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# Time course of discriminative stimulus effects of bretazenil and chlordiazepoxide in rats

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**Abstract**

The time course of the discriminative stimulus effects of a benzodiazepine partial agonist, bretazenil, and a benzodiazepine full agonist, chlordiazepoxide, were determined in rats after administration of two doses of either drug. As in man, bretazenil was considerably shorter-acting than chlordiazepoxide, with 0% drug-appropriate responding at the 2-h time point after the training dose of 7 mg/kg and $< 20\%$ drug-appropriate responding at 9 h after the 14 mg/kg dose. With chlordiazepoxide, responding on the water-appropriate lever did not occur in all rats until 7 h after administration of the training dose of 7 mg/kg, compared to 17 h after administration of 14 mg/kg. Although there was considerable individual variability with both drugs, it would appear that the drug discrimination procedure can be a valuable tool for studying the time course of the interoceptive effects of psychoactive drugs.

*Keywords:* Benzodiazepine; Drug distrimination; Time course; Bretazenil; Chlordiazepoxide; (Rat)

## 1. Introduction

One purpose of this study was to determine if the time course of the discriminative stimulus effects of two benzodiazepines with very different profiles and half-lives in man could be reliably determined in the rat using the drug discrimination procedure. The benzodiazepines of interest were bretazenil and chlordiazepoxide. Bretazenil (formerly known as RO16-6028), a partial agonist, is a potent anxiolytic and anticonvulsant with very little or no sedative activity (Haefely et al., 1990), and it has been reported to have an elimination half-life of approximately 2.5 h in man (Betty Riely, Hoffman-LaRoche, personal communication). Chlordiazepoxide, a full agonist, is also an anxiolytic and anticonvulsant, but unlike bretazenil, it has considerable sedative effects. It also has a much longer elimination half-life than bretazenil, i.e., approximately 10 h in man (Boxenbaum et al., 1977).

Several laboratories have used the drug discrimination procedure to characterize the time course of psychoactive drugs. For example, Silverman et al. (1994) used drug discrimination to determine the onset of caffeine effects in humans, while others (e.g., Ator, 1990; Jarbe, 1993; Schechter, 1987; Winger and Herling, 1982) have used drug discrimination to determine how long the discriminative stimulus effects of a particular drug last. Winger and Herling (1982), for example, determined the time course of the discriminative stimulus effects of pentobarbital in rhesus monkeys by conducting a single test after varying pretreatment times. Ator (1990), however, showed that the time course for pentobarbital was similar in baboons that were repeatedly tested after administration of a single dose. Jarbe (1993) found that the time courses of the discriminative stimulus effects of amphetamine and cocaine were similar, whether a single test was done at varying intervals or whether rats were tested repeatedly after a single dose of either drug. Rats have also been tested repeatedly after a single dose of 3,4-methylenedioxymethamphetamine (MDMA), and it was found that the time course of the discriminative stimulus effects in rats was similar to that of the subjective effects in humans (Schechter, 1987).

## 2. Materials and methods

### 2.1. Subjects

Eight adult male Harlan Sprague-Dawley (Indianapolis, IN) rats weighing 300–320 g served as subjects. They

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were maintained at 80% of their free-feeding weights by supplemental feeding (Purina Rat Chow). All rats were individually housed in stainless steel cages 18 × 29 × 13 cm in a temperature- and humidity-controlled room on a 12 h light-dark cycle with water ad libitum.

### 2.2. Apparatus

Experimental sessions were conducted in standard operant chambers 22 × 22 × 28 cm (Med-Associates, St. Albans, VT, USA). Each chamber was equipped with 3 response levers located 8 cm above the floor and separated 1 cm from each other. Three 7-W white lights were mounted on the intelligence panel 5 cm above each response lever. A pan for food pellet delivery was located on the opposite wall. One 45-mg food pellet (BioServ, Frenchtown, NJ, USA) was delivered upon completion of the schedule requirements. All chambers were enclosed in sound-attenuating boxes equipped with white noise and an exhaust fan for ventilation. The reinforcement schedule and data collection were controlled by a microcomputer with Med-Associates interface and software. Daily sessions were conducted Monday through Saturday.

