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The reinforcing effects of acetaldehyde in the posterior ventral tegmental area of alcohol-preferring rats

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Abstract

Acetaldehyde (ACD), the first metabolite of ethanol, is a biologically active compound, which may mediate some of the reinforcing, behavioral and neurotoxic effects of ethanol. The objective of this study was to test the hypothesis that ACD is reinforcing within the mesolimbic system. The intracranial self-administration (ICSA) technique was employed to determine whether ACD was reinforcing in the posterior ventral tegmental area (VTA), a site that supports the reinforcing actions of ethanol. Adult female alcohol-preferring (P) rats were implanted with guide cannulae aimed at the posterior VTA. Subjects were placed in two-lever operant chambers 7–10 days after surgery. Responding on the "active lever" on a fixed ratio 1 (FR1) schedule of reinforcement caused the delivery of 100 nl of infusate, whereas responses on the "inactive lever" were without consequences. Rats were assigned to one of five groups that self-administered either artificial cerebrospinal fluid (aCSF) throughout all eight sessions (4 h in duration) or 3- and 6-, 11- and 23-, 45- and 90- or 180- and 360-μM ACD for the eight sessions, with the lower concentration of ACD given for the initial four sessions and the higher concentration of ACD given for the last four sessions. A second experiment examined the acquisition (first four sessions), extinction (aCSF in sessions 5 and 6) and reinstatement using 90-μM ACD. A third experiment examined the effects of extending the time-out period (from 5 to 55 s) on the number and pattern of infusions of 23-μM ACD. Adult P rats readily self-administered 6–90-μM ACD and discriminated between the active and inactive levers. Furthermore, rats self-administering 90-μM ACD also demonstrated extinction behavior when aCSF was substituted for ACD and gradually reinstated active lever responding when ACD was reintroduced. P rats maintained similar numbers of infusions and infusion patterns under both time-out schedules. Overall, the data indicate that ACD is a potent reinforcer within the posterior VTA of the P rat.

Keywords: Intracranial self-administration; Ventral tegmental area; Acetaldehyde; Reinforcement; Alcohol; Ethanol; Alcohol-preferring rats

1. Introduction

Acetaldehyde (ACD), the first metabolite of ethanol, is a biologically active compound, which may mediate some of the behavioral and neurotoxic effects of ethanol (Hunt, 1996; Smith et al., 1997). ACD derived from peripheral metabolism of ethanol does not readily cross from blood into brain because of the metabolic barrier imposed by aldehyde dehydrogenase (Sippel, 1974; Smith et al., 1997; Westcott et al., 1980; Zimatkin, 1991). Several studies have demonstrated a slow but measurable accumulation of ACD in rat brain, which does not involve alcohol dehydrogenase or cytochrome *P*450 (Aragon and Amit, 1992; Gill et al., 1992) but may involve catalase (Aragon et al., 1992; Hamby-Mason et al., 1997). Recently, evidence for the involvement of other metabolic processes, in addition to the catalase reaction, for the production of ACD within the brain has been reported (Zimatkin et al., 1998). Thus, these studies support the idea that ACD can be formed from ethanol within the brain.

Several studies, attempting to provide a role for ACD in ethanol reinforcement and high-alcohol drinking behavior,

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have been conducted. Alcohol-preferring (P) rats have significantly higher brain catalase activity than either alcohol-nonpreferring (NP) or Long–Evans rats (Gill et al., 1996). Individuals with a family history positive for alcoholism had higher blood catalase activity than did family history negative individuals (Koechling et al., 1995). Decreases in alcohol intake following inhibition of catalase activity have been reported for Long–Evans rats (Aragon and Amit, 1992). Data from a conditioned taste-aversion paradigm indicated that ACD might be involved in mediating both the reinforcing and aversive effects of alcohol (Aragon et al., 1986). Furthermore, Brown et al. (1979) reported that rats would self-administer 1–5% (v/v) ACD directly into the cerebral ventricles, and Smith et al. (1984) reported that multiple intracerebroventricular infusions of ACD produced conditioned place preference, suggesting to these investigators that centrally administered ACD could produce positive reinforcing effects. Overall, these studies lend support for a potential involvement of ACD, formed from ethanol within the brain, in the rewarding properties of alcohol.

The intracranial self-administration (ICSA) technique has been employed to identify specific brain regions involved in the initiation of response-contingent behaviors for the delivery of a reinforcer (Bozarth and Wise, 1980; Goeders and Smith, 1987). Studies utilizing the ICSA procedure have successfully isolated discrete brain regions where opioids (Bozarth and Wise, 1981; Devine and Wise, 1994; Goeders et al., 1984), amphetamine (Hoebel et al., 1983) and cocaine (Goeders et al., 1986; Goeders and Smith, 1983, 1986; McKinzie et al., 1999) are self-infused and may initiate their rewarding effects. Gatto et al. (1994) reported that the selectively bred P rat self-administered ethanol directly into the ventral tegmental area (VTA) at concentrations ranging from 50 to 200 mg %. A recent study indicated that common stock Wistar rats will self-administer 100–400-mg % ethanol into the VTA and that the posterior but not anterior part of the VTA is mediating this behavior (Rodd-Henricks et al., 2000).

