Selective Breeding for High Alcohol Preference is Associated with Increased Sensitivity to Cannabinoid Reward within the Nucleus Accumbens Shell

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Abstract

Rationale: The rate of cannabinoid intake by those with alcohol use disorder (AUD)exceeds that of the general public. The high prevalence of co-abuse of alcohol and cannabis has been postulated to be predicated upon both a common predisposing genetic factor and the interaction of the drugs within the organism. The current experiments examined the effects of cannabinoids in an animal model of AUD.

Objectives: The present study assessed the reinforcing properties of a cannabinoid receptor 1 (CB1) agonist self-administered directly into the nucleus accumbens shell (AcbSh) in female Wistar and alcohol-preferring (P) rats.

Methods: Following guide cannulae surgery aimed at AcbSh, subjects were placed in an operant box equipped with an 'active lever' (fixed ratio 1; FR1) that caused the delivery of the infusate and an 'inactive lever' that did not. Subjects were arbitrarily assigned to one of seven groups that self-administered either artificial cerebrospinal fluid (aCSF), or 3.125, 6.25, 12.5, or 25 pmol/100nl of O-1057, a water-soluble CB1 agonist, dissolved in aCSF. The first four sessions of acquisition are followed by aCSF only infusates in sessions 5 and 6 during extinction, and finally the acquisition dose of infusate during session 7 as reinstatement.

Results: The CB1 agonist was self-administered directly into the AcbSh. P rats self-administered the CB1 agonist at lower concentrations and at higher rates compared to Wistar rats.

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Conclusions: Overall, the data indicate selective breeding for high alcohol preference has produced rats divergent in response to cannabinoids within the brain reward pathway. The data support the hypothesis that there can be common genetic factors influencing drug addiction.

Keywords

Intracranial Self-Administration; Nucleus Accumbens Shell; reward; cannabinoid; reinforcement; alcohol-preferring

1. INTRODUCTION

Marijuana is the most commonly used illicit drug in America. Approximately 43.5% of all Americans have used marijuana in the past year (SAMHSA, 2019). Marijuana use is common in young Americans with 34.8% of those aged 18-25 years old using marijuana in the past year. Additionally, its use is at a historic high among college-age Americans with approximately 42.6% using marijuana in the last year (Schulenberg et al., 2019). Marijuana use is usually paired with other drug use/abuse (polydrug abuse), in particular alcohol.

Epidemiological data indicate that 58% of subjects who use alcohol or have alcohol use disorders (AUD) also abuse marijuana (Martin et al., 1996). The high prevalence of alcohol and cannabis co-abuse may be predicated upon both a common predisposing genetic factor and the interaction of the drugs within the organism (Uhl, 2004, 2006, Uhl et al., 2008). The common predisposing genetic factor could be based upon innate differences in the endogenous cannabinoid (eCB) system. A single nucleotide polymorphism (SNP) in the human gene encoding an eCB inactivating enzyme, fatty acid amide hydrolase (FAAH), has been strongly associated with drug and alcohol abuse (Sipe et al., 2002) and reduced central nervous system (CNS) FAAH protein levels have been found in individuals with AUD (Vinod et al., 2010). The interrelationship between eCB and alcohol use is also indicated by findings that cannabinoid agents can alter alcohol consumption (Caille and Parsons, 2006; Getachew et al., 2011). Therefore, it is possible that genetic variation in the eCB system may predispose individuals to abuse alcohol and/or be diagnosed with AUD.

Many of the rewarding effects of eCB and Δ^9- tetrahydrocannabinol (Δ^9-THC), the principal psychoactive component of marijuana, are mediated primarily through the CB1 receptors (CB1Rs) (Monory et al., 2007). CB1Rs are predominantly localized in the brain and are expressed in the mesocorticolimbic (MCL) dopaminergic (DAergic) reward system [i.e., ventral tegmental area (VTA), nucleus accumbens (Acb), and medial prefrontal cortex (mPFC)]. The CB1Rs in the Acb mediate food and drug reward (Oleson and Cheer, 2012). It has been reported that intra-accumbal administration of CB1R agonist can induce a conditioned place preference (CPP; Karimi et al., 2013). Numerous studies have also indicated that the eCB system regulates alcohol consumption. For example, CB1R knockout (KO) mice do not develop ethanol (EtOH) conditioned place preference (Houchi et al., 2005, Thanos et al., 2005) and exhibit significantly reduced voluntary alcohol consumption (Hungund et al., 2003, Poncelet et al., 2003; Naassila et al., 2004; Thanos et al., 2005). Furthermore, the ability of systemic administration of EtOH to increase dopamine (DA) levels in the Acb is not apparent in CB1R knockout mice (Hungund et al., 2003).

Neurochemical systems that are modified by alcohol and cannabinoids converge within the mesolimbic DA system. Like all drugs of abuse, alcohol and cannabinoids elevate extracellular levels of DA in the Acb shell (AcbSh; Koob, 2000; Di Chiara et al., 2004). The CB1R system appears to be one pathway in which alcohol produces reinforcement within the mesolimbic DA system (Mechoulam and Parker, 2003). There is a positive correlation between CB1R expression levels within the MCL DA pathway and alcohol preference (Wang et al., 2003). CB1R antagonism prevents EtOH-induced elevations in DA levels of the Acb, as well as DA cell-firing in the Acb and VTA (Perra et al., 2005; Cheer et al., 2007). The ability of CB1R agents to mediate EtOH consumption appears to be limited to CNS areas that support EtOH and cannabinoid self-administration. A series of studies have confirmed that infusions of a CB1R antagonist in the Acb and posterior, but not anterior, VTA decreases EtOH self-administration in rats (Caille and Parsons, 2006, Caille et al., 2007, Alvarez-Jaimes et al., 2009b). In addition, EtOH consumption can also increase CNS extracellular levels of the eCB 2-arachidonoylglycerol (2-AG; Caille et al., 2007). In general, multiple sources indicate that the CB1R system may be one of the platforms in which alcohol acts within the brain.

Systemic administration of CB1R agonists increases (Gallate et al., 1999, Colombo et al., 2002), while CB1R antagonists reduce alcohol consumption (Arnone et al., 1997, Colombo et al., 1998, Gallate and McGregor, 1999). Increasing eCB levels by systemic administration of an FAAH inhibitor enhances EtOH preference and intake in mice (Blednov et al., 2007) and heightened the 2-AG response to EtOH in the Acb of EtOH naïve rats (Alvarez-Jaimes et al., 2009a). In alcohol-preferring (P) rats (c.f., Bell et al., 2006, 2016; McBride et al., 2014), a CB1R antagonist reduced EtOH-seeking and EtOH self-administration, whereas a CB1R agonist increased EtOH-seeking and EtOH self-administration during relapse (Getachew et al., 2011). An additional observation indicated that the P rat may be more susceptible to the sedative properties of CB1 agonists at higher concentrations, suggesting a heightened CB1R system (Getachew et al., 2011). Thus, activation of CB1 receptors is involved in regulating EtOH-seeking as well as the reinforcing effects of EtOH.