### 2.3. Drugs

Chlordiazepoxide HCl (Sigma Chemical Co., St. Louis, MO, USA) was dissolved in 0.9% saline and bretazenil base (kindly supplied by Hoffmann-La Roche, Basel, Switzerland) was suspended in distilled water with 1–2 drops of Tween 80 per 10 ml.

### 2.4. Discrimination training

Lever-pressing was initiated by increasing the interval in a fixed time procedure (i.e., pellet delivery occurred non-contingently at increasing intervals). During this time, a response on any lever automatically resulted in immediate delivery of a food pellet. When rats began pressing the lever for food, a fixed ratio 1 (FR1) was implemented. The lever associated with food delivery was alternated from left to right daily, and the FR requirement was doubled each day until an FR20 was in effect. The left lever and the right lever were illuminated by white lights situated over the levers. Responses on the center lever were recorded but had no programmed consequences. During this time, discrimination training was also initiated, such that drug (7 mg/kg) or water administration alternated from day to day. Right lever responses were designated chlordiazepoxide-appropriate for the 4 rats in group I, while left lever responses were designated vehicle-appropriate. For the 4 rats in group II, left lever responses were designated bretazenil-appropriate and right lever responses were designated vehicle-appropriate. Responses on the injection-appropriate lever were reinforced, whereas responses on the injection-inappropriate lever were counted but not reinforced. The dose of chlordiazepoxide is within the range of doses commonly used in drug discrimination in rats (cf. Tomie et al., 1995; Stephens et al., 1984). The dose of bretazenil was chosen to be the same because, although bretazenil is a more potent anxiolytic and anticonvulsant than chlordiazepoxide (Haefely et al., 1990), it has traditionally been harder to train animals to discriminate a partial agonist (Andrews and Stephens, 1991; Sanger and Zivkovic, 1994) and it was felt that increasing the dose would shorten the training time. During training, drug or water was injected i.p. 15 min before the start of the session. The training sessions lasted for 15 min or 25 reinforcers, whichever occurred first. When responding was stable on the FR20 schedule, such that rate of responding did not vary more than 5% from day to day, training drug or vehicle was administered on a double alternation sequence, i.e., drug-drug-vehicle-vehicle, etc.

### 2.5. Substitution tests

Testing did not start until the percentage of responses before delivery of the first reinforcer was at or above a mean of 80% over 10 consecutive training sessions. Tests were separated by at least one water and one training drug session and rats were only tested if they made > 80% responses on the injection-appropriate lever on the preceding drug and water days. During tests, completion of 20 responses on either lever resulted in pellet delivery. Test sessions ended after 5 reinforcers or 5 min, whichever occurred first.

Dose-effect curves for individual drugs were obtained by using a cumulative dosing procedure. All cumulative dose-effect curves were determined as follows: 15 min after the first injection rats were placed in the behavioral chambers for the first component. Immediately after each component rats were injected with the next highest dose and returned to their cages for a 5-min timeout. Each component consisted of 5 reinforcers or 5 min, whichever occurred first. Cumulative dose-effect curves were initially obtained for chlordiazepoxide (0.1, 1, 7 and 10 mg/kg) in group I and bretazenil (0.01, 0.1, 1 and 7 mg/kg) in group II. For a comparison of potency, Group I was then tested with the same doses of bretazenil, while group II received the same doses of chlordiazepoxide.

### 2.6. Time course of discriminative stimulus effects

To compare the half-lives of chlordiazepoxide and bretazenil, a time course of the discriminative stimulus effects of both compounds was determined. Rats were injected first with an injection of water and were tested 15 min later. This test, and all following tests consisted of 5 reinforcers or 5 min, whichever occurred first, during which time rats were reinforced with a food pellet after 20 responses on any lever. Immediately after the water test, rats were injected with the training dose and then tested