Although the mechanisms underlying the self-administration of ethanol into the VTA are unknown, they most likely involve activation of dopamine (DA) neurons within this structure (Brodie et al., 1990; Gessa et al., 1985; Verbanck et al., 1990). The activating effects of ethanol on DA neurons within the VTA appear to be mediated in part by serotonin receptors (Brodie et al., 1995;Campbell et al., 1996), although the involvement of other receptors in mediating the actions of ethanol within the VTA has not been adequately explored. In addition to mechanisms involving an action of ethanol at one or more receptors, it is possible that local formation of ACD may be a factor contributing to the rewarding actions of alcohol.

The present study was undertaken to test the hypothesis that ACD is reinforcing within the mesolimbic system of the rat. Because the posterior VTA is a site that supports the self-administration of ethanol (Rodd-Henricks et al., 2000), the ICSA technique was used to determine whether P rats would self-administer physiologically relevant concentrations of ACD into this region.

2. Methods

2.1. Animals

Experimentally and alcohol naive, female P rats (from the 44th and 45th generations) weighing 250–320 g at the time of surgery were used. Animals were double housed upon arrival and maintained on a 12-h reverse light/dark cycle (lights off at 09:00 h). Female P rats were used in the present study because they retain their body weight and size better than male rats for more accurate cannula placements. Female rats have been used previously in ICSA experiments (Gatto et al., 1994; Ikemoto et al., 1997a, 1998; Rodd-Henricks et al., 2000). Although not systematically studied, the estrous cycle did not appear to have a significant effect on ICSA behavior in the present study or in previous studies (Gatto et al., 1994; Ikemoto et al., 1997a, 1998; Rodd-Henricks et al., 2000), as indicated by the lack of obvious fluctuations in ICSA behavior in rats given similar doses of the same agent for two or more sessions conducted every other day. Food and water were freely available except in the test chamber. The animals used in this study were maintained in facilities fully accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care (AAALAC). All research protocols were approved by the institutional animal care and use committee and are in accordance with the guidelines of the Institutional 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.

Data for rats that did not complete all experimental test sessions were eliminated from the analyses. The number of animals indicated for each experiment represents approximately 90% of the total number that underwent surgery. About 10% of the animals were not included for analyses mainly due to the loss of the guide cannula before completion of all experimental sessions. The data for these animals were not used because their injection sites could not be verified.

2.2. Drug and vehicle

The artificial cerebrospinal fluid (aCSF) consisted of 120.0-mM NaCl, 4.8-mM KCl, 1.2-mM KH_2PO_4, 1.2-mM $MgSO_4$, 25.0-mM $NaHCO_3$, 2.5-mM $CaCl_2$ and 10.0-mM D-glucose. ACD (Sigma) was dissolved in the aCSF solution. When necessary, 0.1-M HCl or 0.1-M NaOH was added to the solutions to adjust pH levels to 7.4 ± 0.1.

2.3. Apparatus

The test chambers (30 × 30 × 26 cm; l × h × w) were situated in sound-attenuating cubicles (64 × 60 × 50 cm, Coulbourn Instruments, Allentown, PA) and illuminated by a dim house light during testing. Two identical levers (3.5 × 1.8 cm) were mounted on one wall of the test chamber, 15 cm above a grid floor, and were separated by 12 cm. Levers were raised to this level to avoid accidental brushing against the lever and to reduce responses as a result of general locomotor activation. Directly above each lever was a row of three different colored cue lights. Only the red light over the active lever was illuminated during resting conditions. A desktop computer equipped with an operant control system (L2T2 system, Coulbourn Instruments) recorded the data and controlled the delivery of infusate in relation to lever response.

An electrolytic microinfusion transducer system (Bozarth and Wise, 1980) was used to control microinfusions of drug or vehicle. Briefly, two platinum electrodes were placed in an infusate-filled cylindrical container (28 mm in length × 6 mm in diameter) equipped with a 28-G injection cannula (Plastic One, Roanoke, VA). The electrodes were connected by a spring-coated cable (Plastic One) and swivel (Model 205, Mercotac, Carlsbad, CA) to a constant current generator (Model CIC 36, MNC, Shreveport, LA) that delivered 6 μA of quiescent current and 200 μA of infusion current between the electrodes. Depression of the active lever delivered the infusion current for 5 s, which led to the rapid generation of H_2 gas (raising the pressure inside the gas tight cylinder) and, in turn, forcing 100 nl of infusate through the injection cannula. During the 5-s infusion and additional 5-s time-out period (or 55-s time-out period), the house light and red cue light were extinguished and the green cue light over the active lever flashed on and off at 0.5-s intervals.

2.4. Animal preparation

With the use of 2–3% isoflurane anesthesia, a unilateral 22-G guide cannula (Plastic One) was stereotaxically implanted in the right hemisphere of each subject and aimed 1.0 mm above the target region. Coordinates for placements into the posterior VTA were 5.4 mm posterior to bregma, 2.1 mm lateral to the midline and 8.5 mm ventral from the surface of the skull at a 10° angle to the vertical. A 28-G stylet, which extended 0.5 mm beyond the tip of the guide, was placed in the guide cannula between experimental sessions. Following surgery, rats were individually housed and allowed to recover 7–10 days before being placed in the operant chambers. Animals were handled for at least 5 min daily following the fourth recovery day. Subjects were not acclimated to the operant chamber prior to the commencement of data collection or did they receive any prior operant training.

