the training phase for ethanol self-administration took approximately three-and-a-half months.

Ethanol Self-administration under a PR Schedule

Following reliable responding (i.e., no increasing or decreasing trends detected by visual inspection) for the 10% ethanol, 0% sucrose solution under the RR 20 schedule, ethanol presentations (0.1 ml) were determined by lever presses under a PR schedule of reinforcement. Under the PR schedule, requirements for reinforcement (ethanol) were increased within session after each dipper presentation. An arithmetic ratio increase was used such that the values of the steps were 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, and so on. Each experimental condition consisted of a minimum of 15 sessions. Each session under the

PR schedule was programmed to end after 3 h or when 15 min had passed with no response on the active lever. Responding was considered stable in all conditions when the breakpoints during the final 5 sessions of a condition did not exceed or fall below the range of the breakpoints recorded during the previous sessions in the condition (e.g., the breakpoints for sessions
11 through 15 fell within the breakpoints recorded during sessions 1 through 10; Stafford & Branch, 1998).

CP 55,940 Exposure

During Condition B, subjects received intraperitoneal (i.p.) injections of CP 55,940 two times a day at approximately 8:00 a.m. and 8:00 p.m. for 7 days. 0.03 mg/kg CP 55,940 was administered in a volume of 1.0 ml/kg of the 80% free-feeding weight. This dose has been acutely administered in previous research and was found to increase breakpoint for responding for alcohol under a PR schedule (Gallate et al., 1999). All subjects were removed from their home cages for injections and returned immediately following the procedure. During condition A, subjects received an equivalent volume of vehicle administered in the same manner as the CP 55,940.

Drugs

CP 55,940 (Sigma) arrived as 10 mg solid that was suspended in dimethylsulfoxide (DMSO) and diluted in 0.9% saline. CP 55,940 was refrigerated between chronic administration periods. Ethanol solution was prepared with 95% ethanol,

distilled water and table sugar (when specified). Ethanol solutions were kept at room temperature during the entire experiment.

Dependent Measures

During the initial training sessions, the rate of responding as well as the intake of ethanol or sucrose solution was measured. Rate of responding was measured as the number of lever presses per minute. The number of presses on the active and inactive levers was recorded during the length of the experiment to assess the specificity of any observed effects. Ethanol intake was measured as the number of dipper presentations per session.

During PR schedule sessions, the breakpoint, or number of lever presses emitted to obtain ethanol, was defined as the last ratio completed that corresponded with the number of dipper presentations. Ethanol consumption was measured as g/kg determined by the g/kg of ethanol available in each 0.1 ml dipper (i.e., 0.00793 g/kg). Sessions automatically ended when no responses occurred on the active lever for 15 min. All sessions for each subject during the entire experiment were less than 3 hr in length and generally did not last more than 1 hr (cf. Gomez & Meisch, 2003; Solinas et al., 2005). Responding was considered stable when the breakpoints during the final 5 sessions of a condition did not exceed or fall below the range of the breakpoints recorded during the previous sessions in the condition.

RESULTS

Figure 1 shows the pre- and postdeprivation breakpoints for all rats that received chronic injections of CP 55, 940 during the periods of ethanol deprivation. The panels on the left show breakpoints for rats that received chronic injections of CP 55,940 during the first period of ethanol deprivation and the panels on the right show breakpoints for rats that received chronic injections of CP 55,940 during the second period of ethanol deprivation. Following the first and second deprivation periods, breakpoints were lower than the last breakpoint recorded during baseline. Breakpoint recovered to general baseline levels for all subjects by approximately the third session. No trend of increasing or decreasing breakpoint relative to baseline was seen during sessions 7 through 21. Once breakpoints reached approximate baseline levels, they remained relatively stable with one exception. Following the first deprivation, breakpoint for N67 was fairly variable through session 21.