O-1057 is a potent water-soluble CB1R agonist (Pertwee, 1999; Pertwee et al., 2000; Lichtman et al., 2000; Martin et al., 2006) and its effects can be blocked by SR141716A, a CB1R antagonist (Pertwee et al., 2000; Lichtman et al., 2000). O-1057 is significantly more potent at CB1Rs (Martin et al., 2006) and a more potent inhibitor of forskolin-stimulated cyclic AMP production than Δ^9-THC (Pertwee et al., 2000). Thus, the objectives of the present study were to test the hypotheses that the activation of the CB1R receptor within the AcbSh is reinforcing and the AcbSh of the P rats is more sensitive to the reinforcing effects of a CB1R agonist, O-1057, than the AcbSh of outbred Wistar rats.

2. MATERIALS AND METHODS

2.1 Animals

Experimentally naïve, female Wistar (Envigo, Indianapolis, IN, USA) and P rats (bred in-house, Indianapolis, IN, USA) weighing 250-320 g at the time of surgery were used. They were double-housed upon arrival and maintained on a 12 h reverse light-dark cycle (lights off at 0900). The P rats were bred at the school of dentistry building across campus and

shipped in Envigo transport boxes and provided with the same transport items as Envigo deliveries. In order to avoid oversampling, the P rats are taking from multiple unrelated families (i.e., the pups come from several litters rather than just one or two). Then, the animals are divided into treatment groups, to make sure the animals from each family are spread evenly between the groups. Although not systematically studied, the estrus cycle did not appear to have a significant effect on ICSA behavior in the present study, or in previous studies (Gatto et al., 1994; Ikemoto et al., 1997, 1998). This was indicated by no obvious fluctuations in ICSA behavior in rats given similar doses of the same agent for two or more operant sessions conducted every other day. Food and water were freely available except in the test chamber. Protocols were approved by the institutional animal care and use committee of the Indiana University School of Medicine and are in accordance with the NIH Guide for the Care and Use of Laboratory Animals (Research Institute for Laboratory Animal Research, 2011).

Data for rats that did not complete all experimental test sessions were excluded from the analyses. The number of animals indicated for each experiment represents approximately 96% of the total number that underwent surgery. Approximately 3% of the animals were not included for analyses due to the loss of the guide cannula before completion of all experimental sessions. The data for these animals were not used because their injection sites could not be verified.

2.2   Drug and Vehicle

The artificial cerebrospinal fluid (aCSF) consisted of 120.0 mM NaCl, 4.8 mM KCl, 1.2 mM KH_2PO_4, 1.2 mM Mg SO_4, 25.0 mM $NaHCO_3$, 2.5 mM $CaCl_2$, and 10.0 mM d-glucose. O-1057 (Organix Inc, Woburn, MA, USA) was dissolved in the aCSF solution. When necessary, 0.1 M HC1 or 0.1 M NaOH was added to the solutions to adjust pH levels to 7.4 ± 0.1.

2.3   Apparatus

The test chambers (30 X 30 X 26 cm; w x h x d) were situated in a sound-attenuating cubicle (64 X 60 X 50 cm, Coulboum Instruments, Allentown, PA, USA) and illuminated by a dim house-light during testing. Two identical levers (3.5 X 1.8 cm) were mounted on a single wall of the test chamber, 15 cm above a grid floor, and were separated by 12 cm. Details can be found in previous publications (e.g., Rodd –Henricks et al., 2002; Hauser et al., 2014).

An electrolytic microinfusion transducer (EMIT) system (see Bozarth and Wise, 1980) was used to control microinfusions of drug or vehicle (Rodd-Henricks et al., 2002, Hauser et al., 2014). Depression of the active lever delivered the infusion current for 5 s, which led to the rapid generation of H_2 gas and raised the pressure inside the airtight cylinder, in turn, forcing 100-nl of infusate through the injection cannula. During the 5 s infusion and an additional 5 s timeout period, the house light and red inactive lever cue light were extinguished, while the green cue light over the active lever flashed at 0.5 s intervals.

2.4 Animal Preparation

While under isoflurane anesthesia, rats were prepared for unilateral stereotaxic implantation of 22-gauge guide cannula (Plastic One, Roanoke, VA, USA) into the AcbSh. The guide cannula was aimed 1.0 mm above the target region. Coordinates for placements to target the AcbSh were +1.7 mm AP, + 2.4 mm lateral to the midline, and −7.5 mm ventral from the surface of the skull at a 10° angle to the vertical (Paxinos and Watson, 1998). A representative of the placements of injection sites within the AcbShell (defined as +1.7 to + 1.0 mm bregma) is shown in Figure 1. A 28-gauge stylet was placed into the guide cannula and extended 0.5 mm beyond the tip of the guide. All rats were individually housed and allowed to recover from surgeries for 7-10 days. Animals were handled for at least 5 min daily following the fourth recovery day. Subjects were not acclimated to the test chamber prior to the commencement of data collection, nor were they trained on any other operant paradigm.

2.5 General Test Condition

For testing, subjects were brought to the testing room, the stylet was removed, and the injection cannula screwed into place. Rats were placed individually into the test chamber. To avoid trapping air at the tip of the injection cannula, the infusion current was delivered for 5 s during insertion of the injector that resulted in a non-contingent administration of the CB1R agonist or aCSF at the beginning of the session. The test chamber was equipped with two levers. Depression of the 'active lever' [fixed ratio 1 (FR1) schedule of reinforcement] caused the delivery of a 100 nl bolus of infusate over 5 s followed by a 5 s time-out period. During both the 5 s infusion and 5 s time-out period, responses on the active lever did not produce further infusions. Responses on the 'inactive lever' were recorded, but did not result in infusions. The assignment of active and inactive lever with respect to the left or right position was counterbalanced among subjects. However, the active and inactive levers remained the same for each rat throughout the experiment. No shaping technique was used to facilitate the acquisition of lever responses. The number of infusions and responses on the active and inactive lever were recorded. The duration of each test session was 4 h and sessions occurred every other day.

2.6 Treatment Procedures

Animals were randomly assigned to one of five groups (n = 6-8/group/line: Wistar 7-8/group N = 39; P – 6-8/group N = 35). A vehicle group received infusions of aCSF for all seven sessions. The other groups received infusions of 3.125, 6.25, 12.5, or 25 pmol/100nl O-1057 for the first four sessions. During the fifth and sixth sessions, all animals received infusions of aCSF. On the seventh session day, rats were allowed to respond for their originally assigned infusate. A total of 74 rats completed these procedures.

2.7 Histology

At the termination of the experiment, 1% bromophenol blue was injected into the infusion site. Subsequently, the animals were given a fatal dose of Nembutal and then decapitated. Brains were removed and immediately frozen at −80° C. Frozen brains were equilibrated at −15° C in a cryostat microtome and then sliced into 40 um sections. Sections were then

stained with cresyl violet and examined under a light microscope for verification of the injector site using the rat brain atlas of Paxinos and Watson (1998).