2.5. Operant procedures

Subjects were brought to the test room, the stylet was removed and the injection cannula locked onto the guide cannula. Rats were placed individually in the operant chambers. To avoid trapping air at the tip of the injection cannula, the infusion current was delivered for 5 s during insertion of the injector. However, little if any of this 100 nl of solution likely reaches the target site but rather most of it diffuses up the guide cannula due to the time delay during insertion and locking the injector into place. The injection cannula extended 1.0 mm beyond the tip of the guide. The operant chamber was equipped with two levers. Depression of the "active lever" on a fixed ratio 1 (FR1) schedule of reinforcement caused the delivery of a 100-nl bolus of infusate over a 5-s period followed by a 5-s (or 55-s) time-out period. During both the infusion and time-out periods, responses on the active lever were recorded but did not produce further infusions. The responses recorded during the different time out periods are important to help establish reinforcement responding versus reflexive responding. On a FR1 schedule of reinforcement, an excessive high ratio of active lever responses to number of infusions could indicate a possible reflexive or stereotypic behavior (Goeders and Smith, 1987). Responses on the "inactive lever" were recorded but did not result in infusions. The assignment of active and inactive levers with respect to the left or right position was counterbalanced among subjects. However, the active and inactive levers remained the same for each rat throughout the experiment. No shaping technique was used to facilitate the acquisition of lever responses. The number of infusions and responses on the active and inactive levers was recorded throughout each session. The duration of each test session was 4 h and sessions occurred every other day.

For the ACD dose–response experiment, rats were assigned to one of five groups ($n = 6–8$/group) that self-administered either (a) aCSF for all eight sessions or (b) 3- and 6-, (c) 11- and 23-, (d) 45- and 90- or (d) 180- and 360-μM ACD. For the groups given ACD, the lower concentration was available for the initial four sessions and the higher concentration was available for the last four sessions.

For the extinction and reinstatement experiment, rats ($n = 8$) were given 90-μM ACD for the first four sessions, aCSF during sessions 5 and 6 and 90-μM ACD again in sessions 7 and 8. The standard 5-s time-out period between infusions was used for these two experiments. The four acquisition sessions were selected because the initial dose–response experiment indicated that stable responding on the active lever and lever discrimination appeared to be reached in the third and fourth sessions. A previous study (Rodd-Henricks et al., 2000) indicated that two extinction sessions were sufficient for rats to reduce responding on the active lever to the low levels of responding on the inactive lever when vehicle was sub-

stituted for ethanol. With this paradigm, only one or two reinstatement sessions can be tested because of the limitation on the number of microinjection sessions that can be conducted.

A concern of the ICSA paradigm with short time-out periods is the possibility of reflexive responding on the active lever following administration of the test compound (Goeders and Smith, 1987). Therefore, a separate experiment was conducted using an extended time-out period between infusions to examine the possibility of reflexive responding. For the extended time-out experiment, rats ($n=7$) were given 23-μM ACD for the first four sessions with the standard 5-s time-out period between each infusion. During sessions 5–7, the time-out period between infusions was increased to 55 s.

To examine the possibility that ACD self-administration was a result of ACD being oxidized within the injector unit, the self-infusion of acetic acid was examined using standard protocols. For this experiment, rats ($n=8$) were given 11- and 23-M acetic acid. The higher concentration was tested in the initial four sessions, and the lower concentration was tested in the last four sessions. This order was tested because of a concern that the 23-μM acetic acid may be too high (i.e., on the tail end of an inverted "U-shaped" curve) when no significant responding was observed in the first four sessions.

2.6. Verification of injection site

After completing all test sessions, a standard injection cannula was substituted for the cylindrical injector and 1% bromophenol blue (0.5 μl) was infused into the site. Subsequently, the animals were given a fatal dose of Nembutal and then decapitated. Brains were removed, frozen in dry ice and stored at −70 °C. Subsequently, frozen brains were equilibrated at −15 °C in a cryostat microtome and then sliced into 40-μm sections. Sections were then stained with cresyl violet and examined under a light microscope for verification of the injection site using the rat brain atlas of Paxinos and Watson (1986).

