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Discriminative-stimulus and time-course effects of kava-kava (*Piper methysticum*) in rats

Natalie R. Bruner, Karen G. Anderson *

West Virginia University, Department of Psychology, P.O. Box 6040, Morgantown, WV 26506, United States

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ABSTRACT

Kava is a widely available and used herbal medicine that is not regulated in many countries. There are many questions concerning kava's stimulus properties, potential for therapeutic use, and potential for abuse. Although there is evidence that kava may possess some anxiolytic properties, kava's mechanism of action and the extent to which it may serve as an alternative to pharmaceutical anxiolytics are not fully known. The current study was designed to evaluate whether kava shares discriminative-stimulus properties with the anxiolytic chlordiazepoxide (CDP). Effects of different doses of kava extract were evaluated in two groups of rats trained to discriminate either a high or low training dose of CDP (i.p.). In order to assess time-course effects, two tests were conducted/session at 60 (Test One) and 90 (Test Two) min following oral administration of kava, CDP, or *d*-amphetamine. Dose-dependent substitution of CDP was found in both training groups in both tests. Kava (560 mg/kg, p.o.) occasioned responding indicative of partial substitution in both groups during Test One and only the low-dose group during Test Two. Partial substitution of kava extract for CDP suggests that the herbal compound may share a mechanism of action similar to CDP, but is less potent.

1. Introduction

For hundreds of years, the people of the South Pacific have consumed the beverage kava-kava (kava) for ceremonial, medicinal and social purposes (Singh, 1992). The importance of kava in the life of the South Pacific islanders is analogous to the use of alcohol in other cultures. Cultivated from the tropical shrub *Piper methysticum* (meaning "intoxicating pepper"), the traditional preparation consists of grinding down the thick kava root into an intoxicating murky beverage. Consuming the beverage induces a relaxed state and can help to improve social interaction. Kava has also been traditionally used as a natural anti-anxiety or sedative medicine.

It was not until the 1990's that kava gained significant attention as an herbal alternative to pharmaceutical drugs for treatment of stress, anxiety, and pain. By 1994, kava had become one of the top eight herbal remedies in the $18 million herbal remedy industry (O'Sullivan and Lum, 2004). In 2002, several European countries banned the sales of the herb due to cases of severe hepatic toxicity in users of kava. Because it is classified as an herbal supplement, the Food and Drug Administration (FDA) of the United States does not regulate the quality nor approve preparations of kava prior to its marketing. However, following the European ban on kava in 2002, the FDA did issue a consumer advisory pertaining to the potential harmful effects of the herb. Subsequently, several herbal remedy retailers voluntarily withdrew kava-containing items from their stores (O'Sullivan and Lum, 2004), but the drug is still readily available. The potential toxic effects as well as the potential benefits of kava must be more closely examined to properly assess its usefulness as an herbal medicine.

Kava's mechanism of action is not well known. Some studies purport that kavalactones (the active ingredients in the kava extract) appear to interact with $GABA_A$ receptors, the same receptor sites that anti-anxiety drugs such as CDP and diazepam act upon, but the reports are mixed. Jussofie, Schmiz, and Heimke (1994) as well as Boonen and Haberlein (1998) found that both kava extract and kavalactones increase binding of agonists and antagonists at $GABA_A$ receptors. Dinh et al. (2001), however, found that kava extract inhibits the binding of muscimol, a GABA agonist, to the GABA binding site on the $GABA_A$ receptor. In contrast to the previous studies, both Davies et al. (1992) and Garrett et al. (2003) concluded that kava had no effect on the $GABA_A$ receptor. Due to these conflicting results, further research is necessary to determine whether kava extract acts upon $GABA_A$ receptors. If kava affects the same receptors as benzodiazepines, it may be expected to have shared stimulus properties and anxiolytic effects.

Research investigating the use of kava in human participants supports kava as an effective treatment for anxiety. Geier and Konstantinowicz (2004) used the Hamilton Anxiety Scale as a primary dependent variable to assess self-rated subjective level of anxiety before and after kava treatment. It was found that patients had a therapeutically relevant reduction in anxiety in the kava-extract group

* Corresponding author. Fax: +1 304 293 6606.
E-mail address: karen.anderson@mail.wvu.edu (K.G. Anderson).

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compared to the placebo-control group. Another study with human participants supports the finding that kava is beneficial for treating anxiety symptoms in anxiety patients terminating treatment with benzodiazepines (Malsch and Kieser, 2001). Taken together, these findings suggest that kava may be an effective substitute for pharmaceutical anxiolytics such as benzodiazepines (Carlini, 2003). Ernst (2006) conducted a systematic review of controlled clinical trials summarizing the anxiolytic efficacy of herbal medicines. It was reported that kava is the only herbal medicine that has been shown to have anxiolytic effects in humans.