2.8 Statistical Analysis

Data analysis consisted of a group X day mixed ANOVA, with the repeated measure of 'day', performed on the number of infusions/session. Additionally, for each individual group, lever discrimination was determined by type (active or inactive) X day mixed ANOVA with a repeated measure of 'day'. Lever discrimination is a key factor when a stimulant is self-administered (e.g., EtOH, cocaine, amphetamine, nicotine). Without a detailed analysis of lever discrimination, it is impossible to distinguish between reinforcement-contingent behavior and random drug-stimulated locomotor activity. It is also important to note that an ANOVA performed on a dependent variable with only two levels (e.g., between two rat lines), the F value is identical to the product of a t-test. Therefore, all post-hoc analyses performed with ANOVAs between Wistar and P rats at specific concentrations of O-1057 are reported as t-values to avoid the misrepresentation of the statistical test.

3. RESULTS

The initial analysis examined the average number of reinforcers self-administered during the first 4 test sessions of acquisition with between group factors of 'concentration' and 'line' (Fig. 2). There was a significant 'concentration' X 'line' interaction ($F_{4,64} = 3.31$; $p = 0.016$). Reducing the interaction term by examining the effect of 'concentration' within each line revealed significant dose-response effects in Wistar ($F_{4,34} = 36.3$; $p < 0.0001$) and P ($F_{4,30} = 17.5$; $p < 0.0001$) rats. Post-hoc comparisons (Tukey's HSD) revealed that Wistar rats self-administering 12.5 and 25 pmol/100 nl O-1057 directly into the AcbSh self-infused more infusate than all other groups. In P rats, post-hoc comparisons indicated that 3.125, 6.25, 12.5, and 25 pmol/100 nl O-1057 self-infused more than aCSF controls, that the 12.5 group self-infused more than the 3.125 pmol/100 nl group, and that the 25 pmol/100 nl group self-infused significantly more than all other groups. Reducing the significant interaction term by examining the number of self-infusions between the rat lines at each concentration of O-1057 was also performed. Independent t-tests indicated that P rats self-administered greater amounts of 3.125, 6.25, and 25 pmol/100 nl O-1057 than Wistar control rats (significant p-values were $p < 0.01$).

An examination of the number of active lever responses as a function of rat 'line' and O-1057 'concentration' across all 7 sessions was performed. There was a significant 'line' X 'concentration' X 'session' interaction term ($F_{24,384} = 2.43$; $p < 0.001$; Figs. 3 and 4). In Wistar rats there was a significant 'concentration' X 'session' interaction ($F_{24,204} = 2.0$; $p < 0.0001$). Wistar rats given 12.5 or 25 pmol/100 nl O-1057 to self-infuse into the AcbSh responded on the active lever significantly more than all other Wistar groups during sessions 1-4 and 7 (p values < 0.02). In addition, Wistar rats self-administering 12.5 or 25 pmol/100 nl O-1057 reduced responding during aCSF substitution ($p < 0.032$; Fig 4). Similar to the infusion data, responding on the active lever across sessions indicated a significant 'concentration' X 'session' interaction term ($F_{24,180} = 17.5$; $p < 0.0001$). P rats self-

administering 3.125, 6.25, and 25 pmol/100 nl O-1057 responded on the active lever significantly more than aCSF controls, displayed level discrimination, extinction of responding during aCSF substitution, and a reinstatement of responding when O-1057 was returned to the infusate. In addition, in P rats self-administering 3.125 or 6.25 pmol/100 nl O-1057 displayed an increase in active lever responding during the reinstatement session (7) compared to the responding during session the last session of acquisition (p < 0.01).

4. DISCUSSION

The major findings of this study are that a CB1R agonist within the AcbSh is reinforcing and that genetic selection for high alcohol consumption includes an AcbSh that is more sensitive to the reinforcing properties of CB1R agonists than its progenitor stock (Figs. 2-4). This was indicated by the findings that P rats will self-infuse lower concentrations of O-1057 (Figs. 2 and 3) by readily discriminating the active from the inactive lever at 3.125 and 6.25 pmol doses (Fig. 3, middle and bottom panel). Conversely, Wistar rats self-administer these concentrations of O-1057 at the same level as aCSF, thus not demonstrating lever discrimination at these doses. Furthermore, P rats received more self-infusions of O-1057 than did Wistar rats at the 3.125, 6.25, and 25 pmol concentrations (Figs. 3 and 4). The combination of increased responsiveness to the effects of O-1057 and a higher number of self-infusions suggest that O-1057 may be a stronger reinforcer in the AcbSh of P rats than Wistar rats.

Similar differences in sensitivity of the AcbSh between P and Wistar rats have been reported in EtOH (Engleman et al., 2009) and cocaine (Katner et al., 2011) ICSA studies. For example, P rats were shown to self-administer cocaine at lower concentrations (200 pmol vs 800 pmol) and have higher cocaine infusions rates (59 infusions/session vs 38 infusions/ session) compared to Wistar rats (Katner et al., 2011). Thus, providing evidence that selection for high alcohol preference increase the sensitivity of AcbSh to other drugs of abuse. To our knowledge the current study is the first study to examine ICSA differences of a CB1 agonist between a high alcohol preferring animal model and its progenitor control. However, innate differences in the metabolism of eCBs have been associated with differential sensitivity to alcohol in rodents. Alko, Alcohol (AA) rats have lower expression of FAAH compared to Alko, Non-Alcohol (ANA) rats in the prefrontal cortex and decreased CB1 receptor density and coupling (Hansson et al., 2007). In addition, genetic deletion of FAAH results in a reduction in alcohol consumption in mice (Basavarajappa and Hungund, 2005, Blednov et al., 2007). Collectively, these findings support the idea that there may be a genetic linkage between selective breeding for high alcohol preference and increased sensitivity of the AcbSh to the reinforcing actions of cannabinoids.

The current results are in line with Zangen et al. (2006) findings that showed [9]THC is self-administered directly into the AcbSh, but not the AcbC, and in the posterior, but not anterior, VTA (Zangen et al., 2006). However, [9]THC is both an agonist and an antagonist on CB1R (Bergman et al., 2008), thus the current study is the first to look at the reinforcing properties of a selective CB1R agonist within the AcbSh. Collectively these findings were also similar to EtOH ICS A studies that found animals will self-administer EtOH into the AcbSh, but not the AcbC (Engleman et al., 2009), and posterior, but not anterior, VTA (Rodd-Henricks et

al., 2000). Therefore, the neurocircuitry of alcohol and cannabinoid reinforcement incorporates identical structures.

