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Simple behavioral methods to assess the effect of drugs or toxins on sensory experience

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Abstract

When behavioral pharmacologists/toxicologists study conditioned taste aversions (CTAs), or other conditioned responses, as a means to investigate the effects of various drugs or toxins on a learned response, failure to discover a CTA is frequently attributed to the treatment's influence on the associative process. This kind of analysis may fail to identify drug-induced sensory changes that may influence conditioned stimulus (CS) or unconditioned stimulus (US) saliency. The current paper outlines a simple method by which a drug's influence on CS or US sensation may be determined. Further, illustrative data are provided regarding how *N*-methyl-D-aspartate (NMDA) receptor blockade modulates taste and the sensation of malaise. Ketamine (an NMDA receptor antagonist) has been reported to block CTAs in both neonatal and adult rats. The current experiments evaluated ketamine's ability to modulate the taste of a frequently employed CS (saccharin HCl = SAC) or the aversive aspects of a common US (Lithium Chloride = LiCl). Rats normally exhibit a preference for 0.3% SAC over 0.6% SAC and will suppress consumption of these liquids following an injection of LiCl. We report that ketamine did not markedly antagonize these consummatory patterns nor did it disrupt spontaneous locomotor movements. Taken together, these findings point to ketamine's limited ability to change the sensory capacities required for CTA formation. Investigators interested in determining the underlying causes of drug-induced CTA blockade may choose to employ paradigms similar to the one used here.

Keywords: Conditioned taste aversion; Ketamine; Rat; Saccharin; Lithium chloride; Sensation; Taste; Malaise; *N*-methyl-D-aspartate; Drinking

1. Introduction

Conditioned taste aversions (CTAs) may be acquired when an animal consumes a novel taste (conditioned stimulus, CS) and then experiences the symptoms of poisoning (unconditioned stimulus, US) (Garcia et al., 1955). When later given a choice between the CS and some more-familiar taste (typically water), the animal will avoid the taste that it previously associated with the poison. The CTA paradigm has been used extensively to study learning and memory (Domjan, 1993) and, more specifically, to assess the effects of a particular drug or toxin on memory formation.

In these types of studies the drug or toxin of interest is typically administered before (Aguado et al., 1994; Concannon and Freda, 1980; Mickley et al., 1998; Weldon et al., 1997; Shobi and Goel, 2001) or soon after (Risinger et al., 1999; Yasoshima et al., 2000) the CS. The US is also typically given while the subjects are under the influence of the chemical of interest (Aguado et al., 1994; Concannon and Freda, 1980; Mickley et al., 2000; Olszewski et al., 2000). Thus, data suggesting that a drug or toxin can impair CTA memory may actually be interpreted in several ways. The substance may be (a) altering the sensory experience of the CS; (b) reducing the salience of the US, and/or (c) disrupting the CS–US association. Therefore, before it may be assumed that a drug or toxin impairs the ability to make associative connections in the brain, the influence of the substance on CS and US sensation must also be explored.

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This issue has been addressed in several ways. In some experiments the drug of interest has been administered directly into the brain with the expectation that sensory systems are bypassed by this method (Gutierrez et al., 1999). Likewise, comparisons of drugs that freely penetrate the blood–brain-barrier and those that penetrate only to a limited degree have allowed investigators to differentiate sensory vs associative effects (Evenden et al., 1992). In other circumstances, state-dependent controls or tests of the drug's effects on habituation to a taste (Aguado et al., 1994) have been utilized in an attempt to ascertain sensory vs associative effects of a particular substance. The running of additional controls necessarily carries with it additional time investment and expense. The current experiments illustrate a relatively simple method by which some of the sensory effects of a drug or toxin may be evaluated.

