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The contribution of the central nucleus of the amygdala to individual differences in amphetamine-induced hyperactivity

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Abstract

Rats classified as high responders (HR) based on their response to an inescapable novel environment self-administer more amphetamine and have greater amphetamine-induced sensitization than rats classified as low responders (LR). Recent research suggests that the central nucleus of the amygdala (ACe) contributes to the elevated self-administration in HR rats. Therefore, the current study examined the role of the ACe in the expression of both amphetamine-induced sensitization and conditioned hyperactivity in HR and LR rats. Male Sprague-Dawley rats were screened for their response to inescapable novelty and classified as HR or LR rats. Rats were implanted with bilateral cannulae into the ACe and received amphetamine (1.0 mg/kg, s.c.) or saline injections immediately prior to 1-hr locomotor sessions. Following five training sessions, all rats received an infusion of muscimol (0.5 µg/0.5 µl) or phosphate buffered saline (PBS) followed by a saline injection to measure conditioned hyperactivity. HR rats displayed conditioned hyperactivity, while LR rats did not, suggesting that HR and LR rats differ in the expression of conditioned hyperactivity. While ACe inactivation attenuated the expression of conditioned hyperactivity, it did not differentially affect HR and LR rats. Following additional training and a 10 day rest period, all rats were then tested for amphetamine-induced sensitization (1.0 mg/kg) following an infusion of muscimol or PBS. Inactivation of the ACe attenuated the expression of sensitization only in HR rats. These results suggest the ACe contributes to the greater amphetamine sensitization in HR rats.

Keywords

central nucleus of the amygdala; locomotor; conditioned hyperactivity; sensitization; amphetamine; novelty; muscimol

Introduction

Numerous factors contribute to individual differences in vulnerability to drug abuse, including personality traits. One personality trait that correlates with vulnerability to drug abuse is “novelty seeking” or “sensation seeking”. Using Zuckerman’s sensation seeking scale or

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Disclosure/Conflict of Interest

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Cloninger's novelty seeking scale, high novelty seekers have been shown to use drugs more frequently than low novelty seekers [23,68,69,71]. In order to better understand how this personality trait influences drug abuse vulnerability, an animal model of sensation seeking has been developed. Piazza and colleagues (1989) categorized rats as either high or low "responders" based on the amount of locomotor activity in an inescapable novel environment. High responder (HR) rats are more sensitive than low responder (LR) rats to amphetamine-induced locomotor activity [26,32,34,54]; however, see [59], and to amphetamine self-administration [42,54,59]. These differences appear to be greater at low unit doses of amphetamine [9,32,41]. Recently, the ability of the response to inescapable novelty to predict compulsive cocaine-taking has been examined. While the response to inescapable novelty reliably predicts the acquisition of self-administration of low unit doses of cocaine, impulsivity appears to be a better predictor of compulsive cocaine-taking [5,21]. These findings suggest that different personality traits may contribute to different aspects of the response to drugs of abuse.

While it is widely accepted that the response to inescapable novelty predicts the locomotor response to amphetamine, the biological mechanisms for these differences are not fully understood. One explanation for these differences is that HR rats may have greater sensitivity in the mesolimbic dopamine system than LR rats. In support of this hypothesis, HR rats exhibit greater accumbal dopamine (DA) release following either inescapable novelty or stimulant drugs [7,58]. In addition, HR rats have greater DA uptake, greater cocaine evoked DA release [13], and greater firing rates and bursting activity in DA neurons than LR rats [43], suggesting that the responsiveness of DA neurons in HR rats may account for the enhanced responsiveness to novelty and psychostimulants. While there appears to be enhanced dopaminergic function in the nucleus accumbens (NA) of HR rats, the opposite appears to be true in the prefrontal cortex (PFC). HR rats, when compared to LR rats, have less DAT expression and less DOPAC content in the PFC [70], suggesting that the role of DA sensitivity in HR and LR rats varies by brain region. Another explanation may be related to individual differences in the stress hormone corticosterone [8,57]. HR rats have a longer duration of corticosterone secretion after exposure to a novel environment [55] and higher levels of corticotrophin releasing hormone in the paraventricular nucleus of the hypothalamus [36]. Since glucocorticoids have the ability to stimulate dopamine release [56], it is also possible that the interaction between DA and corticosterone contribute to these individual differences.