CB1Rs are expressed on multiple neurotransmitter targets within the Acb (Herkenham et al., 1991; Tsou et al., 1998; Murray et al., 2010). CB1Rs are localized on GABAergic axon terminals of local medium-spiny neurons and parvalbumin-positive interneurons in the Acb (Mato et al., 2005; Robbe et al., 2001; Uchigashima et al., 2007) as well as excitatory prefrontal cortex-Acb synaptic terminals (Robbe et al., 2001). An aspect of the reward circuitry of cannabinoids include activation of CB1R within Acb which disinhibits GABAA receptor-control of dopamine release (cf., Wenzel and Cheer, 2018; Sperlágh et al., 2009). Also, CB1R are expressed in glutamatergic and cholinergic cells within the Acb that regulate Acb DA activity (Wenzel and Cheer, 2018; Fusco et al., 2004). In general, there is a lack of characterization of the cannabinoid system in rats selectively bred for high alcohol preference/consumption, and there are no reported findings that would suggest the biological basis for the increase in sensitivity to the reinforcing properties of O-1057 within the AcbSh of P rats.

The current findings also extend previous findings that CB1R agonists are reinforcing. Animals have been shown to intravenously self-administer the CB1R agonist WIN 55,212-2 (Lefever et al., 2014; Lecca et al., 2006; Fattore et al., 2001; Martellotta et al., 1998), which was associated with a preferential increase of DA in the AcbSh compared to the AcbCo (Lecca et al., 2006). The CB1 agonist CP55940 is intracerebroventricularly (ICV) self-administered, with self-administration blocked by the CB1R antagonist SR141716A (Braida et al., 2001a). Systemic administration of CP55940 (Braida et al. 2001b) and intra-accumbal administration of WIN 55,212-2 (Karimi et al., 2013) also support conditioned place preference.

The results of the present study provide important information regarding how genetic factors influence a predisposition to high alcohol drinking and abuse of cannabinoids, and how genetic factors that influence alcohol drinking can also influence the effects of cannabinoid abuse. These findings are in line with the Ranganathan and colleagues (2014) clinical report which demonstrated that a family history of AUD was correlated with greater euphoria and perceptual alterations induced by low doses of ^{9}–THC. These results suggest a family history of AUD enhances sensitivity to the rewarding effects of ^{9}–THC. This may be due to alterations in CB1R function that could contribute to alcohol misuse and/or vulnerability. These findings in humans support the present results in which rats selectively bred for high alcohol preference are also more sensitive to the reinforcing actions of CB1R agonist, compound with outbred Wistar control rats. Together, these data support the hypothesis that there are some common genetic factors mediating the abuse potential of alcohol and cannabinoids.

In conclusion, the results of the present study indicate an association between selective breeding for high alcohol preference and enhanced sensitivity of the AcbSh to the reinforcing effects of cannabinoids. Moreover, the biological basis for this altered sensitivity within the AcbSh may be due to unknown alterations in CB1R levels and/or function, which resulted from bidirectional selective breeding for divergent alcohol preference over water.

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Acknowledgments

REFERENCES

Alvarez-Jaimes L, Stouffer DG, Parsons LH (2009a) Chronic ethanol treatment potentiates ethanol-induced increases in interstitial nucleus accumbens endocannabinoid levels in rats. J Neurochem. 111:37–48. [PubMed: 19650871]

Alvarez-Jaimes L, Polis I, Parsons LH (2009b) Regional Influence of Cannabinoid CB(1) Receptors in the Regulation of Ethanol Self-Administration by Wistar Rats. Open Neuropsychopharmacol J. 2:77–85. [PubMed: 27974944]

Arnone M, Maruani J, Chaperon F, Thiébot MH, Poncelet M, Soubrié P, Le Fur G (1997) Selective inhibition of sucrose and ethanol intake by SR 141716, an antagonist of central cannabinoid (CB1) receptors. Psychopharmacology (Berl). 132:104–6. [PubMed: 9272766]

Basavarajappa BS, Hungund BL (2005) Role of the endocannabinoid system in the development of tolerance to alcohol. Alcohol Alcohol. 40:15–24. [PubMed: 15550443]

Bell RL, Rodd ZA, Lumeng L, Murphy JM, McBride WJ (2006) The alcohol-preferring P rat and animal models of excessive alcohol drinking. Addict. Biol 11: 270–288 [PubMed: 16961759]

Bell RL, Hauser S, Rodd ZA, Liang T, Sari Y, McClintick J, Rahman S, Engleman EA (2016) A Genetic Animal Model of Alcoholism for Screening Medications to Treat Addiction. Int. Rev. Neurobiol. 126: 179–261 [PubMed: 27055615]

Bergman J, Delatte MS, Paroni CA, Vemuri K, Thakur GA, Makariyannis A (2008) Some effects of CB1 antagonists with inverse agonist and neutral biochemical properties. Physiol Behav 93: 666–70. [PubMed: 18076956]

Blednov YA, Cravatt BF, Boehm SL 2nd, Walker D, Harris RA (2007) Role of endocannabinoids in alcohol consumption and intoxication: studies of mice lacking fatty acid amide hydrolase. Neuropsychopharmacology. 32:1570–82. [PubMed: 17164820]

Bozarth MA, Wise RA (1980) Electrolytic microinfusion transducer system: an alternative method of intracranial drug application. Journal of neuroscience methods. 2: 273–275. [PubMed: 7329097]

Braida D, Pozzi M, Parolaro D, Sala M (2001a) Intracerebral self-administration of the cannabinoid receptor agonist CP 55,940 in the rat: interaction with the opioid system. Eur J Pharmacol. 413(:227–34. [PubMed: 11226397]

Braida D, Pozzi M, Cavallini R, Sala M (2001b) Conditioned place preference induced by the cannabinoid agonist CP 55,940: interaction with the opioid system. Neuroscience. 104:923–6. [PubMed: 11457579]

Caille S, Alvarez-Jaimes L, Polis I, Stouffer DG, Parsons LH (2007) Specific alterations of extracellular endocannabinoid levels in the nucleus accumbens by ethanol, heroin, and cocaine self-administration. J Neurosci. 27:3695–702. [PubMed: 17409233]

Caille S, Parsons LH (2006) Cannabinoid modulation of opiate reinforcement through the ventral striatopallidal pathway. Neuropsychopharmacology. 31:804–13. [PubMed: 16123766]

Cheer JF, Wassum KM, Sombers LA, Heien ML, Ariansen JL, Aragona BJ, Phillips PE, Wightman RM (2007) Phasic dopamine release evoked by abused substances requires cannabinoid receptor activation. J Neurosci. 27:791–5. [PubMed: 17251418]

Colombo G, Serra S, Brunetti G, Gomez R, Melis S, Vacca G, Carai MM, Gessa L (2002) Stimulation of voluntary ethanol intake by cannabinoid receptor agonists in ethanol-preferring sP rats. Psychopharmacology (Berl). 59:181–7.