Prior administration of ketamine [a well known non-competitive *N*-methyl-D-aspartate (NMDA) receptor antagonist (Thompson et al., 1985)] blocks the formation of a CTA in adult (Aguado et al., 1994; Welzel et al., 1990), neonatal (Mickley et al., 1998), and fetal (Mickley et al., 2000, 2001) rats. However, the mechanism of this learning disruption remains controversial (Mickley et al., 1998, 2000). Therefore, we tested ketamine's ability to modulate the taste of a frequently employed CS (saccharin = SAC) or the aversive aspects of a commonly used US (Lithium Chloride = LiCl). We did this by measuring ketamine's effects on the drinking of SAC concentrations of different palatabilities (Experiment 1). Second, we determined the extent to which ketamine could antagonize LiCl's ability to suppress SAC consumption (Experiment 2). Finally, we assessed the motor effects of ketamine as a possible mediator of the changes in consummatory behaviors we recorded (Experiment 3).

It should be noted at the outset that these studies are not aimed at determining the effects of ketamine on the formation of a CTA. Rather, they illustrate ways in which one might determine the extent to which a drug or toxin may modulate and/or prevent sensation of a CS or US. In addition to highlighting a method for evaluating the effects of a drug on taste sensation and LiCl-induced malaise, these studies reveal that ketamine has limited ability to alter sensory experiences when administered in doses that are capable of blocking CTA formation.

2. Methods

2.1. Experiment 1: evaluation of ketamine's influence on taste

The animals involved in these studies were procured, maintained and used in accordance with the Animal Welfare Act and the *Guide for the Care and Use of Laboratory Animals* prepared by the Institute of Laboratory Animal Resources-National Research Council. The subjects were male rats (Mean weight $\pm$SEM = 352.45 $\pm$ 4.69 g) of the Sprague–Dawley strain obtained from Zivic–Miller Laboratories (Zelienople, PA). Throughout the experiment the animals were individually housed in plastic 'shoe box' cages (44.45 cm long $\times$ 21.59 cm wide $\times$ 20.32 cm high). Home cage temperature was maintained at 23–26 °C under a 12/12 h light/dark cycle (lights on at 06:00 h). The numbers of rats in the various treatment groups and the timeline of Experiment 1 are represented in Table 1.

2.2. Ketamine administration and consummatory observations (Experiment 1)

Subjects were placed on a 23.5-h/day water deprivation schedule throughout the study and were given 0.5-h access to a liquid at approximately 14:00 h each day. Rats were allowed to drink tap water on days 1 and 2. On days 3 and 4, rats were allowed access to a single bottle containing either 0.3 or 0.6% saccharin HCl water (SAC). These first exposures to SAC were aimed at reducing neophobia for the novel taste and to establish baseline-drinking patterns for the 2 concentrations of SAC.

On day 5 (test day) rats received ketamine hydrochloride (1.0, 10.0 or 25.0 mg/kg, i.p. mixed in physiological saline) or an equal volume of the vehicle (mean volume of ketamine/vehicle = 0.35 ml). The doses of ketamine were specifically selected based on previous work indicating that CTAs may be blocked with these levels of the drug (Alessandri et al., 1989; Mickley et al., 1998; Wesierska et al., 1990). One-half hour later, rats each had access to a *single* bottle containing either 0.3 or 0.6% SAC (i.e. the same concentration as they experienced on days 3 and 4 of the study). The amount of SAC consumed during a 0.5-h period was recorded.

Note that these procedures were aimed at determining our subject's ability to discriminate between two different palatabilities of SAC while under the influence of the drug ketamine HCl. In this sense, the procedures were unlike a typical CTA paradigm where SAC ingestion is indicative of an association between a CS and US.

2.3. Experiment 2: evaluation of ketamine's influence on LiCl-induced reduction in drinking

Experiment 1 indicated that ketamine had a limited ability to alter taste sensation since rats could continue to adjust their SAC drinking based on concentration factors (see data below). Experiment 2 evaluated the effects of ketamine on LiCl-induced suppression of drinking. The subjects were male rats (Mean weight $\pm$SEM = 366.95 $\pm$ 5.96 g) of the Sprague–Dawley strain obtained from Zivic–Miller Laboratories

Table 1
Experiment 1—Groups, numbers of subjects and timeline

Drug treatments					
Saccharin[a] concentration	Saline	1.0 mg/kg ketamine	10.0 mg/kg ketamine	25.0 mg/kg ketamine	
0.3%	17	7	7	7	
0.6%	7	8	7	9	
Timeline					
Experiment day	Day 1	Day 2	Day 3	Day 4	Day 5
Injection(s)	None	None	None	None	Saline or ketamine
Liquid drunk	H_2O	H_2O	SAC (0.3 or 0.6%)	SAC (0.3 or 0.6%)	SAC (0.3 or 0.6%)

[a] Single-bottle presentations on experimental days 3–5.

