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ORIGINAL INVESTIGATION

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The discriminative stimulus properties of self-administered ethanol are mediated by $GABA_A$ and NMDA receptors in rats

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Abstract *Rationale:* The neurobiological systems that mediate the discriminative stimulus effects of self-administered drugs are largely unknown. The present study examined the discriminative stimulus effects of self-administered ethanol. *Methods:* Rats were trained to discriminate ethanol (1 g/kg, IP) from saline on a two-lever drug discrimination task with sucrose (10% w/v) reinforcement. Test sessions were conducted with ethanol (0 or 10% v/v) added to the sucrose reinforcement to determine if self-administered ethanol would interact with the discriminative stimulus effects of investigator-administered ethanol, or with the ethanol-like discriminative stimulus effects of the $GABA_A$-positive modulator pentobarbital or the non-competitive NMDA antagonist MK-801. *Results:* During a saline test session, ethanol (10% v/v) was added to the sucrose reinforcement. Responding by all animals began accurately on the saline-appropriate lever and then switched to the ethanol-appropriate lever after rats self-administered a mean dose of 1.2±0.14 g/kg ethanol. During cumulative self-administration trials, responding initially occurred on the saline lever and then switched to the ethanol-appropriate lever after ethanol (0.68±0.13 g/kg) was self-administered. Investigator-administered MK-801 (0.01–1.0 mg/kg, cumulative IP) and pentobarbital (0.3–10.0 mg/kg, cumulative IP) dose-dependently substituted for ethanol. When ethanol (10% v/v) was added to the sucrose reinforcer, MK-801 and pentobarbital dose-response curves were shifted significantly to the left. *Conclusions:* Self-administered ethanol substituted for and potentiated the stimulus effects of investigator-administered ethanol, suggesting that the discriminative stimulus effects of self-administered ethanol are similar to those produced by investigator-administered ethanol. Self-administered ethanol enhanced the ethanol-like discriminative stimulus effects of MK-801 and pentobarbital, which suggests that the discriminative stimulus effects of self-administered ethanol are mediated by NMDA and $GABA_A$ receptors.

Keywords Ethanol · $GABA_A$ · NMDA · Rat · Self-administration · Drug discrimination · Investigator-administered

Introduction

Drug self-administration is maintained by the ability of a drug to function as a positive reinforcer. However, drugs of abuse produce reinforcement-independent effects that may also influence drug self-administration. The discriminative stimulus (cue) effects of drugs comprise a second major behavioral process that is thought to influence abuse liability (Haretzen and Hickey1987; Holtzman 1990; Stolerman 1992). For instance, many drugs of abuse can both maintain self-administration and function as discriminative stimuli in experimental animals (Overton 1987; Overton et al. 1986). The discriminative stimulus effects of drugs may reinstate drug-seeking behavior because a history of self-administration repeatedly associates the positive reinforcing aspects of a drug with distinctive stimulus effects. Accordingly, passive administration of low doses of a drug reinstates drug-seeking behavior that has been previously extinguished (Stretch and Gerber 1973; de Wit and Stewart 1983). Thus, the discriminative stimulus properties of drugs may reinstate drug-seeking behavior in ways that are not predicted by the simple positive reinforcement hypothesis (Stolerman 1992).

Pharmacological evidence supports the idea that alcohol self-administration and discrimination are mediated by similar neurobiological mechanisms. Positive modu-

Initial results from this study were presented at the annual meeting of the Research Society on Alcoholism in 1997 (Hodge and Cox 1997)

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lators of $GABA_A$ receptor function decrease alcohol self-administration behavior (Hodge et al. 1995; June et al. 1992, 1994; McBride et al. 1988; Rassnick et al. 1993; Samson et al. 1989). Allosteric $GABA_A$-positive modulators such as barbiturates (Barry 1991; Barry and Krimmer 1978; Kline and Young 1986; Overton 1977; York 1978) and benzodiazepines (Hiltunen and Järbe 1986; Kubena and Barry 1969) produce ethanol-like discriminative stimulus effects. Although systemic administration of the direct $GABA_A$ agonist muscimol does not substitute for ethanol (Shelton and Balster 1994), microinjection of muscimol in the nucleus accumbens produces full substitution for the discriminative stimulus effects of systemic ethanol (Hodge and Aiken 1996; Hodge and Cox 1998) and reduces operant ethanol self-administration (Hodge et al. 1995). Thus, $GABA_A$ receptor activation within mesolimbic pathways may reduce alcohol-seeking behavior by drug substitution.

This hypothesis is complicated, however, by the fact that drug discrimination and drug self-administration procedures require distinct methods of drug administration. Recent evidence indicates that self-administered drugs produce different effects on the central nervous system as compared to investigator-administered drugs (i.e., in drug discrimination procedures). Self-administered cocaine produces greater increases in nucleus accumbens extracellular dopamine as compared to increases seen after investigator-administered cocaine (Hemby et al. 1997). Self-administered ethanol modulates functional brain activity in a manner that is distinct from that observed when equivalent doses of ethanol are administered by the investigator (Eckardt et al. 1988; Porrino et al. 1998). Consequently, the issue of whether drug self-administration and drug discrimination procedures address the same neurobiological effects of drugs of abuse remains open to question.

To address this issue, the present study was designed to investigate whether the discriminative stimulus effects of self-administered and investigator-administered ethanol are mediated by similar neurobiological systems. First, we asked if self-administered ethanol would substitute for and/or potentiate the discriminative stimulus effects of investigator-administered ethanol. Second, to further address the mechanism of action, we asked if self-administered ethanol would enhance the ability of a $GABA_A$-positive modulator or NMDA non-competitive antagonist to substitute for investigator-administered ethanol.

Materials and methods

Animals

Eight male Long-Evans hooded rats served as subjects. Body weights (mean ± SEM) were maintained at 320±15 g by food regulation. Rats were housed individually in hanging stainless steel cages with ad libitum access to water in the home cage and access to a liquid sucrose (10% w/v) solution during experimental sessions. The animals were maintained on a 12-h light-dark cycle (lights on at 0630 hours). Temperature and humidity were maintained within National Institutes of Health guidelines. All experimental sessions were conducted during the light portion of the cycle. Rats were weighed and inspected daily for general health. All rats were experimentally and drug naive. All animal procedures were conducted according to the "Guide for the Care and Use of Laboratory Animals" (National Research Council, 1996).