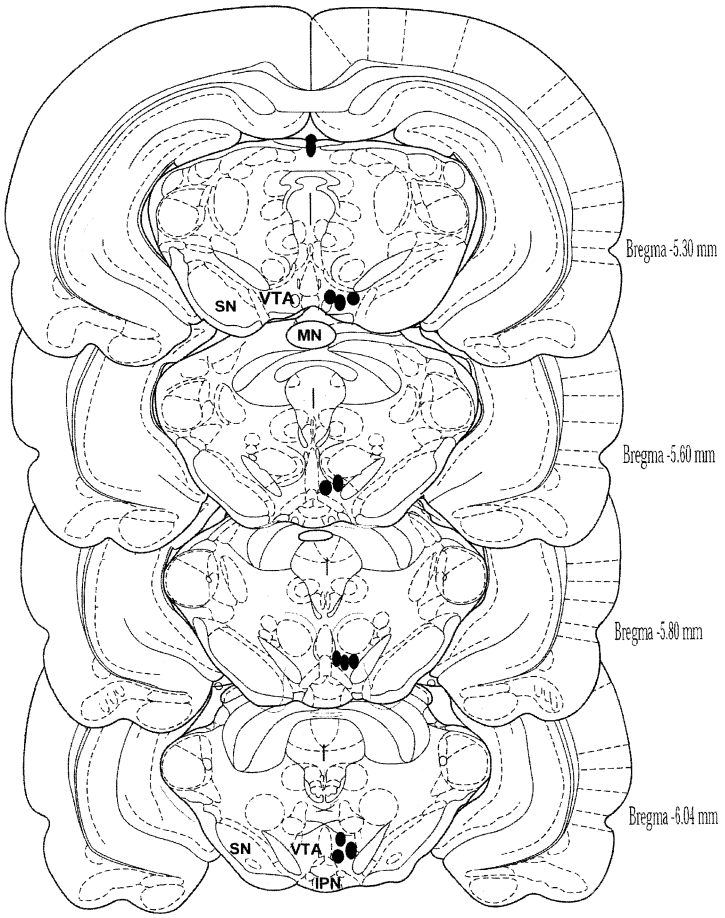

Fig. 1. Location of injection sites for the ICSA of ACD or aCSF. Circles represent placements within the posterior VTA according to the atlas of Paxinos and Watson (1986). Overlapping placements within the posterior VTA are not illustrated. Therefore, this figure does not show a quantitative representation of all placements. Posterior VTA infusion sites were located from −5.3 to −6.04 mm posterior to bregma. SN, substantia nigra; MN, mammillary nuclei; IPN, interpeduncular nuclei.

2.7. Statistical analysis

For the analysis of the ACD dose–response study, data from rats given aCSF throughout the eight sessions were averaged for two consecutive sessions (i.e., session 1 and 2 together, sessions 3 and 4 together, etc.). This was performed to avoid the complexity of a nested design. Data analysis consisted of a Concentration × Session mixed analysis of variance (ANOVA), with a repeated measure of "session" performed on the number of infusions. Additionally, for each concentration, lever discrimination was determined by Type (active or inactive) × Session mixed ANOVA with a repeated measure of "session." Lever discrimination is a key factor to help distinguish between reinforcement-contingent behavior from a general increase in locomotor activity.

Because the extinction–reinstatement, extended time-out and acetic acid experiments were strictly within-subject experiments, statistical analysis focused on within-subject factors, lever discrimination and alterations in active lever responding. Lever discrimination was analyzed by a lever Type (active or inactive) × Session mixed ANOVA with repeated measure of "session." Additionally, changes in active lever responding across sessions were analyzed by a repeated-measure ANOVA.

3. Results

The posterior VTA was defined as the VTA region at the level of the interpeduncular nucleus, coronal sections from −5.3 to −6.04 mm bregma (Fig. 1). Over 90% of the placements were located within the posterior VTA. The few verified placements outside the posterior VTA included injection sites within the substantia nigra and medial lemniscus ($n=2$). These rats did not display significant responding on either the active or inactive lever and were not included in any analysis because of the small sample size.

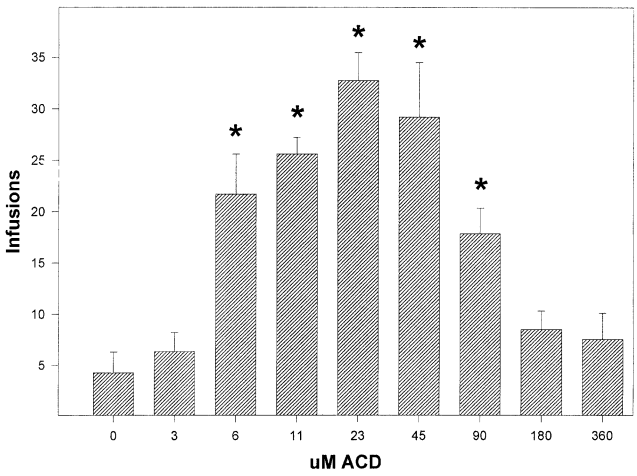

Fig. 2. Average number of infusions during the last two sessions as a function of ACD concentration (0–360 μM) by P rats with placements in the posterior VTA. Asterisks indicate significant difference ($P<.05$) with post hoc Tukey's test. Values for 6-, 11-, 23-, 45- and 90-μM ACD are higher than values for 0-, 3-, 180- and 360-μM ACD. Data are means ± S.E.M. ($n=6$ for 0-μM ACD; $n=7$ for 3-, 6-, 11-, 23-, 180- and 360-μM ACD; $n=8$ for 45- and 90-μM ACD).