Recently, we observed that inactivation of the central nucleus of the amygdala (ACe) attenuates amphetamine self-administration in HR but not LR rats [9]. These findings suggest that the ACe also contributes to the differences in self-administration in HR and LR rats. In this study, rats were also classified as high or low novelty seekers (HNS or LNS respectively) based on their response to free-choice novelty using novelty place-preference test. Interesting, free-choice novelty does not result in corticosterone release [46], and inactivation of the ACe did not differentially affect HNS and LNS rats. The ACe is a focal point for activation of the hypothalamic pituitary adrenal axis (HPA) [4], and has a role in arousal [20,38] and response to novelty [37,45]. The ACe is comprised of two extended components, the medial-extended amygdala and the central-extended amygdala [1,29]. The cells of the central-extended amygdala continue into the reward-relevant shell of the NA [1,29] and the ACe contributes to the rewarding effects of psychostimulants [2,12,14,50]. Together, these findings suggest the ACe may be contributing to the differences between HR and LR rats through regulation of the HPA axis, connectivity with the NA, or a combination of these neuroanatomical connections.

While it has been observed that the ACe contributes to the elevated amphetamine self-administration in HR rats, no studies to date have examined if it contributes to the differences between HR and LR rats in amphetamine-induced hyperactivity. The current study will examine if the ACe contributes to the expression of amphetamine-induced sensitization and if

the ACe contributes to the differences in amphetamine-induced sensitization in HR and LR rats.

In addition to the role of the ACe in regulating the HPA axis and the response to psychostimulants, it has long been implicated to be critical for Pavlovian conditioning. The ACe is critical for the acquisition of Pavlovian fear conditioning [39,51] and expression of Pavlovian conditioned responses [19,28,48,51]. There is extensive literature from research with both animals and humans that drug effects can form learned associations with the environment and other drug cues [3,15,49]. This association is believed to result from Pavlovian conditioning [17,30,63,66]. While there is some suggestion that these conditioned drug effects may not represent genuine Pavlovian conditioning processes [18,27], it is generally accepted that conditioned stimuli, such as the environment, can gate the expression of sensitization [64] and can reinstate extinguished responding [61,62,65]. Therefore, given the well-established role of the ACe in the acquisition and expression of Pavlovian conditioned behaviors, the current study will also examine if the ACe contributes to the expression of amphetamine-induced conditioned hyperactivity. Relatively few studies have examined if HR and LR rats differ in the expression of conditioned hyperactivity [35]. Given that the ACe contributes to the differences between HR and LR rats, the current study will also examine if the ACe contributes to the differences in conditioned hyperactivity between HR and LR rats.

Methods

Animals

Male Sprague-Dawley rats (175–200 g) were obtained from Harlan Industries (Indianapolis, IN) and Charles River (Portage, MI). Rats were housed individually in standard laboratory cages (20×20×42 cm) with access to food and water throughout the experiment, except during locomotor sessions. The colony room was maintained at 24°C and 45% humidity with lights on 0700–1900h. Before the start of the experiment, rats were handled and acclimated to the colony for 1 week. All behavioral procedures were conducted during the light phase. All procedures were approved by the Institutional Animal Care and Use Committee at Kansas State University and conformed to the National Institute of Health's *Guide for the Care and Use of Laboratory Animals* (1996).

Drugs

D-amphetamine sulfate (Sigma, St. Louis, MO) was dissolved in 0.9% saline and was administered at a dose of 1.0 mg/kg. Treatments were administered in a volume of 1 ml/kg subcutaneously. Muscimol (Sigma), a GABA_A agonist, was dissolved in 0.9% phosphate buffered saline (PBS) at infused bilaterally at a dose of 0.5 µg/0.5 µl per side.

Apparatus

Locomotor activity was measured in a locomotor chamber, measuring 40.64×40.64 ×40.64 cm. The chamber consisted of Plexiglas walls and plastic flooring which was covered with bedding. The photobeam sensor ring consisted of a 16×16 (x-axis) photocell array. These photocells were spaced 2.54 cm apart (Coulbourn Instruments, TruScan 2.01) and linked to a personal computer located outside of the chambers. Photobeam interruptions were continuously recorded for all sessions. Cumulative photobeam interruptions, in 5-min blocks of time, were also recorded within each session. A white-noise generator (~70 dB) was used to create ambient background noise to mask sounds from outside the chamber.

