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# Autonomic activation associated with ethanol self-administration in adult female P rats

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ABSTRACT

The present study examined changes in heart rate (HR) prior to and during limited access ethanol drinking in adult female P rats. P rats were implanted with radio-telemetric transmitters to measure HR. Daily testing involved a 90-min pre-test period (water only available) and a subsequent 90-min test period [either water (W) or ethanol available]. After a week of habituation, one ethanol group had access to ethanol for 7 weeks (CE), and another ethanol group had access for 4 weeks, was deprived for 2 weeks and then had access for a final week (DEP). Analyses of HR revealed that CE and DEP rats had significantly higher HR than W rats during test periods that ethanol was present and that DEP rats displayed higher HR during the early test period of the ethanol deprivation interval, as well. These data indicate that ethanol drinking induces HR activation in adult female P rats, and that this activation can be conditioned to the test cage environment, paralleling reports on contextual conditioning and cue-reactivity in alcoholics exposed to alcohol-associated stimuli. Therefore, this behavioral test may prove advantageous in screening pharmacotherapies for reducing craving and relapse, which are associated with cue-reactivity in abstinent alcoholics.



## 1. Introduction

Autonomic activation, as indicated by increases in heart rate, is associated with reinforcement in rats (Burgess et al., 1993), dogs (Kostarczyk and Fonberg, 1982), and humans (Fowles et al., 1982; Tranel et al., 1982). Moreover, these increases in heart rate are not contingent upon motor activity (Fowles et al., 1982; Miller and DiCara, 1967; Tranel et al., 1982). With regard to ethanol reinforcement, ethanol consumption increases heart rate in nonalcoholic normotensive human subjects (Grassi et al., 1989; Higgins et al., 1993; Ireland, 1984; Iwase et al., 1995). Additionally, in family history positive (FHP) for alcoholism individuals, levels of increased heart rate with ethanol consumption were positively associated with the hedonic properties of ethanol (Assaad et al., 2003) and self-reported alcohol consumption (Peterson et al., 1993; Pihl et al., 1994), with FHP individuals experiencing the greatest increases in heart rate also scoring highest on measures of impulsivity and sensation-seeking (Brunelle et al., 2004). These personality traits are strong predictors for a propensity to abuse alcohol (Andrucci et al., 1989). In a study on alcohol-induced stimulation among heavy drinkers, level of alcohol-induced heart rate increases was positively associated with self-report of alcohol-induced stimulation (Ray et al., 2006). However, active self-administration of alcohol is not required to induce autonomic activation.

Cue-reactivity refers to the phenomenon that paraphernalia and contextual cues associated with drug and alcohol self-administration as well as "mental representation of these cues" induces autonomic arousal in humans (Childress et al., 1993; O'Brien et al., 1992; Payne et al., 1992; Rajan et al., 1998; Yu et al., 2007). Using an alcohol-olfactory stimulus, alcoholics displayed greater increases in heart rate, relative to social drinkers, to a higher, but not lower, concentration of alcohol (Stormark et al., 1995). A similar finding was reported by McCaul et al. (1989b) using gustatory stimuli. These findings indicate the importance of stimulus intensity, with stimulus specificity also influencing level of response (Staiger and White, 1991). Additionally,

*Abbreviations:* ADE, alcohol deprivation effect; ANOVA, analysis of variance; BAC, blood alcohol concentration; BPM, beats per minute; CE, continuous ethanol access group; DEP, ethanol access, deprivation, and re-exposure group; FHP, family history positive for alcoholism; g/kg, grams of ethanol per kilogram body weight; HR, heart rate; IP, intraperitoneal; P, alcohol-preferring rat line; VTA, ventral tegmental area; W, water control group.

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cue-reactivity is often associated with "craving" for drugs and alcohol as well as relapse (Carter and Tiffany, 1999; Cooney et al., 1997; Greeley et al., 1993; Newlin, 1992; Niaura et al., 1988; Payne et al., 2006; Rohsenow et al., 1991; Sinha et al., 2000; Tiffany, 1990; Verheul et al., 1999; Vogel-Sprott, 1995). Compared with controls, alcoholics display greater autonomic arousal, and self-reported desire to drink, in the presence of these cues, (Drummond et al., 1990; Kaplan et al., 1985; Rajan et al., 1998). Moreover, a direct association has been shown between cue-reactivity and dependence, such that when subjects were told a beverage contained alcohol, alcoholics displayed increases in heart rate and their level of dependence determined the duration of this response (Stormark et al., 1998).

Given the utility of animal models, selective breeding has developed the alcohol-preferring (P) line of rats (Li et al., 1987). As reviewed elsewhere (c.f., Bell et al., 2005, 2006a; McBride and Li, 1998; Murphy et al., 2002), the P line of rats satisfies all of the criteria proposed for a valid animal model of alcoholism (Cicero, 1979; Lester and Freed, 1973) including the demonstration of relapse-like behavior (McBride and Li, 1998). Relapse behavior is a ubiquitous problem for individuals "recovering" from alcoholism (Barrick and Connors, 2002; Chiauzzi, 1991; Jaffe, 2002; Weiss et al., 2001). Because alcoholics go through cycles of chronic alcohol drinking and abstinence (Finney and Moos, 1991), relapse type drinking has been proposed as an important criterion for a valid animal model of alcoholism (McBride and Li, 1998). The alcohol deprivation effect (ADE) is defined as a temporary increase in the ethanol to total fluid intake ratio and an increase in voluntary intake of ethanol over baseline conditions (i.e., levels before the ethanol deprivation period), when ethanol is reinstated following a period of deprivation (Sinclair and Senter, 1967). The ADE has been proposed as a model of relapse-like behavior (e.g., Rodd et al., 2004b). P rats will display an ADE after extended periods (2 to 8 weeks) of deprivation (Rodd-Henricks et al., 2000), and concurrent access to multiple concentrations of ethanol enhances and prolongs this ADE (Rodd-Henricks et al., 2001).