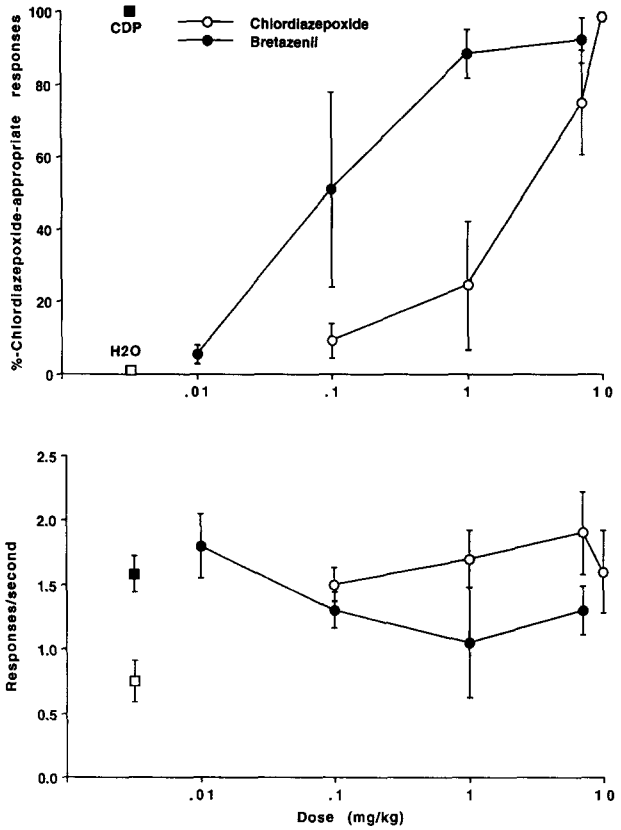


Fig. 1. Discriminative stimulus effects of chlordiazepoxide (open circles) and bretazenil (closed circles) in rats trained to discriminate 7 mg/kg of chlordiazepoxide from water. Ordinate: percentage of chlordiazepoxide-appropriate responding before delivery of the first reinforcer (top panel), rate of responding in responses/s over the entire experimental session (bottom panel); abscissa: dose of chlordiazepoxide or bretazenil in mg/kg. The closed square to the left of the dose-effect curve represent the effects of the training dose of chlordiazepoxide during training sessions, while the open square represents the effects of water administered during training sessions. Each point represents the mean (±S.E.) for 4 rats.

every 15 min for the first hour, then at 90 min and 120 min post-injection, and at hourly intervals thereafter until responding on the drug-appropriate lever was < 20%. Fifteen-minute intervals were chosen for the first hour because of the reported short half-life of bretazenil in the rat (Mandema et al., 1992). Rats were returned to their home cages between tests. Two weeks after completion of the first dose-effect curve, the same rats then received a larger dose (14 mg/kg) of bretazenil or chlordiazepoxide, i.e., double the training dose. Testing proceeded as described above. Rates of responding were recorded during all tests, including the water test.

### 2.7. Data analysis

The percentage of responses on the drug-appropriate lever before delivery of the first reinforcer and rates of responding for the entire session were calculated during training and test sessions.

## 3. Results

### 3.1. Discrimination training

The chlordiazepoxide-water discrimination was acquired by rats in group I within 90 sessions and the bretazenil-water discrimination was acquired by rats in group II within 150 sessions.

### 3.2. Substitution tests

In Fig. 1, it can be seen that chlordiazepoxide and bretazenil dose dependently substituted for the training dose of chlordiazepoxide. Bretazenil was more potent than chlordiazepoxide, however, with bretazenil producing approximately 90% drug-appropriate responding at 1 mg/kg vs. 7 mg/kg for chlordiazepoxide. Fig. 1 also shows that during training sessions, chlordiazepoxide increased rates of responding approximately 100% when compared to water injections. During cumulative dose-effect curves, both chlordiazepoxide and bretazenil increased rates of responding when compared to water control rates.

Bretazenil and chlordiazepoxide also dose dependently substituted for the training dose of bretazenil in