Figure 2 shows the pre- and postdeprivation breakpoints for all rats that received chronic injections of vehicle during the periods of ethanol deprivation. The panels on the left show breakpoints for rats that received chronic injections of vehicle during the first period of ethanol deprivation and the panels on the right show breakpoints for rats that received chronic injections of vehicle during the second period of ethanol deprivation. Following the first deprivation period, breakpoints were lower than approximate baseline levels for the first several sessions. Postdeprivation breakpoints recovered quickly, however, and sessions 7 through 21 following deprivation showed no trend of increasing

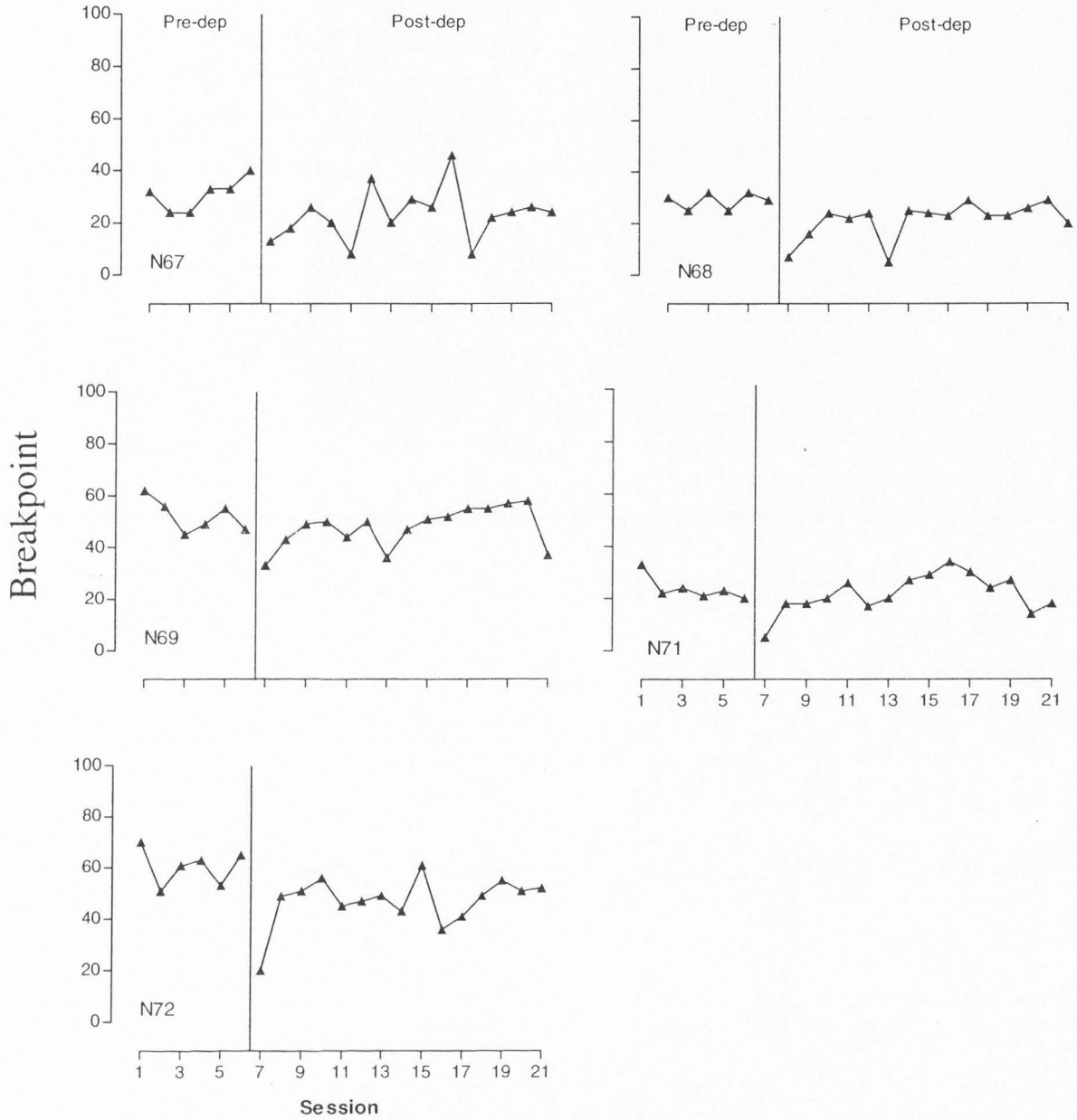

Figure 1. Breakpoint before and after deprivations during which CP 55,940 was chronically administered.