Despite the extensive literature on cue-reactivity in humans, a portion of which was discussed above, there appear to be only three publications examining ethanol self-administration-associated autonomic arousal in rats (Bell et al., 2002; El-Mas and Abdel-Rahman, 2007; Ristuccia and Spear, 2007), with the Bell et al. (2002) study also explicitly examining contextual conditioning of this effect as well. Given that direct associations between level of cue-reactivity and levels of craving, as well as dependence, have been reported in the clinical literature, continued research to refine a behavioral assay of cue-reactivity will prove advantageous for investigating this phenomenon in rats. In comparison with their male counterparts, adult female rodents reportedly consume more ethanol (e.g., Adams, 1995; Juárez and De Tomasi, 1999; Lancaster and Spiegel, 1992; Li and Lumeng, 1984). This effect, although modest, has been found in periadolescent and post-weaning P (Bell et al., 2003; McKinzie et al., 1998a,b) rats, as well. Results from previous studies have indicated that concurrent access to multiple concentrations of ethanol increases ethanol intake in adult outbred rats (Holter et al., 1998; Wolffgramm and Heyne, 1995), as well as periadolescent P (Bell et al., 2003), and adult P (Rodd-Henricks et al., 2001) rats. Additionally, as indicated above, concurrent access to multiple concentrations of ethanol increases the magnitude and duration of the ADE in adult P rats (Rodd-Henricks et al., 2001). Therefore, the effects of these variables on ethanol-associated autonomic and behavioral activation were assessed in the present study.

Previously, our laboratory reported that adult male P rats with limited access to a single concentration of 15% ethanol displayed autonomic (heart rate) activation during the test period (when daily ethanol was available) and during the pre-test period (before daily ethanol was available) after two weeks of ethanol access. The latter finding indicates that autonomic activation can be conditioned to the environment in which the rats had consumed ethanol (Bell et al., 2002). The present study sought to extend our previous findings by determining whether (a) ethanol drinking would result in autonomic and behavioral activation in adult female P rats; (b) increased ethanol consumption due to concurrent access to multiple concentrations of ethanol would alter these responses; (c) relapse-like conditions (i.e., ADE) would alter these responses, as well; and (d) autonomic and behavioral activation could be conditioned to the environment associated with ethanol self-administration during test period extinction-like trials when ethanol, which was usually made available, was replaced with water.

## 2. Materials and methods

### 2.1. Animals

The subjects were 33 ethanol-naïve adult female P rats from the 47th and 48th generations of selective breeding. The animals weighed an average of 280±6 (mean±SEM) g at the beginning of the experiment. Animals were housed two per plastic tub (18×24×45 cm) with wire grid tops in a temperature- (21 °C) and humidity- (50%) controlled vivarium. The vivarium was maintained on a 12/12 h reverse dark light cycle (lights off at 1000 h). Procedures were conducted during the dark cycle (1200–2000 h). All animals had ad lib access to water and food, except during testing when food was absent. Animals used in these procedures were maintained in facilities fully accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care (AAALAC). All experimental procedures were approved by the Institutional Animal Care and Use Committee of the Indiana University School of Medicine (Indianapolis, IN) and are in accordance with the guidelines of the Institutional Animal Care and Use Committee of the National Institute on Drug Abuse, National Institutes of Health, and the *Guide for the Care and Use of Laboratory Animals* (Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council, 1996).

### 2.2. Apparatus and procedures

Testing took place in clear Plexiglas ($W \times H \times D$: 25.5×38×44.5 cm) chambers with the sides and back painted matte white to simulate the rats' home "shoebox" cages. The test chamber was placed on a radio telemetry receiver (ER-4000: Mini Mitter: Bend, OR, USA), which detected heart rate and motor activity from an implanted radio telemetry device. Testing took place in a laboratory room adjacent to the animal vivarium.

The radio telemetry device (E-mitter: PDT-4000HR: Mini Mitter: Bend, OR, USA) was cleaned and sterilized, then surgically implanted in the intraperitoneal (IP) cavity. Briefly, the animal was anesthetized (2% isoflurane) and the ventral surface was shaved and aseptically prepared. A 2 cm incision was made through the skin along the peritoneal midline starting 2 cm below the diaphragm. A second incision was made similarly through the abdominal muscle wall. The E-mitter was inserted into the IP cavity. The heart rate leads, from the E-mitter, were threaded through the muscle wall lateral to the incision. Two 1 cm incisions were made to facilitate heart rate lead attachment (see below). One incision was made through the skin near the right clavicle and the other through the skin at the lower left side of the rib cage. The negative lead was run subcutaneously and sutured to the anterior right side of the chest wall near the clavicle. The positive lead was run subcutaneously and sutured to the anterior left side of the chest wall near the last rib. This placed the sensors (heart rate lead tips) at approximately a 45 to 60° angle to the transverse plane of the heart. The peritoneal cavity was sutured shut as were the skin incisions along the midline and upper right and lower left chest.

### 2.3. Heart rate and motor activity data collection

Animals were allowed to habituate to the home colony until they reached adulthood (>75 days old). During the following week, the animals were handled intermittently and had the E-mitter implanted

at the end of this week. Animals were pseudo-randomly (so that all test groups were represented within each squad of rats tested, 4 squads were tested) assigned to 1 of 3 test groups [water (W): $n=9$; continuous ethanol (CE): $n=10$; or continuous ethanol, followed by an ethanol deprivation period and a subsequent ethanol re-exposure period (DEP): $n=14$] at the time of surgery. The rats were allowed 1 week to recover from the surgeries. After recovery from the surgery, animals were tested daily for 8 weeks. The test room was illuminated by indirect lighting from a single 40-Watt red light bulb. Animals were habituated (water served as the test solution) to the test procedures for the first/habituation week. The procedures involved placing the animals in their respective test chambers with water available for a 90-min pre-test period. After this, a second bottle containing the test solution was presented for an additional 90-min. For the first/habituation week, water served as the test solution for all 3 groups. For weeks 2 through 8, the second bottle contained each animal's respective test solution (W, CE or DEP). The CE group received concurrent access to multiple ethanol concentrations (10%, 20% and 30%), during the 90-min test period, for weeks 2 through 8 (test blocks 2 through 13, the dependent variables were evaluated across 4-day blocks), the DEP group received concurrent access to multiple ethanol concentrations (10%, 20% and 30%), during the 90-min test period, for weeks 2 through 5 (test blocks 2 through 8), then received access to water during the 90-min test period for the 6th and 7th weeks (test blocks 9 through 11), and experienced re-exposure to ethanol access, during the 90-min test period, for the 8th week (test blocks 12 and 13). Body weights and amounts consumed from the pre-test period water bottles and test period test solution bottles were recorded at the end of the experimental procedures each day.