For measuring novelty place preference, a rectangular apparatus was used that had three distinct compartments separated by removable solid partitions. The two end compartments measured 28×35×45 cm, and the smaller, middle compartment measured 19×10×45 cm. One end

compartment consisted of white plexiglass walls, mesh flooring, and newspaper beneath the floor. The other end compartment consisted of black plexiglass walls, a metal rod floor, and pine bedding beneath the floor. The middle compartment consisted of gray plexiglass walls and solid plexiglass flooring. During testing, the solid partitions were replaced with similar partitions containing a 10×10 cm opening, which allowed the rats' access to all three compartments. The apparatus was located in a laboratory room separate from the colony room. A white noise generator was used to create ambient background noise (~70 dB). A video camera, located directly above the apparatus was used to record the experimental sessions.

Novelty Tests

For response to inescapable novelty, rats were brought into the testing room and placed in the locomotor chambers for 30-min. Locomotor activity was measured by recording the total distance traveled (cm). Rats were classified as either HR or LR based on a median split. Rats with activity scores above the median were classified as HR while those with activity scores below the median were classified as LR.

On the day following the inescapable novelty test, each rat began the novelty place preference test. Each rat was habituated to either the white or black compartment for 30-min/day for two consecutive days. The compartment each rat was habituated to was counterbalanced across animals. On the third day, each rat was tested for place preference. Rats were placed in the center gray compartment and were allowed unrestricted access to all three compartments for 15-min. Time in each compartment was scored during the test session by an observer blind to condition. Rats were considered to be in a compartment when both front paws entered the compartment. A preference ratio was calculated as the time spent in the novel end compartment divided by the sum of time in both the novel and familiar end compartments. Rats were classified as high- or low-novelty seekers (HNS; LNS) based on a median split of the place preference ratio.

Surgery

Rats (300–350 g) were anesthetized with ketamine (80 mg/kg, IP) and diazepam (5 mg/kg, IP). Bilateral 26 gauge stainless steel cannula aimed 1 mm dorsal to the region of the ACe were implanted stereotactically (Stoelting Stereotaxic Co.; anterior-posterior, –2.3 mm; medial-lateral, ±4.2 mm; dorsal-ventral, –6.5 mm) [52]. The cannulae were embedded in a cap of dental acrylic affixed to the top of the skull with four stainless-steel jeweler's screws. Following implantation, 33 gauge stainless steel obturators were placed in the cannula until testing. Rats were allowed to recover for at least one week prior to the start of behavioral testing.

Acquisition of Amphetamine-Induced Hyperactivity

Equal numbers of rats classified as HR and LR on the inescapable novelty test were randomly assigned to one of 6 groups: Paired-Muscimol, Paired-PBS, Unpaired-Muscimol, Unpaired-PBS, Control-Muscimol, or Control-PBS. All rats were brought into the testing room at approximately the same time daily. Rats in each group had 5 conditioning sessions in the locomotor chambers with a rest day intervening between each session. The Paired group was administered amphetamine (1.0 mg/kg; s.c.) immediately prior to a 1-h locomotor session. On alternating days, Paired group rats received saline injections in the colony room. The Unpaired group received saline prior to being placed in the locomotor chambers. To control for repeated amphetamine injections, the Unpaired group was administered amphetamine in the colony room on alternating days. The Control group received saline injections for both locomotor session and colony room injections. Following each 1-h session, rats in all groups were removed and returned to the colony room.

Conditioned Hyperactivity Test and Inactivation of the ACe

Rats were brought into the locomotor room and the obturators were removed from the cannulae and a 33 gauge stainless steel injector cut to extend 1mm beyond the cannula tip was inserted bilaterally in the cannulae. Muscimol (0.5 µg/0.5 µl) or PBS was bilaterally infused into the region of the ACe over a 2-min period through the injector. The injector was connected with PE-20 tubing to a 10 µl Hamilton syringe connected to a Stoelting programmable infusion pump. The injector was left in place for 1-min following the infusion to allow diffusion of the muscimol or PBS. The obturators were then replaced. Saline was then administered (s.c.) to all rats prior to being placed in the locomotor chamber for 1-h.