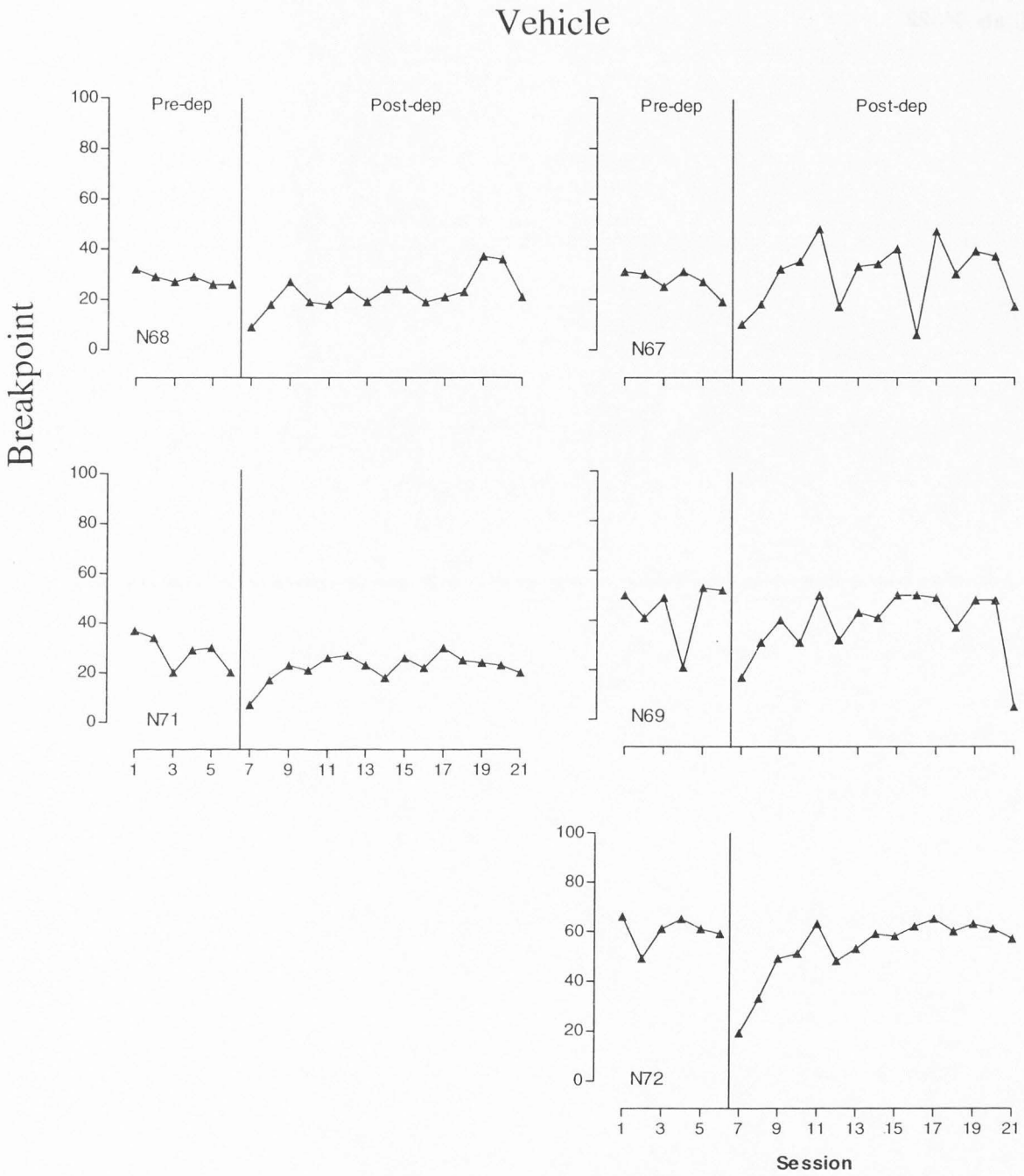

Figure 2. Breakpoint before and after deprivations during which vehicle was chronically administered.

or decreasing breakpoint relative to baseline. Performance for both subjects following the first deprivation was relatively stable with few exceptions of variability. Breakpoints following the second period of deprivation were initially lower than baseline levels. Once breakpoint recovered to approximate baseline levels, performance was relatively variable for subjects N67 and N69, but remained fairly stable for N72 through session 21.