The data concerning kava using animal models of anxiety is limited. Recent studies (Garrett et al., 2003; Rex et al., 2002) have found that kava extract significantly increased changes in behavior, in a manner similar to that of drugs established as anxiolytics in humans, in two animal models of anxiety. In the mirrored-chamber avoidance paradigm, a mouse entering the mirrored chamber is surrounded by its own reflection on six sides. In the elevated-plus maze paradigm, Plexiglas surrounds the arms of one runway of the maze, and the arms of the other runway are open. Animal subjects in these assays typically spend less time in the mirrored chamber or open arms relative to time spent in the enclosed runway leading into the mirrored chamber or closed arms of the maze. However, following administration of a benzodiazepine, time spent in the mirrored chamber or open arms of the maze is increased. Administration of kava extract (e.g., 120–240 mg/kg, p.o., Rex et al., 2002; 88–125 mg/kg, i.p., Garrett et al., 2003) in these animal models of anxiety resulted in behavioral effects similar to those following administration of benzodiazepines.

Kava-kava contains 18 active pharmacological agents, called kavalactones. Six of these kavalactones (kavain, dihydrokavain, methysticin, dihydromethysticin, yangonin, and desmethysticin) account for about 95% of the kava extract (Ganzera and Khan, 1999). Smith et al. (2001) found that of the six major kavalactones tested individually in a chick social separation-stress paradigm, only kava extract (30 mg/kg) and dihydrokavain (30 mg/kg) resulted in behavioral effects similar to those following administration of the benzodiazepine chlordiazepoxide (CDP, 5 mg/kg). Using the same paradigm, Feltenstein et al. (2003) reported that kava fractions containing the highest doses of dihydrokavain moderately suppressed both distress vocalizations and stress-induced analgesia. These studies suggest that some properties of kava may be similar to those of established anxiolytic drugs. More research is needed to determine whether these shared stimulus properties of kava extract are mediated by the total kavalactone content or from particular kavalactones.

Positive results in three experimental paradigms across three species of animals suggest that kava has effects on behavior similar to those produced by drugs classified as anxiolytics in clinical settings with humans. To date, there are no studies examining the discriminative-stimulus properties of kava in animals using a drug-discrimination paradigm, so effects of two different training doses and time-course effects were evaluated in the present study. The dose selected for the training drug (to establish and maintain a discrimination) influences sensitivity to the drug (cf. Stolerman, 1993). A relatively low training dose will generally result in greater sensitivity to test stimuli, and dose-response curves will subsequently be shifted to the left. Discriminations with relatively high doses are generally easier to establish (i.e., requires fewer training sessions), but may result in less sensitivity to discriminative-stimulus properties of test stimuli than that based on lower training doses. This drug-discrimination study was designed to assess substitution of kava extract, at two time points, for CDP in two groups of rats trained to discriminate either a high or low dose of CDP from saline.

2. Method and materials

2.1. Subjects

Sixteen experimentally naïve male Sprague–Dawley rats were used as subjects. Subjects were approximately 2 months of age and weighed an average of 258 g (range, 233–286 g) at the start of the experiment. Rats were housed individually with free access to water in their home cages. Temperature and humidity were maintained at constant levels and there was a reversed 12 h light–dark cycle in effect. All sessions were conducted during the dark phase of the light–dark cycle. The subjects were fed approximately 15 g of food one half hour following each experimental session. This schedule resulted in approximately 22 h of food deprivation prior to the start of each session.

2.2. Apparatus

Experimental sessions were conducted in eight standard operant-conditioning chambers for rats, each enclosed in a melamine sound-attenuating cubicle (Med Associates, VT). Each chamber contained a working area of 30.5 cm by 24.5 cm by 21.0 cm, a grid floor, and a 45 mg pellet dispenser with a pellet receptacle centered between two retractable response levers. The levers were 11.5 cm apart from each other and required at least a force 0.25 N for a response to be recorded. The levers were 4.8 cm wide, protruded 1.9 cm into the chamber, and were elevated 8 cm from the grid floor. Two 28 V stimulus lights that were 2.5 cm in diameter were approximately 7 cm above each lever. Each chamber contained a 28 V houselight on the wall opposite to the wall containing the operandum. A ventilation fan circulated air and served to mask extraneous noise. Equipment was interfaced to a computer and experimental sessions and data collection was programmed and conducted with MedPC-IV (Med Associates, VT).

2.3. Procedure

2.3.1. Initial training

Training sessions were conducted 5 days a week (Monday through Friday) at approximately the same time each day. The subjects were trained to lever press using a free-operant acquisition procedure (cf: van Haaren, 1992; Anderson and van Haaren, 1999). Each rat was placed in a darkened experimental chamber and the ventilation fan was turned on. 10 min later, the houselight and stimulus lights above both levers were illuminated. Food was delivered according to a conjoint fixed-ratio (FR) 1 variable-time (VT) 60 s schedule. Values for the VT were obtained using a Fleshler–Hoffman sequence generator (Fleshler and Hoffman, 1962). Rats received one food pellet either after a lever press or after an average of 60 s had elapsed. If any subjects failed to acquire the response following the lever-press training procedure, the lever-press response was shaped through reinforcement of successive approximations. When subjects were obtaining most food pellets via lever pressing, they then completed an FR 1 schedule that alternated between the left and right levers after the delivery of five food pellets on each lever, for 40 food pellets total. The ratio was increased gradually over consecutive sessions until an FR 10 was reached.