Apparatus

Discrimination sessions were conducted in eight operant chambers (31L×32H×24W cm) located in sound-attenuating cubicles with exhaust fans that helped to mask external noise (Med Associates, Lafayette, Ind., USA). Chambers were equipped with two retractable levers along the right wall separated by a liquid dispenser. Responses on one of two levers activated the liquid dispenser presenting fluid in a 0.1-ml dipper for 4 s during each operation. The operant chambers were interfaced (Med Associates) to a 200-MHz computer (Gateway 2000, North Sioux City, S.D., USA) that was programmed to control sessions and record data. An 8-W light located on the left wall 28 cm above the dipper illuminated the chambers and signaled the start of each session.

Procedure

Rats were allowed to adapt to individual housing conditions and daily handling for 1 week, during which time food and water were always available. When target body weights were obtained, food was restricted to approximately 16 grams/day. Rats were trained to press a single lever on a fixed-ratio 1 (FR 1) schedule of reinforcement that resulted in presentation of 0.1 ml of a liquid sucrose solution (10% w/v). After 3 days, they were then trained to press either the left or the right lever during daily 30-min sessions. The side of the active lever was alternated on a daily basis. Responses on the inactive lever were recorded but produced no programmed consequences. The schedule of reinforcement was gradually increased to FR 10 with only one lever active during each session. All animals received an equal history with each lever at each FR value. Ethanol discrimination training was initiated when response rates stabilized (<10% daily variation).

Discrimination training

Training sessions were conducted 5 days per week (Monday through Friday) during which ethanol 1.0 g/kg (E) or saline (S) was administered IP 10-min prior to the start of 15-min sessions. The animals were placed in the operant chambers and illumination of the house-light signaled the beginning of the session. The lever associated with E or S administration was assigned randomly and counterbalanced between animals. Following E or S injections, completion of ten responses on the appropriate lever produced the sucrose solution. Responses on the inappropriate lever were recorded but produced no programmed consequences. There were an equal number of E and S training days that varied on a double alternation schedule (E, E, S, S...). Training sessions were conducted until the following criteria were met: the percentage of E and S appropriate lever press responses emitted prior to the first reinforcer, and during the entire session, exceeded 80% for 10 consecutive days. These criteria allowed no more than two "errors" prior to completion of the first FR 10. Once the accuracy criteria were met, test sessions were conducted during which an ethanol (0.1–1.5 g/kg, IP) substitution curve was determined.

IP ethanol substitution testing

Ethanol substitution test sessions were identical to training sessions except: (a) they were 2-min in duration, (b) completion of an FR 10 on either lever produced the sucrose solution, and (c) novel doses of ethanol were administered. Test sessions began after per-

formance during training met the accuracy criteria for ten consecutive sessions. A minimum of two training sessions was conducted between test sessions. If performance during these training sessions failed to meet the accuracy criteria, test sessions were postponed until response accuracy was greater than 80% for ten consecutive training sessions. Ethanol was administered in single acute IP injections at doses of 0.1, 0.5, 1.0, and 1.5 g/kg.

Substitution testing in real-time with self-administered ethanol

After demonstration that IP ethanol was functioning as a discriminative stimulus, rats were given a single injection of saline and then tested during two single 30-min sessions with ethanol (0 or 10% v/v) added to the sucrose reinforcement. During these two sessions, behavior was free to vary between the two levers since completion of an FR 10 on either lever produced the ethanol/sucrose solution. Responses were recorded in real-time on each lever to determine if self-administered ethanol would result in responding switching from the saline-associated to the ethanol-associated lever.

Substitution testing with cumulative self-administered ethanol

As a second method to test the ability of self-administered ethanol to substitute for investigator-administered ethanol, four consecutive IP saline test sessions were conducted within the same day with ethanol (0 or 10% v/v) added to the sucrose reinforcement. This allowed the rats to self-administer a cumulative dose of ethanol during four repeated sessions in a manner analogous to cumulative dosing procedures, which have been used to test the discriminative effects of investigator-administered drugs (see, for example, Hiltunen and Järbe 1989; Järbe et al. 1981). The key difference in the present procedure is that the cumulative drug was self-administered during four discrete 2-min trials, not investigator-administered. Prior to each cumulative self-administration session, rats were injected with saline (IP) and placed in the chambers for a 10-min presession delay. Each cumulative self-administration session was 2-min in duration. Thus, the total time required to complete four cumulative ethanol self-administration trials (i.e., one dose-response curve) was 48 min. Again, behavior was free to vary between the two levers since completion of an FR 10 on either lever produced the ethanol/sucrose solution.

Substitution testing with cumulative investigator- and self-administered ethanol

To further explore an interaction between the discriminative stimulus effects of self-administered and investigator-administered ethanol, test sessions were conducted to evaluate whether self-administered ethanol (0 or 10% v/v) would interact with the stimulus effects of cumulative doses of IP ethanol. Thus, two cumulative dose response curves were established. First, the stimulus effects of cumulative IP ethanol (0.1, 0.3, 1.0, and 1.7 g/kg, IP) were determined with sucrose-only reinforcement (ethanol 0% v/v). Then, during a second session, the effects of cumulative ethanol (0.1, 0.3, and 1.0 g/kg, IP) were determined with ethanol (10% v/v) added to the sucrose reinforcement. This procedure tests the ability of cumulative self-administered ethanol to interact with cumulative investigator-administered ethanol. For each cumulative test session, rats were injected with IP ethanol and placed in the operant chambers for a 10-min presession delay. Each cumulative test session was 2 min in duration. Thus, the total time required to complete four cumulative trials (i.e., one dose-response curve) was 48 min. Again, behavior was free to vary between the two levers since completion of an FR 10 on either lever produced the ethanol/sucrose solution.

Substitution testing with MK-801 and pentobarbital

Substitution test sessions were conducted by administering cumulative doses of MK-801 (0.01, 0.03, 0.10, 0.20 mg/kg; IP) or pentobarbital (1.0, 3.0, 10.0 mg/kg; IP) with sucrose-only reinforcement. Then, cumulative dose-response curves for MK-801 (0.01, 0.03, 0.10, 0.20 mg/kg; IP) and pentobarbital (0.30, 1.0, 3.0 mg/kg; IP) were determined with ethanol (10% v/v) added to the sucrose (10% w/v) reinforcement. This procedure tests the ability of cumulative self-administered ethanol to interact with cumulative investigator-administered MK-801 or pentobarbital. Cumulative dosing sessions were performed by conducting sequential 2-min trials, each separated by a 10-min postinjection interval.

Drugs

For peripheral administration, ethanol (95%) was diluted in physiological saline to a concentration of 20% v/v and was administered in varied volumes relative to body weight. The non-competitive NMDA antagonist (5R,10S)-(+)-5-methyl-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5,10-imine hydrogen maleate (MK-801) and the $GABA_A$-positive modulator pentobarbital were dissolved in physiological saline and administered in a constant volume of 1.0 ml/kg. All drugs were obtained from Research Biochemicals International (Natick, Mass., USA). Drug solutions were prepared immediately prior to injection.