Overall, breakpoint decreased relative to baseline for subjects that received chronic injections of CP 55,940 or vehicle during deprivation. Breakpoint was undifferentiated across periods of deprivation and injection type. The degree of decrease in breakpoint and the number of sessions breakpoint remained below baseline varied across subjects. Generally, once breakpoint had reached baseline levels, it remained relatively stable.

Figure 3 shows the mean amount of ethanol made available in g/kg following the first and second periods of ethanol deprivation. Mean alcohol delivery was calculated separately for subjects that received vehicle or CP 55,940. The top panel shows the mean ethanol delivery before and after the first period of ethanol deprivation. Baseline delivery shows that rats that were to receive CP 55,940 during deprivation earned more ethanol than rats that were to receive chronic injections of vehicle. Following deprivation, ethanol delivery decreased relative to baseline for all rats. The amount of ethanol earned postdeprivation by rats that received vehicle, however, was substantially lower than the amount earned by rats that received CP 55,940 during the deprivation period. Ethanol delivery recovered to approximate baseline levels within several sessions and was undifferentiated across CP 55,940 or vehicle exposure.

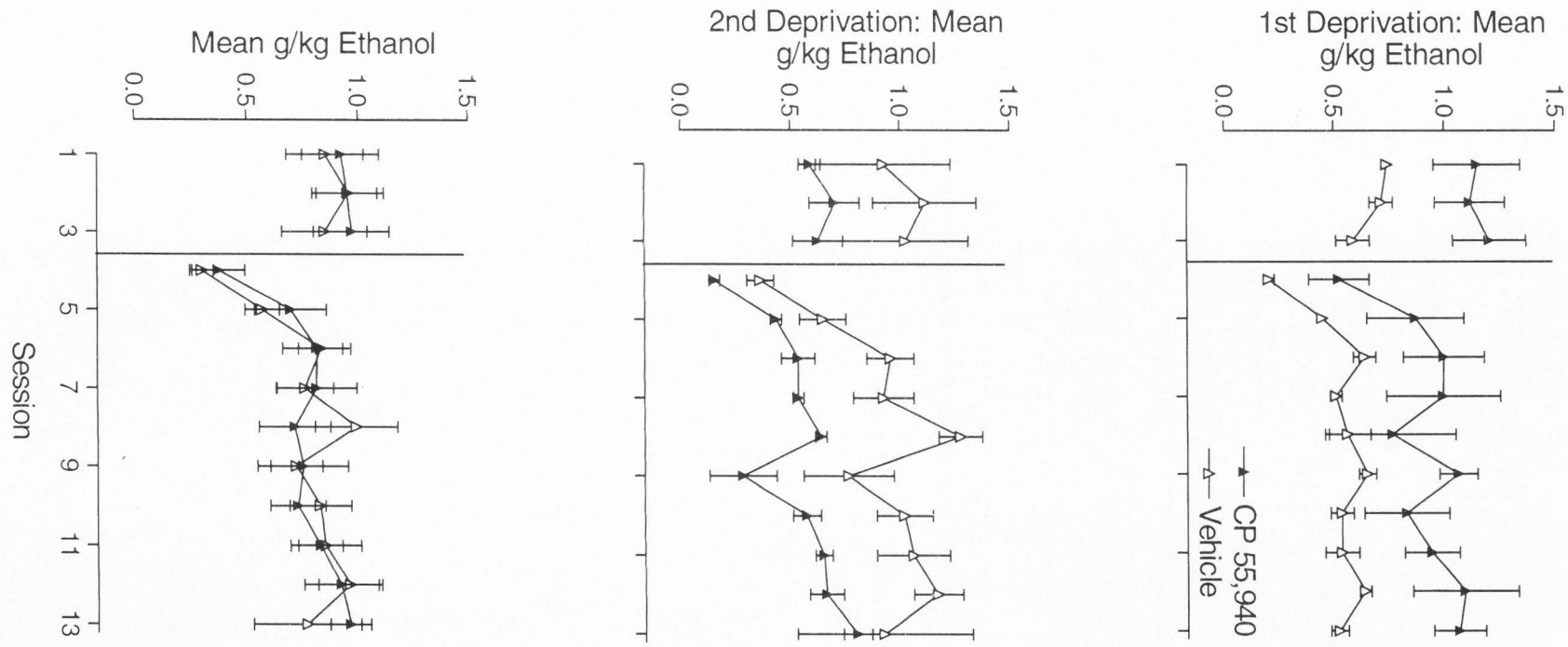

Figure 3. Mean ethanol consumption before and after deprivations calculated separately for vehicle and CP 55,940.