2.3.2. Discrimination training

The subjects were divided into two groups of eight rats (low-dose group and high-dose group). Experimental sessions were generally conducted 7 days a week. One group of rats was trained to discriminate 5.6 mg/kg CDP from saline and the other group was trained to discriminate 13.0 mg/kg CDP from saline. The rats in the low-dose group had prior exposure to 3.0 mg/kg CDP, but subjects failed to acquire the discrimination. The rats in the high-dose group had prior exposure to 17.0 mg/kg CDP, but administration of this dose suppressed responding. Hence, adjustments in training doses were made so that discrimination criteria could be met.

The drug (D) or saline vehicle (V) was administered via i.p. injection prior to each daily session in the following order for each 24 day period (7 days a week): VDVDDDVDVDVV DVDVVVDVDVDD. Following the injection, the subject was placed immediately in the darkened experimental chamber and the ventilation fan was turned on. After a period of 15 min, the houselight and both lever lights were

illuminated, and both levers were extended into the chamber. Food pellet presentation followed responses on the vehicle- or drug-appropriate lever according to an FR 10 schedule of reinforcement. For one half of each group, fulfilling the required responses on the left lever resulted in food pellet delivery following injection of saline, and responses on the right lever resulted in food pellet delivery following injection of CDP. For the other half of each group, the levers were counterbalanced such that reinforced responses were on the left lever following CDP injection and the right lever following saline injection. Responses on the other lever were counted but had no other scheduled consequences (extinction; incorrect lever). Sessions were terminated following the presentation of 40 food pellets or 30 min, whichever occurred first. (Before implementation of the FR 10 schedule, subjects had a prior history of responding under a tandem VI 30 s FR 10 schedule of reinforcement following CDP or saline administration, but did not acquire the discrimination. The change in schedule was necessary for discrimination criteria to be met.)

2.3.3. Generalization testing

Generalization testing began after the subjects emitted at least 80% correct lever presses before the delivery of the first food pellet for five consecutive sessions. Rats that did not successfully complete discrimination training (i.e., three rats in the low-dose group) were not included in the testing phase. Generalization tests were conducted on Tuesdays and Fridays, once following a vehicle injection session and once following a drug injection session. Each dose of CDP, kava, and *d*-amphetamine was tested at least twice. Previous research has demonstrated that time-course effects of a drug may be assessed within a single session by conducting multiple tests in extinction (cf., Anderson and van Haaren, 1999). In the present experiment, time-course effects of kava extract were examined by conducting two tests in extinction within each test session. Due to solubility issues and concerns with absorption following i. p. administration of kava extract, the oral route (via gastric gavage, p.o.) was used during generalization testing of all compounds.

Subjects were administered test compounds 60 min prior to the start of Test One and placed back into their home cage. 45 min following drug administration, subjects were moved into the operant-conditioning chamber and the ventilation fan was turned on. 15 min later, Test One began. Both levers were extended into the chamber and the houselight and both lever lights were illuminated for 5 min or until completion of the response requirement for FR 10. After completing the required responses, instead of delivery of a food pellet, both levers were retracted, and the houselight and stimulus lights were darkened until the beginning of Test Two. During the interval between tests, subjects remained in the operant-conditioning chambers, all lights remained extinguished and the levers remained retracted from the chamber. If the response requirement was not met within 5 min of the onset of the test, the experimental chamber was darkened and the levers were retracted until Test Two began. 90 min following drug administration, a second generalization test commenced. This test was identical to the first and the session was terminated upon its completion. Generalization tests were conducted for CDP (1.0–13.0 mg/kg), *d*-amphetamine (0.3–3.0 mg/kg), saline, kava vehicle, and kava extract (300–560 mg/kg). Doses above 560 mg/kg of kava were not tested because some animals failed to respond following administration of the highest dose of kava. *d*-Amphetamine was incorporated as a negative control in order to establish that responding on the drug-appropriate lever indicated shared discriminative-stimulus properties with the training drug and was not an artifact of general administration of a drug. *d*-Amphetamine was chosen because no existing evidence to date suggests that kava may engender stimulant-like properties. Most subjects received at least two determinations of each dose. However, some subjects experienced doses of kava or *d*-amphetamine only once. In some instances, drug doses (e.g., kava 560 mg/kg, *d*-amphetamine 3.0 mg/kg) suppressed responding almost completely or were not administered due to experimenter error. The data from these determinations are excluded from data analyses.

2.3.4. Drugs

Chlordiazepoxide hydrochloride (Sigma-Aldrich, St. Louis, MO) was dissolved in 0.9% saline vehicle and administered 15 min prior to training (i.p.) and 60 min prior to testing (p.o.). Kava extract and HPLC content analysis were generously supplied by the National Center for Natural Products Research at the University of Mississippi (Oxford, MS). The extract was dissolved in the kava vehicle (91% distilled water, 4% ethanol, 4% Tween 80, and 1% dimethyl sulfoxide) and administered 60 min prior to testing (p.o.). Kavalactones comprised 84% of the kava extract. The kavalactone content was comprised of 49% kavain, 18.6% dihydrokavain, 9.8% methysticin, 8.4% dihydromethysticin, 5.8% yangonin, and 4.8% desmethoxyangonin. *d*-Amphetamine (Sigma-Aldrich Company, St. Louis, MO) was dissolved in 0.9% saline and administered 60 min prior to testing (p.o.).

