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Central vs. peripheral administration of ethanol, acetaldehyde and acetate in rats: Effects on lever pressing and response initiation

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Abstract

The metabolites of ethanol, acetaldehyde and acetate, are biologically active, and different effects may be produced depending upon the particular metabolite and the route of administration. These studies characterized the effects of intraperitoneal (IP) vs. intraventricular (ICV) administration of ethanol, acetaldehyde, and acetate administered to male Sprague-Dawley rats. Operant behavior was assessed by conducting a detailed temporal analysis of lever pressing with rats responding on a fixed ratio 5 schedule of food reinforcement. IP administration of all three drugs produced a rate-decreasing effect on the total number of responses. Acetaldehyde and acetate were much more potent than ethanol at reducing lever pressing. The interresponse time (IRT) distribution also was more potently altered by IP administration of ethanol metabolites than by ethanol itself. The total lever pressing and IRT distributions of ethanol- and acetaldehyde- treated rats were not significantly affected when these drugs were administered ICV, while acetate produced a marked suppression of fast responses and an increase in pausing. The metabolites of ethanol are more potent than ethanol itself in terms of altering patterns of lever pressing. Thus, the effects of ethanol administration could in part be due to the actions of its biologically active metabolites.

Keywords: Alcohol; Acetaldehyde; Acetate; Lever pressing; Inter-response-time

1. Introduction

Although low doses of ethanol in mice (i.e. between 0.5 and 2.0 g/kg; Correa et al., 1999) can have activating effects on behavior, in most strains of rats peripheral administration of ethanol results in a monotonic dose-related decrease in locomotion and various other motor activities (Chuck et al., 2006; Correa et al., 2003a; Duncan and Cook, 1981; Frye and Breese, 1981; Masur et al., 1986). For example, intraperitoneal (IP) administration of ethanol to rats has been shown to produce dose-related decreases in locomotion in several paradigms (Chuck et al., 2006; Correa et al., 2003a; Drugan et al., 2007; Hall et al., 1998; Sanchis-Segura et al., 2005). High doses of ethanol in rats (around 3.0 g/kg and higher) generally produce a loss of righting reflex (Paez and Myers, 1989; Webb et al., 2002). In addition, IP ethanol administration decreased responding on different fixed ratio schedules (FR 5, FR10 and FR20) for food, water or sweetened solutions and increased the latency of responding (Chuck et al., 2006; Gerak et al., 2004; Hiltunen and Jarbe, 1988; Le Foll and Goldberg, 2005; Sobel and Riley, 1997). For several decades, it has been suggested that some of the effects of ethanol are at least partially mediated by the ethanol metabolites, acetaldehyde and acetate (Amit et al., 1980; Carmichael et al., 1991; Hunt, 1996; Israel et al., 1994; Quertemont et al., 2005).

Ethanol is metabolized into acetaldehyde in multiple organs and by several enzymes, including alcohol dehydrogenase (ADH), cytochrome P450 2E1, and catalase (Hunt, 1996). Acetaldehyde is then metabolized mainly by aldehyde dehydrogenase (ALDH)

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into acetic acid. IP administration of acetaldehyde has been shown to decrease locomotion and rearing in an open field (at doses between 10 and 100 mg/kg) (Myers et al., 1987), produce a loss of righting reflex at 100 mg/kg in selected lines of rats (Tampier and Quintanilla, 2002) and reduce lever pressing for food in a discrimination paradigm (at doses of 300 mg/kg) (Quertemont and Grant, 2002). There is evidence that the specific behavioral effects of ethanol and acetaldehyde can differ depending upon the route of administration (i.e., central vs. peripheral; Arizzi et al., 2003; Arizzi-LaFrance et al., 2006; Correa et al., 2003b, 2005a,b). However, studies of the behavioral effects of acetate have shown that either systemic or intracranial administration of this ethanol metabolite reliably suppresses motor activity. For example, ICV injections of acetate decreased operant DRL responding for food (Arizzi et al., 2003) and locomotion in an open field (Correa et al., 2003b), and previous studies have reported motor suppressant effects of peripherally administered acetate (Carmichael et al., 1991, Israel et al., 1994). Furthermore, acetate derived from ethanol has been implicated in effects such as loss of righting reflex and motor incoordination (Carmichael et al., 1991; Kiselevski et al., 2003).

In the present study we were interested in systematically characterizing and comparing the putative motor suppressant effects of ethanol and ethanol metabolites when administered systemically (IP) or into the brain via intraventricular (ICV) injections. Thus, we studied the acute effects of ethanol, acetaldehyde and acetate administered IP or ICV to rats responding on a fixed ratio 5 (FR5) lever pressing schedule for food reinforcement. The FR5 lever pressing schedule was used because it generates a high rate of responding (i.e. greater than 1000 lever presses in 30 min) that is very sensitive to the response-suppressant properties of drugs, but not to the stimulating effects, as animals typically press at near-maximal levels during baseline performance (Chuck et al., 2006; Salamone et al., 1993). This schedule has been shown to be highly sensitive to the rate-decreasing effects of several classes of drugs: dopamine antagonists (Salamone et al., 1993, 1996, 2002), the acetylcholinesterase inhibitor tacrine (Carriero et al., 1997) or drugs of abuse, such as the cannabinoids (Arizzi et al., 2004; Carriero et al., 1998; McLaughlin et al., 2005) and ethanol (Chuck et al., 2006). In addition to providing a measure of total number of lever press responses, this task can be analyzed to illustrate detailed temporal parameters of operant responding such as interresponse times (IRTs), pausing and local rate in order to provide a more specific characterization of the behavioral effects of the drugs being tested (McLaughlin et al., 2005; Salamone et al., 1993). This research was undertaken in order to provide a further characterization of the behavioral effects of ethanol metabolites, and to compare effects across different routes of administration.