Data analyses

Response accuracy was expressed as the percentage of ethanol-appropriate lever presses upon delivery of the first reinforcer. Response rate (responses/min) was analyzed for the entire session as a measure of possible non-specific effects of drugs on behavior. Group averages for the saline and ethanol training sessions from 10 days immediately prior to the beginning of testing represented control performance for the effects of IP ethanol in Fig. 1. Complete ethanol substitution was defined as >80% choice of the ethanol lever upon completion of the first FR 10 during test sessions, whereas partial substitution was defined as between 40% and 80% ethanol lever responding. Response accuracy and response rate data were tested for statistical differences with one- or two-way repeated measures analysis of variance (ANOVA). When significant main effects were observed, post hoc comparisons were conducted with Tukey *t*-tests. When ANOVA was used to compare dose response curves, analyses were conducted only on doses that were tested under both conditions. Mean (± SEM) ED50 values for the dose effects on response accuracy were determined by log-dose probit analysis of data from individual animals where appropriate and compared by paired *t*-test. All data are presented as mean (± SEM).

Results

IP ethanol discrimination

One rat failed to acquire the ethanol discrimination task and was excluded from the study. All data are presented for n=7 animals. Performance during control conditions and IP ethanol substitution test sessions is shown in Fig. 1. The percentage of ethanol-appropriate lever presses upon completion of the first FR 10 was approximately 90% during ethanol control sessions and less than 10% during saline control sessions indicating that the procedures established reliable stimulus control (Fig. 1A). Both the 1.0 and 1.5 g/kg doses of ethanol substituted fully for the 1.0 g/kg training dose. The be-

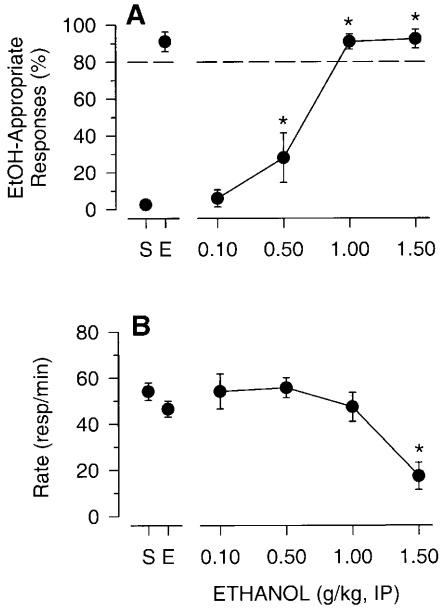

Fig. 1A,B Discriminative stimulus function of investigator-administered ethanol. **A** Mean (± SEM) percentage of ethanol appropriate lever presses upon completion of the first fixed-ratio 10 (FR 10) and **B** mean (± SEM) total session response rate plotted as a function of ethanol dosage. Data points to the left of the *x*-axis break represent performance during the last ten saline (*S*) or ethanol (*E*) training sessions prior to the start of the test sessions. Data points to the right of the *x*-axis break represent test-session performance following IP ethanol administration. Training and test sessions began 10 min after IP ethanol administration. The *horizontal dashed line* (at 80%) represents full substitution for the discriminative stimulus effects of ethanol (1.0 g/kg, IP). All points represent mean performance of n=7 animals. *Error bars* represent ± SEM. * Significantly different from saline, P<0.05 Tukey test

havior of all individual animals demonstrated dose-dependent substitution to the training dose of at least one test-dose of ethanol. The ED50 value for ethanol substitution was 0.68 (±0.10 g/kg). Repeated measures ANOVA indicated that ethanol significantly increased the percentage of ethanol-appropriate responses [F(4,23)=49.9, P<0.001] in a dose-dependent manner (Fig. 1A). Ethanol (1.5 g/kg) significantly reduced the response rate [F(4,23)=8.01, P<0.001] during test sessions (Fig. 1B).

Self-administered ethanol substituted for investigator-administered ethanol

After the discriminative stimulus function of IP ethanol was verified, saline test sessions (30-min in duration) were conducted with sucrose (10% w/v) reinforcement or with ethanol (10% v/v) added to the sucrose reinforcement. During these test sessions, responses on both levers produced the available reinforcer. The left column of Fig. 2 shows that after IP saline injection, responding occurred almost entirely on the saline lever with sucrose reinforcement (Fig. 2A top left). Virtually no responses occurred on the ethanol lever after saline injection (Fig. 2A bottom left). However, when ethanol was added to the sucrose reinforcer, responding accurately began on the saline-appropriate lever (Fig. 2B top right) and then switched to the ethanol-appropriate lever after rats self-administered an average of 1.2±0.14 g/kg ethanol during an average period of 14.9±2.9 min (Fig. 2B bottom right). Under these conditions, responding of all seven rats switched from the saline-appropriate to the ethanol-appropriate lever.

To further address the ability of self-administered ethanol to substitute for investigator-administered ethanol, cumulative saline test sessions were conducted during which sucrose (10% w/v) or sucrose (10% w/v) plus ethanol (10% v/v) were used as reinforcers. Results indicated that sucrose (10% w/v) reinforcement resulted in less than 2% ethanol-appropriate responding during all four sessions (data not shown) and produced no changes in response rate (Fig. 3B). Self-administered ethanol during discrete cumulative 2-min test sessions produced full substitution for the discriminative stimulus effects of investigator-administered ethanol (Fig. 3A). Full substitution for the stimulus effects of investigator-administered ethanol (1.0 g/kg, IP) occurred when the cumulative dose of self-administered ethanol reached an average of 0.68±0.13 g/kg, which was upon completion of the first FR 10 during the fourth cumulative session (Fig. 3A). Repeated measures ANOVA of lever choice data (Fig. 3A) indicated that self-administered ethanol increased the percentage of ethanol-appropriate responses [F(1,6)=48, P<0.001] as a function of cumulative session [F(3,18)=6.1, P<0.005] and that the effect of the reinforcer was dependent on cumulative intake [F(3,18)=6.3, P<0.004]. Self-administered ethanol (g/kg) increased linearly over the four cumulative trials to a total self-administered dose of 0.96±0.18 g/kg, which produced a significant effect of cumulative trial on ethanol intake [F(3,18)=24.4, P<0.001]. The discriminative stimulus effects of this dose of ethanol were not tested since it would have required a fifth session. Self-administered ethanol produced no effects on response rate (Fig. 3B).

