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Conditioned Taste Reactivity in Rats After Phenylpropanolamine, d-Amphetamine or Lithium Chloride

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DAVIES, B. T. AND P. J. WELLMAN. *Conditioned taste reactivity in rats after phenylpropanolamine, d-amphetamine or lithium chloride.* PHARMACOL BIOCHEM BEHAV 36(4) 973–977, 1990.—That an aversive property of phenylpropanolamine (PPA) in part contributes to its anorexic capacity is suggested by the demonstration of conditioned taste avoidance to PPA doses ranging from 10–40 mg/kg. In order to further evaluate the putative aversive property of PPA, the present experiment compared the effects of PPA on multiple measures of aversion (chin rubs, gaping) in the taste reactivity (TRT) paradigm with those produced by the classic agent lithium chloride and by amphetamine. Male rats were infused via an intraoral cannula with 0.5 M sucrose followed by injection with either vehicle, 127 mg/kg lithium chloride (LiCl), 1.5 or 3.0 mg/kg amphetamine or by 10, 20 or 40 mg/kg PPA. LiCl and 40 mg/kg PPA induced significant chin rub responses during conditioning but only the aversive response induced by 40 mg/kg PPA persisted during extinction trials. In contrast, lower doses of PPA (10 mg/kg, 20 mg/kg) were not aversive in the TRT paradigm. These results suggest that an aversive component is not contributing to anorexia induced by PPA within the dose range of 10–20 mg/kg, but that higher doses may further suppress appetite via an aversive action.

Feeding Taste aversion Conditioned taste reactivity Phenylpropanolamine Amphetamine Lithium chloride Gaping Chin rubbing

PHENYLPROPANOLAMINE (PPA), the racemic mixture of d- and l-norephedrine, suppresses feeding behavior in a variety of species (11,15). The mechanism by which PPA suppresses appetite has been linked in past studies to activation of lateral hypothalamic adrenergic receptors [(8), but see (17)], to inhibition of gastric emptying (16) and to the induction of an aversive state that interferes with feeding. The latter was supported by the demonstration that PPA doses of 10, 20 and 40 mg/kg induce conditioned taste avoidance (19).

In the conditioned taste avoidance (CTA) paradigm, rats are treated with some drug agent immediately following ingestion of a novel solution, such as saccharin. Upon subsequent testing, the rats are offered a choice between water and the saccharin solution. Reduced consumption of the saccharin solution in drug-treated rats is often taken as evidence of an aversive property of that treatment. However, it is presently unclear as to what a positive finding derived from a CTA test really means. The list of agents that induce CTA includes a number of substances that do not induce an aversive state in humans (6).

Booth (3) has cogently argued that satiety may be conditioned; a concept which leads to the idea that substances such as PPA may produce a positive CTA finding because of the conditioning of an anorexic property rather than an aversive property. The typical CTA paradigm involves fluid deprivation in order to evoke adequate consumption of the saccharin solution during conditioning. Administration of a drug that induces satiety upon consumption of a novel saccharin solution may result in conditioning of satiety. On subsequent test trials, a conditioned satiety state may be elicited upon tasting the saccharin flavor and consequently may reduce consumption of that solution. Therefore, the traditional CTA paradigm may be inappropriate for the examination of the potential aversive effects of drugs that suppress appetite.

The taste reactivity test (TRT) involves the infusion of a sucrose solution through an intraoral cannula followed by treatment with drug agents or vehicle (7). The responses elicited by subsequent sucrose infusions can be categorized as ingestive, aversive, or neutral. Because the TRT paradigm does not involve the use of deprivation to motivate consummatory behavior, this paradigm may be more appropriate for the assessment of the putative aversive nature of anorexic compounds, such as PPA and amphetamine. The present experiment therefore compared the motivational properties of PPA (10, 20 or 40 mg/kg) and d-amphetamine (1.5 or 3.0 mg/kg) with that of lithium chloride (127 mg/kg) using the TRT paradigm.

METHOD

Subjects

Forty-nine male Sprague-Dawley albino rats (Harlan Indus-

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tries, Houston, TX), weighing between 250–350 g, served as subjects. The animals were maintained on ad lib rodent pellets (Teklad) and tap water, except as required by the experimental protocol. The animal holding room was maintained at 20°C with a 12-hour light:dark schedule (lights on at 0800 hr). The animals were randomly assigned to one of seven drug Treatment Groups (n = 7 per group).