Colombo G, Agabio R, Fà M, Guano L, Lobina C, Loche A, Reali R, Gessa GL (1998) Reduction of voluntary ethanol intake in ethanol-preferring sP rats by the cannabinoid antagonist SR-141716. Alcohol Alcohol. 33:126–30. PubMed PMID: 9566474. [PubMed: 9566474]

Di Chiara G, Bassareo V, Fenu S, De Luca MA, Spina L, Cadoni C, Acquas E, Carboni E, Valentini V, Lecca D (2004) Dopamine and drug addiction: the nucleus accumbens shell connection. Neuropharmacology. 47:227–41. [PubMed: 15464140]

Egan KL, Cox MJ, Suerken CK, Reboussin BA, Song EY, Wagoner KG, Wolfson M (2019) More drugs, more problems? Simultaneous use of alcohol and marijuana at parties among youth and young adults. Drug Alcohol Depend 202: 69–75. [PubMed: 31319362]

Engleman EA, Ding ZM, Oster SM, Toalston JE, Bell RL, Murphy JM, McBride WJ, Rodd ZA (2009) Ethanol is self-administered into the nucleus accumbens shell, but not the core: evidence of genetic sensitivity. Alcohol Clin Exp Res. 33:2162–71. [PubMed: 19764930]

Fattore L, Cossu G, Martellotta CM, Fratta W (2001) Intravenous self-administration of the cannabinoid CB1 receptor agonist WIN 55,212-2 in rats. Psychopharmacology (Berl). 156:410–6. [PubMed: 11498718]

Fusco FR, Martorana A, Giampà C, De March Z, Farini D, D'Angelo V, Sancesario G, Bernardi G (2004) Immunolocalization of CB1 receptor in rat striatal neurons: a confocal microscopy study. Synapse. 53:159–67. [PubMed: 15236348]

Gadzicki D, Müller-Vahl K, Stuhrmann M (1999) A frequent polymorphism in the coding exon of the human cannabinoid receptor (CNR1) gene. Mol Cell Probes. 13:321–3. [PubMed: 10441206]

Gallate JE, McGregor IS (1999) The motivation for beer in rats: effects of ritanserin, naloxone and SR 141716. Psychopharmacology (Berl). 142:302–8. [PubMed: 10208323]

Gallate JE, Saharov T, Mallet PE, McGregor IS (1999) Increased motivation for beer in rats following administration of a cannabinoid CB1 receptor agonist. Eur J Pharmacol. 370:233–40. [PubMed: 10334497]

Gatto GJ, McBride WJ, Murphy JM, Lumeng L, Li TK (1994) Ethanol self-infusion into the ventral tegmental area by alcohol-preferring rats. Alcohol. 11:557–64. [PubMed: 7865158]

Getachew B, Hauser SR, Dhaher R, Katner SN, Bell RL, Oster SM, McBride WJ, Rodd ZA (2011) CB1 receptors regulate alcohol-seeking behavior and alcohol self-administration of alcohol-preferring (P) rats. Pharmacol Biochem Behav. 97:669–75. [PubMed: 21110997]

Hansson AC, Bermúdez-Silva FJ, Malinen H, Hyytiä P, Sanchez-Vera I, Rimondini R, Rodriguez de Fonseca F, Kunos G, Sommer WH, Heilig M (2007) Genetic impairment of frontocortical endocannabinoid degradation and high alcohol preference. Neuropsychopharmacology. 32:117–26. [PubMed: 16482090]

Hauser SR, Bracken AL, Deehan GA Jr, Toalston JE, Ding ZM, Truitt WA, Bell RL, McBride WJ, Rodd ZA (2014) Selective breeding for high alcohol preference increases the sensitivity of the posterior VTA to the reinforcing effects of nicotine. Addict Biol. 19:800–11. [PubMed: 23496648]

Heller D, Schneider U, Seifert J, Cimander KF, Stuhrmann M (2001) The cannabinoid receptor gene (CNR1) is not affected in German i.v. drug users. Addict Biol. 6:183–187. [PubMed: 11341859]

Herkenham M, Lynn AB, Johnson MR, Melvin LS, de Costa BR, Rice KC (1991) Characterization and localization of cannabinoid receptors in rat brain: a quantitative in vitro autoradiographic study. J Neurosci. 11:563–83. [PubMed: 1992016]

Houchi H, Babovic D, Pierrefiche O, Ledent C, Daoust M, Naassila M (2005) CB1 receptor knockout mice display reduced ethanol-induced conditioned place preference and increased striatal dopamine D2 receptors. Neuropsychopharmacology. 30:339–49. [PubMed: 15383833]

Hungund BL, Szakall I, Adam A, Basavarajappa BS, Vadasz C (2003) Cannabinoid CB1 receptor knockout mice exhibit markedly reduced voluntary alcohol consumption and lack alcohol-induced dopamine release in the nucleus accumbens. J Neurochem. 84:698–704. [PubMed: 12562514]

Ikemoto S, Glazier BS, Murphy JM, McBride WJ (1998) Rats self-administer carbachol directly into the nucleus accumbens. Physiol Behav. 63:811–4. [PubMed: 9618003]

Ikemoto S, Murphy JM, McBride WJ (1997) Self-infusion of GABA(A) antagonists directly into the ventral tegmental area and adjacent regions. Behav Neurosci. 111:369–80. [PubMed: 9106676]

Karimi S, Azizi P, Shamsizadeh A, Haghparast A (2013) Role of intra-accumbal cannabinoid CB1 receptors in the potentiation, acquisition and expression of morphine-induced conditioned place preference. Behav Brain Res. 247:125–31. [PubMed: 23523958]

Katner SN, Oster SM, Ding ZM, Deehan GA Jr, Toalston JE, Hauser SR, McBride WJ, Rodd ZA (2011) Alcohol-preferring (P) rats are more sensitive than Wistar rats to the reinforcing effects of

cocaine self-administered directly into the nucleus accumbens shell. Pharmacol Biochem Behav. 99:688–95. [PubMed: 21723879]

Koob GF (2000) Neurobiology of addiction. Toward the development of new therapies. Ann N Y Acad Sci.909:170–85. [PubMed: 10911930]

Lecca D, Cacciapaglia F, Valentini V, Di Chiara G (2006) Monitoring extracellular dopamine in the rat nucleus accumbens shell and core during acquisition and maintenance of intravenous WIN 55,212-2 self-administration. Psychopharmacology (Berl). 188:63–74. [PubMed: 16850116]

Lefever TW, Marusich JA, Antonazzo KR, Wiley JL (2014) Evaluation of WIN 55,212-2 self-administration in rats as a potential cannabinoid abuse liability model. Pharmacol Biochem Behav. 118:30–5. [PubMed: 24412835]

Lichtman AH, Peart J, Poklis JL, Bridgen DT, Razdan RK, Wilson DM, Poklis A, Meng Y, Byron PR, Martin BR (2000) Pharmacological evaluation of aerosolized cannabinoids in mice. Eur J Pharmacol. 399:141–9. [PubMed: 10884513]