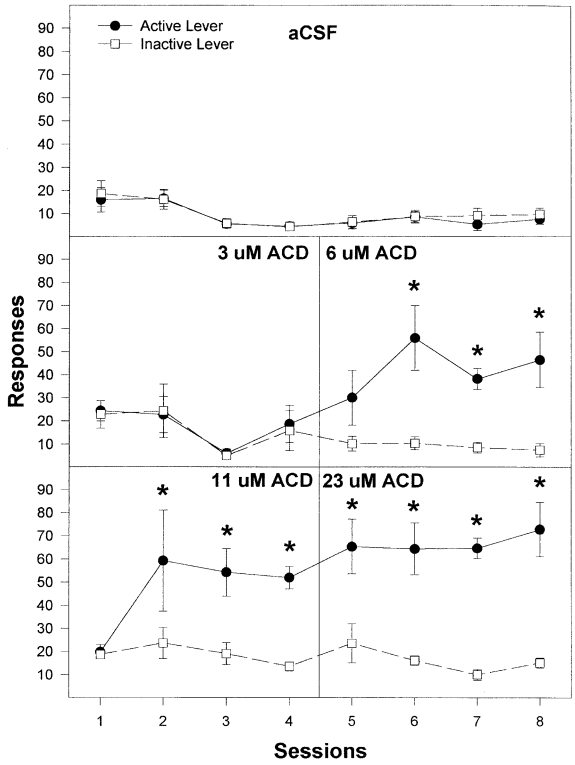

Fig. 3. Responses per session on the active and inactive levers by P rats given 0 (aCSF), 3- and 6- or 11- and 23-μM ACD to self-infuse into the posterior VTA. One group received aCSF for all eight sessions. A second group was given 3-μM ACD for the first four sessions and 6-μM ACD for the second four sessions. A third group was given 11-μM ACD for the first four sessions and 23-μM ACD for the second four sessions. Asterisks indicate significant ($P<.05$; Tukey's) difference in responding on the active lever versus responding on the inactive lever. Rats that showed significantly higher responding on the active than inactive lever also had higher responding than the P rats given aCSF (determined by one-way ANOVAs performed on individual sessions contrasting active and inactive lever presses). Data are means ± S.E.M. ($n=6$ for aCSF and $n=7$ for 3/6- and 11/23-μM ACD).

3.1. ACD dose–response

A select range of ACD concentrations infused into the posterior VTA supported response-contingent behaviors. For rats receiving infusions into the posterior VTA, an ANOVA on the average number of infusions (Fig. 2) received during the four test sessions revealed a significant effect of concentration [$F(8,53)=8.7$, $P<.001$]. Post hoc comparisons (Tukey's) indicated that the 6-, 11-, 23-, 45- and 90-μM ACD groups received significantly more infusions ($P<.05$) than the aCSF control group and the groups given 3-, 180- and 360-μM ACD.

For the groups given aCSF or 3-, 180- or 360-μM ACD (Figs. 3 and 4), the number of response on the active and inactive lever did not differ throughout the sessions [Lever: F's(1,5) < 0.14, P's > .72; Session: F's(3,15) < 3.12, P's > .06; Lever × Session: F's(3,15) < 1.51, P's > .25]. For rats given 6- and 11-μM ACD to self-administer (Figs. 3 and 4), lever discrimination [Lever: F's(1,5) > 34.41, P's < .001; Session: F's(3,15) < 1.21, P's > .34; Lever × Session: F's(3,15) > 4.68, P's < .01] occurred during the second to fourth sessions of administration [Lever: F's(1,5) > 13.52, P's < .014]. For rats given 23-, 45- and 90-μM ACD to self-administer (Figs. 3 and 4), lever discrimination [Lever: F's(1,6) > 52.14, P's < .001; Session: F's(3,18) < 0.52, P's > .68; Lever × Session: F's(3,18) < 0.55, P's > .66] was present for all four sessions [Lever: F's(1,6) > 8.06, P's < .03].

3.2. ACD extinction–reinstatement

During the acquisition sessions for 90-μM ACD (Fig. 5), P rats responded significantly more on the active than inactive lever [Lever: $F(1,5) = 17.44$, $P < .009$; Session: $F(3,15) = 0.93$, $P = .45$; Lever × Session: $F(3,15) = 3.85$, $P = .043$]. In particular, rats discriminated the active from the inactive lever during the second to fourth session [F's(1,5) > 15.9, P's < .01] but not in session 1 (Fig. 5). In session 5, when aCSF was substituted for ACD, responding on the inactive lever increased while responding on the active lever was maintained at the same level as session 4 [lever discrimination in session 5: $F(1,5) = 2.12$, $P = .21$]. In session 6 (the second session with aCSF alone), responses on both levers decreased [Session: $F(2,10) = 4.37$, $P = .04$] and were lower than session 5 ($P < .05$). In addition, lever discrimination was not observed [$F(1,5) = 1.82$, $P = .24$]. Restoring ACD in sessions 7 and 8 did not result in increased responding on the active lever [Session: $F(2,10) = 12.78$, $P = .018$] and lever discrimination [$F(1,5) = 13.91$ $P = .014$] until session 8 (Fig. 5).