The middle panel shows the mean ethanol delivery before and after the second period of ethanol deprivation. Baseline delivery shows that rats that received vehicle during deprivation earned more ethanol than rats that were to receive CP 55,940 during deprivation. Following deprivation, ethanol delivery decreased relative to baseline for all rats. The decrease in the amount earned by rats that received vehicle, however, was greater than the decrease in the amount earned by the rats that received CP 55,940. Approximate baseline delivery was recovered by the third session for rats that received vehicle and by the fifth session for rats that received CP 55,940.

The bottom panel shows the overall mean ethanol delivery following both periods of ethanol deprivation. Ethanol delivery was calculated separately for CP 55,940 and vehicle. Results show that the amount of ethanol earned was similar across deprivations, subjects and vehicle or CP 55,940 administration.

Figure 4 shows the mean proportion of baseline for breakpoint following the first and second period of ethanol deprivation. Proportion of baseline was calculated because baseline measures of breakpoint and g/kg ethanol earned varied substantially across subjects. The top panel shows the proportion of baseline following the first deprivation. The breakpoint decreased similarly for groups that received vehicle and CP 55,940 during deprivation and remained relatively undifferentiated between groups through session 15. Breakpoint for both groups recovered to levels similar to baseline by the third session. There was some variability through session 15.

The bottom panel shows the proportion of baseline following the second deprivation. Similar to the first session following the first deprivation, breakpoint was