Self-administered ethanol enhanced the effects of investigator-administered ethanol

When sucrose-only was the reinforcer, cumulative ethanol (1.7 g/kg, IP) substituted fully for the training dose of ethanol (1.0 g/kg) in six of seven animals tested (Fig. 4A). The mean (± SEM) ED50 for cumulative ethanol substitution was 1.06±0.16 g/kg with sucrose (10% w/v) reinforcement. This ED50 was not statistically different [t(6)=–2.338, P=0.06] from the ED50 obtained from non-cumulative ethanol administration (see Fig. 1). For the one rat that failed to show full substitution, ethanol-lever selection was 71% upon completion of the first FR 10 and 91% during the total session. Further inspection of individual data showed that the cumulative ethanol (1.0 g/kg) substituted fully for the training dose in

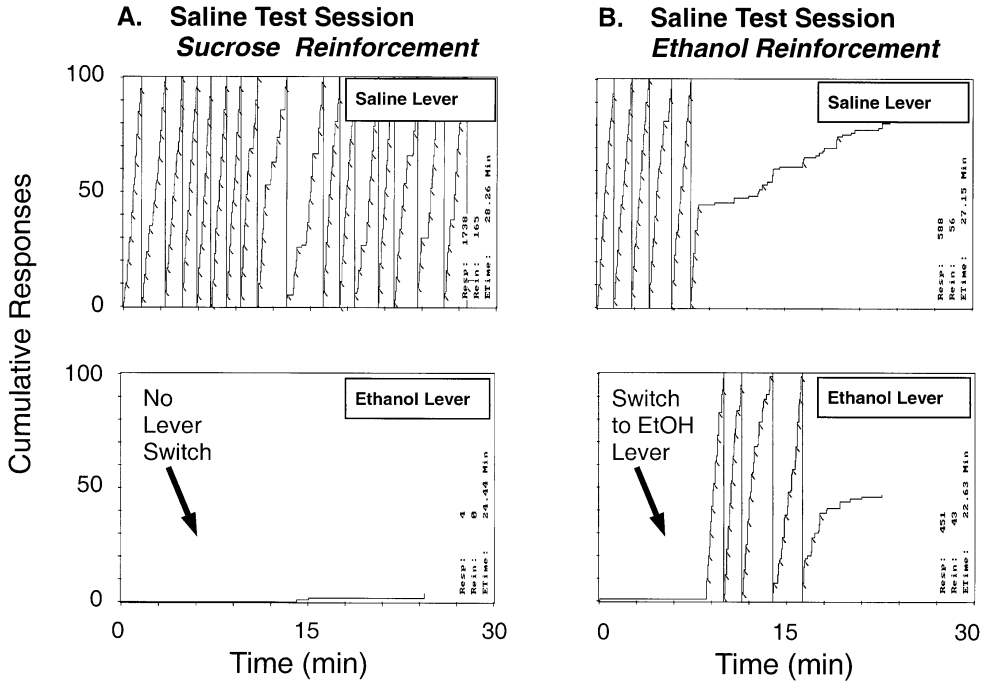

Fig. 2A,B Emergence of the discriminative stimulus function of self-administered ethanol in real-time. **A** The *left side* of the figure shows representative cumulative response records, which depict the temporal pattern of responding on the saline-appropriate (*top left*) and ethanol-appropriate (*bottom left*) levers during a saline test session with sucrose (10% w/v) reinforcement. Responding occurred almost exclusively on the saline-appropriate lever indicating saline discrimination. **B** Cumulative response records showing temporal pattern of responding on saline-appropriate (*top right*) and ethanol-appropriate (*bottom right*) levers during a saline test session in which ethanol (10% v/v) was added to the sucrose reinforcement. Responding accurately began on the saline lever, but switched to the ethanol-appropriate lever as an increasing amount of ethanol was self-administered. The slope of the line indicates response rate and the *angled "pips"* on each line indicate presentation of a reinforcer. Data are from a single animal following saline injection (IP) with sucrose-only reinforcement (**A** *left column*) or with ethanol (10% w/v) added to the sucrose reinforcement (**B** *right column*)

three of seven animals but failed to produce any substitution in the other four animals.

When 10% ethanol was added to the sucrose reinforcer a lower dose of cumulative ethanol (1.0 g/kg, IP) substituted fully for ethanol in all seven animals tested (Fig. 4A). The addition of ethanol (10% v/v) to the sucrose reinforcement significantly shifted the ED50 for cumulative ethanol substitution over fourfold to the left to 0.25±0.06 g/kg as compared to sucrose-only reinforcement [$t(5)=3.4$, $P=0.02$; paired t-test]. Two-way repeated measures ANOVA on the lever choice data showed a significant effect for reinforcer [$F(1,6)=22.1$, $P<0.01$], cumulative dose of IP ethanol [$F(2,12)=15.4$, $P<0.001$], and a significant interaction between reinforcer and ethanol dosage [$F(2,12)=5.7$, $P<0.05$], which indicates that self-administered and IP ethanol interactively influenced ethanol discrimination. There was no main effect of reinforcer on response rate; however, ethanol

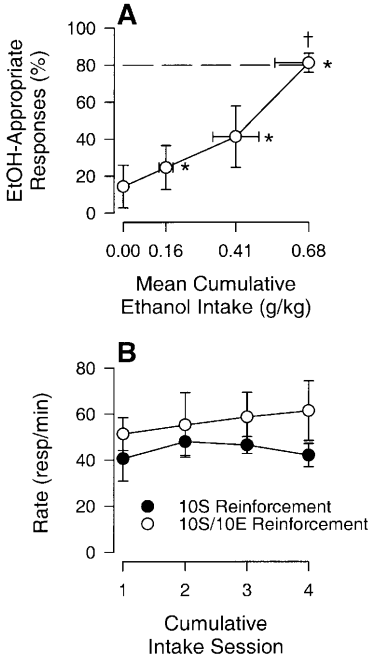

Fig. 3A,B Ethanol self-administered during cumulative 2-min trials substituted fully for the discriminative stimulus effects of investigator-administered ethanol (1.0 g/kg). **A** Mean (± SEM) percentage of ethanol-appropriate responses plotted as a function of mean (± SEM) self-administered ethanol (g/kg) upon completion of the first FR 10 of each cumulative session. **B** Response rate plotted as a function of each cumulative saline test session. Dose-response curves were determined by conducting sequential 2-min trials, each separated by a 10-min intersession interval. *Horizontal dashed line* indicates threshold for ethanol substitution (i.e., >80%). *Vertical and horizontal error bars* are ± SEM. * Significant increase in ethanol intake (g/kg) as compared to the first session, † significant increase in ethanol-lever selection as compared to the first session, $P<0.05$ Tukey test