2.3.5. Data analysis

The percentage of correct (injection-appropriate) lever presses in training was determined by dividing the number of responses made on the injection-appropriate lever by the number of total responses made on both levers preceding the delivery of the first food pellet. Response rate (responses/min) in training was determined by dividing the total number of lever presses during the session on the both levers by the duration (min) of each session. Training data were represented graphically in which the percent choice of injection-appropriate lever prior to the first reinforcer delivery was plotted as a function of injection type (drug or saline).

Calculations were the same for the generalization tests, except data collection ended when the required responses were made or when the session timed out. Only data following oral administration of the test drugs are presented. In testing, percentage of total responses on the CDP-appropriate lever and response rate on both levers were calculated and plotted. Test data were not included if subjects failed to fulfill the schedule requirement (i.e., ten responses were not emitted on a single lever). In the case where partial substitution of a test compound was found, the percentage of total responses emitted on the CDP-appropriate lever was tested for significance with a 3-way repeated measures ANOVA. Occasionally, subjects did not receive a test dose or failed to respond following a test dose. In these cases, data were interpolated from the group mean for that dose. In no instance was more than one dose interpolated for any subject. No interpolated data were used for kava evaluation. Three doses were interpolated for CDP data. (Two were for 10.0 mg/kg in two of the rats in the low-dose training group after 5.6 mg/kg had already resulted in full substitution. The other interpolated data point was for 1.0 mg/kg in a rat in the high-dose training group after 3.0 mg/kg did not substitute, i.e., 0% drug-lever responding.) *d*-Amphetamine 0.3 mg/kg data were interpolated for two subjects in the low-dose training group and one subject in the high-dose training group. In the case that main effects (training group, test time, drug dose) were found, Tukey tests were conducted as post-hoc analyses. If applicable, e.g., full or at least greater than 50% substitution, the mean effective dose (ED_{50}) and 95% confidence interval for each drug was calculated by log-linear interpolation of the ascending portion of the dose-response curve. For all statistical tests, $p<0.05$ was considered to be significant. The percentage of responses emitted greater than or equal to 80% on the CDP-appropriate lever was considered to be full substitution, 21–79% partial substitution, and less than or equal to 20% no substitution.

3. Results

3.1. Discrimination training

The CDP discrimination required an average of 40 training sessions on FR 10 schedule of reinforcement for the low-dose group (range=25–62 sessions) and an average of 32 sessions (range=23–49 sessions) for the high-dose group. For the last ten sessions of the

training phase, the mean percent correct lever responding was 93.7% (range=86.6–100%) for the low-dose training group and 95.9% (range=88.6–100%) for the high-dose training group. The mean response rate on both levers was 64.5 rsp/min (range=56.6–82.0 rsp/min) and 62.3 rsp/min (range=52.9–73.4 rsp/min) for the low-dose and high-dose group, respectively. Subsequent *t*-tests revealed no significant differences between groups for mean number of sessions to complete training, mean percentage of total responses on the stimulus-appropriate lever, or response rate on both levers.

3.2. Generalization of CDP

In general, full substitution of CDP was found at doses equivalent to and above that of the training dose for both groups in Test One (Fig. 1, upper panels, filled circles). In both groups, doses smaller than the training dose only partially substituted for the training dose. The percentage of total responses emitted on the CDP-appropriate lever increased in a dose-dependent manner. During Test One, the ED_{50}s and 95% confidence limits for the low-dose and high-dose groups were CDP 2.9 mg/kg (2.34–3.49 mg/kg) and CDP 5.9 mg/kg (4.91–6.85 mg/kg), respectively. The lower ED_{50} value in the low-dose group (especially in Test One) suggests greater sensitivity to the discriminative-stimulus effects of CDP administration. Subsequent *t*-tests, however, did not reveal any significant differences in ED_{50} values between groups.

On average, full substitution of the training dose of CDP was found in the high-dose group, but only partial substitution was found in the low-dose group during Test Two (Fig. 1, lower panels, filled circles). Three of the rats in the low-dose group and only one in the high-dose group emitted at least 80% of total responses on the CDP-appropriate lever during Test Two following administration of the training dose. In both groups, the average percentage of total responses emitted on the CDP-appropriate lever was functionally related to CDP dose, such that the average percentage increased as the dose size increased. The ED_{50}s and 95% confidence intervals for the low-dose and high-dose groups were CDP 3.5 mg/kg (1.09–5.86 mg/kg) and CDP 3.3 mg/kg (1.98–4.63 mg/kg), respectively. Subsequent t-tests did not reveal any significant differences in ED_{50} values between subjects.