2. Methods

2.1. Subjects

Male Sprague-Dawley rats (Harlan Sprague-Dawley, Indianapolis, IN; a total of 57, were housed in a colony maintained at 23 °C with a 12 h dark-light cycle (lights on at 7:00 h). All animals weighed between 300 and 430 g at the beginning of the study. Animals were initially food deprived to 85% of their free-feeding body weight, and allowed modest growth over the course of the study. All animals had *ad libitum* access to water in their home cages. During the course of the experiment, rats acquired all their food by lever pressing except for the 2 day-weekend periods when they were fed in the home cage with measured amounts of lab chow. Animal protocols were approved by the Institutional Animal Care and Use Committee, and the methods were in accord with the Guide for the Care and Use of Laboratory Animals, National Research Council, National Academy Press, 1996.

2.2. Drugs

Ethanol (100%, 200 proof, USP (United States Pharmacopea); AAPER Alcohol and Chemical Co.), acetaldehyde (Fisher Scientific), and anhydrous sodium acetate (hereafter referred to as acetate, Fisher Scientific) were dissolved in physiological saline for the IP studies and in artificial cerebrospinal fluid (aCSF) for the ICV studies. The aCSF was prepared by mixing sodium chloride, potassium chloride and calcium chloride (147.2 mM NaCl, 2.4 mM CaCl2, 4.0 mM KCl) in purified water. For IP injections, the stock solutions from which the different doses were obtained were: ethanol 20% v/v, acetaldehyde 2% v/v and acetate 10% w/v. Xylazine, Ketamine and Metacam were purchased from Phoenix Pharmaceutical, Inc. (St. Joseph, Mo).

2.2.1. Selection of doses

For the IP studies, the ethanol dose range was selected to show the potency differences between ethanol and ethanol metabolites. The dose range for acetaldehyde was not extended into higher doses due to toxic effects found at 200 mg/kg (Quertemont and De Witte, 2001). Due to the solubility properties of acetate it was not possible to administer it at higher doses than 400 mg/kg. Pilot studies revealed that these dose ranges for acetaldehyde and acetate had response suppressing effects. For ICV administration, ethanol, acetaldehyde and acetate were dissolved in aCSF, and the vehicle control procedure consisted of injections of 1.0 µl of aCSF. Ethanol, acetaldehyde and acetate were injected in doses of 0.7, 1.4, 2.8 or 5.6 µmol, in 1.0 µl total volume (Ethanol: 0.0, 32.24, 64.96, 128.8 or 257.93 µg; Acetaldehyde: 0.0, 30.83, 61.67, 123.34 or 246.68 µg; Acetate; 0.0, 42.03, 84.07, 168.14 or 336.28 µg). These dose ranges were chosen based upon previous studies (Arizzi et al., 2003, Arizzi-LaFrance et al., 2006; Correa et al., 2003a,b).

2.2.2. Surgical procedure

For ICV drug injections, rats were implanted with unilateral guide cannulae (10.0 mm length, 23 ga.). Rats were anesthetized with a solution (1.0 ml/kg, IP) that contained ketamine (100 mg/ml) and xylazine (20 mg/ml), and after surgery received an oral dose of the analgesic and anti-inflamatory drug Metacam. The stereotaxic coordinates for the cannulation into the lateral ventricle were as follows: AP −0.5 mm (from bregma), DL +1.3 mm lateral (from midline), and DV −3.0 mm ventral (from the surface of the skull). The incisor bar on the

stereotax was set to 0.0 mm above the interaural line. All animals were single housed following surgery, and were allowed to recover for 7–10 days before behavioral retraining and drug testing. Stainless steel stylets were kept in each guide cannulae to maintain its integrity.

ICV injections were made via 30 ga. stainless steel injection cannulae extending 1.5 mm below the guide cannulae. The injectors were attached to 10.0 μl Hamilton syringes by PE-10 tubing, and were driven by a syringe pump (Harvard Apparatus) at a rate of 0.5 μl/min for a total volume of 1.0 μl. Following the infusion period the injectors were left in place for 1 min to allow for diffusion of the drug, after which the injectors were removed, stylets were replaced, and animals were immediately placed into the behavioral chambers for testing.

2.2.3. Apparatus and general procedure

The operant chambers (28 cm × 23 cm × 23 cm, Med Associates) were equipped with one operant response lever and a pellet delivery cup attached to a mechanical feeder. Animals had one day of magazine training during which a 45 mg pellet (Research Diets, Inc., New Brunswick, NJ) was delivered every 30 s as well as after every lever press. For the next four days the animals were placed on a continuous reinforcement (CRF) schedule of operant responding, in which every lever press was reinforced with the delivery of one pellet. After 4 days of CRF training rats were shifted to a FR5 schedule (i.e., every fifth lever press was reinforced with a food pellet). As with the CRF schedule, animals were tested in 30 min sessions each day, five days per week, for at least two weeks, until they reached a criterion of at least 1000 responses per day over a 3-day period. Drug testing took place once a week, allowing a seven-day drug washout period between injections for each group. Drug treatments were administered in a pseudorandom order, with each subject receiving all doses of one drug plus a single vehicle treatment. No significant effects were found for dosing order. Each rat was measured for total number of lever presses and detailed temporal parameters over the 30-min FR5 operant session. IRTs were analyzed in terms of their relative distribution in discrete time bins. Each of the first 20 time bins was 250 ms in length, accounting for all IRTs < 5.0 s. One additional time bin (i.e., bin 21) used was for IRTs > 5.0 s, and this time value served as the operational definition of a "pause"; time elapsed in IRTs < 5.0 s was defined as "time spent responding", while time elapsed in IRTs > 5.0 s was defined as "time spent not responding". "Average pause length" was defined as the total time spent not responding divided by number of pauses (i.e., number of IRTs in the >5.0 s bin). "Average rate of responding" provides a measure of the local rate of responding, and was defined as the number of IRTs occurring in bins < 5.0 s divided by the total time spent responding. These analyses of operant responding were based upon previous studies (McLaughlin et al., 2005).