Sensitization Training

Following the conditioned hyperactivity test, all rats received 5 additional training sessions in the locomotor chambers. Procedures were identical to those described for acquisition of amphetamine-induced hyperactivity. Following the last day of sensitization training, rats rested for 10 days.

Sensitization Test and Inactivation of the ACe

Procedures for inactivation of the ACe were identical to those described for conditioned hyperactivity testing. Following bilateral infusions of muscimol or PBS, all rats received an amphetamine (1.0 mg/kg; s.c) injection prior to being placed into the locomotor chamber for 1-h.

Histology

Rats were administered an overdose of sodium pentobarbital (150 mg/kg, ip). The brain was removed and 60 µm frozen serial sections were taken and stained with thionin (Sigma). The sections were examined under a light microscope to determine the location of cannula placement. The most ventral point of each injection track was mapped onto the appropriate plate from the rat brain atlas of Paxinos and Watson (1998).

Data Analysis

Bivariate correlations were conducted to determine if there was a relationship between the two novelty tests. The effect of Treatment (Paired, Unpaired, or Control) on locomotor activity across sessions was analyzed using a mixed factorial analysis of variance (ANOVA) with Session as the within-subjects factor. The effects of ACe inactivation on locomotor activity during the conditioned hyperactivity and sensitization tests were analyzed in separate between-subjects ANOVAs with Treatment and Infusion (Muscimol vs. PBS) as the between-subjects factors. Individual differences in the two novelty tests were analyzed in separate ANOVAs, with Novelty Classification (HR vs. LR; HNS vs. LNS), Treatment, and Infusion as between-subjects factors and Session as a within-subject factor where appropriate. For all ANOVAs, the alpha level was set at .05. Where appropriate, median split groups (HR vs. LR; HNS vs. LNS) were compared using Bonferroni corrected simple effects or planned comparisons with the alpha level set at .01 and the *F* values from these tests are reported.

Results

Histological Verification

Only rats with accurate bilateral cannulae placement were included in the analyses. A total of 89 rats had accurate bilateral cannulae placement within the ACe (Figure 1). This resulted in a minimum of 6 rats with accurate bilateral cannulae placement in each treatment group with the exception of the LR-Paired Muscimol group, which consisted of 5 rats.

Novelty Tests

The total distance traveled (cm) for the 30-min inescapable novelty test ranged from 3019.9 – 7866.6, with a median score of 5583.1. The preference ratio for the novelty place preference test ranged from 0.18 – 0.75, with a median score of 0.55. A bivariate correlation between locomotor scores and novelty place preference ratios was not significant.

Acquisition of Amphetamine-Induced Hyperactivity

HR-Paired rats had significantly greater locomotor activity than LR-Paired rats during the majority of the conditioning sessions (Figure 2A). During conditioning sessions there was a main effect of Session, $F(4, 288) = 4.89, p < .001$, and a significant Session X Treatment interaction, $F(8, 288) = 10.89, p < .001$. HR and LR rats in the Paired group significantly differed from Unpaired and Control groups on all five acquisition sessions, $F_s(1, 72) = 75.88$ to $191.32, p_s < .001$. Planned comparisons revealed that HR-Paired rats had significantly greater locomotor activity than LR-Paired rats during sessions 1 to 4 $F_s(1, 72) = 4.04$ to $6.06, p_s < .05$.

While repeated amphetamine administration increased locomotor activity in HNS and LNS rats in the Paired group, HNS and LNS rats were not found to differ (Figure 2B). A main effect of Session was found, $F(4, 280) = 7.99, p < .001$. There was also a Session X Treatment interaction, $F(8, 280) = 8.90, p < .001$, and a Session X Novelty Classification interaction, $F(4, 280) = 2.23, p < .001$. HNS and LNS rats in the Paired groups significantly differed from Unpaired and Control groups on all five acquisition sessions, $F_s(1, 70) = 66.20$ to $189.12, p_s < .001$. While there was a Session X Novelty Classification interaction, simple effect tests did not reveal any significant differences between HNS and LNS rats.