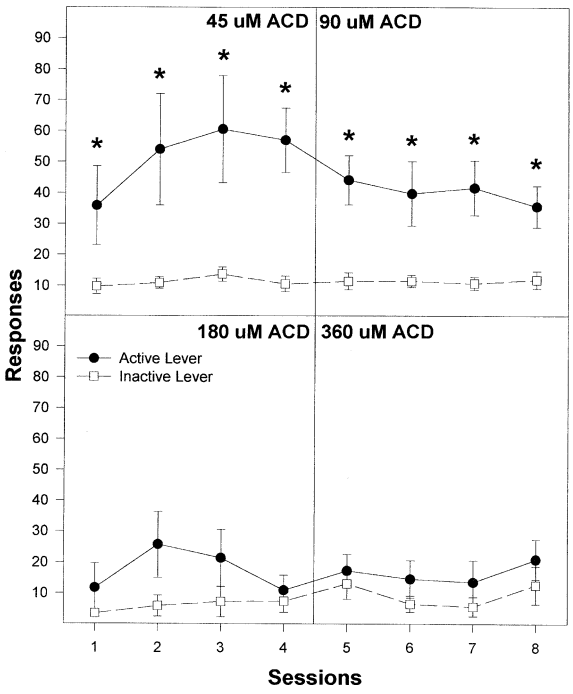

Fig. 4. Responses per session on the active and inactive levers by P rats given 45- and 90- or 180- and 360-μM ACD to self-infuse into the posterior VTA. One group was given 45-μM ACD for the first four sessions and 90-μM ACD for the second four sessions. A second group was given 180-μM ACD for the first four sessions and 360-μM ACD for the second four sessions. Asterisks indicate significant ($P < .05$; Tukey's) difference in responding on the active lever versus responding on the inactive lever. Rats that showed significantly higher responding on the active than inactive lever also had higher responding than the P rats given aCSF (determined by one-way ANOVAs performed on individual sessions contrasting active and inactive lever presses). Data are means ± S.E.M. ($n = 8$ for 45/90-μM ACD and $n = 7$ for 180/360-μM ACD).

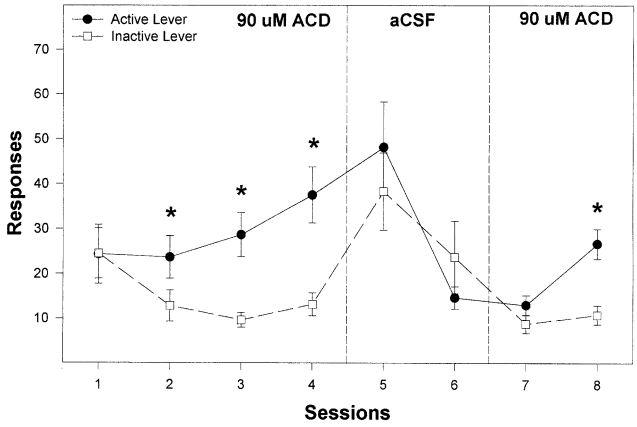

Fig. 5. Responses per session on the active and inactive levers by P rats given 90-μM ACD to self-infuse into the posterior VTA for the first four sessions (acquisition), aCSF for sessions 5 and 6 (extinction) and 90-μM ACD again in sessions 7 and 8 (reinstatement). Asterisks indicate significantly ($P < .05$) higher responding on the active than inactive lever (determined by one-way ANOVAs performed on individual sessions contrasting active and inactive lever presses). Data are means ± S.E.M. ($n = 8$).

3.3. ACD-extended time-out

Extending the time-out period between infusions from 5 to 55 s resulted in an almost twofold increase in responding on the active lever without a significant change in responding on the inactive lever (Fig. 6, top). In examining active lever responses across sessions 3–7, there was a significant effect of session [$F(4,24) = 5.3$, $P < .003$], and post hoc contrasts revealed that active lever responses were elevated ($P < .05$) during sessions 5–7 compared to sessions 3 and 4. In addition, the number of infusions of 23-μM ACD under both time-out conditions did not change significantly during sessions 3–7 [$F(4,24) = 0.5$, $P = .73$; Fig. 6, bottom].

The pattern of responding on the active lever and infusions under both time-out conditions was evaluated in 30-min time blocks (Fig. 7). In the first session with the 5-s time-out condition, there were low levels of responding throughout the session, with the highest

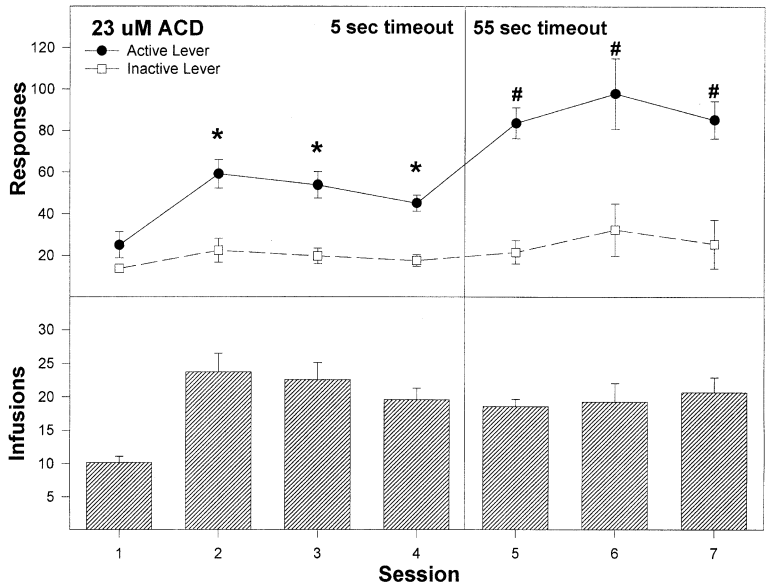

Fig. 6. Responses on the active and inactive levers and infusions/session for rats self-administering 23-μM ACD into the posterior VTA under 5- or 55-s time-out periods. The 5-s time-out was used in the first four sessions and the 55-s time-out was used in the last three sessions. Asterisks indicate significant ($P<.05$) lever discrimination (determined by one-way ANOVAs performed on individual sessions contrasting active and inactive lever presses). Pound symbols indicate elevated levels of responses on the active lever compared to responses in session 3 and 4 ($P<.05$). Infusion levels in sessions 3–7 were not significantly different from each other. Data are means ± S.E.M. ($n=7$).