2.3. Experiments

2.3.1. Experiment 1

Effect of IP administration of ethanol, acetaldehyde or acetate on FR5 operant responding.

We used a repeated measures design, with each animal receiving all treatments in a randomly varied order and serving as its own control. Experiment 1A: IP administration of ethanol ($n = 10$) at doses of 0 (saline vehicle; 1.0 ml/kg), 200, 400, 800 and 1600 mg/kg. Experiment 1B: IP administration of acetaldehyde ($n = 7$) at doses of 0 (saline vehicle; 1.0 ml/kg), 25, 50, 100 and 200 mg/kg. Experiment 1C: IP administration of acetate ($n = 12$) at doses of 0 (saline vehicle; 1.0 ml/kg), 50, 100, 200 and 400 mg/kg.

2.3.2. Experiment 2

Effect of ICV administration of ethanol, acetaldehyde or acetate on FR5 operant responding.

Experiment 2 used a repeated measures design, with each animal receiving all treatments in a random order and serving as its own control. Experiment 2A: ICV administration of ethanol ($0 = 10$) at doses of 0.0 (aCSF vehicle), 0.7, 1.4, 2.8, and 5.6 μmol in 1.0 μl total volume. Experiment 2B: ICV administration of acetaldehyde ($n = 9$) at doses of 0.0 (aCSF vehicle), 0.7, 1.4, 2.8, and 5.6 μmol in 1.0 μl total volume. Experiment 2C: ICV administration of acetate ($n = 9$) at doses of 0.0 (aCSF vehicle), 0.7, 1.4, 2.8, and 5.6 μmol in 1.0 μl total volume. The rats used in this study also contributed to a previously published report; the data on total lever presses were published in Arizzi et al. (2003), however, the present paper includes the analyses of the IRT distribution and the detailed parameters of operant responding from the same test sessions, and these data were not published previously.

2.3.3. Histology

For the ICV experiments, the placements of the injectors were verified histologically by collecting consecutive 50 μm slices through the relevant brain areas. Slices were mounted on glass slides and stained with cresyl violet to aid in the detection of the injector tracts. Coverslipped slides were viewed under

Table 1A
Effects of IP ethanol on FR5 responding

Dose (mg/kg)	Vehicle	200	400	800	1600
Total lever presses	1773.0 ± 134.8	1829.9 ± 103.6	1662.6 ± 142.2	1428.10 ± 132.2	284.5 ± 144.9**
Time not responding (s)	399.1 ± 54.6	429.8 ± 46.5	480.6 ± 30.3	679.3 ± 104.6	941.6 ± 200.0*
Average pause length (s)	17.4 ± 2.1	17.7 ± 2.7	18.1 ± 3.2	12.6 ± 0.7	362.4 ± 177.6**
Local response rate (responses per s)	1.35 ± 0.07	1.42 ± 0.08	1.35 ± 0.09	1.27 ± 0.08	0.84 ± 0.179 #

*Different from vehicle, $p < 0.05$; **different from vehicle, $p < 0.01$; #regression analysis indicates overall significant dose/response function. Mean ± SEM for every parameter.

microscopic examination to assess accuracy of implantation. All slides were examined for the degree of damage and gliosis in the vicinity of the injector. Only animals with correct ICV implantations and minimal damage were included in the study. A total of six animals were excluded from the analyses based upon histological examinations.

2.4. Statistical analysis

Data from each experiment were analyzed by repeated measures ANOVA. If there was a significant overall drug effect, non-orthogonal planned comparisons were used, which involved the overall error term from the ANOVA (Keppel and Zedeck, 1989). The number of comparisons was limited to the number of doses minus one, and each dose was compared to the vehicle control. The data for time-based measures (total time not responding and average pause length) were log transformed prior to ANOVA to reduce heterogeneity of variance. For cases in which the between-treatments ANOVA of specific response parameters was not statistically significant, data also were analyzed by regression analyses that tested for the relation between a behavioral measure (e.g., local response rate) and drug dose. This analysis, which takes into account the numeric nature of the independent variable (i.e., dose), was performed to determine if there were changes in that particular variable that occurred as a function of increasing dose. Effects on the distribution of IRT bins were analyzed with a two-way ANOVA in which dose and bin were entered as within-subjects factors. Although the percent transformation acts to eliminate differences between treatments in the factorial ANOVA of the IRT bin data, this analysis allows for the interaction term of the ANOVA to provide a measure of how the relative distribution of IRTs across bins is affected by drug treatment, independently of the total number of responses (e.g. Sokolowski and Salamone, 1998; McLaughlin et al., 2005). For cases in which treatment × bin interactions were found, two-way dose × IRT bin analyses were performed comparing each drug dose with vehicle, and separate one-way ANOVAs were performed for each bin across all doses. A computerized statistical program was used to analyze these data (SPSS 10.0).

3. Results

3.1. Experiment 1

Effect of IP administration of ethanol, acetaldehyde or acetate on FR5 operant responding.