### 2.4. Treatment of heart rate data

Heart rate, in beats per min (BPM), was detected by the ER-4000 receiver and averaged every 30-s by a personal computer program. The voltage created by the R-wave of the QRS complex is detected as a change in voltage across the heart rate leads. The time between consecutive R-waves is computed and heart rate is determined from this. The 30-sec averages were screened for artifacts (i.e., acute increases or decreases in heart rate that lasted for up to two-and-a-half min and were not part of an upward or downward trend in heart rate were deleted). It has been reported that the "orienting response" involves a dramatic reduction in heart rate, presumably to heighten attention in preparation for "fight or flight" behavior (Barry, 1990; Graham and Clifton, 1966). Our laboratory has found that loud noises (e.g., from an adjoining room or the ventilation system) can induce this pronounced reduction in heart rate. When observed, it is a time-locked event that occurs in multiple animals of a test cohort. Additionally, rare occurrences of heart rate readings of 600 BPM have also been observed, which is the upper limit of the heart rate range that our laboratory measures. These abnormally high heart rate readings appear to occur when the transmitter is in a certain orientation, mostly during rearing behavior, relative to the receiver's antenna array. It may be that the transmitter's signal is detected by two "activity zones" in the receiver's antenna array (discussed below) and summed by the computer program. When observed, it is an isolated event in only one animal. The 30-sec averages were then combined for 5-min averages. A maximum of 15-min in missing data was allowed for the 75-min time periods and a maximum of 5-min in missing data was allowed for the 15-min time periods (see below for time period descriptions). If an animal's heart rate data exceeded these criteria for missing data, the respective animal's data (heart rate, motor activity and drinking data) were removed from the study in its entirety. The animal's nearest four data points before and after missing data points were averaged and used to replace these data points. Approximately 15% of the data were either removed from analysis or replaced with averaged heart rate. The majority of the removed data were from five animals that met criteria for excessive missing data.

### 2.5. Treatment of motor activity data

Activity counts were detected by the ER-4000 receiver and totaled every minute by a personal computer program. Each change in signal strength (i.e., loss or reacquisition of signal), detected by the ER-

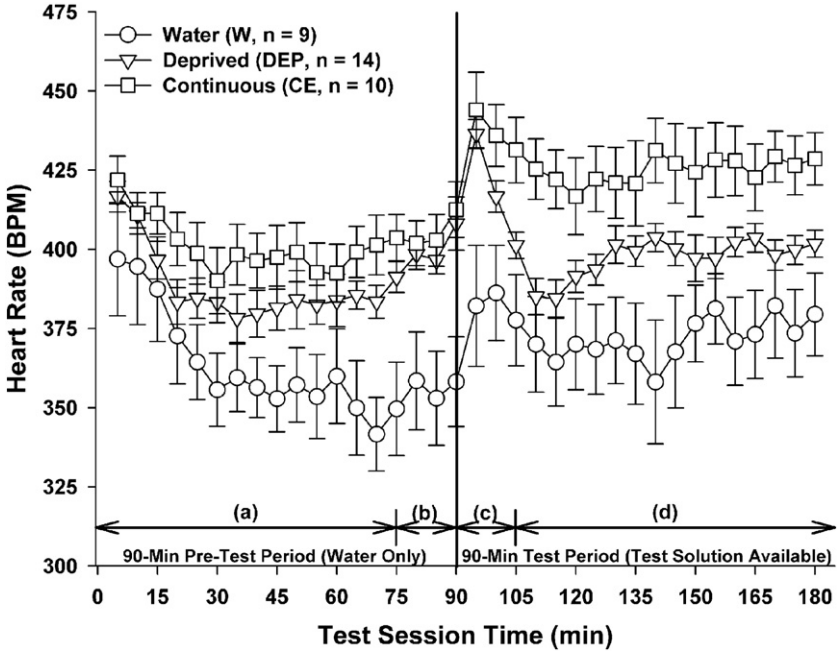


**Fig. 1.** The time and test periods represented in 5-min increments. The data depict heart rate changes for the third ethanol deprivation block (test block 11). (a) designates the early pre-test period, (b) designates the late pre-test period, (c) designates the early test period and (d) designates the late test period.

4000, was recorded as an activity count. The receiver-(ER-4000) transmitter (E-Mitter) system involves a radio-telemetric device that is not battery operated. Therefore, the transmitter must collect sufficient RF energy, from the receiver, to energize its circuits and then transmit the collected data (i.e., heart rate) back to the receiver. The ER-4000 receiver contains an antenna "array" that is not continuous, such that the rat moves from one section (activity zone described below) to another as it moves across the receiver's surface. Each "activity zone" emits a weak RF signal, to power the transmitter, and collects data from the transmitter. Therefore, when an animal moves from one activity zone to another there is a loss of the transmitter's signal and a subsequent reacquisition of the signal, which is recorded as two activity counts. The ER-4000 contains three activity zones to detect lateral movement, five activity zones to detect longitudinal movement and four activity zones to detect changes in vertical orientation of the E-4000 telemetry device. The data stream from the receiver does not allow for differentiating lateral, longitudinal and vertical movement. The data were totaled into 15-min intervals, which were combined, where appropriate, for the 75-min early pre-test period and the 75-min late-test period (see below for time period description).

The ER-4000 Mini Mitter system measures and records body temperature in addition to heart rate and motor activity. This data was analyzed, but no significant changes in body temperature were detected. Regarding estrous cycle, because the design of the present study sought to limit handling of the animals to procedures directly related to the context of ethanol self-administration, stages of the estrous cycle were not charted for the subjects in this study. Within the animal vivarium, the female P rats were housed in close proximity to male P rats, which suggests that individual rat estrous cycles were not synchronized. Given the above and the fact that four different squads of rats were tested, it is likely that the effects of stage of estrous were randomized across animals, treatment groups and test days.