responding and infusions occurring in the first and fourth 30-min blocks. In session 4, under the 5-s time-out, the number of active lever presses and infusions increased relative to the first session. Highest responding occurred during the first two and last two 30-min time blocks. Throughout the fourth session under the 5-s time-out period, the ratio of active lever responses to infusions was approximately 2. When rats were switched to the 55-s time-out period, highest responding was observed in the first 30-min period and in the last two 30-min blocks, with nearly equal active lever presses occurring among the 30-min blocks between these time periods. In the first 30-min period and last two 30-min blocks, the ratio of active lever responses to ACD infusion was approximately 6 (Fig. 7, bottom panel). The pattern for infusions of ACD under both time-out conditions was similar (Fig. 7, compare session 4 with session 6).

3.4. ICSA of acetic acid

To examine the possibility that acetic acid formed from the oxidation of ACD in the injection reservoir may contribute to or be responsible for the self-infusion of ACD into the posterior VTA, two concentrations of acetic acid were tested. Concentrations of 11- and 23-μM acetic acid were chosen because these concentrations are close to the optimal range of ACD that is self-infused but are well below the maximal dose of ACD that was reliably self-administered (Fig. 2). At the concentrations tested, the number of acetic acid self-infusions into the posterior VTA was not significantly different than the number of infusions of aCSF alone (average number of infusions/session of acetic acid ranged from 4.6 ± 1.0 to 9.2 ± 1.3). Active lever responding resembled that typically observed during self-administration of aCSF, i.e., a low level of responding that gradually declined across sessions (from 31.1 ± 10.4 to 9.3 ± 1.7 responses/session). In addition, lever discrimination was not displayed with either concentration of acetic acid (P's > .68).

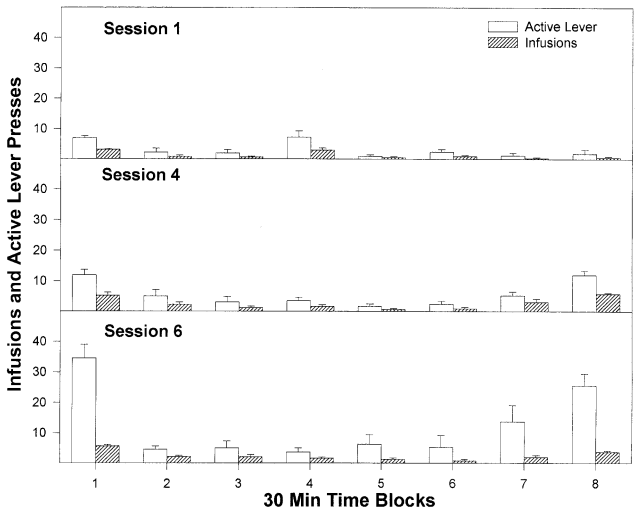

Fig. 7. Temporal pattern of responding on the active lever and infusions for P rats self-administering 23-μM ACD into the posterior VTA under the 5- and 55-s time-out periods. Data are the means ± S.E.M. of 30-min time blocks ($n=7$). Sessions 1 and 4 are with the 5-s time-out and session 6 is with the 55-s time-out.

4. Discussion

The results of this study indicate that ACD is a potent reinforcer within the posterior VTA of P rats. Evidence to support the interpretation that ACD is reinforcing within the posterior VTA comes from (a) the dose–response effects for self-infusions of ACD (Fig. 2), (b) the demonstration of lever discrimination at relevant concentrations of ACD (Figs. 3 and 4), (c) the extinction behavior and loss of lever discrimination when vehicle was substituted for ACD (Fig. 5), (d) the reinstatement of active lever responding and lever discrimination when ACD was restored after extinction training (Fig. 5) and (e) the maintenance of infusion levels and patterns of infusion when the time-out was extended (Figs. 6 and 7).

The dose–response plot for the self-infusions of ACD indicated an inverted "U-shaped" curve (Fig. 2). Concentrations at 3 μM or lower were not self-infused and concentrations of 180 μM and higher also were not self-infused. These data indicate that the lower limit of producing a reinforcing effect of ACD is around 6 μM (or 0.6 pmol/100 nl infused). This concentration of ACD could be theoretically reached with brain ethanol concentrations in the range of 45–200-mg % ethanol. In vitro studies (Zimatkin et al., 1998) indicated that 10–50-mM ethanol (45–225-mg % ethanol) could generate up to 4 nmol/mg wet wt/h of ACD (approximately 4-μM ACD). These brain alcohol concentrations could be attained under certain self-administration conditions (Murphy et al., 1986; Waller et al., 1984). Consequently, the concentrations of ACD self-infused are pharmacologically relevant. Moreover, previous studies with female P (Gatto et al., 1994) and Wistar (Rodd-Henricks et al., 2000) rats indicated that concentrations in the range of 100–200-mg % ethanol are readily self-administered into the VTA, whereas concentrations below 50 mg % are not reliably self-infused. Overall, the results of the present study indicate that ACD is a 1000-fold more potent reinforcer than ethanol in the VTA and that the concentrations of ACD self-infused could be locally formed from the concentrations of ethanol, which were self-administered into the VTA.