The total response data for the ethanol experiment (i.e., total number of lever presses in 30 min) are shown in Table 1A. There was a significant overall effect on FR5 performance (i.e., total lever presses; [$F(4,36)=26.56$, $p<0.01$]). Planned comparisons revealed that the 1600 mg/kg dose significantly decreased the number of lever presses from vehicle ($p<0.01$). Table 1A also shows the results of the analyses of additional parameters of responding. There was a significant effect of ethanol on time spent not responding [$F(4,36)=2.80$, $p<0.05$] and average length of pauses [$F(4,36)=10.64$, $p<0.01$], and in both cases the highest dose (1600 mg/kg) was significantly different from vehicle ($p<0.05$). Analysis of the local rate of responding did not reveal a significant effect of ethanol treatment, however, regression analyses indicated that there was a significant dose related tendency for local response rate to decrease as a function of increasing dose ($r=0.519$, $p<0.05$). The relative IRT distributions for the systemic ethanol experiment are shown in Fig. 1A. Since IRTs in each bin were expressed as a percentage of total session IRTs, the IRT bins within each session summed to 100% for all treatments. Thus, factorial ANOVA revealed that the dose factor was not significant. However, there was a significant overall effect of bin [$F(20,180)=22.44$, $p<0.01$],

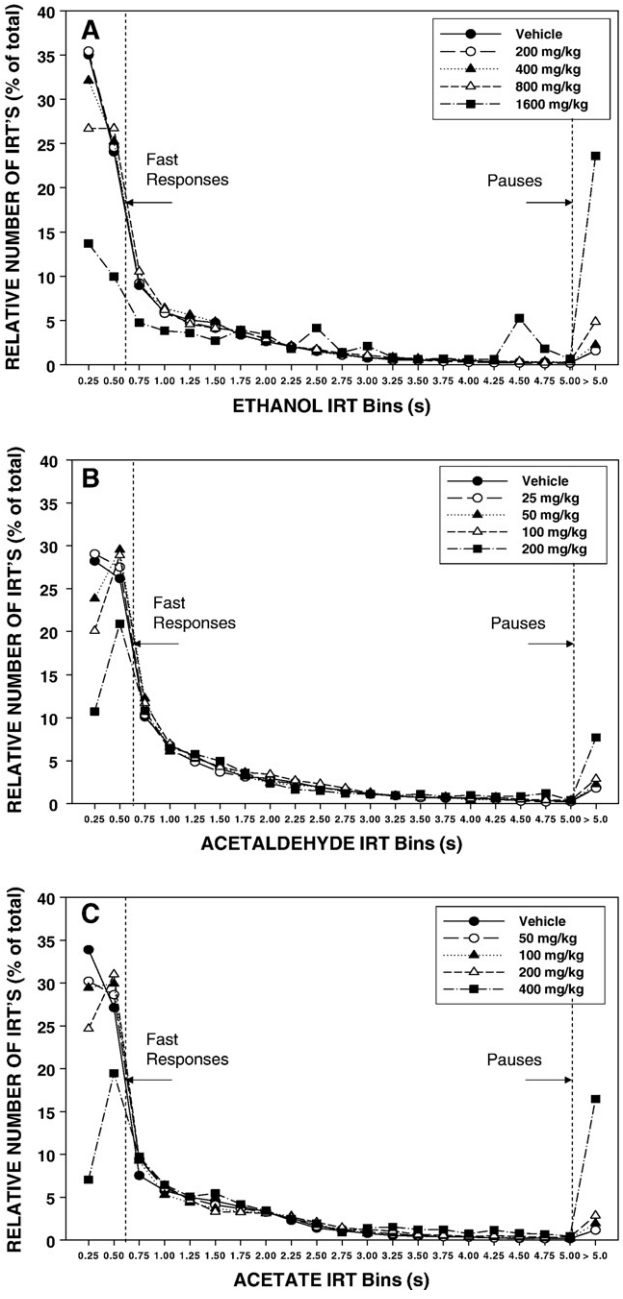

Fig. 1. Effect of IP injections of ethanol (A), acetaldehyde (B) or acetate (C), on the IRT distribution in rats responding on an FR5 operant schedule. Mean percentage of IRTs in each bin are shown. (See text for statistical details).

Table 1B
Effects of IP acetaldehyde on FR5 responding

Dose (mg/kg)	Vehicle	25	50	100	200
Total lever presses	1677.0±112.1	1645.7±121.8	1497.0±126.7	1405.1±129.8	503.4±141.1**
Time not responding (s)	350.9±44.9	428.8±91.6	458.1±49.1	474.3±73.8	791.5±192.5**
Average pause length (s)	13.0±1.2	16.1±2.7	15.8±1.9	13.9±2.4	277.1±252.9
Local response rate (responses per s)	1.21±0.08	1.27±0.09	1.21±0.07	1.10±0.06	0.93±0.08**

**Different from vehicle, $p<0.01$. Mean±SEM for every parameter.

and a significant dose×IRT bin interaction [$F(80,720)=5.90$, $p<0.01$], indicating that the shape of the IRT distribution differed across doses. Interaction comparisons showed that IRT distributions for the 800 and 1600 mg/kg ethanol significantly differed from the IRT distribution for vehicle (i.e., there were significant dose×IRT bin interactions); vehicle vs. 800 mg/kg ethanol [$F(20,180)=5.70$, $p<0.01$], vehicle vs. 1600 mg/kg ethanol [$F(20, 180)=6.66$, $p<0.01$]. One-way ANOVAs performed on individual bins showed that there were significant overall drug treatment effects ($p<0.05$) in bins 1, 2, 3, 4 and 6. The effects in bins 1 and 2 in particular reflect a decrease in the relative number of fast responses in the ethanol treated groups. There also was an effect for bin 21, which reflects a significant drug-induced increase in the relative number of pauses (i.e., percentage of IRTs>5.0 s).