### 2.6. Statistical analyses

Four time periods were of interest: the first 75-min of the (early) pre-test period, the last 15-min of the (late) pre-test period, the first 15-min of the (early) test period and the last 75-min of the (late) test period. Oral reinforcers are primarily self-administered at the beginning of a limited access period (e.g., Bell et al., 2006a; Murphy et al., 1986). Therefore, we expected activation to take place within the first 15-min of the test period, and conditioning of autonomic and/or behavioral responses to take place in the 15-min before access to the test solutions. For illustrative purposes, Fig. 1 depicts 5-min heart rate averages during the last block of the ethanol deprivation interval (test block 11). The figure displays the characteristic habituation of heart rate across the 75-min time periods and the activation displayed during the first 15-min of the test period. Consequently, these time frames were treated as single units in order to analyze the data (heart rate and motor activity) concisely. Our laboratory has found that averaging across 2-day blocks results in excessive within-groups variance compared with that seen when data is averaged into 4-day blocks. Additionally, an initial examination of the data, from the present study, revealed that there was limited habituation across the

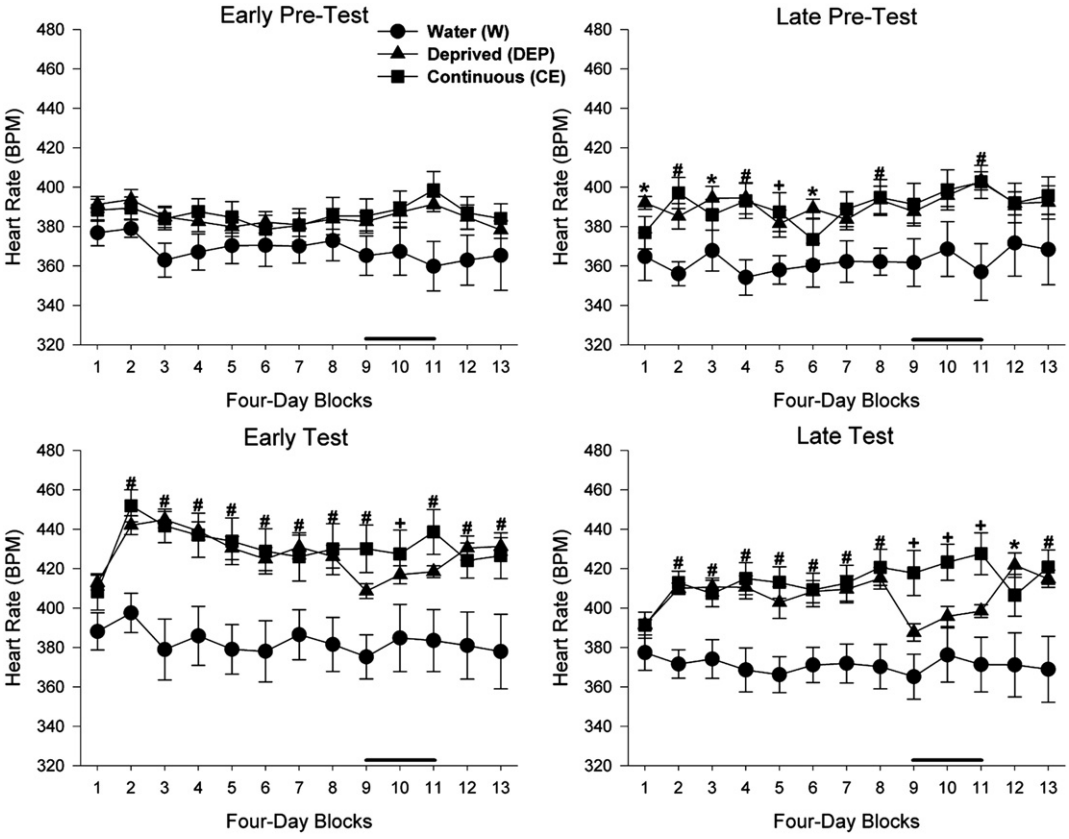


**Fig. 2.** Effects of drinking condition [W: water; DEP: rats given concurrent access to multiple concentrations of ethanol (10%, 20% and 30%) during test blocks 2 through 8 as well as test blocks 12 and 13; CE: rats given concurrent access to multiple concentrations of ethanol (10%, 20% and 30%) during test blocks 2 through 13] and test block [during the habituation block (test block 1), water served as the test solution for all groups] on the mean (±SEM) heart rate, in beats per min (BPM), (upper left panel) for the first 75-min of the (early) pre-test period, (upper right panel) for the last 15-min of the (late) pre-test period, (lower left panel) for the first 15-min (early) test period, and (lower right panel) for the last 75-min (late) test period. The solid black line designates when the DEP group experienced the ethanol deprivation interval. *, indicates a significant ($p<0.05$) difference between the DEP group and the W group. +, indicates a significant ($p<0.05$) difference between the CE group and the W group. #, indicates a significant ($p<0.05$) difference between both the DEP and CE groups and the W group.

first 3 days of the habituation week but significant habituation across the last 4 days of the habituation week. Therefore, to balance across-days temporal resolution (i.e., sensitivity) with day-to-day variability in the data, the data (heart rate, motor activity and solution intakes) were averaged into 4-day blocks starting with the last 4 days of the habituation week. Because our laboratory's standard ethanol deprivation protocol involves 2 weeks of deprivation, 2 weeks of ethanol deprivation were used here for the ethanol deprivation interval, which was averaged into a 5-day, a 4-day, and a final 5-day test block. Additionally, the 8th week of test was split into a 4-day (test block 12) and a 3-day (test block 13) test block. Analyses were not conducted across time (5-min or 15-min bins), since the habituation across the 75-min time periods (Fig. 1) is a common phenomenon and these analyses would not add significantly to interpretation of the results. One-way ANOVAs were conducted on the heart rate, motor activity and solution intake data for each time period during the first (habituation) test block to determine if group baseline differences were present. Omnibus 12×3 (test block×group) mixed ANOVAs were performed on the data (heart rate, motor activity and solution intake, except for ethanol intake which was limited to the 9 test blocks during which both ethanol groups had access to ethanol), with the within-subjects factor being test block and the between-subjects factor being group. *A priori* analyses for group effects were conducted for each time period within each test block followed by planned comparisons using the Dunnett's *t*-test (2-sided, $p<0.05$), with the W group serving as the control group. The Dunnett's *t*-test controls for Type 1 error when the multiple comparisons are limited to comparisons to a control condition, the W group for this study, (Keppel, 1991). Alpha was set at $p<0.05$ for all analyses.