Conditioned Hyperactivity Test

During the conditioned hyperactivity test, rats in all treatment groups received a saline injection. Inactivation of the AChE significantly attenuated the expression of conditioned hyperactivity in the Paired, Unpaired, and Control groups (Figure 3). A two-way ANOVA of Infusion by Treatment revealed a main effect of Infusion, $F(1, 81) = 55.08, p < .001$, and a main effect of Treatment, $F(2, 810) = 19.41, p < .001$. Rats pretreated with PBS in the Paired group displayed significant conditioned hyperactivity when compared to Unpaired-PBS rats, $F(1, 81) = 17.29, p < .01$ and Control-PBS rats, $F(1, 81) = 12.83, p < .001$. Muscimol treatment was found to significantly decrease locomotor activity in Paired, Unpaired, and Control groups relative to PBS-treated rats, $F_s(1, 81) = 16.92$ to $20.15, p_s < .01$.

Analysis of the expression of conditioned hyperactivity in HR and LR rats revealed a main effect for Infusion, $F(1, 77) = 59.15, p < .001$, a main effect of Treatment, $F(2, 77) = 21.64, p < .001$, and a Treatment X Novelty Classification interaction, $F(2, 77) = 3.72, p < .05$. LR rats in the Paired group receiving muscimol displayed a decrease in locomotor activity compared to HR rats, $F(1, 77) = 5.63, p < .05$.

When treated with PBS, HR rats in the Paired group displayed significant conditioned hyperactivity when compared to Unpaired, $F(1, 77) = 18.28, p < .001$, and Control groups, $F(1, 77) = 15.02, p < .001$ (Figure 4A). Similarly, when treated with muscimol, HR rats in the Paired displayed significant conditioned hyperactivity when compared to both Unpaired, $F(1, 77) = 18.74, p < .001$, and Control groups, $F(1, 77) = 10.98, p < .01$. However, LR rats in the Paired group, regardless of infusion, were not found to display significant conditioned hyperactivity relative to Unpaired and Control groups (Figure 4B). Rats classified as HNS and LNS did not significantly differ during the conditioned hyperactivity test (data not shown).

Sensitization Training

Following the conditioned hyperactivity test, rats received an additional 5 training sessions in the locomotor chambers. Across sensitization training sessions, rats in the Paired group displayed greater locomotor activity than rats in the Unpaired and Control groups (Figure 5). A main effect of Treatment was found, $F(2, 75) = 201.06, p < .001$. Rats in the Paired groups displayed significantly greater activity when compared to the Unpaired and Control groups across all five sensitization training sessions, $F_s(1, 72) = 83.13$ to $186.18, p_s < .001$. A main effect of Novelty Classification, $F(1, 75) = 4.89, p < .05$ and an Treatment X Novelty Classification interaction, $F(2, 75) = 3.85, p < .05$, were found. HR rats in the Paired group displayed significantly greater locomotor activity than LR rats in the Paired group only during the first session of sensitization training, $F(1, 75) = 10.95, p < .01$. No significant differences were found between HNS and LNS rats during sensitization training (data not shown).

Sensitization Test

During the sensitization test, all rats were treated with amphetamine. Inactivation of the ACe with muscimol did not significantly decrease sensitization (Figure 6). A main effect of Infusion, $F(1, 86) = 7.09, p < .01$, and Treatment, $F(2, 86) = 8.45, p < .001$, were found. Rats in the Paired-PBS, $F(1, 86) = 9.28, p < .01$, and Paired-Muscimol, $F(1, 86) = 7.55, p < .01$, groups displayed significant sensitization when compared to Controls.

Analysis of the response to inescapable novelty revealed a 3-way interaction between Novelty Classification, Infusion, and Treatment, $F(2, 80) = 3.53, p < .05$. When comparing HR-PBS rats and HR-muscimol rats, the HR Paired-Muscimol group was observed to display marginally less sensitization than HR Paired-PBS rats, $F(1, 80) = 5.09, p = .053$. Additionally, the HR rats in the Unpaired-Muscimol group were also found to display significantly less sensitization than HR rats in the Unpaired-PBS treated rats, $F(1, 80) = 7.47, p < .01$ (Figure 7A). LR rats were not found to differ between infusion groups (Figure 7B).