(Zelienople). Up to day 5 of the study, the animals were treated as described in Experiment 1. On day 5 (test day) rats received an injection of ketamine hydrochloride (either 1.0, 10.0 or 25.0 mg/kg, i.p. mixed in physiological saline) or an equal volume of the vehicle (mean volume of ketamine/vehicle = 0.37 ml). Immediately following the ketamine or saline injection on day 5, the subjects received a second injection consisting of either LiCl (81 mg/kg, i.p.) or an equal volume of saline. One-half hour later, rats each had access to a *single* bottle containing either 0.3 or 0.6% SAC (i.e. the same concentration as they experienced on days 3 and 4 of the study). The amount of SAC consumed during a 0.5-h period was recorded. See Table 2 for an outline of the experimental design, timeline and the number of subjects in each treatment group of Experiment 2.

Note that the procedures of Experiment 2 were aimed at determining the ability of ketamine to modulate the malaise induced by LiCl (a drug typically used as a US in CTA paradigms). Although SAC consumption was measured, it was SAC consumed *while* the animals were under the influence of the LiCl. Therefore, unlike a typical CTA paradigm, SAC ingestion is not indicative of an association between a CS and US.

2.4. Experiment 3: evaluation of ketamine's influence on locomotor behavior and water drinking

It is possible that the changes in SAC drinking measured in these experiments may be influenced by ketamine-induced changes in motor responding. Ketamine has well-known anesthetic properties (Menache et al., 1990) and non-linear dose–response effects on locomotion. Low doses frequently cause an increase in locomotor movements, whereas higher doses ($\geqslant$ 25 mg/kg) cause a reduction in movement and 'catalepsy' (Alessandri et al., 1989; Patel and Chapin, 1990). Similar effects have been reported when bar pressing behaviors have been measured (Meliska and Trevor, 1978). In Experiment 3, we measured locomotor responding

Table 2
Experiment 2—Groups, numbers of subjects and timeline

Drug treatments					
Saccharin[a]	Saline[b]	1.0 mg/kg ketamine[b]	10.0 mg/kg ketamine[b]	25.0 mg/kg ketamine[b]	
Concentration	Saline (or LiCl)[c]	Saline (or LiCl)[c]	Saline (or LiCl)[c]	Saline (or LiCl)[c]	
0.3%	17 (7)	7 (7)	7 (8)	7 (8)	
0.6%	7 (7)	8 (9)	7 (6)	9 (8)	
Timeline					
Experiment day	Day 1	Day 2	Day 3	Day 4	Day 5
Injection(s)	None	None	None	None	Saline or ketamine; followed by Saline or LiCl
Liquid drunk	H_2O	H_2O	SAC (0.3 or 0.6%)	SAC (0.3 or 0.6%)	SAC (0.3 or 0.6%)

[a] Single-bottle presentations on experimental days 3–5.
[b] First i.p. injection on experimental day 5. Drug treatments were all administered 0.5 h before the bottle test.
[c] Second i.p. injection on experimental day 5.

following the same doses of ketamine that were used in Experiments 1 and 2. Further, we determined the extent to which our highest dose of ketamine altered drinking of tap water.