## 3. Results

### 3.1. Heart rate data

*Pre-test period*. The one-way ANOVAs of the first (habituation) block pre-test period data revealed no group differences during the early pre-test period, but did reveal a marginally significant effect of group for the late pre-test period: $F(2, 30)=3.42$, $p=0.046$, with the DEP group having slightly higher ($p<0.05$) heart rate than the W control group (Fig. 2, upper right panel). Examination of the early pre-test period heart rate data (first 75-min) revealed the main effect of test block approached significance ($p=0.068$). As seen in Fig. 2 (top left panel), there was a modest decrease in heart rate of the W control group across test blocks 2–13, which was absent in both of the ethanol groups. Examination of the late pre-test period heart rate data (last 15-min) revealed a significant main effect of group: $F(2, 30)=5.65$, $p=0.008$. As seen in Fig. 2 (upper right panel), heart rate was higher in the DEP and CE groups, compared with the W control group. *A priori* analyses for group differences revealed that the CE group displayed significantly ($p<0.05$) greater heart rate than the W group during the 2nd, 4th, 5th, 8th, and 11th test blocks, whereas the DEP group displayed significantly ($p<0.05$) greater heart rate than the W group

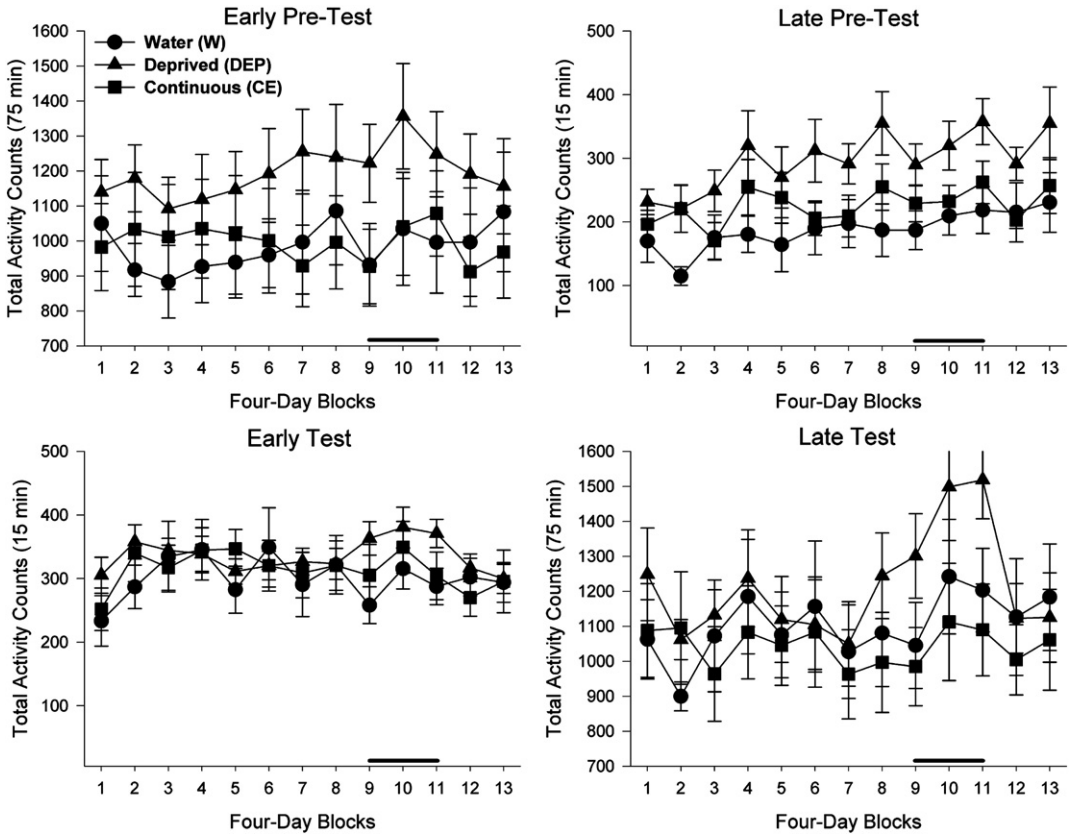


**Fig. 3.** Effects of drinking condition [W: water; DEP: rats given concurrent access to multiple concentrations of ethanol (10%, 20% and 30%) during test blocks 2 through 8 as well as test blocks 12 and 13; CE: rats given concurrent access to multiple concentrations of ethanol (10%, 20% and 30%) during test blocks 2 through 13] and test block [during the habituation block (test block 1), water served as the test solution for all groups] on the mean (±SEM) motor activity (total activity counts), (upper left panel) for the first 75-min of the (early) pre-test period, (upper right panel) for the last 15-min of the (late) pre-test period, (lower left panel) for the first 15-min (early) test period, and (lower right panel) for the last 75-min (late) test period. The solid black line designates when the DEP group experienced the ethanol deprivation interval. While there were no statistically robust differences [the main effect of group was marginally significant ($p=0.054$) during the late pre-test period, and the group by test block interaction approached significance ($p=0.069$) for the early test period] among the test solution groups, probably due to substantial within-group variance, the DEP group did have elevated motor activity during the test blocks when they experienced the ethanol deprivation interval.

during the 1st, 2nd, 3rd, 4th, 6th, 8th, and 11th test blocks (Fig. 2, upper right panel).