Martellotta MC, Cossu G, Fattore L, Gessa GL, Fratta W (1998) Self-administration of the cannabinoid receptor agonist WIN 55,212-2 in drug-naive mice. Neuroscience. 85:327–30. [PubMed: 9622233]

Martin CS, Kaczynski NA, Maisto SA, Tarter RE (1996) Polydrug use in adolescent drinkers with and without DSM-IV alcohol abuse and dependence. Alcohol Clin Exp Res. 20:1099–1108. [PubMed: 8892534]

Martin BR, Wiley JL, Beletskaya I, Sim-Selley LJ, Smith FL, Dewey WL, Cottney J, Adams J, Baker J, Hill D, Saha B, Zerkowski J, Mahadevan A, Razdan RK (2006) Pharmacological characterization of novel water-soluble cannabinoids. J Pharmacol Exp Ther. 318:1230–9. [PubMed: 16757541]

Mato S, Robbe D, Puente N, Grandes P, Manzoni OJ (2005) Presynaptic homeostatic plasticity rescues long-term depression after chronic Delta 9-tetrahydrocannabinol exposure. J Neurosci. 25:11619–27. [PubMed: 16354920]

McBride WJ, Rodd ZA, Bell RL, Lumeng L, Li TK (2014) The alcohol-preferring (P) and high-alcohol-drinking (HAD) rats--animal models of alcoholism Alcohol. 48: 209–215 [PubMed: 24268381]

Mechoulam R, Parker L (2003) Cannabis and alcohol--a close friendship. Trends Pharmacol Sci. 24:266–8. [PubMed: 12823949]

Monory K, Blaudzun H, Massa F, Kaiser N, Lemberger T, Schütz G, Wotjak CT, Lutz B, Marsicano G (2007) Genetic dissection of behavioural and autonomic effects of Delta(9)-tetrahydrocannabinol in mice. PLoS Biol. 5:e269. [PubMed: 17927447]

Murray JE, Bevins RA (2010) Cannabinoid conditioned reward and aversion: behavioral and neural processes. ACS Chemical Neuroscience. 1:265–78. [PubMed: 20495676]

Naassila M, Pierrefiche O, Ledent C, Daoust M (2004) Decreased alcohol self-administration and increased alcohol sensitivity and withdrawal in CB1 receptor knockout mice. Neuropharmacology. 46:243–53. [PubMed: 14680762]

Oleson EB, Cheer JF (2012) A brain on cannabinoids: the role of dopamine release in reward seeking. Cold Spring Harb Perspect Med. 2: pii: a012229. [PubMed: 22908200]

Paxinos G and Watson C (1998) The rat brain in stereotaxic coordinates. 4th Edition, Academic Press, New York.

Perra S, Pillolla G, Melis M, Muntoni AL, Gessa GL, Pistis M (2005) Involvement of the endogenous cannabinoid system in the effects of alcohol in the mesolimbic reward circuit: electrophysiological evidence in vivo. Psychopharmacology (Berl). 183:368–77. [PubMed: 16228194]

Pertwee RG (1999) Cannabis and cannabinoids: pharmacology and rationale for clinical use. Forsch Komplementarmed. Suppl 3:12–5.

Pertwee RG, Gibson TM, Stevenson LA, Ross RA, Banner WK, Saha B, Razdan RK, Martin BR (2000) O-1057, a potent water-soluble cannabinoid receptor agonist with antinociceptive properties. British journal of pharmacology. 129: 1577–1584. [PubMed: 10780961]

Poncelet M, Maruani J, Calassi R, Soubrié P (2003) Overeating, alcohol and sucrose consumption decrease in CB1 receptor deleted mice. Neurosci Lett. 343:216–8. [PubMed: 12770700]

Ranganathan M, Sewell RA, Carbuto M, Elander J, Schnakenberg A, Radhakrishnan R, Pittman B, D'Souza DC (2014) Effects of 9 tetrahydrocannabinol in individuals with a familial vulnerability to alcoholism. Psychopharmacology (Berl). 231:2385–93. [PubMed: 24424782]

Research Institute for Laboratory Animal Research (2011) Guide for the Care and Use of Laboratory Animals. 8th Ed. The National Academies Press, Washington, D.C.

Robbe D, Alonso G, Duchamp F, Bockaert J, Manzoni OJ (2001) Localization and mechanisms of action of cannabinoid receptors at the glutamatergic synapses of the mouse nucleus accumbens. J Neurosci. 21:109–16. [PubMed: 11150326]

Rodd-Henrick ZA, McKinzie DL, Crile RS, Murphy JM, McBride WJ (2000) Regional heterogeneity for the intracranial self-administration of ethanol within the ventral tegmental area of female Wistar rats. Psychopharmacology. 149: 217–224. [PubMed: 10823401]

Rodd-Henricks ZA, McKinzie DL, Li TK, Murphy JM, McBride WJ (2002) Cocaine is self administered into the shell but not the core of the nucleus accumbens of Wistar rats. J Pharmacol Exp Ther. 303:1216–26. [PubMed: 12438546]

Schulenberg JE, Johnston LD, O'Malley PM, Bachman JG, Miech RA, Patrick ME (2019) Monitoring the Future national survey results on drug use, 1975–2018: Volume II, College students and adults ages 19–60. Ann Arbor: Institute for Social Research, The University of Michigan Available at http://monitoringthefuture.Org/pubs.html#monographs

Substance Abuse and Mental Health Services Administration (2019) Key substance use and mental health indicators in the United States: Results from the 2018 National Survey on Drug Use and Health (HHS Publication No. PEP19-5068, NSDUH Series H-54). Rockville, MD: Center for Behavioral Health Statistics and Quality, Substance Abuse and Mental Health Services Administration Retrieved from https://www.samhsa.gov/data/

Sipe JC, Chiang K, Gerber AL, Beutler E, Cravatt BF (2002) A missense mutation in human fatty acid amide hydrolase associated with problem drug use. Proc Natl Acad Sci U S A. 99:8394–9. [PubMed: 12060782]

Sperlagh B, Windisch K, Andó RD, Sylvester Vizi E (2009) Neurochemical evidence that stimulation of CB1 cannabinoid receptors on GABAergic nerve terminals activates the dopaminergic reward system by increasing dopamine release in the rat nucleus accumbens. Neurochem Int. 54:452–7. [PubMed: 19428788]

Terry-McElrath YM, O'Malley PM, Johnston LD (2014) Alcohol and marijuana use patterns associated with unsafe driving among US high school seniors: high use frequency, concurrent use, and simultaneous use. J Stud Alcohol Drugs 75: 378–89. [PubMed: 24766749]

Thanos PK, Dimitrakakis ES, Rice O, Gifford A, Volkow ND (2005) Ethanol self-administration and ethanol conditioned place preference are reduced in mice lacking cannabinoid CB1 receptors. Behav Brain Res. 164:206–13. [PubMed: 16140402]