Response rates on both levers were also calculated during both CDP tests and are presented in Fig. 2 (filled circles). In Test One (see Fig. 2, upper panels), response rates increased as the dose of CDP increased for the low-dose group, but not the high-dose group. The average response rate for the low-dose group (58.6 rsp/min) was lower than that of the high-dose group (92.2 rsp/min) following administration of the training dose (5.6 and 13.0 mg/kg, respectively) in Test One. During Test Two, the average response rate was lower than that found during Test One for both groups (see Fig. 2, lower panels). As found in Test One, the average response rate for the low-dose group (33.3 rsp/min) was lower than that found for the high-dose group (50.7 rsp/min) following administration of the training dose (5.6 and 13.0 mg/kg, respectively).

3.3. Generalization of saline vehicle

The average percentage of total responses on the CDP-appropriate lever and response rate on both levers during both tests following saline administration were also calculated (see Figs. 1 and 2, open circles). Saline served as the vehicle for both CDP and negative control *d*-amphetamine. For both groups, no substitution following saline administration was found in either test as mean percent CDP-appropriate responding was always below 20%.

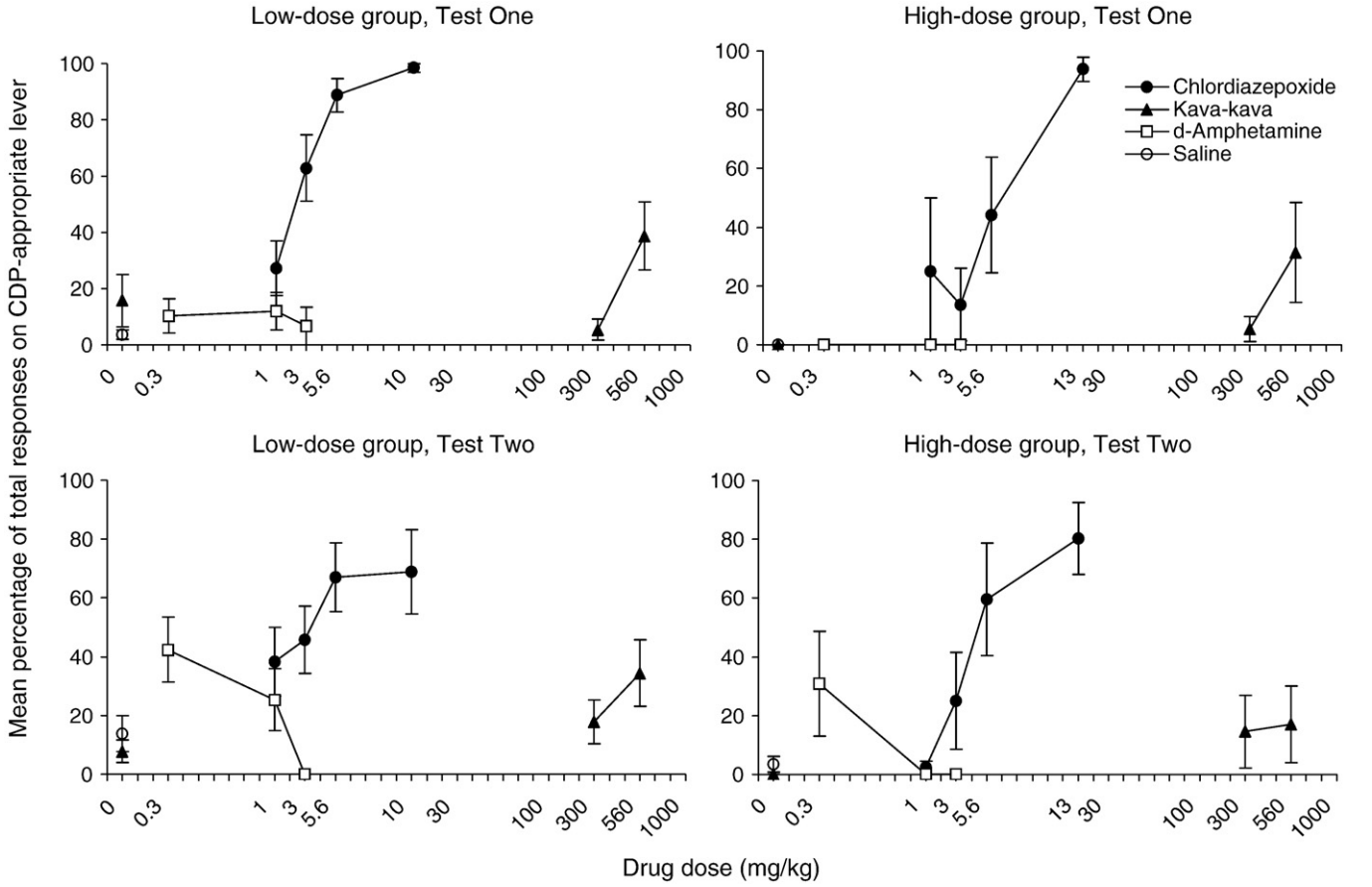

Fig. 1. Mean percent and *SEM* of total responses emitted on the CDP-appropriate lever for each of the test doses of CDP, *d*-amphetamine, kava, kava vehicle, and saline for the low-dose training group (CDP 5.6 mg/kg, left panels) and high-dose training group (CDP 13.0 mg/kg, right panels) as a function of test dose. The graphs in the upper panels display data obtained 60 min following administration of the test drug (Test One), and the lower graphs reflect responding 90 min post-administration (Test Two).