Drugs

A vehicle solution was prepared using sterile distilled water and 0.9% (w/v) sodium chloride. A lithium chloride solution was prepared by dissolving 127 mg lithium chloride per ml of sterile distilled water, whereas two amphetamine solutions were similarly prepared using 1.5 or 3.0 mg/ml d-amphetamine sulfate. The phenylpropanolamine solutions consisted of 10, 20 or 40 mg phenylpropanolamine hydrochloride per ml sterile distilled water. All drugs were obtained from Sigma Chemical Company (St. Louis, MO) and all solutions were calculated as the weight of chemical (base and salt) per volume.

Intraoral Cannula Implantation

The animals were allowed a one-week adaptation period before being surgically implanted with intraoral cannulae, as previously described by Parker (13). Following a 12-hour food and water deprivation period, each rat was injected (IP) with 0.4 mg/kg atropine sulfate, followed by injection (IP) of 60 mg/kg ketamine hydrochloride (Ketaset). Five minutes later, each rat was injected (IP) with 20 mg/kg sodium pentobarbital. Upon anesthetization, the back of the rat's neck was shaved and swabbed with alcohol. A 15-gauge stainless steel needle was inserted into the rat's skin at the base of the neck, and brought subcutaneously behind and below the ear. With one finger placed inside the rat's mouth to retract its cheek, the needle then pierced the wall of the oral cavity near the first molar. With the needle in place, a 6-inch length of PE-90 tubing was inserted through the barrel, and the needle was withdrawn. The metal end of a 20-gauge Intramedic adapter (Beckton-Dickinson) was filed with sandpaper to facilitate its insertion into the tubing. The tubing was secured at the base of the neck by the adapter, and a 5 mm plastic washer was used to hold the tubing in place inside the rat's mouth. The tubing was then heat-flanged to secure the washer. Postoperatively, the rats received 125,000 units penicillin (IM), and were allowed one week to recover before beginning the adaptation trials. During the recovery period, the cannulae were flushed with water every other day to prevent blockage from food.

Apparatus

The taste reactivity test employed a Plexiglas chamber (26 × 26 × 26 cm), located in a room adjacent to the colony room. A color video camera (Panasonic PK-452B) was focused on a mirror which hung at an angle below the chamber, thus allowing viewing of the rat's ventral surface. The rat's image was transmitted through a video cassette recorder (Panasonic AG-1230) to a color monitor (NEC model No. PM1271A). A video counter timer (TEL model No. 436) was used to superimpose the subject number and an elapsed time record onto each video frame. Videotapes of the rat's orofacial and somatic responses were later scored using an event recorder program created for use on a Macintosh SE microcomputer.

Procedure

The rats received two adaptation trials, five conditioning trials, and four extinction trials. During the adaptation trials, each rat was individually transported into the room containing the test chamber. The rat was placed into the chamber and a 30-cm infusion tube (PE 90) was inserted through the ceiling of the chamber and connected to the adapter of the intraoral cannula. A 5-ml syringe was connected to the infusion tube and placed into the holder of a Razel (Model A) infusion pump. After allowing the rat 1 min to adapt to the chamber, the infusion pump delivered water through the tube into the rat's mouth at the rate of 1 ml/min for 2 min. The rat was then returned to its home cage. The chamber was wiped clean following each occupancy by a subject.

During each of the first four conditioning trials, the procedure was identical to that outlined above, except that each rat received 0.5 M sucrose solution delivered at the rate of 1 ml/min for 2 min, and the rat's orofacial and somatic responses elicited by exposure to the sucrose solution were videotaped for 2 min. Upon removal from the chamber, the rat's cannula was flushed with water, and the rat was injected with the appropriate drug solution before being returned to its home cage. During conditioning trial 5 and each of the 4 extinction trials, the procedure was identical to that outlined for the first 4 conditioning trials, except that all rats received injections of 0.9% saline before being returned to their home cages.

Data Scoring

Videotapes of the taste reactivity conditioning/extinction trials were scored by raters blind to the experimental conditions. The orofacial and somatic responses recorded have previously been described by Berridge and Grill (2). The patterns of responses reported below were scored for the occurrence of ingestive, aversive, and neutral response components.

The ingestive responses that were recorded and analyzed included tongue protrusions (rhythmic protrusions of the tongue), and mouth movements (low amplitude, rhythmic openings of the mandible). These ingestive responses were scored in terms of the amount of time the rat engaged in the activity (duration), and were combined to produce a composite ingestive response score. The ingestive response of paw-licking was also recorded, but showed no evidence of conditioning and was not included in the data analysis.