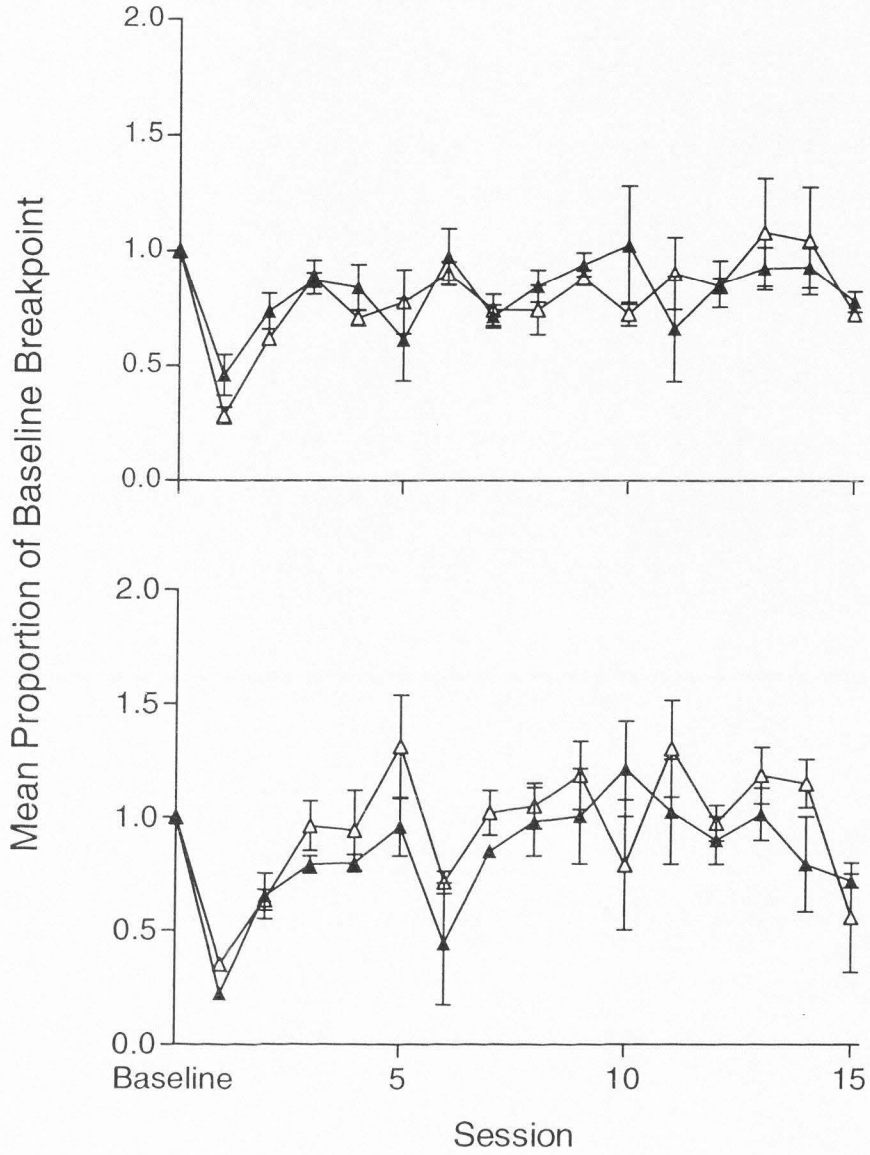

Figure 4. Breakpoint following each deprivation relative to baseline breakpoint.

initially lower than baseline following either vehicle or CP 55,940 administration. Breakpoint for both groups recovered to levels similar to baseline by the second session, but was relatively variable through session 15. Breakpoint was fairly undifferentiated between groups across sessions.

DISCUSSION

The present results show an initial decrease in breakpoint relative to baseline following a period of ethanol deprivation. Additionally, responding for ethanol was not different following chronic administration of CP 55,940 or vehicle during ethanol deprivation. Therefore, these results indicate a decrease in the reinforcing efficacy of ethanol following deprivation, and that chronic administration of a cannabinoid receptor agonist during deprivation did not facilitate responding for ethanol following deprivation.

Based on previous research, results of the present experiment were not what were expected. The alcohol deprivation effect (ADE) is a widely used animal model of alcoholism (see Rodd, Bell, Sable, Murphy, & McBride, 2004; Spanagel & Holter, 1999, for review). Research has shown that depriving a laboratory rat of access to ethanol for a period of time following extended, reliable ethanol consumption results in a temporary increase in ethanol intake when it is made available again. The traditional laboratory methodology involves comparing ethanol intake in rats that have had 24 h free-access to ethanol before and after a period of ethanol deprivation. For example, Sinclair and Senter (1967) first demonstrated the ADE in which rats were given 24 h free home-cage access to a 7% v/v ethanol solution for weeks 2, 4, 6, and 8 of the experiment. Rats in one group (i.e., Group D) were deprived of ethanol during weeks 1, 3, 5, and 7 and rats in a second group (i.e., control) were not deprived of ethanol. The amount of ethanol consumed by Group D following periods of deprivation was substantially greater than the amount consumed by control rats, although the effect was short lived. Humans, however, are required to work in some capacity to obtain alcohol. Therefore, it is unclear how free

access to ethanol can provide an accurate model of human alcohol abuse when no work is required of the subject(s).