In order to determine the extent to which ketamine changes an animal's ability to move to a bottle spout (and thereby influence our measures of drinking), we measured locomotor movements in a separate set of male, Sprague–Dawley rats (Mean weight $\pm$SEM = 372.90 $\pm$ 6.14 g). Animals were housed in the same manner as specified above. On the experimental day rats received saline ($N = 12$), 1.0 mg/kg ketamine, i.p. ($N = 9$), 10.0 mg/kg ketamine, i.p. ($N = 11$), or 25.0 mg/kg ketamine, i.p. ($N = 8$). Injection volumes averaged 0.37 ml. Subjects received the injections 0.5-h before the locomotor observations were begun. At the start of the test, rats were placed in the middle of an open-field (i.e. a plastic box: 64 cm long $\times$ 46 cm wide $\times$ 42 cm high) with an open top and opaque walls. Light intensity 30 cm above the floor of the activity chamber was 57 $\mu W/cm^2$. A grid design (consisting of nine, 20.5 $\times$ 14.5 cm^2 rectangles) was drawn on the floor of the open field. Rats were video taped during 2, sequential 5-min periods. Later, two independent observers (blind to the experimental condition of the animal) viewed the tapes and counted the number of lines crossed (horizontal activity) and number of rears (vertical activity; i.e. when 2 front feet were taken off the floor). The means of these two independent observations (of each behavioral component) were used in this data analysis. The inter-rater reliability was statistically significant [$r\ (28) = 0.98,\ P < 0.01$].

The decision to analyze just 10 min of locomotor activity was motivated by a preliminary analysis suggesting that the first 10 min of combined horizontal and vertical movements was highly correlated with 30-min activity [$r\ (22) = 0.94,\ P < 0.001$]. Likewise, we determined from the data collected in Experiment 1, that the majority of SAC drinking takes place in the first 10 min and is well correlated with the amount of SAC drinking that occurs over 30 min [$r\ (15) = 0.95,\ P < 0.001$].

In order to determine the extent to which a high dose of ketamine could alter the drinking of water-deprived animals we measured water drinking in a separate set of male, Sprague–Dawley rats (Mean weight $\pm$SEM = 361.05 $\pm$ 4.61 g). Animals were obtained and housed in the same manner as specified above. Subjects were placed on a 23.5-h/day water deprivation schedule throughout the study and were given 0.5-h access to a liquid at approximately 14:00 h each day. Rats were allowed to drink tap water on days 1 and 2. On day 3, the animals received either 25.0 mg/kg ketamine, i.p. ($N = 5$) or an equal volume of saline (i.p.) ($N = 5$). Injection volumes averaged 0.36 ml. One-half hour later rats had access to tap water for 0.5 h.

2.5. Statistical analyses

The data for Experiment 1 were evaluated through the use of a 2-way ANOVA [Drug pre-treatment (0.0, 1.0, 10.0 or 25.0 mg/kg ketamine) $\times$ Saccharin concentration (0.3 or 0.6%)]. In all the analyses we used a general linear model (SPSS Inc., Chicago, IL 60606) compensating for unequal N values (Kirk, 1982; Winer, 1971) and an $\alpha = 0.05$. Baseline SAC drinking was analyzed via a repeated measures ANOVA (Saccharin concentration (0.3 or 0.6%) $\times$ Baseline day (day 3 or day 4)).

Experiment 2 was focused on evaluating ketamine's ability to alter LiCl-induced suppression of drinking. In this context, the concentration of SAC was a less important variable than in Experiment 1. Here, we were less concerned with the animal's preference for different SAC concentration. Rather, the critical data were the extent to which LiCl would suppress drinking. In fact, we were aware (based on Experiment 1) that rats prefer 0.3% SAC over 0.6% SAC. In order to adjust for this preference, we computed a ratio of SAC drinking on Day 5 (test day)/Day 4 (baseline). These percent-of-baseline ratios for the animals drinking the 2 concentrations of SAC were not significantly different and were then combined for this analysis. The ratio data were then evaluated through the use of a 2-way ANOVA [Drug pre-treatment (0.0, 1.0, 10.0 or 25.0 mg/kg ketamine) $\times$ Malaise induction (saline or 81 mg/kg LiCl)].

In our movement analysis (Experiment 3), locomotor activity after 0-, 1.0-, 10-, or 25-mg/kg ketamine was analyzed within a one-way ANOVA repeated measures design over the 2, 5-min time blocks. A t-test was used to compare the water drinking of animals following ketamine (25 mg/kg) or saline.