The inverted "U-shaped" plot for ACD is somewhat similar to the dose–response plot for ethanol (Rodd-Henricks et al., 2000) in that self-infusions of ACD were significantly reduced at the two highest concentrations tested (Fig. 2). It is possible that at these higher concentrations, ACD may be producing effects on DA neurons within the VTA to reduce their activity. This could occur through ACD reacting with membrane proteins to alter their normal function. This effect appears to occur early because low levels of responding on the active lever were observed throughout all the sessions with 180- and 360-μM ACD (Fig. 4). Studies with 400-mg % ethanol indicated that high responding on the active lever and lever discrimination were observed during the first two sessions but, thereafter, responding on the active lever decreased to the low levels observed for the inactive lever (Rodd-Henricks et al. 2000). In this latter study, it is possible that, at the 400-mg % concentration of ethanol, high levels of ACD accumulated and resulted in reduced ethanol self-infusions.

Without any prior operant experience, the P rats readily learned to discriminate the active from the inactive lever. At concentrations between 6 and 90 μM, the P rats responded more on the active than inactive lever by the second session of the initial ACD acquisition period (Figs. 3, 5 and 6). A similar rapid acquisition of responding for the self-infusion of ethanol (Gatto et al., 1994; Rodd-Henricks et al., 2000), opioids (Bozarth and Wise, 1981; Devine and Wise, 1994) and $GABA_A$ antagonists (Ikemoto et al., 1997b) into the VTA has been reported. The neurobiological bases for the rapid acquisition of operant responding for direct self-infusions into the VTA or other CNS regions (Carlzon et al., 1995; Goeders et al., 1984; Ikemoto et al., 1997b) are unknown. Moreover, the rapid acquisition of lever discrimination appears to be related to the reinforcing actions of ACD rather than a result of reflexive motor behavior because (a) infusions appear to follow a distinct temporal pattern with the highest responding and infusions occurring at the beginning and end of the acquisition session (Fig. 7, middle panel), (b) extinction responding was observed in the first session when vehicle was substituted for ACD (Fig. 5) and (c) a similar temporal pattern of responding on the active lever and number and pattern of infusions were observed when the time-out period was increased from 5 to 55 s (Figs. 6 and 7). In addition, the temporal pattern of infusions of 23-μM ACD observed with P rats on the 5-s time-out schedule (Fig. 7, middle panel, session 4) was similar to the infusion pattern observed for 200-mg % ethanol with Wistar rats (Rodd-Henricks et al., 2000). In both cases, the highest infusions were found at the beginning and end of the 4-h session.

The neurobiological mechanisms underlying the reinforcing effects of ACD within the posterior VTA are not known. However, it is possible that the reinforcing actions of ACD may involve activation of DA neurons in the VTA, either directly through an interaction of ACD with receptors or ion channels and/or indirectly through an interaction of ACD with neurotransmitter systems regulating the activity of VTA DA neurons (Kalivas, 1993). Evidence using isolated cardiac myocytes indicates that ACD can increase calcium channel currents (Chen et al., 2000). If a similar effect occurred on DA neurons within the VTA, then this could be a mechanism underlying the reinforcing actions of ACD. Additional studies need to be undertaken to examine the mechanisms underlying the reinforcing actions of ACD in the posterior VTA.

ACD is a biologically active compound that is considered to be highly toxic (Hunt, 1996). However, under the conditions utilized in the present study, concentrations of 90 μM and lower of ACD, when microinjected into the VTA, do not appear to be toxic. For one, the effective tissue concentration is likely to be lower than the concentration

injected due to diffusion and dilution within the brain site. Secondly, stable responding on the active lever is still evident after eight sessions of ACD exposure (Fig. 3, bottom panel;, top panel; Fi, top panel)4, top panel; Fi, top panel)6), suggesting that significant neuronal damage has not been produced with repeated microinjections of ACD. On the other hand, the finding that neither 180- nor 360-μM ACD was self-administered into the VTA (Fig. 4, bottom panel) may indicate that, at these concentrations, ACD could be producing neuronal damage.

Overall, the ICSA data suggest that ACD can produce reinforcing effects within the posterior VTA of P rats and that ACD is a more potent reinforcer in this region than is ethanol. Although it has not been clearly established that similar pharmacologically relevant concentrations of ACD could be regionally formed within the CNS following alcohol drinking, the concentrations of ACD that were found to be reinforcing could, theoretically, be attained with brain levels of alcohol, which were found to be reinforcing. Additional studies need to be undertaken to determine the role of ACD in the rewarding effects of alcohol and alcohol addiction.

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