The aversive (rejection) responses that were recorded and analyzed included chin rubbing (bringing the lower jaw in direct contact with the floor or a wall of the chamber and projecting the body forward), and gaping (rapid, large-amplitude opening of the jaw with concomitant retraction of the corners of the mouth). These aversive responses were scored in terms of frequency of occurrence. The aversive responses of head shaking and limb-flicking were also scored, but showed no evidence of conditioning and were not included in the data analysis.

The neutral response of passive dripping (passive opening of the mouth with fluid dripping from the oral cavity) was recorded and analyzed in terms of frequency of drips.

Data Analysis

The taste reactivity responses were analyzed separately as 7 × 5 mixed ANOVA's using the factors of Treatment Group (LiCl, PPA-40, PPA-20, PPA-10, AMP-3.0, AMP-1.5, and VEH) and trials (conditioning trials: C1–C5, or extinction trials: C5 and E1–E4). Subsequent single-factor ANOVA's for each trial were conducted upon indication of a significant Treatment × Trials interaction during either the conditioning or extinction phase of the study. Newman-Keuls analyses were used to compare the different Treatment Groups if a significant Treatment effect was indicated

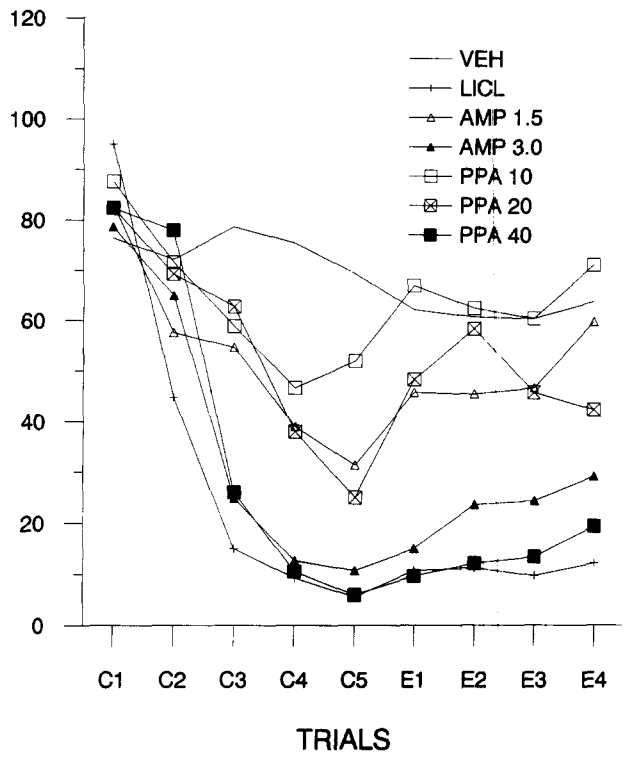

FIG. 1. Mean duration (seconds) of ingestive responding for rats treated with either vehicle (VEH), 127.2 mg/kg lithium chloride (LICL), 1.5 or 3.0 mg/kg d-amphetamine (AMP 1.5, AMP 3.0) or 10, 20 or 40 mg/kg PPA (PPA 10, PPA 20, PPA 40) during conditioning trials (C1–C5) and for these groups during the extinction trials (E1–E4).

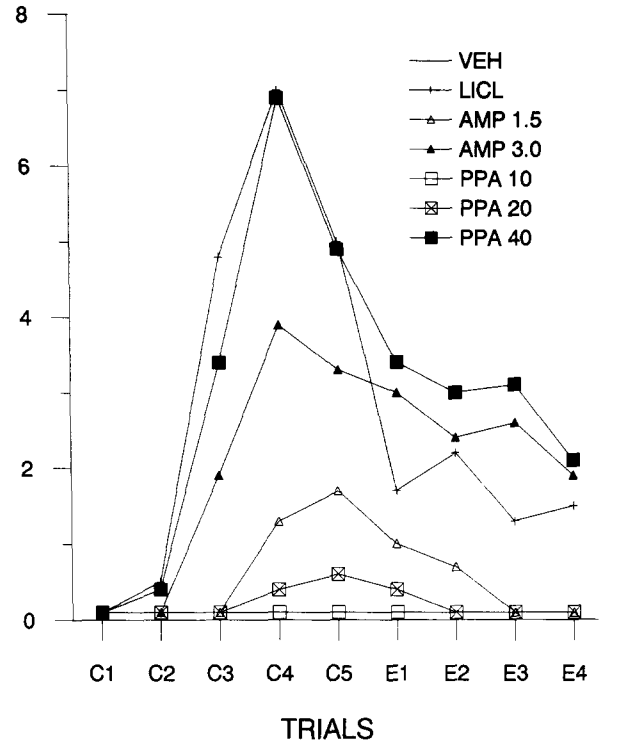

FIG. 2. Mean frequency of chin rubs for the seven treatment groups during conditioning and extinction trials. Group designations are as in Fig. 1.