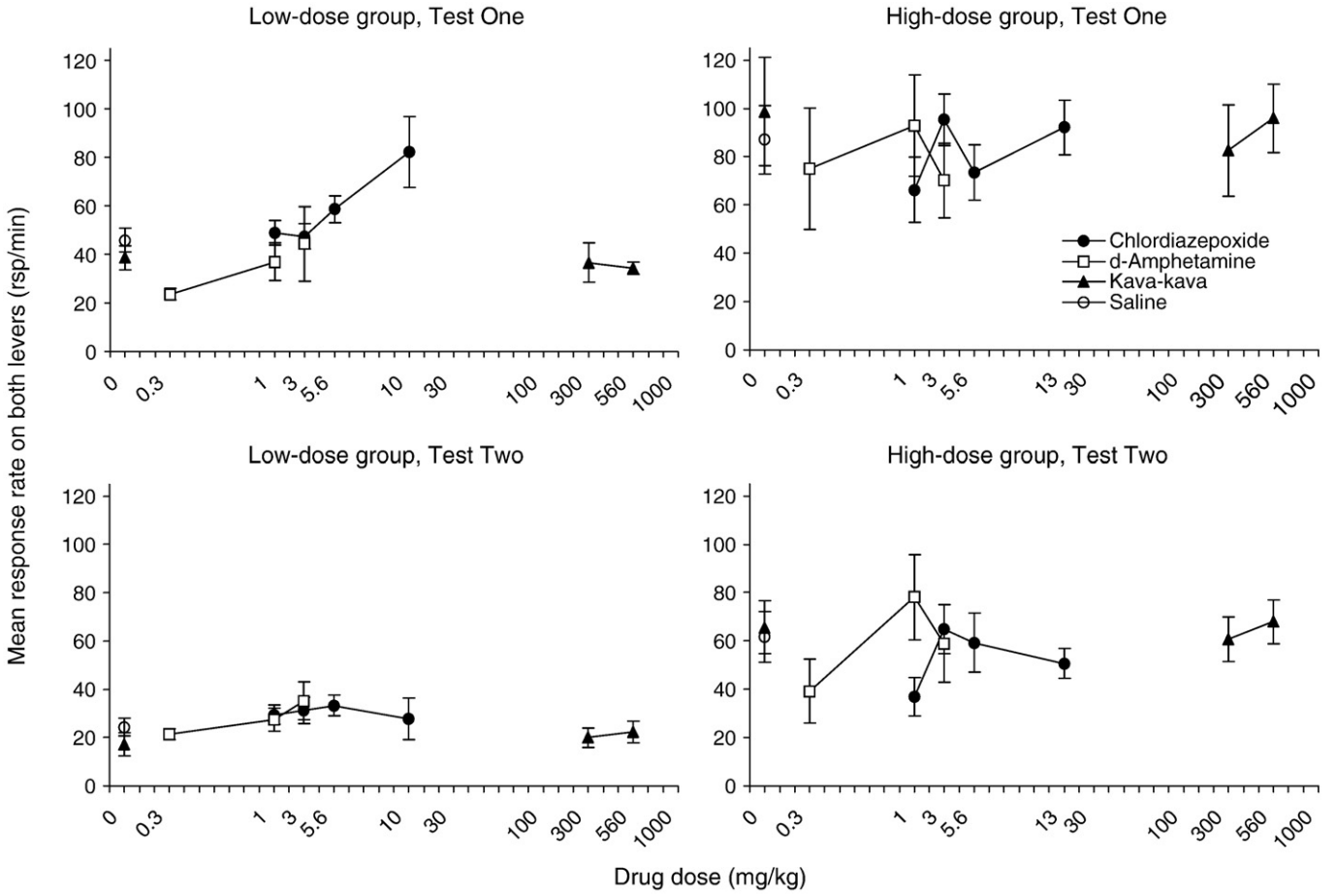

Fig. 2. Mean response rate and *SEM* on the CDP-appropriate lever for each of the test doses of CDP, *d*-amphetamine, kava, kava vehicle, and saline for the low dose training group (CDP 5.6 mg/kg, left panels) and high-dose training group (CDP 13.0 mg/kg, right panels) as a function of test dose. The graphs in the upper panels display data obtained 60 min following administration of the test drug (Test One), and the lower graphs reflect responding 90 min post-administration (Test Two).

3.4. Generalization of d-amphetamine

Evidence of substitution of *d*-amphetamine was not found during Test One for either group, as mean percent CDP-appropriate responding was below 20% for all doses evaluated (see Fig. 1, upper panels, open squares). The low-dose group emitted a higher average percentage of responses (mean range=6.7–16.3%) than the high-dose group (mean range=0.0–0.0%) on the CDP-appropriate lever following administration of *d*-amphetamine during Test One.