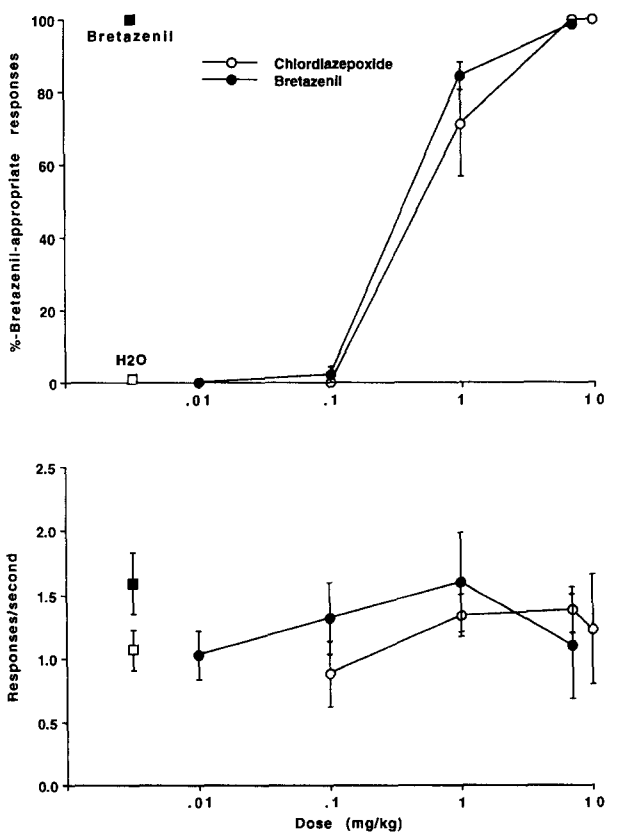


Fig. 2. Discriminative stimulus effects of chlordiazepoxide (open circles) and bretazenil (closed circles) in rats trained to discriminate 7 mg/kg of bretazenil from water. Other details are the same as in Fig. 1 except that the closed square to the left of the dose-effect curve represents the effects of bretazenil during training sessions.

bretazenil-trained rats (Fig. 2), but in contrast to Fig. 1, the dose-effect curves in Fig. 2 were almost identical. Bretazenil increased rate of responding in bretazenil-trained rats, whereas chlordiazepoxide had very little effect on responding in these rats compared to rats trained on chlordiazepoxide.

### 3.3. Time course

Fig. 3 shows the time course for 7 and 14 mg/kg chlordiazepoxide in rats trained to discriminate 7 mg/kg chlordiazepoxide from water. All rats were still responding solely on the drug lever 2 h after the training dose of 7 mg/kg, and the effects gradually wore off over the hour until by 7 h responding was < 20% on the drug-appropriate lever. Although it is difficult to determine from Fig. 1, it is worth noting that once a rat switched to the vehicle lever (i.e., less than 20% drug-appropriate responding), the rat's responses from that time on were vehicle-appropriate. In other words, rats did not switch back and forth from one lever to the other. After the higher dose of 14 mg/kg chlordiazepoxide, there was a great deal of variability in the time course. Three out of 4 rats were still responding entirely on the drug lever at 6 h, but from 7 to 12 h, 2 rats were responding entirely on the drug lever, while the other 2 were responding entirely on the water lever. During h 13–16, only 1 rat was still responding 100% on the drug lever, and he only switched to the water lever at 17 h. As can be seen in the bottom panel of Fig. 1, both doses of chlordiazepoxide increased rates of responding, and the offset of the discriminative stimulus effects generally corresponded to the return of response rates to normal.