3. Results

3.1. Experiment 1

Rats preferred 0.3% SAC over 0.6% SAC and ketamine did not significantly alter this preference (Fig. 1). An analysis of the amount of SAC drunk on the test day (day 5) revealed a significant effect of SAC concentration [$F(1,61) = 19.29;\ P < 0.001$] and a significant drug effect [$F(3,61) = 16.76;\ P < 0.001$]. Drinking of 0.3% SAC was consistently higher that 0.6% SAC although, in a dose-dependent manner, ketamine seemed to influence overall consumption of both concentrations of SAC. There was a trend towards the highest dose of ketamine (25 mg/kg) to reduce the preference for 0.3% SAC over 0.6% SAC. However, this was not a reliable effect (based on the lack of a significant interaction between SAC concentration and ketamine dose).

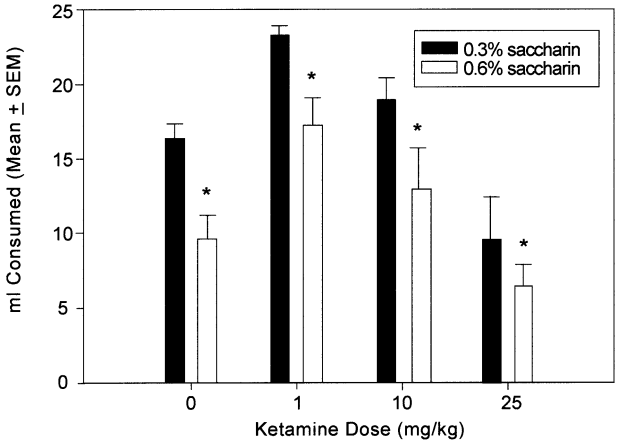

Fig. 1. Rats from Experiment 1 drank more 0.3% SAC than 0.6% SAC and ketamine HCl (1.0, 10.0, or 25.0 mg/kg) did not significantly alter this pattern. An ANOVA revealed significant main effects of SAC concentration and drug treatment but not a significant interaction (see text). Therefore, while ketamine modulated the overall drinking of the subjects, there remained a persistent difference between the 0.3 and 0.6% consumption of SAC. There was a tendency for the highest dose of ketamine (25 mg/kg) to reduce the differential consumption of 0.3 and 0.6% SAC but (based on the lack of a significant interaction term) this was not statistically significant. An * immediately above the bars indicates a significant difference ($P \leqslant 0.05$) between the drinking of the two saccharin concentrations. Variance indicators are the standard error of the mean (SEM).

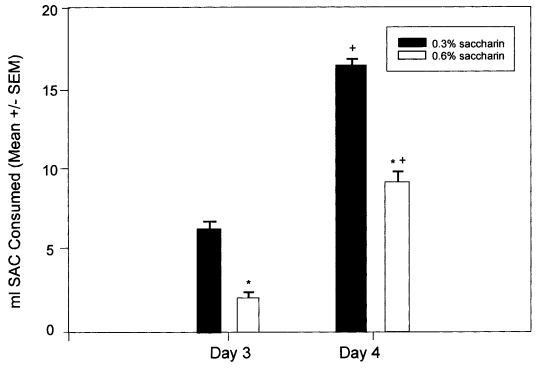

Fig. 2. Baseline SAC drinking on days 3 and 4 of Experiment 1. Rats exhibited a neophobia upon their first exposure to SAC on day 3. There was a significant increase in SAC drinking on day 4. Subjects consumed more 0.3% SAC than 0.6% SAC on both baseline drinking days (see text). An * immediately above the bars indicates a significant difference ($P \leqslant 0.05$) between the drinking of the two saccharin concentrations on a particular test day. An + indicates a significant increase in the amount of drinking of a particular concentration of SAC from day 3 to day 4. Variance indicators are the SEM.