for a given Trial (9). The criterion level for all statistical tests was $p<0.05$.

RESULTS

Ingestive Responses

Figure 1 presents the mean duration of ingestive responding (mouth movements and tongue protrusions) for the seven Treatment Groups during the conditioning and extinction trials. A 7×5 mixed ANOVA for the conditioning trials (C1–C5) revealed a significant Treatment effect, $F(6,42)=9.7$, $p<0.001$, a significant Trials effect, $F(4,168)=160.3$, $p<0.001$, and a significant Treatment × Trials interaction, $F(6,168)=9.0$, $p<0.001$. While there were no significant differences among the Treatment Groups on Trials C1 and C2, single-factor ANOVA's for the remaining conditioning trials indicated significant Treatment effects on Trials C3–C5, F's(6,42)>12.4, p's<0.001. Groups LiCl, PPA-40 and AMP-3.0 followed very similar patterns of ingestive responding, declining from an average of about 80 sec on Trials C1 and C2 to about 10 sec on Trials C4 and C5. Subsequent Newman-Keuls analyses revealed that, on Trials C3–C5, Groups LiCl, PPA-40, and AMP-3.0 demonstrated significantly less ingestive responding than Group VEH (p's<0.05). Although the lower doses of PPA or AMP produced suppressions of ingestive responding that were intermediate between VEH and the higher drug dose values, these groups were not significantly different from one another. Thus, only those Treatment Groups receiving the highest drug dosages showed a significant suppression of ingestive responding upon oral infusion of the sucrose solution.

A 7×5 mixed ANOVA for the extinction period (C5 and E1–E4) revealed a significant Treatment effect, $F(6,42)=21.0$, $p<0.001$, a significant Trials effect, $F(4,168)=12.5$, $p<0.001$, and a significant Treatment × Trials interaction, $F(6,168)=3.1$, $p<0.001$. Single-factor ANOVA's for the extinction trials indicated significant Treatment effects for all trials, F's(6,168)>11.5, p's<0.001. Subsequent Newman-Keuls analyses revealed that, throughout the extinction period, Groups LiCl, PPA-40, and AMP-3.0 continued to suppress ingestive responding relative to Group VEH (p's<0.05).

Chin Rubs

Figure 2 presents the mean frequency of chin rubs for the seven Treatment Groups during the conditioning and extinction trials. While Groups VEH and PPA-10 failed to demonstrate the chin rub response on any trial, Groups LiCl and PPA-40 followed similar response patterns during the conditioning trials, with both groups reaching an asymptote of about 7 chin rubs on Trial C4. An overall 7×5 mixed ANOVA for the conditioning trials (C1–C5) revealed a significant Treatment effect, $F(6,42)=9.1$, $p<0.001$, a significant Trials effect, $F(4,168)=20.7$, $p<0.001$, and a significant Treatment × Trials interaction, $F(6,168)=3.6$, $p<0.001$. Single-factor ANOVA's for each conditioning trial indicated significant Treatment effects on Trials C3–C5, F's(6,42)>3.2, p's<0.05. Subsequent Newman-Keuls analyses for each of these trials revealed that, on Trials C3–C5, Groups LiCl and PPA-40 differed significantly from Group VEH in frequency of chin rubs (p's<0.05). Although Group AMP-3.0 reached an asymptote of almost 4 chin rubs on Trial C4, this value was not statistically significant from Group VEH or Groups LiCl and PPA-40, most