Table 1B shows the total response data for the acetaldehyde experiment. A significant overall treatment effect [$F(4,24)=16.09$, $p<0.01$] on lever pressing was found. The planned comparisons demonstrated that the 200 mg/kg dose significantly decreased the number of lever presses compared to vehicle ($p<0.01$). The results of the analyses of additional parameters are shown in Table 1B. Time spent not responding was significantly increased across dose of acetaldehyde [$F(4, 24)=5.76$, $p<0.01$], and the 200 mg/kg dose was significantly different from vehicle ($p<0.05$). Acetaldehyde treatment did not produce a significant increase in average length of pauses, although there was a trend in this direction [$F(4,24)=2.25$, $p<0.1$], and regression analysis between dose and pause length approached significance [$F(1,33)=3.59$, $p=0.067$]. There was a significant effect of acetaldehyde on local rate of responding [$F(4, 20)=6.40$, $p<0.01$]. For this measure, the 200 mg/kg condition significantly differed from vehicle ($p<0.01$). Fig. 1B depicts the distributions of the relative number of IRTs after IP vehicle or acetaldehyde administration. Factorial ANOVA revealed that although the dose factor was not significant there was a significant overall effect of bin [$F(20,120)=44.10$, $p<0.01$], and a significant dose×IRT bin interaction [$F(80,480)=4.48$, $p<0.01$], indicating that the shape of the IRT distribution differed across treatments. Interaction comparisons revealed that IRT distributions for the 50, 100, and 200 mg/kg significantly differed from the IRT distribution for vehicle (i.e., there were significant dose×bin interactions); vehicle vs. 50 mg/kg acetaldehyde [$F(20,120)=2.26$, $p<0.05$, vehicle vs. 100 mg/kg acetaldehyde [$F(20,120)=7.26$, $p<0.01$, vehicle vs. 200 mg/kg acetaldehyde [$F(20,120)=7.77$, $p<0.01$]. Analyses of individual bins showed that there was a significant overall drug treatment effect ($p<0.05$) in bin 1, which indicates that there was an acetaldehyde-induced decrease in the relative number of fast responses. As was the case with ethanol, there also was an effect in bin 21 ($p<0.01$), indicating that acetaldehyde increased the number of pauses as a percent of all IRTs.

Effects of IP acetate on total lever presses are shown in Table 1C. There was a significant overall treatment effect [$F(4,44)=55.10$, $p<0.01$]. The planned comparisons test revealed that both the 400 and 200 mg/kg doses significantly decreased the number of lever presses compared to vehicle ($p<0.01$). The results of the analyses of additional parameters of responding at different doses of acetate are shown in Table 1C. Time spent not responding was not significantly modified across dose of acetate, and neither was pause length. However, analysis of the local rate of responding revealed a significant effect of dose of acetate [$F(4, 36)=22.08$, $p<0.01$], with the 200 and 400 mg/kg doses significantly differing from vehicle ($p<0.01$). The relative IRT distributions after IP administration of vehicle or acetate are shown in Fig. 1C. The ANOVA demonstrated that the drug treatment factor was not significant, although there was a significant overall effect of bin [$F(20,220)=72.74$, $p<0.01$], and a significant dose×IRT bin interaction [$F(80,880)=7.39$, $p<0.01$], indicating that the shape of the IRT distribution differed across treatments. Interaction comparisons between each dose and vehicle revealed significant differences between all doses of acetate and vehicle (dose×IRT bin interactions: vehicle vs. 50 mg/kg [$F(20,220)=2.18$, $p<0.05$]; vehicle vs. 100 mg/kg [$F(20,220)=2.61$, $p<0.01$]; vehicle vs. 200 mg/kg [$F(20,220)=6.45$, $p<0.01$]; vehicle vs. 400 mg/kg [$F(20,220)=10.93$,

Table 1C
Effects of IP acetate on FR5 responding

Dose (mg/kg)	Vehicle	50	100	200	400
Total lever presses	1962.9±67.1	1913.8±94.9	1696.8±145.1	1493.9±83.1**	368.5±102.9**
Time not responding (s)	319.9±42.0	309.6±48.7	446.3±72.4	481.8±55.2	409.4±101.7
Average pause length (s)	15.4±1.9	15.5±1.6	19.6±3.5	12.5±0.6	103.9±87.3
Local response rate (responses per s)	1.38±0.05	1.33±0.05	1.33±0.06	1.20±0.04**	0.84±0.10**

**Different from vehicle, $p<0.01$. Mean±SEM for every parameter.

Table 2A
Effects of ICV ethanol on FR5 responding

Dose (μmol)	Vehicle	0.7	1.4	2.8	5.6
Total lever presses	1746.1±69.8	1687.2±69.1	1582.4±78.8	1691.71±13.1	1728.2±93.7
Time not responding (s)	408.8±38.3	448.8±52.5	452.1±47.7	437.2±59.2	409.9±49.0
Average pause length (s)	11.5±1.1	14.1±2.0	14.9±1.7	13.8±1.7	12.1±1.2
Local response rate (responses per s)	1.26±0.05	1.25±0.04	1.18±0.06	1.24±0.06	1.24±0.06

Mean±SEM for every parameter.