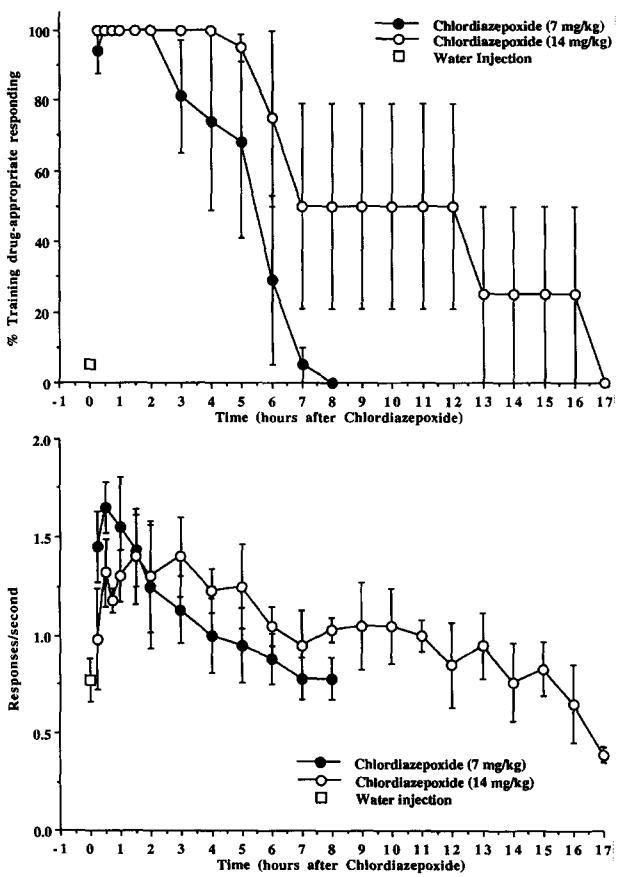


Fig. 3. Time course of the discriminative stimulus effects of 7 (open circles) and 14 mg/kg (closed circles) chlordiazepoxide in rats trained to discriminate 7 mg/kg chlordiazepoxide from water. In the top graph, the ordinate represents the mean (±S.E.) percentage of responding on the drug-appropriate lever before delivery of the first reinforcer. The abscissa represents time, in hours, since injection of chlordiazepoxide. The bottom graph represents rate of responding at the various time points. The open square to the left represents the effects of a water injection administered 15 min before a test. This test occurred immediately prior to injection of chlordiazepoxide.

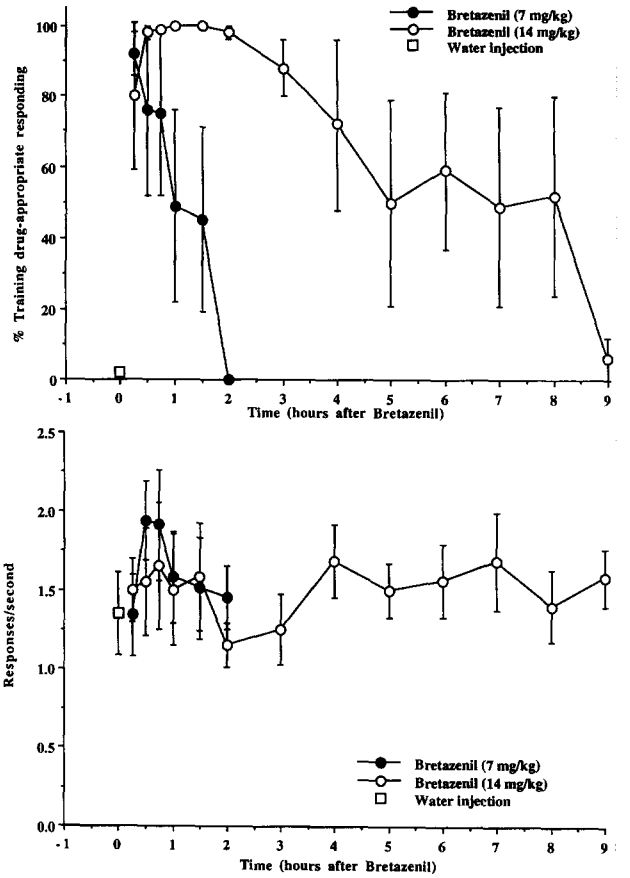


Fig. 4. Time course of the discriminative stimulus effects of 7 (open circles) and 14 mg/kg (closed circles) bretazenil in rats trained to discriminate 7 mg/kg bretazenil from water. The open square to the left represents the effects of a water injection administered 15 min before a bretazenil test. Other details are the same as in Fig. 3, except that the abscissa represents time, in hours, since injection of bretazenil.

The effects of 7 and 14 mg/kg bretazenil are shown in Fig. 4. Both doses of bretazenil had a shorter time course than chlordiazepoxide, but as with chlordiazepoxide, there was a great deal of individual variability. If all rats are included, the 14 mg/kg dose of bretazenil lasted at least 4 times as long as the 7 mg/kg dose, in that after the low dose, all rats were responding on the water-appropriate lever at 2 h, whereas this was not the case with the high

dose until 9 h. Comparing the point for bretazenil at which 2/4 rats switched entirely to the water lever (1 h for 7 mg/kg versus 5 h for 14 mg/kg), the high dose of bretazenil was still much longer lasting than would be expected, given its relatively short half-life. If the same time points are compared for the two doses of chlordiazepoxide, it can be seen that 2/4 rats switched entirely to the water lever at 5–6 h, whereas the same rats switched to the water lever only 7 h after the 14 mg/kg dose. Unlike chlordiazepoxide, the discriminative stimulus effects of bretazenil were not correlated with alterations in response rate. In fact, at the 7 mg/kg dose, rates of responding were similar to control at 15 min post injection, despite the fact that this was the only time point when all rats were responding entirely on the drug lever. Furthermore, rates of responding were increased at the 30- and 45-min time points when some rats were starting to respond on both the drug and the water lever.