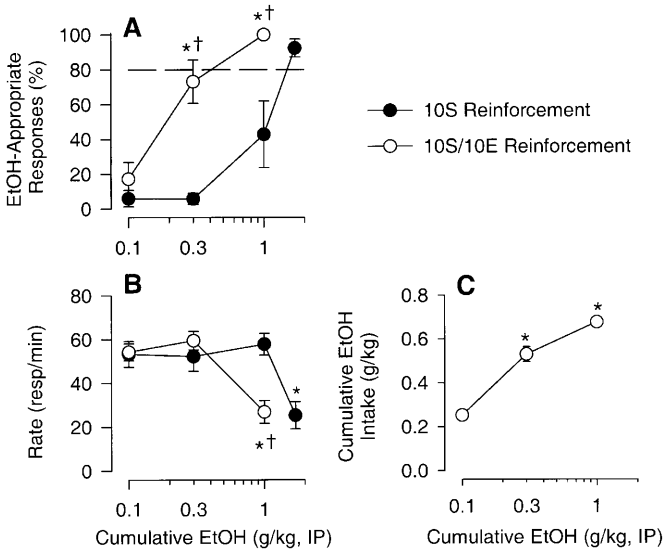

Fig. 4A–C Self-administered ethanol enhanced the discriminative stimulus effects of investigator-administered ethanol. Mean (± SEM) percentage of ethanol appropriate responses (**A**), total session response rate (**B**), and cumulative ethanol intake (**C**) plotted as a function of cumulative ethanol dosage. Dose response curves were determined with sucrose (10% w/v) reinforcement (*10S Reinforcement*) or with ethanol (10% v/v) added to the sucrose reinforcement (*10S/10E Reinforcement*). Each cumulative dose-response curve was determined by conducting sequential 2-min trials, each separated by a 10-min postinjection interval. *Horizontal dashed line* indicates threshold for ethanol substitution (i.e., >80%). * Significantly different from lowest dose of IP ethanol, † significantly different from 10S reinforcement at corresponding dose of IP ethanol, $P<0.05$ Tukey test

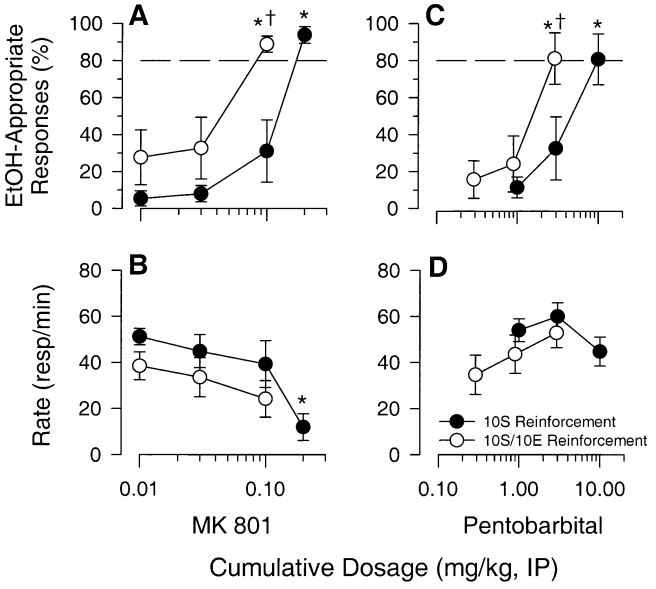

Fig. 5A–D Self-administered ethanol enhanced the ethanol-like discriminative stimulus effects of the NMDA antagonist MK-801 and the $GABA_A$-positive modulator pentobarbital. Mean (± SEM) percentage of ethanol appropriate responses (**A**) and total session response rate (**B**) plotted as a function of cumulative drug dosage of MK-801. Mean (± SEM) percentage of ethanol appropriate responses (**C**) and total session response rate (**D**) plotted as a function of cumulative drug dosage of pentobarbital. Each cumulative dose-response curve was determined by conducting sequential 2-min trials, each separated by a 10-min postinjection interval. *Horizontal dashed line* indicates threshold for ethanol substitution (i.e., >80%). * Significantly different from the lowest dose of each drug, † significantly different from 10S reinforcement at corresponding dose of IP ethanol, $P<0.05$ Tukey test

concentration [$F(2,12)=7$, $P<0.01$] and the interaction between reinforcer and concentration [$F(2,12)=10.7$, $P<0.01$] reached statistical significance indicating that self-administered ethanol interacted with cumulative IP ethanol to reduce response rate (Fig. 4B).

Figure 4C shows the dosage of ethanol that was self-administered during cumulative IP ethanol test sessions. Self-administered ethanol increased significantly over the three sessions [$F(2,12)=161$, $P<0.001$] reaching a maximal mean dosage of 0.68±0.01 g/kg. The total ethanol dosage (IP + self-administered) at the time of full substitution shown in Fig. 4A was 1.0+0.53=1.53 g/kg. This dosage was obtained from three cumulative IP injections and two cumulative self-administration opportunities since the data in Fig. 4A represent performance upon delivery of the first reinforcer during the third cumulative test session.

Self-administered ethanol enhanced the ethanol-like effects of NMDA and $GABA_A$ ligands

Investigator-administered MK-801 substituted fully for the discriminative stimulus effects of IP ethanol with sucrose-only reinforcement (Fig. 5A). Cumulative MK-801 substituted for ethanol in six of the seven animals tested with an ED50 of 0.12±0.02 mg/kg. The addition of ethanol to the sucrose reinforcement shifted the MK-801 ED50 value for ethanol substitution significantly to the left to a value of 0.05±0.01 mg/kg [$t(5)=3.4$, $P=0.02$, paired *t*-test] and produced full substitution in five of seven animals tested. MK-801 failed to substitute for ethanol in one rat under both reinforcement conditions. One-way ANOVA showed that MK-801 significantly increased the percentage of responses on the ethanol-associated lever [$F(3,18)=25$, $P<0.001$]. Two-way ANOVA indicated that the addition of ethanol to the sucrose solution resulted in a significant difference in lever choice [$F(1,6)=26$, $P=0.002$] that was dependent on the dosage of MK-801 [$F(2,12)=9.2$, $P=004$]. The highest dose of MK-801 alone produced a significant decrease in response rate [$F(3,18)=11$, $P<0.001$] but there were no reductions in response rate at any doses of MK-801 tested in conjunction with ethanol added to the reinforcer (Fig. 5B).