Thoma RJ, Monnig MA, Lysne PA, Ruhl DA, Pommy JA, Bogenschutz M, Tonigan JS, Yeo RA (2011) Adolescent substance abuse: the effects of alcohol and marijuana on neuropsychological performance. Alcohol Clin Exp Res.35:39–46. [PubMed: 20958330]

Tsou K, Brown S, Sañudo-Peña MC, Mackie K, Walker JM (1998) Immunohistochemical distribution of cannabinoid CB1 receptors in the rat central nervous system. Neuroscience. 83:393–411. [PubMed: 9460749]

Uchigashima M, Narushima M, Fukaya M, Katona I, Kano M, Watanabe M (2007) Subcellular arrangement of molecules for 2-arachidonoyl-glycerol-mediated retrograde signaling and its physiological contribution to synaptic modulation in the striatum. J Neurosci. 27:3663–76. [PubMed: 17409230]

Uhl GR, Drgon T, Johnson C, Li CY, Contoreggi C, Hess J, Naiman D, Liu QR (2008) Molecular genetics of addiction and related heritable phenotypes: genome-wide association approaches identify "connectivity constellation" and drug target genes with pleiotropic effects. Ann N Y Acad Sci. 1141:318–81. [PubMed: 18991966]

Uhl GR, Ishiguro H, Onaivi ES, Zhang PW, Akinshola BE, Lin Z, Hope B, Leonard CM, Liu QR (2006) Molecular neurobiological methods in marijuana-cannabinoid research. Methods Mol Med. 123:1–17. [PubMed: 16506399]

Uhl GR (2004) Molecular genetics of substance abuse vulnerability: remarkable recent convergence of genome scan results. Ann N Y Acad Sci. 1025:1–13.

Vinod KY, Kassir SA, Hungund BL, Cooper TB, Mann JJ, Arango V (2010) Selective alterations of the CB1 receptors and the fatty acid amide hydrolase in the ventral striatum of alcoholics and suicides. J Psychiatr Res. 44:591–7. [PubMed: 20015515]

Wang L, Liu J, Harvey-White J, Zimer A, Kunos G (2003) Endocannabinoids signaling via cannabionoid receptor 1 is involved in ethanol preference and its age-dependent decline in mice. PNAS 100:1393–1398. [PubMed: 12538878]

Wenzel JM, Cheer JF (2018) Endocannabinoid Regulation of Reward and Reinforcement through Interaction with Dopamine and Endogenous Opioid Signaling. Neuropsychopharmacology. 43:103–115. [PubMed: 28653666]

Zangen A, Solinas M, Ikemoto S, Goldberg SR, Wise RA (2006) Two brain sites for cannabinoid reward. J Neurosci. 26:4901–7. [PubMed: 16672664]

HIGHLIGHTS

- O-1057, a CB1 agonist, is self-administered directly into the AcbSh of female rats

- P rats self-administered O-1057 at lower concentrations compared to Wistar rats

- P rats received more self-infusions of O-1057 than Wistar rats

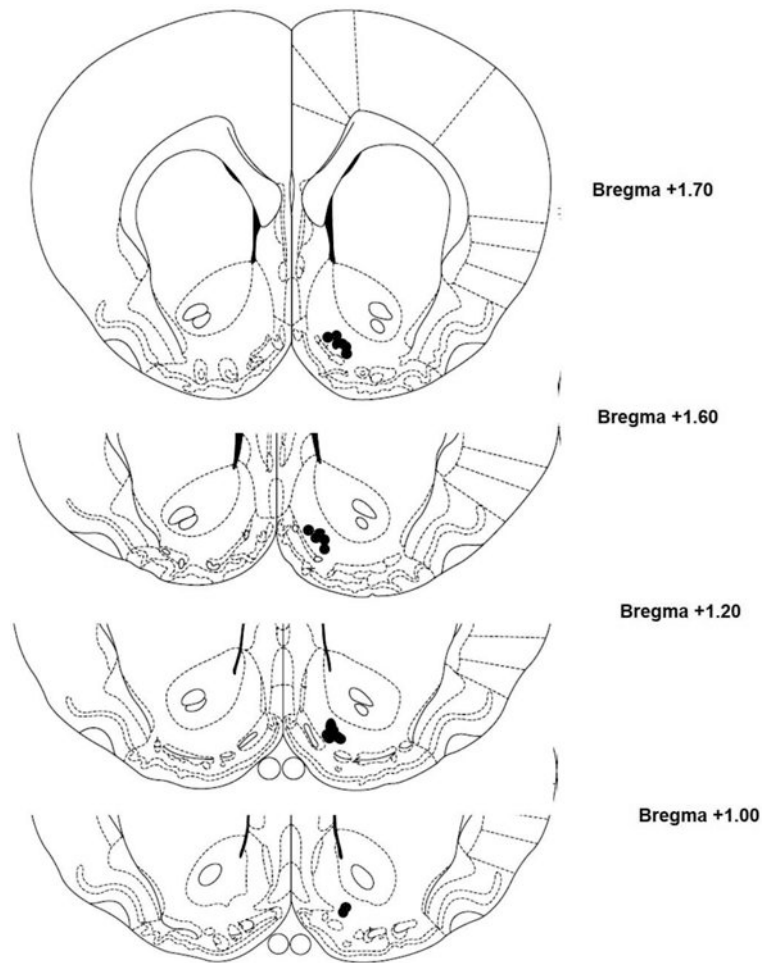- Alcohol preference may enhance sensitivity to the rewarding effects of cannabinoids