On the baseline drinking days (Day 3 and Day 4) rats consistently drank more 0.3% SAC than 0.6% SAC [$F(1,121) = 85.78$; $P < 0.001$] (Fig. 2). As expected, the animals exhibited a neophobia as evidenced by a low level of SAC consumption during the first exposure to this novel taste on Experimental day 3. There was a significant increase in SAC drinking on day 4 [$F(1,121) = 796.45$; $P < 0.001$]. In fact, by day 4, the animals were drinking approximately the same amount as saline-treated rats will drink on the test day (day 5) (Fig. 1). These data indicate that the SAC consummatory patterns were stable by the test day.

3.2. Experiment 2

LiCl-induced malaise reduced consumption of SAC and this LiCl-induced suppression of drinking was not significantly changed by ketamine (Fig. 3). The ANOVA revealed significant main effects of the malaise-inducing treatment [$F(1,121) = 49.39$; $P < 0.001$] and ketamine treatment [$F(3,121) = 7.47$; $P < 0.001$]. The interaction term was not statistically significant. We may therefore conclude that (at higher doses) ketamine caused a suppression of SAC consumption. This suppression was seen in rats irrespective of whether they had received LiCl or a control saline injection. Moreover, rats that had received LiCl exhibited a significantly greater suppression of SAC consumption than did saline-treated animals. Most important to the hypothesis of interest, the lack of interaction may be interpreted as an indication that ketamine does not disrupt LiCl's suppression of drinking. Said another way, while increasing doses of ketamine causes consumption of SAC by LiCl-treated animals to decrease, these animals drink significantly less SAC than saline-treated rats irrespective of the dose of ketamine administered.

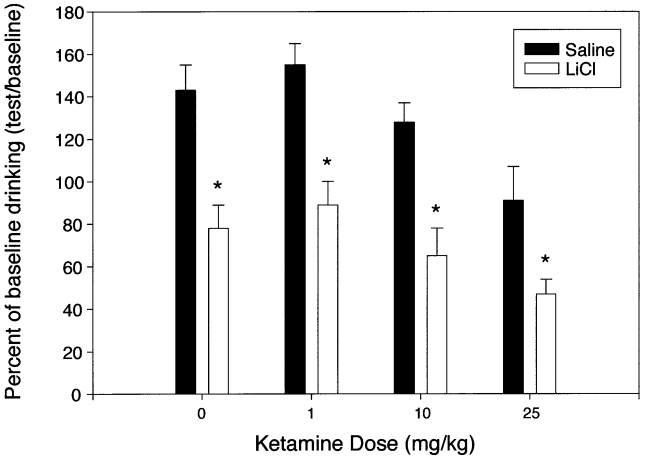

Fig. 3. Lithium Chloride (LiCl; 81 mg/kg) suppressed SAC drinking and ketamine HCl (1.0, 10.0, or 25.0 mg/kg) did not significantly alter this pattern. The figure shows data from Experiment 2. Presented here are the means of the ratios of SAC drunk on the test day (day 5) divided by SAC drunk on experimental day 4 (baseline). The ANOVA revealed significant main effects of LiCl treatment and ketamine treatment but not a significant interaction (see text). Therefore, while ketamine modulated the overall drinking of the subjects, there remained a persistent difference between the SAC drinking of the saline-treated and the LiCl-treated rats. An * immediately above the bars indicates a significant difference ($P \leqslant 0.05$) between specific saline-treated and LiCl-treated groups. Variance indicators illustrate $\pm$SEM.

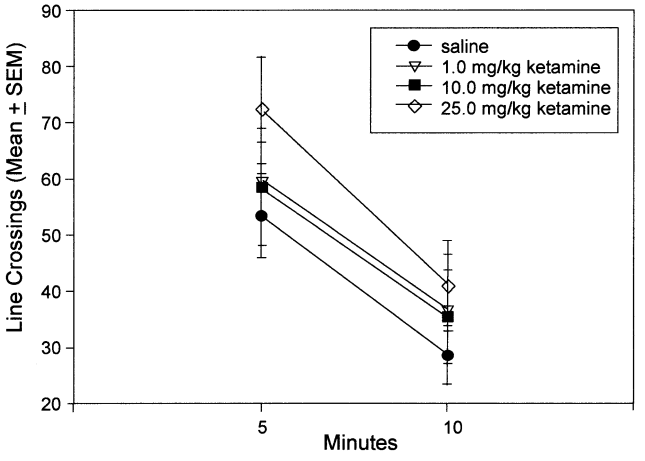

Fig. 4. Spontaneous open-field activity exhibited by rats after either ketamine or saline control injections. Ketamine HCl (1.0, 10.0 or 25 mg/kg, i.p.) did not significantly alter the horizontal locomotor movements of rats. Variance indicators illustrate ±SEM.