With sucrose reinforcement, pentobarbital substituted fully for the discriminative stimulus effects of ethanol (1.0 g/kg, IP) in six of seven animals tested with an ED50 of 4.7±1.1 mg/kg (Fig. 5C). Self-administered ethanol shifted the pentobarbital ED50 significantly to the left [$t(5)=4.2$, $P<0.01$, paired *t*-test] to a value of 1.28±0.35mg/kg. One-way ANOVA indicated that pen-

tobarbital significantly increased the percentage of ethanol-lever responses [$F(2,12)=9.9$, $P=0.003$]. Two-way ANOVA showed that self-administered ethanol produced a significant change in the percentage of response on the ethanol-associated lever [$F(1,6)=7.5$, $P<0.05$] that was dependent on dosage of pentobarbital [$F(1,6)=13.6$, $P<0.01$]. No significant changes in response rate were seen at any dosage of pentobarbital either with sucrose or sucrose/ethanol reinforcement (Fig. 5D).

Discussion

The results of the present study indicate that self-administered ethanol substitutes for the discriminative stimulus effects produced by investigator-administered ethanol. These data support and extend a similar study in which rats were trained to discriminate ethanol from saline and then subsequently trained to self-administer ethanol (Shelton and Macenski 1998). In that study, ethanol (mean = 1.1 g/kg) self-administered prior to a discrimination test session substituted for the discriminative stimulus effects of investigator-administered ethanol (1.0 g/kg). In the present study, ethanol was self-administered during discrimination test sessions. Under these conditions, self-administered ethanol substituted for the stimulus effects of investigator-administered ethanol (1.0 g/kg) in real-time after 14.9 min at an average dose of 1.2 g/kg. Self-administered ethanol (0.68 g/kg) also substituted for investigator-administered ethanol (1.0 g/kg) when self-administration occurred during four cumulative 2-min sessions. These findings demonstrate that self-administered ethanol substitutes for a comparable dose of investigator-administered ethanol.

The present study also sought to determine if self-administered ethanol would interact with the discriminative stimulus effects of investigator-administered ethanol. This was accomplished by combining a cumulative dosing procedure (see, for example, Hiltunen and Järbe 1989) with cumulative self-administration sessions. Results indicated that cumulative investigator-administered ethanol substituted fully for non-cumulative investigator-administered ethanol (1.0 g/kg, IP) with sucrose (10% w/v) reinforcement. When ethanol (10% v/v) was added to the sucrose (10% w/v) reinforcement, a lower dose of cumulative investigator-administered ethanol (1.0 g/kg, IP) produced full substitution. Moreover, the ED50 for cumulative IP ethanol substitution was shifted significantly to the left when ethanol was added to the sucrose reinforcer (Fig. 4). These data demonstrate that self-administered ethanol enhanced the discriminative stimulus effects of investigator-administered ethanol in a dose-dependent and additive manner.

When administered in cumulative doses, ethanol (1.7 g/kg, IP) substituted fully for the training dose of ethanol (1.0 g/kg, IP). This dose was higher than the dose of ethanol (1.0 g/kg, IP) that produced full substitution when administered by single acute injection. These data are in contrast with results from other studies that reported equal efficacy of ethanol (1.0 g/kg) when administered in cumulative or acute doses (see, for example, Green and Grant 1998; Hiltunen and Järbe 1989). Analysis of the data from individual animals showed that partial substitution was a function of divided performance among the seven animals with three rats responding at an average of 97% on the ethanol lever and the other four rats responding at approximately 2%, which might reflect intersubject variability in response to the cumulative dosing regimen (Colpaert 1987). In agreement with these previous studies, however, the ED50 for ethanol substitution did not differ as a function of cumulative or acute dosing, which indicates that the potency of ethanol was not different between the two procedures. Equal potency of ethanol in the cumulative and acute dosing procedures suggests comparable discriminative stimulus effects.

In this study, ethanol pharmacokinetics appeared to be an important determinant of ethanol discrimination. First, when ethanol was self-administered during free operant conditions (Fig. 2), lever press behavior switched from the saline-appropriate lever to the ethanol-appropriate lever after rats self-administered an average dose of 1.2±0.14 g/kg, indicating substitution of self-administered ethanol. This dose of ethanol was similar to the training dose of ethanol (1.0 g/kg, IP). In view of that, evidence suggests that the brain ethanol levels obtained immediately following free operant self-administration of 1.2 g/kg would be similar to the brain levels of ethanol obtained 10 min after IP injection of 1.0 g/kg (see, for example, Ferraro et al. 1990, 1991). Second, differential pharmacokinetics of oral versus IP ethanol administration appeared to influence the dose of ethanol that substituted for the training dose. Our results indicated that cumulative self-administered ethanol (0.68±0.13 g/kg) substituted fully for investigator-administered ethanol (1.0 g/kg; Fig. 3A). However, a twofold higher dose of cumulative investigator-administered ethanol (1.7 g/kg) was required to produce full substitution for the training dose (Fig. 4A). Substitution by both self-administered ethanol and investigator-administered ethanol occurred during the fourth cumulative dosing trial. Although these data are consistent with increased sensitivity to the discriminative stimulus effects of self-administered ethanol, evidence indicates that the obtained brain ethanol levels would be similar under these two conditions. Specifically, brain ethanol levels 40 min after self-administration of ethanol (0.79 g/kg) are approximately twofold greater than brain ethanol levels at the same time after IP injection of ethanol (0.80 g/kg; Ferraro et al. 1990, 1991). This corresponds to our finding that substitution by cumulative investigator-administered ethanol occurred at a dose twofold greater than the dose of cumulative self-administered ethanol that produced full substitution.

There is very little evidence that directly addresses the relative ability of a self-administered drug to interact with its discriminative stimulus effects. However, interactions between the discriminative and reinforcing effects of midazolam have been reported in two baboons

trained to discriminate midazolam (0.32 mg/kg, IV) from saline (Ator and Griffiths 1993). A history of IV midazolam self-administration shifted the midazolam substitution curve to the left as compared to the substitution curve determined before self-administration. Alternatively, after a history of investigator-administered midazolam, the midazolam substitution curve was shifted to the right. These data suggest that sensitivity to the discriminative stimulus effects of a drug can be enhanced by experience self-administering the drug. In the present study, ethanol was not independently established as a reinforcer and all of the effects of self-administered ethanol appeared to be additive.

Another manner in which the discriminative stimulus effects of drugs are thought to interact with drug-seeking behavior is through "drug priming" mechanisms (Pickens and Harris 1968). That is, when intravenous drug self-administration is extinguished by substituting saline for the drug, administering small doses of the drug prior to behavioral sessions can reinstate drug-seeking behavior. Drug priming has been observed with most drugs of abuse including amphetamine (Stretch and Gerber 1973), heroin (de Wit and Stewart 1983), barbiturates (Slikker et al. 1984), and cocaine (Gerber and Stretch 1975). Consistent with the importance of drug discrimination to this effect, the priming efficacy of drugs is positively correlated with the similarity between the discriminative stimulus effects of the priming drug and the self-administered drug (Gerber and Stretch 1975; Slikker et al. 1984; de Wit and Stewart 1983). The data from the present study confirm that self-administered drug enhances the discriminative stimulus effects of investigator-administered drug via similar neurobiological mechanisms. Thus, it appears that the discriminative effects of self-administered drugs have the potential to induce drug-seeking behavior, but additional studies that utilize methods like those in the present study are required to further examine the relationship between these two behavioral processes.