Table 2B
Effects of ICV acetaldehyde on FR5 responding

Dose (μmol)	Vehicle	0.7	1.4	2.8	5.6
Total lever presses	1628.1±63.6	1755.2±70.4	1764.0±79.6	1754.2±88.6	1784.0±53.1
Time not responding (s)	504.5±29.4	404.4±35.3	419.7±51.0	425.0±72.8	384.5±35.5
Average pause length (s)	19.6±3.3	13.6±1.4	16.4±2.9	12.1±1.4	14.5±3.0
Local response rate (responses per s)	1.26±0.05	1.26±0.05	1.28±0.03	1.28±0.04	1.26±0.03

Mean±SEM for every parameter.

$p<0.01$]). A drug treatment effect [$p<0.05$] was also found for IRT bins 1, 2, 13, 14, 15, 16, 18, and 21; these analyses indicated that, as was the case with ethanol and acetaldehyde, IP administration of acetate tended to decrease the relative number of fast responses (bins 1 and 2) and increase the relative number of pauses (bin 21). Moreover, as with ethanol, acetate generally affected the normal pattern of distribution of IRTs.

3.2. Experiment 2

Effect of ICV administration of ethanol, acetaldehyde or acetate on FR5 operant schedule.

As described above, the data on total number of lever presses for these animal were published in Arizzi et al. (2003); for the present paper these data also are shown in Tables 2A, 2B and 2C. ANOVAs for each drug effect revealed a significant decrease in lever presses after acetate [$F(4,32)=4.20$, $p<0.01$], but not after ethanol or acetaldehyde. Planned comparisons revealed that the acetate doses of 2.8 μmol ($p<0.05$) and 5.6 μmol ($p<0.01$) significantly decreased lever presses compared to vehicle. Table 2A shows the results of the analyses of additional parameters of responding after ethanol administration. In addition to a lack of effect on overall responding, ethanol also had no effect on time spent not responding, average length of pauses, or local response rate. The relative IRT distributions are shown in Fig. 2A. Two-way factorial ANOVA on the IRT distribution revealed an effect of bin [$F(20,180)=106.87$, $p<0.01$], but not for dose, and as no dose×bin interaction was found, no interaction comparisons were performed. Table 2B depicts the results of the analyses of acetaldehyde data in terms of the additional parameters of responding. Acetaldehyde treatment did not affect time spent not responding, average pause length, or the local response rate. The relative IRT distributions are shown in Fig. 2B. As with ICV ethanol, an effect of IRT bin [$F(20,160)=113.29$, $p<0.01$] was found; however, neither the main effect of dose, nor the dose×IRT bin interaction were significant. Table 2C shows the results of the analyses of additional parameters of responding after administration of acetate. Time spent not responding was significantly increased across dose of acetate [$F(4,32)=3.91$, $p<0.05$]; the 1.4, 2.8 and 5.6 μmol doses were significantly different from vehicle. However, there were no effects upon average pause length or local rate of response. The relative IRT distributions for the various treatments are shown in Fig. 2C. A main effect of IRT bin [$F(20,160)=52.24$, $p<0.01$], but not of dose was found. In contrast to ICV ethanol and acetaldehyde, a significant dose×bin interaction was found [$F(80,640)=1.69$, $p<0.01$]. Interaction comparisons revealed significant differences in the IRT distribution between vehicle and both the 2.8 and 5.6 μmol doses (dose×IRT bin interactions: vehicle vs. 2.8 μmol [$F(20,160)=3.36$, $p<0.01$]; vehicle vs. 5.6 μmol [$F(20,160)=2.34$, $p<0.05$]). Although there was a tendency for the relative numbers of IRTs in bin 1 to be reduced by acetate, this effect was not significant. Nevertheless ICV acetate was found to have a significant ($p<0.05$) effect on the relative number of IRTs in bin

Table 2C
Effects of ICV acetate on FR5 responding

Dose (μmol)	Vehicle	0.7	1.4	2.8	5.6
Total lever presses	1706.3±57.4	1516.3±102.5	1401.5±114.6	1353.2±121.1*	1039.5±176.6**
Time not responding (s)	336.6±74.5	423.9±66.5	591.8±88.6*	565.3±45.1*	767.4±136.4**
Average pause length (s)	11.7±2.7	10.7±2.2	13.2±3.0	11.1±1.2	18.7±5.4
Local response rate (responses per s)	1.18±0.04	1.10±0.04	1.16±0.05	1.09±0.07	0.98±0.07

*Different from vehicle, $p<0.05$; **different from vehicle, $p<0.01$. Mean±SEM for every parameter.