3.3. Experiment 3

As expected, both horizontal and vertical (rearing) movement decreased over the 10-min observation period as the animals habituated to the open field environment [Horizontal: $F(1,40) = 54.85$; $P < 0.0001$; Vertical: $F(1,40) = 25.08$; $P < 0.0001$]. However, ketamine-injected animals exhibited horizontal (Fig. 4) and vertical activity that was not statistically distinguishable from saline-injected rats (*Drug* effects and *Drug* × *Time* effects were not significant).

In Experiment 3, we also investigated the extent to which ketamine may alter an animal's ability to make movements required for consummatory behaviors (i.e. licking from a water tube). During the 0.5-h baseline water drinking period on days 1 and 2 our 23.5 h-deprived rats drank 20.97 ± 1.61 ml (Mean ± SEM) and 20.42 ± 0.89 ml, respectively. On the drug-injection day the water drinking of saline-injected rats (Mean ± SEM = 24.24 ± 1.67 ml) and 25 mg/kg ketamine-injected rats (19.43 ± 3.41 ml) was not significantly different. These data suggest that rats treated with our highest dose of ketamine were still able to move to, and drink from, a waterspout.

4. Discussion

The data presented here reveal that saline-injected control rats drank more 0.3% SAC than 0.6% SAC replicating the well-known preference–aversion function for saccharin (Young, 1959). Pretreatment with ketamine (1.0, 10.0 or 25.0 mg/kg) did not disrupt this taste discrimination. Likewise, pretreatment with ketamine did not attenuate the ability of LiCl to reduce SAC drinking. These data are consistent with the interpretation that ketamine, at least at the doses employed (1–25 mg/kg), has a very limited ability to alter taste or the sensation of LiCl's effects.

Our data are consistent with other findings suggesting that ketamine does not alter the ability to taste sweet substances. For example, Aguado et al. (1994) reported that ketamine (25 mg/kg) did not alter the process of habituation to the neophobia observed upon exposure to novel sucrose. A similar failure to disrupt neophobic effects was observed when the NMDA receptor antagonist MK-801 was used (Robinson et al., 1989).

Our data are also consistent with the literature indicating that ketamine does not reduce the US properties of LiCl. In fact, under some circumstances, ketamine (and other NMDA receptor blocking agents) can act as a US and produce a (albeit mild) taste aversion when injected after exposure to a novel taste (Etscorn and Parson, 1979; Jackson and Sanger, 1989; Welzel et al., 1990). However, in the current study (where CTAs were not established/measured), ketamine neither consistently strengthened nor weakened the ability of LiCl to suppress the drinking of SAC.

Since ketamine has well-known anesthetic properties (Evenden et al., 1992), we were concerned that the drug might produce significant changes in motor capacities and, therefore, any drug-induced alterations in drinking might be secondary to an inability to move or to ingest a liquid. However, neither the horizontal nor the vertical locomotor activity of our ketamine-treated rats was significantly different from that of saline-treated controls. Likewise, our highest dose of ketamine (25 mg/kg) did not significantly reduce our rat's ability to consume tap water.