When treated with PBS, HR rats in the Paired group displayed significant sensitization relative to the Control group, $F(1, 80) = 9.70, p < .01$. There were no differences between HR rats in the muscimol treatment group. When treated with PBS, LR rats did not differ between injection treatment groups, however, when treated with muscimol, both Paired, $F(1, 80) = 9.55, p < .01$, and Unpaired, $F(1, 80) = 10.56, p < .01$, groups displayed significantly greater sensitization than LR rats in the Control group. Analysis of rats classified as HNS and LNS revealed no significant differences (data not shown).

Unilateral Microinjection Sites

A total of 13 rats (6 Paired and 7 Control) had unilateral cannula placements and their data were analyzed to determine if the observed effects of inactivation were specific to the ACe. For each rat, one cannula tip was located within the region of the ACe and the placement of the other tip varied from the adjacent basolateral nucleus, the caudate putamen, or the interstitial nucleus of the posterior limb of the anterior commissure. ANOVA was performed on the total distance traveled during the conditioned hyperactivity test and revealed no main effects of Novelty Classification, Infusion, or Treatment (all $F_s > 1$). Analysis of the total distance traveled during the sensitization test revealed a main effect of Treatment, $F(1, 5) = 6.76, p < .05$, but no main effects of Novelty Classification or Infusion ($F_s > 1$), suggesting that the observed effects of ACe inactivation were regionally specific.

Discussion

This experiment examined the role of the ACe in the expression of amphetamine-induced conditioned hyperactivity and sensitization in HR and LR rats. Inactivation of the ACe

attenuated the expression of sensitization in HR, but not LR rats, suggesting that the ACe contributes to the elevated sensitization observed in HR rats. Interestingly, when the role of the ACe was examined regardless of HR and LR classification, inactivation of the ACe had no effect on the expression of sensitization, suggesting the ACe is only critical for the expression of sensitization in HR rats. In the current study, only HR rats expressed conditioned hyperactivity. However, while HR and LR rats differed in the expression of conditioned hyperactivity, inactivation of the ACe did not affect conditioned hyperactivity differentially in HR and LR rats. While inactivation of the ACe attenuated the expression of conditioned hyperactivity, it also attenuated activity in the control groups, so we cannot conclude that the attenuation is specific to expression of conditioned hyperactivity. These results suggest that while the ACe contributes to the elevated sensitization in HR rats, it is not clear if it contributes to conditioned hyperactivity.

While HR rats had significantly greater locomotor activity than LR rats during some sessions, locomotor activity did not consistently differ between HR and LR rats following repeated amphetamine injections. This finding is consistent with previous literature suggesting that the effect of repeated amphetamine injections in HR and LR rats varies with the dose administered. When a low dose (0.5 mg/kg) of amphetamine is administered repeatedly, HR rats generally have greater amphetamine-induced hyperactivity than LR rats [32,33,35]. When a moderate dose (1.0 mg/kg) is administered repeatedly, one study observed robust differences between HR and LR rats [32]. When higher doses (1.5 mg/kg or higher) are administered repeatedly, some studies have reported greater amphetamine-induced hyperactivity in HR than LR rats [31,54], while other studies have not observed differences between HR and LR rats [26,59]. The current study administered a 1.0 mg/kg to ensure robust hyperactivity and to examine conditioned hyperactivity using a dose of amphetamine that has not previously been examined. Since only one study has examined the 1.0 mg/kg dose, it is possible that the 1.0 mg/kg dose is similar to the 1.5 mg/kg in that it sometimes yields differential locomotor activity in HR and LR rats. Despite the fact that we did not observe consistent HR and LR differences following repeated injections, it is important to note that we did observe differences between HR and LR rats during initial training and during the expression of conditioned hyperactivity and sensitization.

Only HR rats expressed conditioned hyperactivity, suggesting that the rats most vulnerable to drug abuse may also be most vulnerable to Pavlovian conditioned drug cues. This finding is consistent with previous literature in which a 0.5 mg/kg dose of amphetamine resulted in conditioned hyperactivity in HR rats, but not LR rats [35]. Similarly, administration of methamphetamine results in greater conditioned hyperactivity in HR than LR rats [6]. While several studies now suggest that HR and LR rats differ in expression of conditioned hyperactivity, HR and LR rats only differ in amphetamine conditioned place preference when a low dose of amphetamine is used [25,53]. Interestingly, the response to inescapable novelty does not predict conditioned hyperactivity to all drugs of abuse. HR and LR rats do not differ in conditioned hyperactivity when nicotine [16] or bupropion hydrochloride [67] are administered, suggesting that the ability of the response to inescapable novelty to predict conditioned hyperactivity varies both as a function of the dose and drug administered.