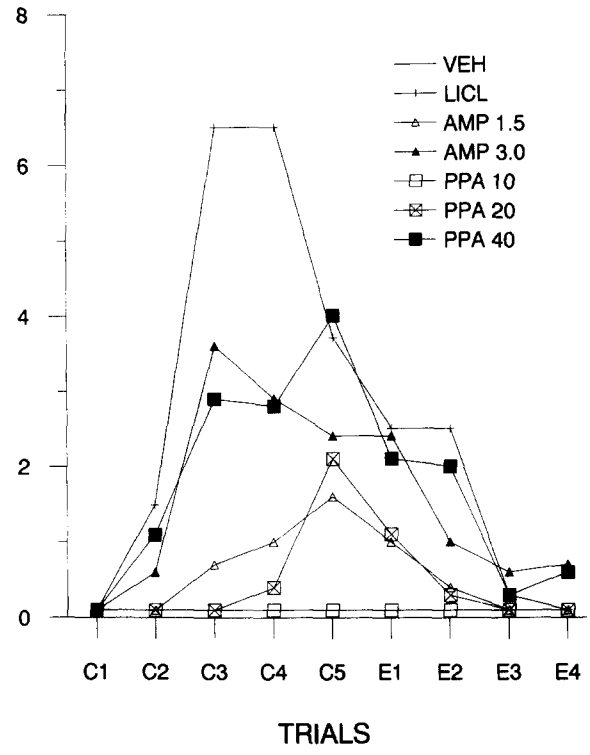

FIG. 3. Mean frequency of gaping responses for the seven treatment groups during conditioning and extinction trials. Group designations are as in Fig. 1.

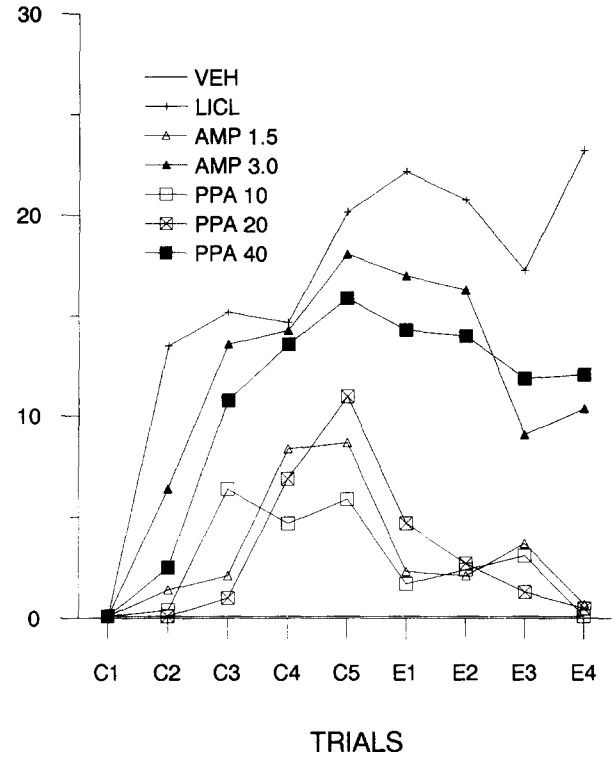

FIG. 4. Mean frequency of passive drips for the seven treatment groups during conditioning and extinction trials. Group designations are as in Fig. 1.

likely due to large within-group variability.

Although Group LiCl showed a sharp decline in chin rub frequency on the first extinction trial, falling from a value of about 5 on Trial C5 to less than 2 on Trial E1, an overall 7 × 5 mixed ANOVA for the extinction period (C5 and E1–E4) did not indicate any significant group differences ($p>0.05$), and no further analyses were conducted.

Gaping

Figure 3 presents the mean frequency of gaping for the seven Treatment Groups during the conditioning and extinction trials. A 7 × 5 mixed ANOVA for the conditioning trials (C1–C5) revealed a significant Treatment effect, $F(6,42)=14.2$, $p<0.001$, a significant Trials effect, $F(4,168)=13.7$, $p<0.001$, and a significant Treatment × Trials interaction, $F(6,168)=2.9$, $p<0.001$. While there were no significant differences among the Treatment Groups on Trials C1 and C2, single-factor ANOVA's for the remaining conditioning trials indicated significant Treatment effects on Trials C3 and C4, F's>6.8, p's<0.001. Group LiCl reached an asymptote of about 6.5 gapes on Trials C3 and C4, and subsequent Newman-Keuls analyses revealed that, on Trial C3, Groups LiCl and AMP-3.0 differed significantly from Group VEH (p's<0.05). On Trial C4, Group LiCl differed significantly from Group VEH, and by Trial C5 there were no statistically significant differences among the groups. Groups VEH and PPA-10 did not demonstrate the gaping response on any of the conditioning or extinction trials.