*Test period.* The one-way ANOVAs of the heart rate data from the first (habituation) test block's test period did not reveal significant effects of group for either the early or late test periods (Fig. 2, lower panels). Examination of the early test period heart rate data (first 15-min) revealed a significant test block by group interaction: $F(22, 330) = 1.70$, $p = 0.027$. As seen in Fig. 2 (lower left panel), there was a robust increase in heart rate for the CE and DEP groups across the 12 test blocks when ethanol was available, relative to the W group, with a modest reduction in this increase exhibited by the DEP across the test blocks of ethanol deprivation (test blocks 9 through 11). There were also significant main effects of test block: $F(11, 330) = 5.74$, $p < 0.001$ and group: $F(2, 30) = 9.27$, $p = 0.001$. As seen in Fig. 2 (lower left panel), heart rate values for the W group were relatively constant across the 12 test blocks, whereas there was a marked increase in the heart rate of the CE and DEP rats when ethanol was made available. *A priori* analyses for group differences for each test block revealed CE animals had significantly ($p < 0.05$) greater heart rate than W animals for all test blocks during which ethanol was available (Fig. 2, lower left panel). Similarly, DEP animals displayed significantly ($p < 0.05$) higher heart rates than W animals during all test blocks when ethanol was available, as well as during the 1st and 3rd test blocks of the deprivation interval (Fig. 2, lower left panel). Examination of the late test period heart rate data (last 75-min) revealed a significant test block by group interaction: $F(22, 330) = 2.65$, $p < 0.001$. As seen in Fig. 2 (lower right panel), the CE group's heart rate values were higher than that seen in the W group during 11 of the 12 test blocks when ethanol was available. Similarly, the DEP group displayed significantly higher heart rates than the W group during test blocks when ethanol was available with a reduction in heart rate, toward levels seen in the W group, across the 3 test blocks of the ethanol deprivation interval. There was also a significant main effect of group: $F(2, 30) = 10.18$, $p < 0.001$. As seen in Fig. 2 (lower right panel), in general, animals with access to ethanol displayed significantly higher heart rates than that seen in the W group. *A priori* analyses for group differences for each test block confirmed that whenever ethanol was made available, the CE and DEP animals displayed significantly ($p < 0.05$) higher heart rates than the W animals, with the exception of the 12th test block for the CE rats (Fig. 2, lower right panel).

### 3.2. Motor activity data

*Pre-test period.* The one-way ANOVAs of the first (habituation) test block's motor activity data during the pre-test period did not reveal any significant group effects for either the early or late pre-test period (Fig. 3, upper panels). Whereas the mixed ANOVA examining motor activity data from the early pre-test period did not reveal any significant main effects or interactions (Fig. 3, upper left panel), the mixed ANOVA examining the late pre-test period revealed a significant main effect of test block: $F(11, 330) = 4.45$, $p < 0.001$, and a marginally significant main effect of group ($p = 0.054$). In general, there was a modest increase in motor activity across test blocks, with the rats having ethanol access displaying greater motor activity compared to the water group (Fig. 3, upper right panel).

*Test period.* The one-way ANOVAs of the motor activity data from the first (habituation) test block's test period did not reveal any significant effects of group during either the early or late test periods. The mixed ANOVA examining motor activity from the early test period revealed a significant main effect of test block: $F(11, 330) = 2.11$, $p = 0.019$; and a test block by group interaction that approached significance ($p = 0.069$). In general, there was a modest increase in motor activity across test blocks and the DEP group had slightly higher motor activity than the CE and W control groups during the test blocks that it was deprived of ethanol access (Fig. 3, lower left panel). The mixed ANOVA examining motor activity from the late test period revealed a significant main effect of test block: $F(11, 330) = 3.22$, $p < 0.001$, again with a general, modest increase in motor activity across test blocks and no significant differences between groups (Fig. 3, lower right panel).

### 3.3. Body weight

The mixed ANOVA of the body weight data revealed a significant main effect of test block: $F(12, 360) = 24.07$, $p < 0.001$, with a general increase in body weight across test blocks, and no significant differences between groups (Fig. 4, top panel).

### 3.4. Measures of fluid intake

All fluid intakes were measured at the end of the test session to limit animal disturbances (i.e., to reduce artifactual changes in heart rate). While this may limit direct statements about amounts of solutions consumed during the four respective time periods, previous research indicates that under limited access conditions most of the

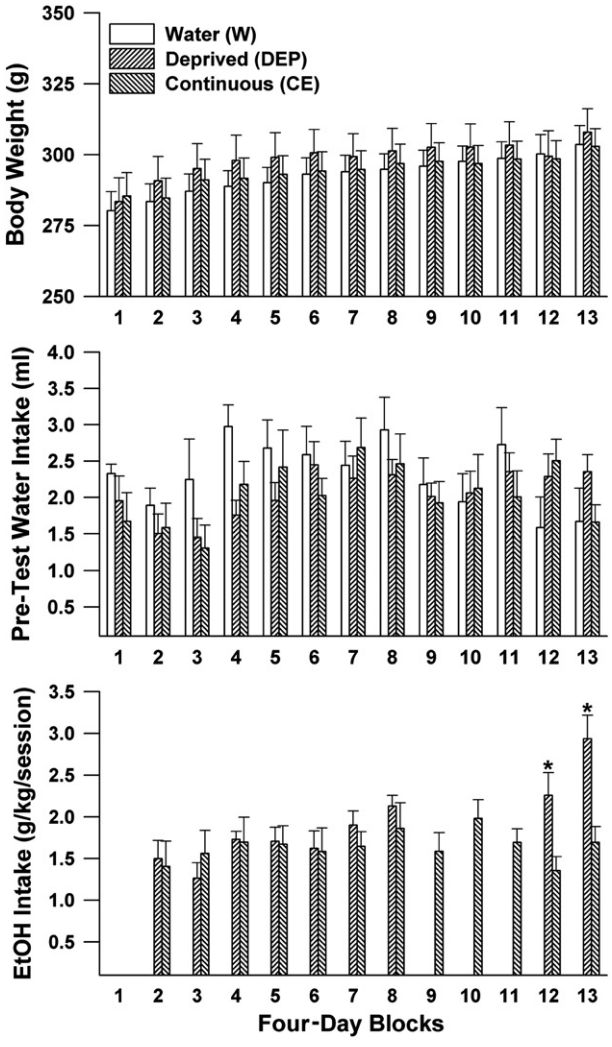


**Fig. 4.** Effects of drinking condition [W: water; DEP: rats given concurrent access to multiple concentrations of ethanol (10%, 20% and 30%) during test blocks 2 through 8 as well as test blocks 12 and 13; CE: rats given concurrent access to multiple concentrations of ethanol (10%, 20% and 30%) during test blocks 2 through 13] and test block [during the habituation block (test block 1), water served as the test solution for all groups] on the mean (±SEM) body weight in g (top panel), pre-test period water intake in ml (middle panel), and ethanol intake in g/kg/session (bottom panel). *, indicates a significant ($p < 0.05$) difference between test solution conditions for the respective test block.

consumption takes place at the beginning of the limited access period (e.g., Bell et al., 2006a; Murphy et al., 1986).