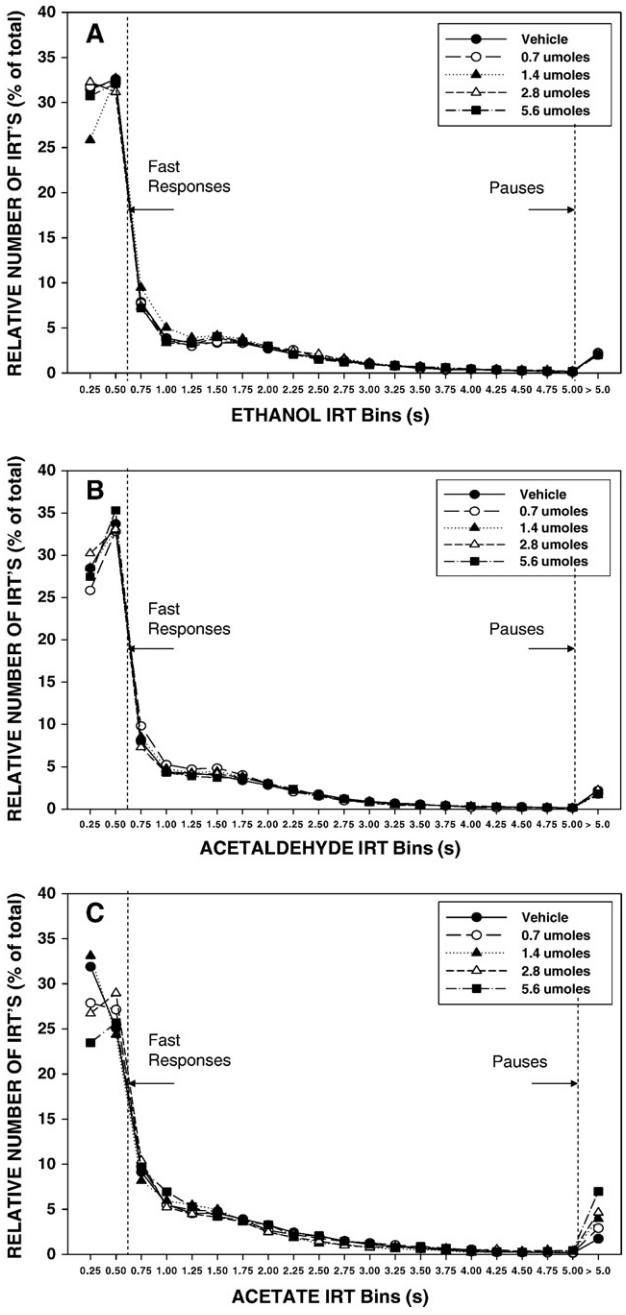

Fig. 2. Effect of ICV injections of ethanol (A), acetaldehyde (B) or acetate (C), on the IRT distribution in rats responding on an FR5 operant schedule. Mean percentage of IRTs in each bin are shown. (See text for statistical details).

21, indicating an increase in the relative number of pauses (i.e., IRTs < 5.0 s).

4. Discussion

Consistent with previous studies (Chuck et al., 2006; Gerak et al., 2004; Hiltunen and Jarbe, 1988; Le Foll and Goldberg, 2005; Sobel and Riley, 1997), systemic administration of ethanol was shown to suppress lever pressing reinforced by an FR schedule (Experiment 1). Of particular interest in the present work was the effect of systemic ethanol on detailed temporal patterns of lever pressing. Ethanol-induced decreases in lever pressing were marked by substantial increases in total pause time. This effect was dependent upon two factors. First of all, ethanol increased the relative (but not the absolute) number of pauses (i.e., IRTs > 5.0 s), which means that responding was more fragmented (i.e., interrupted by breaks in responding). Additionally, and perhaps most importantly, ethanol increased the average length of pauses, and the combination of these effects led to a substantial overall increase in total pause time, thereby reducing the time spent pressing the lever. Another effect of IP ethanol was the tendency to reduce the relative number of fast responses (i.e, IRTs in bins 1 and 2). This action probably contributed to the tendency of ethanol to reduce the local rate of responding. Systemic administration of the first ethanol metabolite, acetaldehyde, produced effects that were more potent than those induced by ethanol. Acetaldehyde reduced responding and altered parameters of lever pressing at doses as low as 200 mg/kg, which is considerably lower than the 1600 mg/kg dose of ethanol that was required to produce reductions in responding. This observation of the greater potency of acetaldehyde relative to ethanol is consistent with previous studies in mice involving locomotor activity (Font et al., 2005). Overall, acetaldehyde produced effects on the temporal patterns of lever pressing that were similar to ethanol. Like ethanol, acetaldehyde decreased total time spent responding, increased the relative number of pauses, and reduced the relative number of fast IRTs.

Systemic administration of the second metabolite of ethanol, acetate, also decreased FR5 lever pressing. As was the case for acetaldehyde, acetate was much more potent than ethanol in reducing lever pressing. This has been observed previously in measures of motor coordination and anesthetic potency in which peripherally administered acetate was shown to be more potent than peripherally administered ethanol (Campisi et al., 1997; Carmichael et al., 1991, 1993; Israel et al., 1994). Nevertheless, the pattern of effects shown by acetate differed substantially from those induced by ethanol and acetaldehyde. Although IP injections of acetate decreased total number of responses, this effect appears to have been largely dependent upon drug-induced changes in the local rate of responding. These changes in local rate are consistent with the observation that acetate administration decreased the relative number of fast responses (i.e., IRTs in bins 1 and 2). Analyses of IRT distributions also showed that acetate increased the relative number of pauses (i.e., bin 21). Nevertheless, acetate failed to affect total pause time, and in this regard acetate appears to differ substantially from ethanol and acetaldehyde. Taken together, it appears as though ethanol, acetaldehyde and acetate all suppress lever pressing, but do so in different ways. Ethanol and acetaldehyde suppressed responding through a combination of effects that increased pause time and decreased fast responding. In contrast, acetate suppresses responding in a manner largely dependent upon decreases in fast responses, and the resulting reductions in local rate of responding, without having significant effects on total pause time. Thus, acetate-treated rats continue to spend appreciable amounts of time responding, but they do so at lower rates. These distinct patterns of responding that can be observed serve to emphasize that an

analysis of the temporal characteristics of responding can yield information that is useful for distinguishing between the actions of different drugs.