It was hypothesized that inactivation of the ACe would attenuate the expression of conditioned hyperactivity due to the well-established role of the ACe in the acquisition and expression of Pavlovian conditioned behaviors [19,28,39,48,51]. In addition, given that the ACe contributes to the elevated responding in HR rats during amphetamine self-administration [9], it was expected that the ACe would contribute to the expression of conditioned hyperactivity in HR rats. While inactivation of the ACe did attenuate the expression of conditioned hyperactivity, it also attenuated locomotor activity in our Unpaired and Control groups, so we cannot conclude from the current results that the ACe is critical for the expression of amphetamine-induced

conditioned hyperactivity. In addition, we did not observe a differential effect of inactivation in HR and LR rats, perhaps because locomotor activity was attenuated in the Control groups. It is important to note that inactivation of the ACe did not attenuate locomotor activity in the Control groups during the sensitization test when amphetamine was present, suggesting that inactivation only attenuated activity during the saline conditioned hyperactivity test.

One possible explanation for the decreased locomotor activity in the Control groups during the conditioned hyperactivity test is that microinjections of muscimol into the ACe have been shown to result in an “anxiolytic-like” response in the social interaction and elevated plus maze tests [47,60]. In addition, repeated social defeat stress abolishes the differences in response to a single injection of amphetamine in HR and LR rats [22] suggesting that stress can alter amphetamine-induced hyperactivity differences between HR and LR rats. Thus, it is possible that the muscimol microinfusions had an “anxiolytic-like” effect in the HR and LR rats and this decreased locomotor activity. While a direct measure of anxiety would be needed to address the issue fully, this explanation for the current results is not likely because the rats had five training sessions prior to the conditioned hyperactivity test and thus were habituated to the experimental procedures. Further, locomotor activity was not decreased by muscimol during the sensitization test and muscimol did not differentially affect HNS and LNS rats. Therefore, it seems unlikely that the effects obtained in HR rats during the sensitization test simply reflect a nonspecific effect of muscimol on locomotor activity or anxiety. While the current results suggest the ACe may contribute to the expression of conditioned hyperactivity, additional studies will be necessary to determine the extent of the contribution of the ACe.

Interestingly, while inactivation of the ACe attenuated the expression of sensitization in HR Paired and Unpaired rats, it had no effect in LR rats. Additionally, inactivation of the ACe had no effect on the expression of sensitization in HNS or LNS rats or when sensitization was examined regardless of novelty classification. These findings suggest that the ACe contributes to the expression of sensitization only in HR rats. As HR rats have greater expression of amphetamine-induced sensitization than LR rats [32,33,54], this finding suggests that the ACe contributes to the elevation in sensitization in HR rats.

While the current study only inactivated the ACe, the involvement of brain sites outside of the point of injection needs to be considered, as muscimol may have spread to areas adjacent to the ACe. Previous research suggests that a 1.0 µg/1.0 µl infusion of muscimol diffuses in a sphere of at least 1.0 mm around the site of injection [44] and more recent research suggests the spread may be greater [24]. In an attempt to minimize the spread of muscimol to other regions, in particular the basolateral complex (BLA), the cannulae placements were targeted anterior and dorsomedial to the BLA. Based on previous work using neurotoxin injections, it is known that drug spread into the BLA from the ACe is inhibited due to fiber encapsulation of the ACe on its lateral and ventral borders [40]. While it is possible some of the observed effects were due to diffusion of muscimol to neighboring amygdaloid nuclei, the lack of effect observed with our unilateral infusions demonstrates that the observed effects in HR rats shows some regional specificity.