The paradigm described here did not detect a significant effect of ketamine on taste or illness sensitivity. These negative findings raise an issue of test sensitivity. Are the methods employed here sensitive enough to detect effects if they exist? Will any dose of ketamine (or some other drug) cause a change in the behavioral measures described here? Experiment 1 revealed significant main effects of 'SAC concentration' (i.e. 0.3% SAC was preferred over 0.6% SAC) and 'drug' (i.e. higher doses of ketamine tended to suppress drinking of both SAC concentrations). Although there was not a statistically significant interaction between these factors, there was a trend towards the highest dose of ketamine to reduce the preference for 0.3% SAC over 0.6% SAC (Fig. 1). Would this trend become a reliable effect at higher doses of ketamine and therefore illustrate the method's ability to detect drug-induced disruptions in sensation? Unfortunately, higher doses of this drug also tend to suppress most movements (Alessandri et al., 1989) and therefore a 'floor effect' may make the data more difficult to interpret. How much sensitivity is required to make our procedure useful in a practical sense? This is a basic issue in

measurement and one that should be addressed in the context of the potential use of the paradigm we propose.

It may be noted that Aguado et al. (1994) reported a disruption of a CTA when 25 mg/kg ketamine was presented before a CS and US pairing. Thus, under dosing parameters similar to the ones employed here, ketamine blocked CTA formation. Our data provide evidence suggesting that the rats in these studies were able to sense the CS and US at the time of presentation. This information makes more tenable Aguado's conclusion that ketamine was acting to disrupt the association between the CS and US rather than masking the sensation of the stimuli.

Thus, it may be the case that the paradigm we suggest here may be most useful as a control procedure in the context of evaluating the salience of a specific CS and US following a particular drug or toxin of interest. Based on our data we cannot say if the paradigm will, or will not, be able to detect the subtlest of changes in CS/US sensation. However, it may be quite useful in detecting the magnitude of changes in sensation that will allow it to serve as a practical control for a variety of CTA studies.

How applicable are the current data to other experiments that employ the CTA paradigm but use other CSs, taste combinations, USs and/or drugs/toxins? The SAC and LiCl stimuli used in our study were selected based on their common usage in a variety of CTA experiments (Domjan, 1993). Likewise, ketamine is a drug representative of a class of NMDA receptor blocking agents (Thompson et al., 1985) and the role of NMDA receptors in memory formation has received substantial attention in recent years (Alessandri et al., 1989; Welzel et al., 1990; Wesierska et al., 1990; Weldon et al., 1997). Thus, the data presented here may be generalizable to a number of other studies utilizing similar gustatory, malaise-producing and NMDA-receptor blocking substances (Aguado et al., 1994; Welzel et al., 1990; Yasoshima et al., 2000).

Would our paradigm be useful in testing tastes more-complex than SAC? Although the data presented here do not speak directly to this issue, information from other laboratories suggests that responses to taste mixtures are usually highly correlated with the response to single-component stimuli in the mixture (Travers and Smith, 1984). Human subjects usually have no difficulty in analyzing the components present in taste mixtures (for review see Travers and Smith (1984)). Likewise, animal psychophysical studies indicate that mixtures of gustatory stimuli evoke responses similar to the component tastes (Theodore, 1977; Nowlis and Frank, 1977). The neural mechanisms that subserve complex tastes and their component parts may also be similar (Travers and Smith, 1984). Additional studies will determine if the methods proposed here are equally effective in determining how drugs or toxins modulate the sensation of complex as well as simple tastes. It should be recognized, however, that the extent to which the paradigm reported here is applicable to *different* tastes, taste combinations, US doses or other drugs/toxins has not been established as of this writing.

Within the fields of behavioral neuroscience and neurotoxicology, it will continue to be important to determine the extent to which disruption of a CTA, or other classically conditioned responses, may be interpreted as a disruption of sensation vs a disruption in CS–US association. Typically, the finding that a drug or toxin blocks the formation of a CTA is taken as an indicator that that substance impairs the ability of the animal to make the CS–US association (for example, Fenu et al., 2001; Concannon and Freda, 1980; Weldon et al., 1997; Risinger et al., 1999; Yasoshima et al., 2000). Before this conclusion can be made with confidence, it is important to examine the extent to which the substance under investigation alters sensation of the CS and US (Kunin et al., 2001). The methods employed here may be useful to investigators attempting to discover the extent to which drugs or toxins modulate the experiencing of taste CSs or malaise-inducing USs typically used in CTA paradigms.

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