During Test Two, administration of the lower doses of *d*-amphetamine resulted in responding on the CDP-appropriate lever indicative of partial substitution in both groups (Fig. 1, lower panels, open squares), particularly following administration of the lowest dose (0.3 mg/kg). As was found in Test One, the average percentage of responses on the CDP-appropriate lever following *d*-amphetamine administration was slightly higher in the low-dose group (mean range=0.0–42.4%) than the high-dose group (mean range=0.0–30.9%). The 3-way repeated measures ANOVA revealed a main effect of percentage of total responses emitted on the CDP-appropriate lever for *d*-amphetamine of test time, $F(1, 7)=39.5$, $p<0.01$, and group, $F(1, 7)=20.6$, $p=0.05$. No main effect was found for dose, $F(2, 14)=2.5$, $p=0.11$. Because only one subject from the high-dose group and two subjects from the low-dose group fulfilled the response requirement following administration of *d*-amphetamine 3.0 mg/kg, these data were not included in the analyses. It should be noted that no substitution was observed in the few subjects that did respond. In general, the high-dose group responded at higher rates than the low-dose group following administration of *d*-amphetamine during both tests (see Fig. 2, open squares).

3.5. Generalization of kava

Partial substitution of kava 560 mg/kg was found in both groups during Test One (Fig. 1, upper panels, filled triangles). The average percent responding on the CDP-appropriate lever was slightly higher in the low-dose group (maximum 38.7%) than the high-dose group (maximum 31.4%) following administration of 560 mg/kg. For two rats in the low-dose group, full substitution of kava 560 mg/kg was observed, and for two rats, partial substitution was observed. However, for two rats in that same group, no substitution was observed following administration of that same dose. Partial substitution of kava 560 mg/kg was found for two of the three subjects in the high-dose group. Partial substitution was not found following administration of kava 300 mg/kg, except for one rat in the low-dose group. No substitution was found in either group during Test One for kava vehicle. Subjects in the low-dose group emitted a significantly higher average percentage of total responses on the CDP-appropriate lever following administration of kava 560 mg/kg (range=0.0–95.5%, mean=38.7%) than kava 300 mg/kg (range=0.0–23.1%, mean=5.4%). The 3-way repeated measures ANOVA revealed a significant main effect of kava dose, $F(2, 14)=6.1$, $p=.01$ for mean percent of total responses on the CDP-appropriate lever. No main effects were found for test time, $F(1, 7)=.03$, $p=.86$ or group, $F(1, 7)=1.76$, $p=.23$. A subsequent Tukey post-hoc test revealed a significant difference of

percentage of total responses on the CDP-appropriate lever between administration of kava vehicle and kava 560 mg/kg ($p<.01$), as well as between administration of kava 300 mg/kg and kava 560 mg/kg ($p<.01$). Mean response rates following administration of kava were higher in the high-dose group than in the low-dose group during Test One. Group data for response rates during Test One are presented in Fig. 2 (upper panels, filled triangles).

Partial substitution of 560 mg/kg kava was found during Test Two in the low-dose group only (Fig. 1, lower panels, filled triangles). In this group, the average percentage of total responses on the CDP-appropriate lever was slightly lower in Test Two than Test One following administration of kava 560 mg/kg. On average, administration of kava 300 mg/kg did not result in partial substitution for either group during Test Two, but some individual subjects did show partial substitution up to 33.3% CDP-appropriate lever responding. No substitution was observed following administration of kava vehicle in either group during Test Two (Fig. 1, lower panels, filled triangle).

4. Discussion

Dose-dependent substitution of CDP was found in both the low- and high-dose training groups. Although the low-dose group appeared to be more sensitive to the effects of CDP because of the lower ED_{50} values, no significant differences were found between groups. The negative control *d*-amphetamine did not substitute for CDP in either group during Test One. During Test Two, however, partial substitution was found in both groups at the lower doses of *d*-amphetamine. Kava 560 mg/kg was found to occasion partial substitution in both groups during Test One and only the low-dose group during Test Two.

The extent to which kava 560 mg/kg generalized to the training dose was modest (i.e., the means were 31.4%–38.7%). However, this was likely an artifact of averaging across subjects as full substitution for CDP was observed for two rats in the low-dose group following administration of kava 560 mg/kg and two rats showed no substitution. Individual differences in sensitivity to methods and drugs used in the present experiment may have contributed to the variability in substitution observed. Several procedural variables may have influenced the present results. One potential variable that may have influenced the findings is that the training doses of CDP may have engendered poor stimulus control for other potential $GABA_A$ agonists like kava. Perhaps utilizing a shorter-acting benzodiazepine (e.g., midazolam) for discrimination training would enhance stimulus control. It is possible that administration of kava results in effects that are more sedative than anxiolytic (see discussion below). Therefore, it may be worthwhile to investigate the discriminative-stimulus effects of kava in rats trained to discriminate a short-acting barbiturate from saline.