*Pre-test period*. Analysis of the pre-test water consumption data for the habituation test block revealed no differences between groups ($p=0.44$, Fig. 4, middle panel). Analysis of the pre-test water consumption data across test blocks 2 through 13 revealed a significant test block×group interaction: $F(22, 330)=2.27$, $p=0.001$, and a significant main effect for test block: $F(11, 330)=5.08$, $p<0.001$. As seen in Fig. 4 (middle panel), there was a modest increase in water intake across test blocks; and, whereas the W group consumed more than the ethanol groups at the beginning of the experiment, the ethanol groups consumed more water than the W group at the end of the experiment.

*Test period*. Analysis of the test water consumption data for the habituation test block revealed no differences between the groups ($p=0.72$): average water intakes were (mean±SEM) 1.0±0.1, 1.2±0.2, 1.1±0.2 ml for the W, DEP, CE groups, respectively. Analysis of ethanol consumption across test blocks during which ethanol was available to both the CE and DEP groups (i.e., ethanol consumption of the CE animals during the deprivation interval was not included in the analysis) revealed a significant test block by group (CE vs. DEP) interaction: $F(8, 176)=4.42$, $p<0.001$, and a main effect of test block: $F(8, 176)=5.83$, $p<0.001$. As seen in Fig. 4 (bottom panel), whereas ethanol intakes did not differ between the CE and DEP groups prior to the deprivation interval, the DEP animals consumed significantly ($p<0.05$) more ethanol than the CE animals during the 2 test blocks following the deprivation interval.

## 4. Discussion

The present results indicate that, similar to our previous findings with adult male P rats (Bell et al., 2002), oral ethanol self-administration results in autonomic (heart rate) activation in adult female P rats (Fig. 2, lower panels). However, our hypothesis that oral ethanol self-administration results in behavioral (motor activity) activation received limited support [the group by test block interaction approached significance ($p=0.069$), Fig. 3, bottom panels]. Again, similar to our previous findings (Bell et al., 2002), the present results indicate that autonomic (heart rate) activation can be conditioned to the environment associated with ethanol self-administration in adult female P rats, both when assessed during the pre-test period before ethanol access (Fig. 2, upper right panel) and during the test period of the deprivation interval, with these deprivation blocks serving as extinction-like trials (Fig. 2, lower right panel). Although, there were no significant differences between the DEP and W group during the late test period of the deprivation interval. Similar to the above limited support for ethanol-induced behavioral activation, results from the present study provided modest [main effect of group was marginally significant ($p=0.054$)] support for our hypothesis that there would be a conditioned increase in motor activity to the environment associated with oral ethanol self-administration, with a nonsignificant increase in motor activity in the DEP animals, compared with the W group, during the ethanol deprivation interval (Fig. 3, solid line indicates the test blocks associated with ethanol deprivation in the DEP rats).

The present results of autonomic activation during ethanol self-administration are in agreement with previous studies examining this phenomenon in rats (Bell et al., 2002; El-Mas and Abdel-Rahman, 2007; Ristuccia and Spear, 2007). The present findings also parallel clinical reports of ethanol consumption-induced autonomic activation (Assaad et al., 2003; Brunelle et al., 2004; Grassi et al., 1989; Higgins et al., 1993; Ireland, 1984; Iwase et al., 1995). Regarding our laboratory's present, and previous (Bell et al., 2002), findings and parallels with clinical work on cue-reactivity, these animal studies of the phenomenon are limited to examining the effects of contextual conditioning (i.e., the environment in which ethanol was self-administered), whereas most of the clinical work has examined the effects of alcohol-, and drug-associated paraphernalia (e.g., the subject's favorite drink). While the ethanol bottle was replaced with a water bottle during the deprivation interval in the present study, this stimulus (the ethanol bottle) shared substantial characteristics with the pre-test and home-cage water bottles, being identical in the case of the pre-test water bottle. Therefore, the replacement water bottle served more as a stimulus for extinction-like trials, than a representation of alcohol-associated paraphernalia. Future studies are needed to determine whether a more explicit "cue" results in a more robust effect on autonomic activation within an animal model of cue-reactivity.

The DEP group did not display an enhanced heart rate response to ethanol consumption during the ethanol re-exposure week (test blocks 12 and 13; Fig. 2, lower panels), even though this group displayed a significant ADE (Fig. 4, bottom panel). This may have stemmed from a ceiling effect, such that average heart rate in the 430 to 450 BPM range may be the maximal response, for adult female P rats, to orally self-administered ethanol. It is noteworthy that in our previous study with adult male P rats (Bell et al., 2002) the average peak heart rate response was in the 430 to 450 BPM range, as well. Another possibility is the development of tolerance to ethanol consumption's effect on autonomic activity. Our laboratory's previous report (Bell et al., 2002) and that of El-Mas and Abdel-Rahman (2007) indicated there was a modest decrease in heart rate activation across weeks, suggesting the development of tolerance to this effect. Similarly, there was a modest decrease in heart rate during the first 15 min of the test period across the early test blocks of the present study (Fig. 2, lower left panel). Context-specific tolerance to the autonomic activating effects of ethanol also occurs in humans (Dafters and Anderson, 1982.) The expression of this tolerance may have been due to a conditioned compensatory response at the autonomic level as suggested by other clinical investigations (McCaul et al., 1989a; Newlin, 1985, 1986; White and Staiger, 1991). Therefore, the fact that enhanced ethanol intake by the DEP group during the ADE did not result in further increases in heart rate indicates that the relationship between ethanol self-administration and autonomic activation is not linear. Thus, further research is required to delineate factors (e.g., tolerance) modulating this relationship.

Regarding conditioning of ethanol-associated heart rate activation, the present findings with adult female P rats replicate the report of this conditioning in adult male P rats (Bell et al., 2002). In both studies, repeated limited access sessions to ethanol in a non-home-cage setting resulted in elevated heart rates, compared with W control rats, during the pre-test period. A striking difference between the two studies was the display of this effect within the first 4 days of ethanol access in the present study, whereas male P rats, in our laboratory's previous study, did not display this effect until the third week of ethanol access (Bell et al., 2002). This difference may have been due to sex-of-animal differences and/or the fact that, rather than having access to a single concentration (15%) of ethanol as in the Bell et al. (2002) study, the female P rats in the present study had concurrent access to multiple concentrations (10%, 20% and 30%) of ethanol. As indicated above, female rats often consume more ethanol than their male counterparts (Adams, 1995; Bell et al., 2003; Juárez and De Tomasi, 1999; Lancaster and Spiegel, 1992; Li and Lumeng, 1984; McKinzie et al., 1998a,b); and concurrent access to multiple concentrations of ethanol increases intake over that seen when a single concentration of ethanol is available (Bell et al., 2003, 2004; Holter et al., 1998; Rodd-Henricks et al., 2001; Wolffgramm and Heyne, 1995). Regarding this, the female P rats (1.5 to 2.5 g/kg/session) in the present study drank significantly more ethanol than the male P rats (~1.1 g/kg/session) of the Bell et al. (2002) study. The design of the present study allowed for examining conditioned heart rate activation not only prior to daily ethanol access sessions, during the pre-test period, but also during the test period of an ethanol deprivation interval. Therefore, the fact that the DEP group displayed elevated

heart rate during the test period (Fig. 2, bottom panels), while being deprived of ethanol (test blocks 9 through 11), indicates that female P rats not only display autonomic activation prior to daily ethanol access sessions but also during extinction-like sessions when ethanol access is denied.