Figure 1.
Representative placements for the intracranial self- administration of aCSF and O-1057 into the AcbSh of adult female P rats are shown. Black circles represent placements of injection sites within the AcbSh (defined as +1.7 to + 1.0 mm bregma).
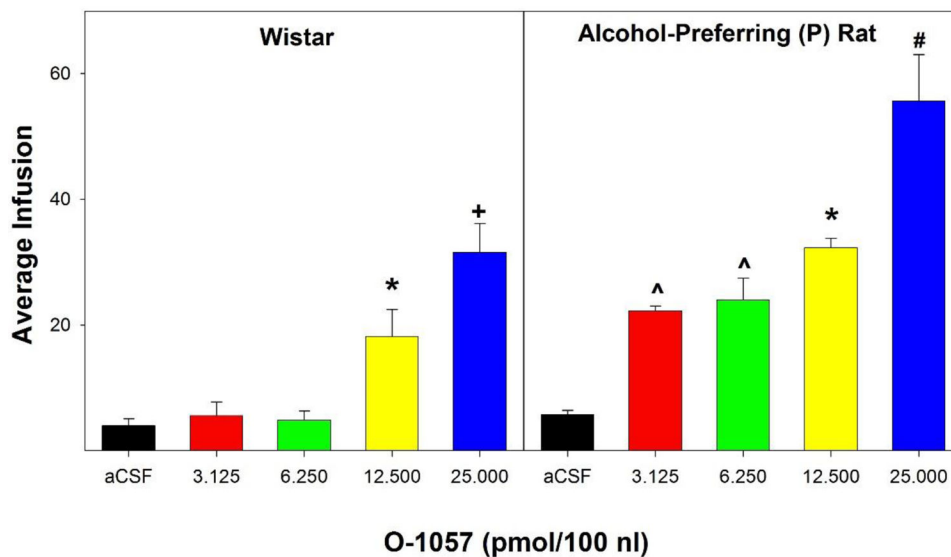
Figure 2.
Depicts the mean (± SEM) average number of O-1057 infusions self-administered directly into the AcbSh during sessions 1-4 in Wistar (left panel) and P (right panel) rats. Asterisk (*) indicates significantly more infusions than aCSF controls. Plus sign (+) indicates significantly more infusions in the Wistar 25 pmol group compared to all other Wistar groups. Carrot (^) indicates significantly more infusions than aCSF controls and P > Wistars. Number sign (#) indicates significantly more self-infusions than all other groups.
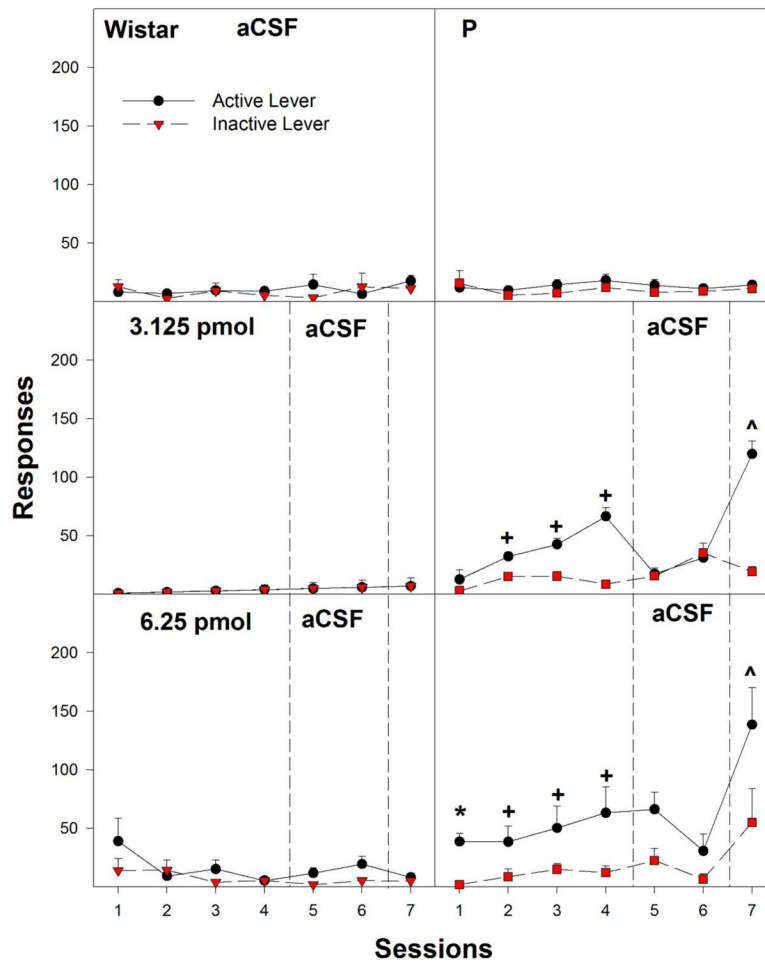
Figure 3.
Depicts the mean (± SEM) number of lever responses in Wistar (left panels) and P (right panels) rats given aCSF (top panels), 3.125 (middle panels), or 6.5 pmol of O-1057 (bottom panels) to self-administer directly into the AcbSh. Asterisk (*) indicates significantly more responding on the active lever than that observed in aCSF controls and discrimination between levers. Plus sign (+) indicates more responding on the active lever than that observed in aCSF controls and P > Wistar. Carrot (^) indicates more responding on the active lever than that observed in aCSF controls, P > Wistar, and responding during sessions 7 > session 4.
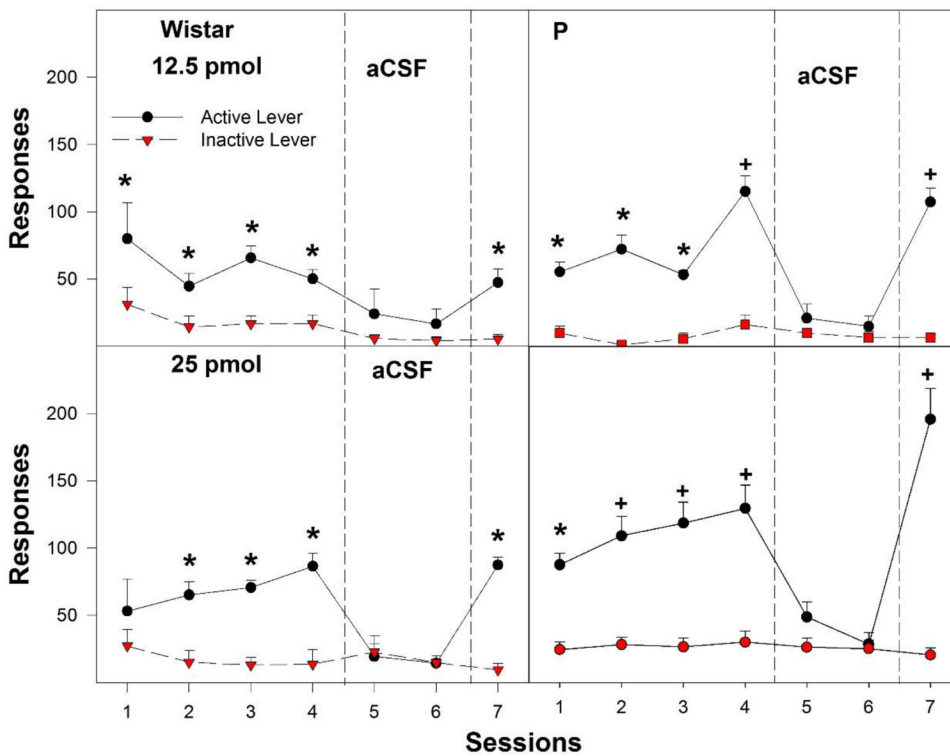
Figure 4.
Depicts the mean (± SEM) number of lever responses in Wistar (left panels) and P (right panels) rats given 12.5 (top panels) or 25 pmol of O-1057 to self-administer directly into the AcbSh. Asterisk (*) indicates significantly more responding on the active lever than that observed in aCSF controls and discrimination between levers. Plus sign (+) indicates more responding on the active lever than that observed in aCSF controls and P > Wistar. Carrot (^) indicates more responding on the active lever than that observed in aCSF controls, P > Wistar, and responding during sessions 7 > session 4.