Operant procedures, in which subjects must fulfill some predetermined work requirement prior to reinforcement, have more recently been implemented in the study of the ADE. Samson and Chappell (2001) conducted two experiments in which they examined the effects of alcohol deprivation on intake of, and responding for, ethanol during a limited access operant procedure. Rats were required to complete an FR 30 prior to 20-min access to ethanol. A predeprivation extinction session was then conducted in which access to ethanol did not follow completion of the FR 30. Once baseline responding was reestablished, rats were deprived of ethanol and kept in their home cages for 15 days. Following deprivation, an extinction session was conducted. Then, rats were allowed access to ethanol again after completion of an FR 4. Responding measured during the postdeprivation extinction session was significantly lower than during the predeprivation extinction session. Additionally, ethanol intake following deprivation was slightly greater than intake before deprivation. The results of this experiment suggest that when work is required of the subjects (i.e., operant condition), neither increased responding nor ethanol intake indicative of an ADE is observed following a period of ethanol deprivation. Conversely, other experiments in which ethanol was available only under operant conditions have observed an ADE following deprivation. The reasons for these conflicting results are largely unclear; however, the imposition of a single period of deprivation may be a factor. The ADE model of alcohol abuse first proposed by Sinclair and Senter (1967) showed a substantial ADE occurring following a single period of

deprivation. Alcoholism in humans, however, is often marked by multiple periods of abstinence (e.g., McMillen, 1997). Therefore, recent experiments have examined the effects of repeated periods of deprivation on the expression of an ADE.

Oster and colleagues (2006) recently examined the effects of multiple periods of alcohol deprivation on responding for ethanol by high-alcohol-drinking (HAD-1 and -2) rat lines. Utilizing an operant procedure, ethanol solution was available during 1 h sessions on an FR 5 schedule. Once responding was stable, rats were deprived of ethanol for 0 (control), 2, 5, or 8 weeks. Rats were then allowed access to ethanol on the operant schedule for 2 weeks followed by 2 weeks of deprivation. Three additional 2-week periods of access and deprivation followed. Responding for ethanol after the first period of deprivation was substantially below baseline levels for all rats. After the second deprivation, responding increased relative to the first deprivation, but was at or below baseline levels. A small increase above baseline levels was observed following the third deprivation; however, responding was not significantly above baseline until after the fourth deprivation. Consequently, three or more periods of deprivation were necessary to elicit an ADE in rats on an operant schedule. Therefore, it is possible that the two periods of deprivation imposed on subjects in the present experiment were not sufficient to result in the expression of an ADE, particularly when alcohol was available only under operant conditions. Related experiments have also shown that repeated periods of alcohol deprivation were necessary to elicit an ADE in two alcohol-preferring rat strains even when 24 h free-access to ethanol was employed (Rodd-Hendricks, McKinzie, Murphy et al., 2000; Rodd-Hendricks, McKinzie, Shaikh, et al., 2000).

Research has indicated that the length, as well as the number, of deprivations may affect the expression of an ADE. Heyser, Schulteis, and Koob (1997) trained rats to respond during 30-min daily sessions for a 10% (w/v) ethanol solution on a continuous reinforcement schedule (CRF). Once responding was stable, rats were deprived from ethanol for 3, 5, 7, 14, or 28 days after which access to ethanol was resumed under previous conditions. Responding for ethanol increased as a function of the length of deprivation. Rats that were deprived of ethanol for 14 or 28 days responded significantly more for ethanol than rats deprived for 5 or 7 days. Periods of deprivation were 9 days long in the present experiment. Therefore, it is possible that the length of deprivation imposed during the present experiment may not have been sufficient to elicit an ADE.

Another factor that may have contributed to the present effects is stress. Different types of stress have been shown to facilitate or attenuate responding for ethanol in laboratory animals. At this time, the reasons for the differential effects are unclear. Research shows that exposure to some types of physical or psychological stressors results in an increase in responding for, or intake of, ethanol in rats. Conversely, research also shows that some types of stress result in a decrease in ethanol-related behavior. For example, van Erp and Miczek (2001) exposed rats to short-term, daily episodes of social defeat stress and examined the effects on subsequent ethanol consumption in operant and free-access conditions. The stress of social defeat resulted in a short-lived suppression of ethanol intake in both the operant and free-access rats. Furthermore, ethanol intake was suppressed both immediately following social defeat stress and several hours after exposure to the stressor.