The motor activity results are in partial agreement with past research indicating P rats are activated by low doses of injected ethanol both during adolescence (Rodd et al., 2004a) and adulthood (Waller et al., 1986). It is also noteworthy that the Melendez et al. (2002), similar to the Bell et al. (2002) study, reported increased motor activity by P rats during ethanol self-administration, which has also been reported by Robledo et al. (1993). In the present study, there was only modest support for the conditioning of behavioral activation to the ethanol self-administration environment, with the DEP group displaying increased motor activity during the ethanol deprivation interval. As seen in Fig. 4, there was substantial within-groups variance, which undoubtedly prevented, at least in part, more of these group effects from reaching statistical significance. The female P rats (~300 counts during the early test period) of the present study displayed significantly more activity compared with the male P rats (~90 counts during the early test period) of the Bell et al. (2002) study. In the Rodd et al. (2004a) study, adolescent male and female P rats did not differ in motor activity at baseline, nor after low-dose ethanol challenges. A possible reason for the differences in motor activity counts between the present study and our laboratory's previous study (Bell et al., 2002) is our use of 3rd and 4th generation E-Mitters (Mini Mitter, Bend, OR, USA) in the present study, whereas 1st and 2nd generation E-Mitters were used in our previous study. The increased "dynamic" range of motor activity observed in the present study may have increased within-groups variance and consequently interfered with our ability to detect significant differences. Additionally, previous studies, from our laboratory, examining motor activity have used either photo beam (Rodd et al., 2004a) or infrared (Melendez et al., 2002) test equipment. These apparatuses have greater sensitivity for detecting changes in motor activity, but are stand alone measures (i.e., the Mini Mitter set-up detects changes in heart rate and body temperature, as well).

It is noteworthy that the observed increases in heart rate were not contingent upon motor activity, since substantial group differences in heart rate, but limited group differences in motor activity, were detected during ethanol self-administration. Moreover, when group differences in motor activity appeared to emerge (e.g., during the DEP group's ethanol deprivation interval), the group with highest motor activity (i.e., the DEP group) displayed decreases in heart rate, compared with the CE group (Figs. 2 and 3). Additionally, despite the DEP group's heart rate levels returning to levels seen in the CE group upon re-exposure to ethanol access, the DEP group's motor activity actually decreased relative to the increased activity seen during the deprivation test blocks (Figs. 2 and 3). The dissociation between heart rate and motor activity was not limited to the DEP group, with the control (W) group displaying a gradual decrease in heart rate, presumably habituation, across test blocks but displayed steady, or increased, levels of motor activity across the same.

Although ethanol can induce tachycardia by interfering with the baroreflex arc, in the basal nucleus tractus solitarius (c.f., Abdel-Rahman et al., 1985, 1987; Zhang et al., 1989), this effect requires a bolus dose of at least 1.0 g/kg ethanol. The ethanol drinking data (Fig. 4, 7 bottom panel) indicate that the amount of ethanol consumed would have been between 1.25 and, at peak, 3.0 g/kg/session, which would yield BACs between 60 and 150 mg% (Bell et al., 2006a,b; Murphy et al., 1986, 2002) during the test session. Previous studies from our laboratory (unpublished observations) indicate that the BACs achieved under the present self-administration protocol, at least during test blocks 2 through 11, would not correspond with peak, and time to reach peak, BACs achieved after an intraperitoneal injection of at least 1.0 g/kg. Also, although ethanol can affect the heart muscle, these effects are small and require high bolus doses of ethanol (c.f., Posner et al., 1984). Interestingly, in our laboratory's previous report (Bell et al., 2002) of heart rate increases during ethanol self-administration, heart rate was increased during saccharin self-administration, as well. This fact, along with the observation that these increases in heart rate could be conditioned to the environment in which self-administration took place suggest that the observed increases in heart rate of the present, and previous (Bell et al., 2002), study were not due to the toxic effects of ethanol.

In summary, the present results provide further support that ethanol self-administration produces rewarding and reinforcing effects in alcohol-preferring P rats (c.f., Bell et al., 2005, 2006a; McBride and Li, 1998; Murphy et al., 2002). This was illustrated by autonomic, increased heart rate, activation in response to self-administered ethanol. Moreover, both of the ethanol groups displayed a "conditioned" increase in heart rate during the pre-test period. Also, the DEP group displayed elevated heart rate and a modest increase in motor activity during its ethanol deprivation interval, with this interval having extinction-like qualities. These latter findings provide confirming evidence that activation associated with the rewarding properties of ethanol can be conditioned to the environment in which rats self-administer ethanol. These and previous findings (Bell et al., 2002; El-Mas and Abdel-Rahman, 2007; Ristuccia and Spear, 2007) extend the indices of ethanol self-administration-induced activation, primarily motor activation (Melendez et al., 2002) or electrophysiological measures (Robledo et al., 1993) in the past, to include increases in heart rate. This allows some parallels to be drawn with the clinical literature examining contextual conditioning, and to some extent cue-reactivity, in alcoholics and those with a family history of alcoholism (FHP individuals). Therefore, information from studies on contextual conditioning in rodent animal models may provide important information for developing treatment modalities (McCusker and Brown, 1995; Staiger and White, 1991) or pharmacotherapies (Ooteman et al., 2007) for the treatment of alcoholism and alcohol abuse, namely for the prevention of craving and relapse, which are directly associated with cue-reactivity (e.g., Ooteman et al., 2006).

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