A key finding of the present study is that self-administered ethanol enhanced the ethanol-like discriminative stimulus effects produced by the non-competitive NMDA antagonist MK-801 and by the $GABA_A$-positive modulator pentobarbital. One implication of this finding is that the neurobiological mechanisms that mediate the discriminative stimulus effects of alcohol are also recruited during alcohol self-administration. NMDA and $GABA_A$ receptor systems are known to mediate the discriminative stimulus effects of ethanol (Barry 1991; Barry and Krimmer 1978; Grant et al. 1991; Hiltunen and Järbe 1986; Hodge and Cox 1998; Kline and Young 1986; Kubena and Barry 1969; Overton 1977; York 1978) and to modify alcohol self-administration behavior (Hodge et al. 1995; June et al. 1992, 1994; McBride et al. 1988; Rassnick et al. 1992, 1993; Samson et al. 1989), suggesting that discrimination and self-administration behavior are jointly mediated by NMDA and $GABA_A$ systems. The results of the present study extend these findings and suggest that self-administered ethanol produces its discriminative stimulus effects via modulation of NMDA or $GABA_A$ receptor systems.

An alternative consideration is that self-administered ethanol might have enhanced the discriminative stimulus effects of MK-801 or pentobarbital by altering the pharmacokinetics of these compounds. Studies have shown differential pharmacokinetic interactions between acute or chronic ethanol and sedative hypnotics. Chronic ethanol exposure can result in reduced plasma drug concentration, shorter elimination half-life, and reduced efficacy of CNS depressants, such as pentobarbital (see Sellers and Bendayan 1987), possibly by metabolic cross-tolerance. It is not likely that self-administered ethanol enhanced drug clearance in the present study as this would have resulted in a rightward shift in pentobarbital or MK-801 dose-response curves, which was not observed. Alternatively, acute ethanol has been shown to produce a 100% increase in blood diazepam concentrations 18-min after diazepam administration as compared to subjects who received diazepam alone (Sellers et al. 1980), which is predictive of supra-additive effects. However, since the effects of self-administered ethanol in the present study were additive, not supra-additive, and the animals received chronic intermittent ethanol treatment, it is unclear to what extent acute pharmacokinetic interactions contributed to the results. A plausible interpretation of additive drug effects is that self-administered ethanol interacted pharmacologically with pentobarbital or MK-801 at $GABA_A$ or NMDA receptors, respectively.

The findings of this study suggest overlap between the neurochemical systems that mediate ethanol self-administration and discrimination. This is in apparent contrast with recent evidence, which indicates that self-administered and investigator-administered drugs produce differential changes in neurochemical (see, for example, Hemby et al. 1997), physiological (Carelli et al. 1993), and functional brain (Eckardt et al. 1988; Porrino et al. 1998) activity. Although these studies suggest that self-administration behavior can change the neurobiological effects of drugs, it is not known if differential biochemical and physiological effects produced by self-administered and investigator-administered drugs produce differential functional effects on behavior. In view of that, the present findings indicate that any differential neurobiological effects that might have resulted from the two different methods of drug administration did not alter the discriminative stimulus function of ethanol.

Accordingly, evidence suggests that the neurobiological mechanisms that mediate ethanol self-administration are similar to those that mediate ethanol discrimination. For instance, administration of the direct $GABA_A$ agonist muscimol in the nucleus accumbens terminates alcohol self-administration at 15-min postinfusion (Hodge et al. 1995), which corresponds exactly with the time-course of intra-accumbens muscimol substitution for the discriminative stimulus effects of systemic ethanol (Hodge and Aiken 1996). These data suggest that normal termination of ethanol self-administration may correspond with the onset of ethanol discrimination via its activity at $GABA_A$

receptors in the nucleus accumbens. A caveat to this notion, however, is that dopamine transmission in the nucleus accumbens also mediates ethanol self-administration (see, for example, Hodge et al. 1992) but has no effect on ethanol discrimination (Hodge unpublished observations). Therefore, it is probable that some portion of the neurobiological control of ethanol self-administration involves important overlapping systems with those that mediate discrimination, but the two are not isomorphic.

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References

Ator NA, Griffiths RR (1993) Differential sensitivity to midazolam discriminative-stimulus effects following self-administered versus response-independent midazolam. Psychopharmacology 110:1–4

Barry H III (1991) Distinctive discriminative effects of ethanol. In: Glennon RA, Järbe TUC, Frankenheim J (eds) Drug discrimination: applications to drug abuse research (NIDA monograph, vol 116). US Government Printing Office, Washington, pp 131–144

Barry H III, Krimmer EC (1978) Similarities and differences in discriminative stimulus effects of chlordiazepoxide, pentobarbital, ethanol and other sedatives. In: Colpaert FC, Rosecrans JA (eds) Stimulus properties of drugs: ten years in progress. Elsevier, Amesterdam, pp 31–55

Carelli RM, King VC, Hampson RE, Deadwyler SA (1993) Firing patterns of nucleus accumbens neurons during cocaine self-administration in rats. Brain Res 626:14–22

Colpaert FC (1987) Drug discrimination: methods of manipulation, measurement, and analysis. In: Bozarth MA (ed) Methods of assessing the reinforcing properties of abused drugs. Springer, Berlin Heidelberg New York, pp 341–372

Eckardt MJ, Campbell GA, Marietta CA, Majchrowicz E, Weight FF (1988) Acute ethanol administration selectively alters localized cerebral glucose metabolism. Brain Res 444:53–58

Ferraro T, Weyers P, Carrozza D, Vogel W (1990) Continuous monitoring of brain ethanol levels by intracerebral microdialysis. Alcohol 7:129–132

Ferraro T, Carrozza D, Vogel W (1991) In vivo microdialysis study of brain ethanol concentrations in rats following oral self-administration. Alcohol Clin Exp Res 15:504–507

Gerber GJ, Stretch R (1975) Drug-induced reinstatement of extinguished self-administration behavior in monkeys. Pharmacol Biochem Behav 3:1055–1061

Grant KA, Kinsely JS, Tabakoff B, Barret JE, Balster RL (1991) Ethanol-like discriminative stimulus effects of noncompetitive *N*-methyl-D-aspartate antagonists. Behav Pharmacol 2:87–95