An overall 7 × 5 mixed ANOVA for the extinction period (Trials C5 and E1–E4) revealed a significant Treatment effect, $F(6,42)=5.6$, $p<0.001$, a significant Trials effect, $F(4,168)=17.0$, $p<0.001$, and a significant Treatment × Trials interaction, $F(6,168)=1.8$, $p<0.05$. However, the gaping response declined for all Treatment Groups during the extinction phase such that a single-factor ANOVA for Trial E4 failed to indicate any differences among the groups.

Passive Dripping

Figure 4 presents the mean frequency of passive drips for the seven Treatment Groups during the conditioning and extinction trials. While Group VEH failed to demonstrate passive dripping on any trial, Group LiCl began to show this response after only one conditioning trial, and continued to surpass all other groups throughout conditioning and extinction. An overall 7 × 5 mixed ANOVA for the conditioning trials (C1–C5) indicated a significant Treatment effect, $F(6,42)=9.3$, $p<0.001$, a significant Trials effect, $F(4,168)=35.8$, $p<0.001$, and a significant Treatment × Trials interaction, $F(6,168)=2.8$, $p<0.001$. Single-factor ANOVA's for each conditioning trial revealed significant Treatment effects on Trials C2–C5, F's>4.3, p's<0.01. Subsequent Newman-Keuls analyses indicated that Group LiCl differed significantly from Group VEH on Trial C2, $p<0.05$, and Groups LiCl, AMP-3.0, and PPA-40 differed significantly from Group VEH on Trials C3–C5, p's<0.05.

An overall 7 × 5 mixed ANOVA for the extinction period (C5 and E1–E4) indicated a significant Treatment effect, $F(6,42)=19.6$, $p<0.001$, a significant Trials effect, $F(4,168)=11.1$, $p<0.001$, and a significant Treatment × Trials interaction, $F(6,168)=2.1$, $p<0.01$. Single-factor ANOVA's revealed significant Treatment effects on all extinction trials, F's(6,42)>5.6, p's<0.001. Subsequent Newman-Keuls analyses showed that Groups LiCl, PPA-40, and AMP-3.0 continued to demonstrate significantly more passive dripping than Group VEH throughout

the extinction phase. By Trial E4, Group LiCl demonstrated significantly more passive dripping than all other groups (p's<0.05).

DISCUSSION

The intent of the present experiment was to evaluate the putative aversive properties of PPA using the TRT paradigm. This paradigm offers multiple indices (e.g., gaping responses, chin rub responses) of aversive properties of a drug without the concomitant use of a deprivation state. In the present study, qualitatively equivalent chin rub responses were observed in rats treated with 40 mg/kg PPA or 127 mg/kg LiCl during the conditioning phase. In contrast, however, chin rub responding declined more rapidly during the extinction phase for rats previously treated with LiCl than for rats treated with 40 mg/kg PPA. These results attest to the strength and persistence of the aversive effect of 40 mg/kg PPA.

These findings, however, must be considered in light of the relative absence of chin rubbing and gaping responses to 10 mg/kg PPA and 20 mg/kg PPA. Dose-response studies of PPA anorexia reveal that the threshold for the induction of PPA anorexia in rats lies at about 5 mg/kg with 20 mg/kg PPA producing nearly a 50% reduction in feeding behavior (5, 10, 12, 18). Thus, although a high dose of PPA (40 mg/kg) clearly induces an aversive motivational state, such is not observed for doses that are within the range used to suppress appetite.

An interesting issue regarding the use of the TRT paradigm relates to an assessment of the convergence of findings for drugs evaluated by the CTA and TRT paradigms. Whereas the classic agent LiCl produces dose-dependent aversive responses in both the TRT and CTA paradigms (20), the results with regard to amphetamine are not as clear. Amphetamine produces dose-dependent reductions in saccharin consumption in the CTA paradigm (1,4). In the present experiment, 3.0 mg/kg amphetamine produced both chin rub and gaping responses. Although the frequency of chin rub responses for amphetamine-treated rats was not significantly different from vehicle-treated rats, the frequency of these responses was also not different from that of rats treated with 127 mg/kg LiCl or 40 mg/kg PPA. Other investigators have not observed such increases in aversive responses to amphetamine in the TRT paradigm (14,20). With regard to PPA, a high dose (40 mg/kg) produces aversive responses in both CTA and TRT paradigms but lower doses (i.e., those doses relevant to feeding behavior) do not produce aversive responses in the TRT paradigm.

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