In contrast to the effects of ACe inactivation during sensitization, when rats were classified based on novelty place preference, inactivation of the ACe did not differentially affect HNS and LNS rats. Given that ACe inactivation did not differentially affect HNS and LNS rats, these results suggest that the effects of ACe inactivation are specific to the inescapable novelty test. Importantly, the tests for inescapable and free-choice novelty are not related to one another and thus they measure different aspects of response to novelty [10]. To our knowledge, this is first study to examine if the novelty place preference test can be used to predict the locomotor response to amphetamine. In the current study, rats classified as HNS and LNS based on the novelty place preference test did not differ in the locomotor response to amphetamine during

any phase of the experiment. This is consistent with previous research that the novelty place preference test does not predict differences in amphetamine self-administration [10,11,41] and that inactivation of the ACe does not differentially affect HNS and LNS rats [9]. While HR rats have a longer duration of corticosterone secretion after exposure to a novel environment [55], the novelty place preference test does not elevate levels of corticosterone [46]. This suggests that the ability of ACe inactivation to decrease sensitization in HR rats may be due to the role of the ACe in activating the HPA axis [4].

The current results suggest that while the ACe is not critical for the expression of amphetamine-induced sensitization, it does contribute to the elevated expression of sensitization in HR rats. The current results further suggest that HR rats are more sensitive to Pavlovian conditioned drug cues than LR rats. While it appears the ACe contributes to the expression of conditioned hyperactivity, it is unclear from the current results if the ACe contributes to the greater conditioned hyperactivity observed in HR rats. The current results, combined with previous literature, suggest that the ACe contributes to both the elevated locomotor response to amphetamine and the elevated self-administration of amphetamine in HR rats.

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Figure 1.

Shaded regions indicate the location of cannulae placements as verified by thionin-stained sections for HR (●) and LR (▨) rats. The shaded regions represent the most ventral point of the injector tracts on coronal sections based on the atlas of Paxinos and Watson (1998). Numbers indicate the distance from Bregma in millimeters.

Figure 2.

Figure 2A & B: The mean ($\pm$ S.E.M.) total distance traveled (cm) for rats in the Paired, Unpaired and Control groups during sessions 1–5 of acquisition training. (A): HR and LR groups. (B) HNS and LNS groups. Asterisks (*) indicate a significant difference ($p < .001$) in Paired groups relative to Unpaired groups. Numerical signs (#) indicate a significant difference ($p < .05$) between HR and LR rats.

Figure 3.

The mean ($\pm$ S.E.M.) total distance traveled (cm) for rats in the Paired, Unpaired and Control groups during the conditioned-hyperactivity test. Asterisks (*) indicate a significant difference ($p < .01$) in Paired groups relative to Unpaired and Control groups. Numerical signs (#) indicate a significant difference ($p < .01$) between Muscimol treatment groups relative to PBS treatment groups.

Figure 4.

Figure 4A & B: The mean ($\pm$ S.E.M.) total distance traveled (cm) for HR and LR rats in the Paired, Unpaired and Control groups during the conditioned-hyperactivity test. (A): HR rats treated with PBS and muscimol. (B). LR rats treated with PBS and muscimol. Asterisks (*) indicate a significant difference ($p < .01$) from the Paired groups. Numerical signs (#) indicate a significant difference ($p < .001$) between PBS and muscimol treatment groups.

Figure 5.

The mean ($\pm$ S.E.M.) total distance traveled (cm) for HR and LR rats in the Paired, Unpaired and Control groups during sessions 1–5 of sensitization training. Asterisks (*) indicate a significant difference ($p < .001$) in Paired groups relative to Unpaired and Control groups. Numerical sign (#) indicates a significant difference ($p < .05$) between HR and LR rats.

Figure 6.

The mean ($\pm$ S.E.M.) total distance traveled (cm) for rats in the Paired, Unpaired and Control groups during the sensitization test. Asterisks (*) indicate a significant difference ($p < .01$) in Paired groups relative to Unpaired and Control groups.

Figure 7.

Figure 7A & B: The mean ($\pm$ S.E.M.) total distance traveled (cm) for HR and LR rats in the Paired, Unpaired and Control groups during the sensitization test. (A): HR rats treated with PBS and muscimol. (B). LR rats treated with PBS and muscimol. Asterisks (*) indicate a significant difference ($p < .01$) from Control groups. Numerical signs (#) indicate a significant difference ($p < .05$) between PBS and muscimol treatment groups.