Kava dose-dependently increased the average percent of total responding on the CDP-appropriate lever to a range considered as partial substitution (i.e., greater than 20%). At higher doses, it is possible that kava may fully substitute for CDP. However, due to potential toxic effects of the herb and suppression of lever pressing in some subjects, this possibility was not evaluated. Four subjects in the high-dose group died or had to be euthanized due to gastrointestinal complications that may or may not have been related to kava administration. When subjects had to be euthanized did not correspond to any particular drug/dose exposure when generating the dose-response functions. For two of the rats, it had been a couple of weeks since they had received kava. Interestingly, GI complications were only observed in rats in the high-dose CDP group. Thus, this symptom may have been due to chronic administration of CDP on training days (i.p.) and not kava administration, or perhaps it was due to a drug interaction. It is interesting to note, however, that in 2002, the FDA issued a warning pertaining to the potential harmful effects of kava. It is possible that the doses of kava used were having harmful effects on the subjects. Because of the potential toxic effects of this drug, future research investigating kava's use as an herbal medicine is warranted. It is also possible that administration of kava had effects on motivation (i.e., kava had anorexic effects). This seems unlikely, however, because subjects consumed all food that was provided in the home cage 30 min following completion of the session.

Characteristics of the particular preparation of kava used in this study may have influenced the present results. Previous research has indicated that particular kavalactones, particularly dihydrokavain, might mediate effects similar to benzodiazepines in behavioral tests (e.g., Feltenstein et al., 2003). It is possible that the preparation of kava extract used in this study did not have a high enough content of a particular kavalactone to occasion full substitution. The samples used by Feltenstein et al. that resulted in anxiolytic effects contained 15.0–67.5% dihydrokavain, while the sample used in this experiment contained 18.6% dihydrokavain. Future work may test effects of different samples of kava extract containing higher concentrations of different kavalactones. Another possibility would be to examine effects of dihydrokavain or other kavalactones administered alone, instead of within the kava extract. There is reason to believe, however, that the extract used in the present study was behaviorally active, as subjects were visibly sedated and sometimes failed to respond following administration of kava 560 mg/kg.

One variable that may have influenced the present results was the change in training doses in both groups, but this appears unlikely because subjects responding met criterion, i.e., they allotted at least 80% of total responses on the stimulus-appropriate lever prior to delivery of the first reinforcer for at least five consecutive sessions, before generalization testing began. Another variable that may have influenced the results of the present experiment is the altered route of administration during testing. Initially, an i.p. route of administration was used for the testing of all compounds. However, no substitution of kava was observed following administration via the i.p. route. Given the difficulty in getting the kava extract into solution, it was believed that solubility issues may have affected substitution of the compound. In order to test substitution of kava, drugs had to be administered orally on test days. Although CDP was still administered i.p. on training days, using the p.o. route of administration on testing days did not seem to affect substitution of CDP. Thus, the training doses of CDP administered orally engendered full substitution in both training-dose groups. The subjective effects of the drugs appeared to be the same, regardless of route of administration. Issues concerning the bioavailability of the compounds administered by different routes may be topics for future research.

Interestingly, during Test Two the negative control *d*-amphetamine 0.3 mg/kg (in both groups) and *d*-amphetamine 1.0 mg/kg (in the low-dose group) partially substituted for CDP. However, the extent of the substitution was only marginal, and may have been due to a couple of factors. First, effects of extinction in Test One (i.e., no food received after fulfilling the response requirement during Test One) might occasion responding on opposite lever during Test Two. It may be useful to examine sessions in which effects of different time courses are examined individually (e.g., Anderson and van Haaren, 1999). These authors investigated the hypothesis that a drug's time-course effects may be evaluated within subjects and within a single session. Two generalization tests were given following cocaine administration, one at 10 min and one at 30 min, and compared to a generalization test at 30 min only. The authors found that effects of exposure to extinction in Test One on responding during Test Two was negligible. There were no differences in the two generalization gradients that were obtained 30 min after cocaine administration, regardless of whether another gradient was obtained earlier in the session. The lack of an impact of an earlier test within the session may not have been the case in the present study. Second, it is possible that responding on the CDP-appropriate lever 90 min post-administration of *d*-amphetamine was due to the general administration of a drug. In both groups, the CDP training dose when tested 90 min following administration

similarly resulted in partial substituted. Some, but not all, of the responding on the drug lever may have been due to the waning subjective effects of CDP.

In conclusion, in the present study it was found that kava 560 mg/kg partially substituted for the training stimuli in both the high- and low-dose training groups 60 min (both groups) and 90 min (low-dose group only) post-administration. At 60 min post-administration, kava 560 mg/kg shared some discriminative-stimulus effects with the training stimuli in both groups. It appears as though in the high-dose group, the discriminative-stimulus effects of kava 560 mg/kg were diminishing 90 min post-administration. However, kava 560 mg/kg still partially generalized to the training stimulus in the low-dose group 90 min after administration. At present, it is unclear why kava failed to fully substitute for the training doses of CDP. Although it is possible that administration of kava may not fully substitute for benzodiazepines, further research is warranted to rule out other variables that may have influenced the present results. Future research may incorporate a different benzodiazepine as a training drug to perhaps engender stronger stimulus control. Another possibility that kava failed to fully substitute may be due to the particular preparation of kava used in this study, or the chemical structure of kava extract itself. In the case that kava acts more as a sedative than anxiolytic, it may generalize to a different drug class (e.g., barbiturate). Future research should also examine the possibility that extinction during Test One may have resulted in responding on the opposite lever during Test Two by incorporating a phase in which subjects are presented with tests at different times.

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