## 4. Discussion

The present study demonstrated that rats can be trained to discriminate 7 mg/kg of both the full benzodiazepine receptor agonist, chlordiazepoxide, or the partial benzodiazepine receptor agonist, bretazenil, from water, although it was more difficult to train the bretazenil stimulus, as evidenced by the fact that it took 90 days to train chlordiazepoxide and 150 days to train bretazenil. Similar results have been found by investigators training animals to discriminate other benzodiazepine partial agonists such as alpidem (Sanger and Zivkovic, 1994) and abecarnil (Andrews and Stephens, 1991).

There was dose-dependent cross substitution between the partial benzodiazepine receptor agonist, bretazenil, and the full agonist, chlordiazepoxide, although bretazenil was more potent than chlordiazepoxide in chlordiazepoxide-trained rats. In chlordiazepoxide-trained rats, 1 mg/kg (2.39 mmol) bretazenil substituted completely for chlordiazepoxide, whereas 7 mg/kg (20.82 mmol) chlordiazepoxide was required for full substitution in the same rats. Similar results with bretazenil were also obtained by Sanger (1987) in rats trained to discriminate 5 mg/kg chlordiazepoxide from saline. In that study, as in the present study, bretazenil had a similar dose-effect curve, with 1 mg/kg bretazenil substituting fully for the training dose of chlordiazepoxide.

The current study confirms the short half-life of bretazenil as compared to chlordiazepoxide. In man, bretazenil is reported to have a half-life of approximately 2.5 h (Betty Riely, Hoffmann-LaRoche, personal communication) versus approximately 10 h for chlordiazepoxide (Boxenbaum et al., 1977). Chlordiazepoxide does have active metabolites (Boxenbaum et al., 1977), but to the authors' knowledge, none of these metabolites have been tested in animals trained to discriminate chlordiazepoxide, and it is not known whether these metabolites contributed to the long half-life of chlordiazepoxide in the present study. The metabolic fate of bretazenil has not been extensively studied at this point.

In the time course study, there was considerable individual variability, with some rats switching to the water lever earlier than other rats. It is important to note that once a rat switched from the drug lever to the water lever, responding from that point on was almost entirely on the water lever, particularly in the case of chlordiazepoxide. These results are similar to those obtained in a recent study using male rats of the same strain, in which the time course of the rate-decreasing effects of 5′-*N*-ethylcarboxamidoadenosine (NECA) was determined (Bronson et al., 1994). In that study, it was visually apparent that half of the rats displayed more pronounced and prolonged effects of NECA at all doses. The time-course of effects was then evaluated by a model which incorporated the Hill equation with a one compartment pharmacokinetic model. Similar $EC_{50}$ (amount of drug producing 50% of maximal effects) values were observed for both groups but the $K_e$ (drug elimination rate constant) and $t_{1/2}$ (drug pharmacokinetic half life) values were significantly different between the two groups. The large intersubject variation observed in the duration and intensity of the effects of NECA in rats appeared to be related to pharmacokinetic differences, and the same may be true with chlordiazepoxide in the present study.

Because the offset of the discriminative stimulus effects of chlordiazepoxide generally corresponded with a return to baseline responding, it is possible that the rate increases produced by chlordiazepoxide were involved in the discriminative stimulus effects of the training dose of 7 mg/kg in this group of rats. This is in contrast, however, to what others have found when rats are trained to discriminate benzodiazepines that decrease rate of responding, i.e., decreased responding was not found to be involved in the discriminative stimulus effects of the training drug, as drugs that did not decrease responding substituted fully for the training drug (Andrews and Stephens, 1990). Furthermore, with bretazenil, rates of responding correlated negatively with the time course of the discriminative stimulus effects of this drug. Thus, it seems unlikely that alterations in response rate were involved in the discriminative stimulus effects of these two benzodiazepines.

Similar results comparing the time course of the discriminative stimulus effects in rats versus the time course of subjective effects in humans have been obtained with MDMA (Schechter, 1987). Thus, it would appear that the drug discrimination procedure can be a valuable tool for studying the time course of the interoceptive effects of psychoactive drugs.

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