Within the last few years, several drugs have been studied for their effects upon specific parameters of operant responding, employing analyses similar to those used in the present study. For example, several studies have investigated the effects of various neurochemical and pharmacological manipulations on IRT distributions. Dopamine antagonists, as well as depletions of neostriatal or nucleus accumbens dopamine, have been shown to reduce the number of IRTs that were relatively short (i.e., fast responses) and increase pausing (Mingote et al., 2005; Salamone et al., 1993), like peripheral ethanol and acetaldehyde did in the present paper. McLaughlin et al. (2005) reported on the effects of the cannabinoid CB1 receptor agonist AM411 on FR5 lever pressing. In that study, the effects of AM411 were marked by alterations in the general shape of the IRT distribution, and by dose-related reductions in the relative number of IRTs in Bin 1 (defined in the same way as in the present study). Although AM411 did not produce an overall effect on the average local rate of responding, there were substantial increases in the relative number of pauses (i.e., Bin 21), average pause length, and total time spent not responding. These analyses indicate that for AM411 there was a slight effect on response speed during periods of responding (i.e., relatively fewer fast responses), but the primary action of AM 411 was to fragment periods of responding and dramatically reduce time spent responding. In that sense, systemic administration of AM411 as described in the McLaughlin et al. (2005) paper appeared to produce effects that resemble those of IP injections of ethanol and acetaldehyde in the present study, but not those produced by acetate. Thus, some drugs belonging to different pharmacological classes can exert similar effects on operant responding, even in terms of the detailed parameters of responding. This could indicate that, despite the distinct neurochemical effects of these drugs, there are some commonalities in terms of how they are ultimately affecting the brain systems that control operant response output.

The ICV administration studies demonstrated that acetate was the only drug that suppressed responding in the dose range tested. Indeed, even when administered at higher doses than the ones used for the present work (Arizzi et al., 2003), ICV injections of ethanol and acetaldehyde failed to suppress FR5 lever pressing. As described in Arizzi et al. (2003), neither ethanol nor acetaldehyde produced rate suppressing effects at doses up to 17.6 μmol, and those results indicated that acetate was at least 6 times more potent than the other compounds for suppressing lever pressing (i.e., the lowest dose of acetate that produced an effect was 2.8 μmol). The detailed parameters analyzed in the present paper were even more sensitive to the acetate suppressant effects, since lower doses of acetate (1.4 μmol) were effective in suppressing total time spent responding. Although it is very likely that higher doses of ethanol and acetaldehyde administered directly into the brain could in fact suppress lever pressing, the present results indicate that, at the very least, acetate is the most potent of the three substances for suppressing lever pressing after ICV administration. This observation is consistent with previous research involving locomotor activity (Correa et al., 2003b); in those studies, in which all three substances were administered in the same dose range, acetate was the only substance that reduced locomotion. Thus, the central formation of acetate from ethanol should be taken into account when interpreting results of ethanol self-administration experiments. Because acetate has a clear impact on operant responding it could affect the operant self-administration of ethanol. Moreover, acetate itself could have pharmacological interactions with other substances that are introduced in the design to assess ethanol intake.

Interestingly, the pattern of effects produced by ICV injections of acetate differed substantially from those effects produced by IP acetate. Although IP injections of acetate failed to affect pauses (time not responding), that was the major parameter of responding affected by ICV injections of acetate. These data suggest that ICV and IP injections of acetate are suppressing responding via different mechanisms. It is not clear why systemic administration of acetate suppresses response rate without exerting much effect upon pause time. It is possible that systemic acetate administration produces motor slowing through peripheral effects such as muscle relaxation or impairments in motor neuron function. At the rat neuromuscular junction, facilitation of acetylcholine release is balanced through tonic activation of pre-synaptic muscarinic M1 and adenosine A2A receptors. The increasing tonic activation of A2A receptors by adenosine counteracts M1 facilitation of ACh release (Oliveira and Correia-de-Sa, 2005). It has been proposed that the extrahepatic metabolism of acetate into acetyl-CoA yields AMP and adenosine (Carmichael et al., 1993). Thus, adenosine derived from acetate could be counteracting the release of ACh at the neuromuscular junction. In contrast, the effects of acetate when injected ICV could be substantially different from those induced by peripheral injection, and may include actions such as akinesia, ataxia or sedation. Evidence indicates that the central mechanism through which acetate produces its potent suppression on motor activity may also involve the formation of adenosine (Dar, 2001, 2002; Kiselevski et al., 2003). Acetate derived from liver ethanol metabolism is delivered into systemic circulation, crosses the blood brain barrier, and is metabolized into adenosine in the brain (Campisi et al., 1997; Kiselevski et al., 2003). Both acetate and adenosine levels are increased in the brain after systemic ethanol administration (Kiselevski et al., 2003), and both have been implicated in the motor suppressant and ataxia-inducing effects of ethanol (Dar, 2001, 2002; Israel et al., 1994; Kiselevski et al., 2003). Adenosine in the cerebellum, striatum and motor cortex of the rat has been implicated in the motor incoordination produced by ethanol (Barwick and Dar, 1998; Dar, 2001, 2002).

In summary, these studies demonstrated that IP administration of ethanol, acetaldehyde and acetate suppressed FR5 responding, with acetaldehyde and acetate being much more potent than ethanol itself. The IRT distributions also were more potently altered by peripherally administered ethanol metabolites. The total lever presses and IRT distributions of ethanol- and acetaldehyde-treated rats were not significantly affected

when these drugs were administered ICV in the dose range tested, while acetate produced a marked suppression of fast responses and an increase in pausing after ICV infusion. These results demonstrate that the metabolites of ethanol are more potent than ethanol itself in terms of altering patterns of lever pressing. Moreover, acetate appears to be more potent than acetaldehyde and ethanol at suppressing lever pressing when these substances are administered centrally. Together with other studies, our results suggest that acetaldehyde and acetate are biologically active metabolites of ethanol that contribute to the behavioral effects of this drug. Also, the detailed analysis of the temporal characteristics of drug effects on operant responding can yield information that is useful for understanding the complex impact that drugs and their metabolites have on behavior.

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