A recent experiment examined the effects of different types of stressors on the ADE (Dayas, Martin-Fardon, Thorsell, & Weiss, 2004). Following ethanol self-administration training on an FR 3 schedule, rats were given a liquid diet containing a 10% ethanol concentration for 21 days. After the liquid diet, rats were returned to the operant sessions to obtain ethanol. Once responding for ethanol was stabilized, rats were deprived of ethanol for 7 days during which they were randomly separated into three groups. During deprivation, one group received daily, chronic, intermittent footshock. A second group received daily injections of a toxin that activates the hypothalamic-adrenal-pituitary axis and is a model for chronic stress. A third group was exposed to no stressor (i.e., control). Upon resumption of ethanol self-administration, responses per session were compared to responses prior to ethanol deprivation. Control rats exhibited an ADE in which responding during the first session was significantly higher than baseline. Rats that received chronic footshock exhibited no significant increase in responding following the deprivation period. Rats that received daily toxin injections responded significantly less than baseline during the first session after deprivation and responding was suppressed for several days following deprivation. Thus, the effects of stressors on responding for ethanol are unclear at this time. The type of stressor, the length of exposure to stress, the context and contingency of access to ethanol, or a number of other variables, may differentially contribute to changes in ethanol-related behavior.

Research has also shown that painful stimuli such as footshock increase ethanol consumption following periods of deprivation (Funk, Vohra, & Le, 2004). Additional research suggests that chronic saline injections can alter later responding for ethanol

(Slamberova, Schindler, & Vathy, 2002). To this author's knowledge, however, no research exists examining the effects of injection pain or stress on the manifestation of an ADE. In the present experiment, all subjects were exposed to two 9-day periods of ethanol deprivation during which time each received an i.p. injection of either CP 55,940 or drug vehicle twice a day for the first 7 days. It is possible that the injections resulted in fear during injection or increased sensitivity of the injection site following multiple injections. Additionally, the removal of access to ethanol may have acted as a stressor and heightened sensitivity to the injections. It is therefore conceivable that the stress of multiple injections in addition to the stress related to the removal of ethanol during deprivation resulted in the initial decrease in breakpoint measures following access to ethanol.

In conclusion, some procedural changes may need to be made in future experiments to observe responding and ethanol intake indicative of a clear alcohol deprivation effect. Oster and colleagues (2006) recently showed that three or more periods of deprivation were necessary for the expression of an ADE when ethanol self-administration was placed on an operant schedule. Additionally, research has suggested that the length of the periods of deprivation affect the expression of an ADE (e.g., Heyser et al., 1997). Therefore, three or more periods of deprivation, each at least 14 days in length should be implemented in future investigations of the ADE.

The differential effects of numerous types of physiological and psychological stressors applied in previous research suggest that it is possible that the present route of administration of CP 55,940 or vehicle during deprivation was aversive enough to

attenuate subsequent responding for ethanol. Intraperitoneal injection is commonly used to administer drugs to rats. It may be necessary, however, to investigate routes of administration that are less aversive, particularly when injections occur multiple times each day for a number of days.

The failure of the cannabinoid receptor agonist to increase the reinforcing efficacy of ethanol might also have been partially due to the dose administered. The dose of CP 55,940 administered (0.03 mg/kg) has been previously shown to increase the reinforcing efficacy of alcohol when it was injected just prior to testing (Gallate et al., 1999). The preparation used in the present experiment, however, in which chronic drug exposure during deprivation was followed by 2 days with no drug may not have been sufficient to produce the neuroadaptive changes that research has suggested are necessary to increase motivation to consume another drug, including ethanol. The occurrence of any neurological changes, however, will remain unclear unless histological procedures are employed to assess the sufficiency of the administered dose. Barring the use of such procedures, it may be necessary to administer larger doses of CP 55,940 in future experiments in which effects of chronic exposure to a cannabinoid receptor agonist are being investigated.

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