Green KL, Grant KA (1998) Evidence for overshadowing by components of the heterogeneous discriminative stimulus effects of ethanol. Drug Alcohol Depend 52:149–159

Haretzen CA, Hickey JE (1987) Addiction research center inventory (ARCI): measurement of euphoria and other drug effects. In: Bozarth MA (ed) Methods of assessing the reinforcing properties of abused drugs. Springer, Berlin Heidelberg New York, pp 489–524

Hemby SE, Co C, Koves TR, Smith JE, Dworkin SI (1997) Differences in extracellular dopamine concentrations in the nucleus accumbens during response-dependent and response-independent cocaine administration in the rat. Psychopharmacology 133:7–16

Hiltunen AJ, Järbe TU (1986) Discrimination of Ro 11–6896, chlordiazepoxide and ethanol in gerbils: generalization and antagonism tests. Psychopharmacology 89:284–290

Hiltunen AJ, Järbe TU (1989) Discriminative stimulus properties of ethanol: effects of cumulative dosing and Ro 15–4513. Behav Pharmacol 1:133–140

Hodge CW, Aiken AS (1996) Discriminative stimulus function of ethanol: role of $GABA_A$ receptors in the nucleus accumbens. Alcohol Clin Exp Res 20:1221–1228

Hodge CW, Cox AA (1997) The discriminative stimulus effects of self-administered ethanol: evidence for common mechanisms of reinforcement and discrimination. Alcohol Clin Exp Res 21:9A

Hodge CW, Cox AA (1998) The discriminative stimulus effects of ethanol are mediated by NMDA and GABA(A) receptors in specific limbic brain regions. Psychopharmacology 139:95–107

Hodge CW, Samson HH, Haraguchi M (1992) Microinjections of dopamine agonists in the nucleus accumbens increase ethanol-reinforced responding. Pharmacol Biochem Behav 43:249–254

Hodge CW, Chappelle AM, Samson HH (1995) GABAergic transmission in the nucleus accumbens is involved in the termination of ethanol self-administration in rats. Alcohol Clin Exp Res 19:1486–1493

Holtzman SG (1990) Caffeine as a model drug of abuse. Trends Pharmacol Sci 11:355–356

Järbe TU, Swedberg MD, Mechoulam R (1981) A repeated test procedure to assess onset and duration of the cue properties of (–) delta 9-THC, (–) delta 8-THC-DMH and (+) delta 8-THC. Psychopharmacology 75:152–157

June HL, Colker RE, Domangue KR, Perry LE, Hicks LH, June PL, Lewis MJ (1992) Ethanol self-administration in deprived rats: effects of Ro15–4513 alone, and in combination with flumazenil (Ro15–1788). Alcohol Clin Exp Res 16:11–16

June HL, Hughes RW, Spurlock HL, Lewis MJ (1994) Ethanol self-administration in freely feeding and drinking rats: effects of Ro15–4513 alone, and in combination with Ro15–1788 (flumazenil). Psychopharmacology 115:332–339

Kline FS, Young AM (1986) Differential modification of pentobarbital stimulus control by d-amphetamine and ethanol. Pharmacol Biochem Behav 24:1305–1313

Kubena RK, Barry H III (1969) Generalization by rats of alcohol and atropine stimulus characteristics to other drugs. Psychopharmacologia 15:196–206

McBride WJ, Murphy JM, Lumeng L, Li TK (1988) Effects of Ro 15–4513, fluoxetine and desipramine on the intake of ethanol, water and food by the alcohol-preferring (P) and -nonpreferring (NP) lines of rats. Pharmacol Biochem Behav 30: 1045–1050

Overton DA (1977) Comparison of ethanol, pentobarbital, and phenobarbital using drug vs. drug discrimination training. Psychopharmacology 53:195–199

Overton DA (1987) Applications and limitations of the drug discrimination method for the study of drug abuse. In: Bozarth MA (ed) Methods of assessing the reinforcing properties of abused drugs. Springer, Berlin Heidelberg New York, pp 291–340

Overton DA, Leonard WR, Merkle DA (1986) Methods for measuring the strength of discriminable drug effects. Neurosci Biobehav Rev 10:251–263

Pickens R, Harris WC (1968) Self-administration of d-amphetamine by rats. Psychopharmacologia 12:158–163

Porrino LJ, Whitlow CT, Samson HH (1998) Effects of the self-administration of ethanol and ethanol/sucrose on rates of local cerebral glucose utilization in rats. Brain Res 791:18–26

Rassnick S, D'Amico E, Riley E, Pulvirenti L, Zieglgansberger W, Koob GF (1992) GABA and nucleus accumbens glutamate neurotransmission modulate ethanol self-administration in rats. Ann NY Acad Sci 654:502–505

Rassnick S, D'Amico E, Riley E, Koob GF (1993) GABA antagonist and benzodiazepine partial inverse agonist reduce motivated responding for ethanol. Alcohol Clin Exp Res 17:124–130

Samson HH, Haraguchi M, Tolliver GA, Sadeghi KG (1989) Antagonism of ethanol-reinforced behavior by the benzodiazepine inverse agonists Ro15–4513 and FG 7142: relation to sucrose reinforcement. Pharmacol Biochem Behav 33:601–608

Sellers EM, Bendayan R (1987) Pharmacokinetics of psychotropic drugs in selected patient populations. In: Meltzer HY (ed) Psychopharmacology: the third generation of progress. Raven Press, New York, pp 1397–1406

Sellers EM, Naranjo CA, Giles HG, Frecker RC, Beeching M (1980) Intravenous diazepam and oral ethanol interaction. Clin Pharmacol Ther 28:638–645

Shelton KL, Balster RL (1994) Ethanol drug discrimination in rats: substitution with GABA agonists and NMDA antagonists. Behav Pharmacol 5:441–450

Shelton KL, Macenski MJ (1998) Discriminative stimulus effects of self-administered ethanol. Behav Pharmacol 9:329–336

Slikker W Jr, Brocco MJ, Killam KF Jr (1984) Reinstatement of responding maintained by cocaine or thiamylal. J Pharmacol Exp Ther 228:43–52

Stolerman I (1992) Drugs of abuse: behavioural principles, methods and terms. Trends Pharmacol Sci 13:170–176

Stretch R, Gerber GJ (1973) Drug-induced reinstatement of amphetamine self-administration behaviour in monkeys. Can J Psychol 27:168–177

Wit H de, Stewart J (1983) Drug reinstatement of heroin-reinforced responding in the rat. Psychopharmacology 79:29–31

York JL (1978) A comparison of the discriminative stimulus effects of ethanol, barbital, and phenobarbital in rats. Psychopharmacology 60:19–23