

## Peripheral vasopressin accelerates extinction of conditioned taste avoidance

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Received 19 December 2003; received in revised form 6 October 2004; accepted 8 November 2004

### Abstract

Both peripheral and central administration of vasopressin improves retention and delays extinction when given before or after acquisition of shock avoidance learning. For conditioned taste avoidance, however, vasopressin prolongs extinction when injected peripherally before acquisition tests and accelerates extinction when infused intracerebroventricularly after acquisition. The following experiments were designed to determine whether this inconsistency is based on the route of administration or timing of vasopressin treatment. Because acquisition of conditioned taste avoidance is strengthened when an agent that is capable of inducing avoidance is administered after LiCl injection, it was determined in experiment 1 that a 6  $\mu\text{g}/\text{kg}$  dose of vasopressin did not induce conditioned taste avoidance when administered 50 min after consumption of a sucrose solution. In experiment 2, it was determined that this dose of vasopressin accelerated extinction of a LiCl-induced conditioned taste avoidance when given 50 min after LiCl injection. These results suggest that the inconsistency is not based on route of administration. In experiment 3, it was determined that there was a tendency for animals to show prolonged extinction when vasopressin was administered 20 min before access to a sucrose solution. All of the results taken together suggest that the differential effects of vasopressin on extinction are due to the timing of administration. It was suggested that vasopressin accelerates extinction when given after acquisition by reducing the effectiveness of LiCl and it prolongs extinction when given before acquisition by altering neural responsiveness in areas mediating conditioned taste avoidance.

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*Keywords:* Arginine vasopressin; Conditioned taste aversion

### 1. Introduction

The neuropeptide, arginine vasopressin, has been shown to facilitate the retention and prolong the display of learned behaviors (see Refs. [1,2]). Although a variety of tasks have been used to study the effects of vasopressin on memory (e.g., sexually and appetitively motivated tasks), shock avoidance has been used most frequently. The enhancing effect of vasopressin in shock avoidance has been demonstrated despite variations within certain parameters, such as route of administration

and timing of the injection. Firstly, peripheral, as well as, central administration of vasopressin results in a prolonged display of learned behaviors. Animals treated with subcutaneous injections, intracerebroventricular infusions, or intracerebral infusions of vasopressin continue to exhibit shock avoidance behaviors when such a display is no longer seen by their saline-injected controls [3–17]. Secondly, the timing of the treatment with respect to before or after acquisition or extinction trials does not appear to be critical. Animals receiving injections before or after acquisition or the first post-acquisition testing session of a one-trial step-through passive shock avoidance task retain the learned behavior longer than controls [3–8]. In shuttle box and pole-jumping shock avoidance tasks, animals maintain a higher level of response during

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extinction testing when vasopressin is administered before or after the first or last acquisition trial, after the first extinction trial, or throughout extinction testing [6,10,12–15].

Although most of the studies have focused on shock avoidance tasks, the effects of vasopressin on conditioned taste avoidance also have been examined. Some of the results for conditioned taste avoidance have been similar to what has been found for shock avoidance. Vawter and Green [18] reported that the vasopressin analogue, desglycinamide lysine vasopressin (DG-LVP) increases resistance to the extinction of the avoidance behavior when given subcutaneously 1 h before each of the three acquisition or eight extinction trials, or before all of the acquisition and extinction trials. In a study by Cooper et al. [19], lysine vasopressin prolonged extinction in both old and young animals when subcutaneous injections occurred shortly before every fourth extinction test. However, a recent study using central injections of vasopressin reported contradictory results. In this study, infusion of arginine vasopressin into the lateral ventricle shortly after the acquisition test produced an accelerated extinction [20].

A number of suggestions focusing on the differences in methodologies used in the studies finding prolonged extinction and that finding accelerated extinction have been made [20]. Two notable differences are the route of administration and the timing of administration. The studies finding prolonged extinction used peripheral administration of vasopressin before the acquisition and/or extinction tests while the study finding accelerated extinction used central administration after the acquisition test. Peripheral administration of vasopressin increases blood pressure, decreases heart rate, inhibits gastric emptying, disrupts spontaneous locomotor activity, and suppresses eating in food-deprived animals [4,21–25]. Central administration of vasopressin also increases peripheral blood pressure [25–31]. However, the effects of central administration of vasopressin on more extensive peripheral changes have not been consistently reported, possibly reflecting a sensitivity to location of infusion [32–34]. Thus, it is conceivable that peripheral and intracerebroventricular administration of vasopressin produce a different array of physiological changes, which in turn differentially alters extinction.

Although the presence of vasopressin before as opposed to after a learning session does not differentially affect behavior in a shock avoidance learning situation, there is some evidence that it does in other learning paradigms. In the learned submission paradigm, peripheral administration of vasopressin produced either impairment or enhancement of a submissive behavior (i.e., defensive upright posture upon contact) depending on when during acquisition training treatment occurred. Peripheral injections of vasopressin before training resulted in the display of less submissive behavior during retention testing than saline injections. However, when peripheral treatment occurred immediately after training, vasopressin-treated animals displayed an

increase in the frequency of the submissive behavior compared to their saline-treated counterparts [35]. This raises the possibility that the differential effect of vasopressin on extinction of conditioned taste avoidance is due to timing of administration.

The following experiments were designed to determine whether the route and/or timing of administration influences the type of effect vasopressin has on extinction of conditioned taste avoidance. To test whether these factors would accelerate or prolong extinction of taste avoidance, experiments were conducted to compliment previous findings by using peripheral administration of vasopressin shortly after the acquisition test (experiment 2) and central administration before the acquisition test (experiment 3). If route of administration is the critical factor, then one would expect to find prolonged extinction in experiment 2 and accelerated extinction in experiment 3. On the other hand, if timing of administration is the critical factor, then one would expect to find accelerated extinction in experiment 2 and prolonged extinction in experiment 3. Before conducting these experiments, it was important to verify that the dose of vasopressin used could not itself induce a conditioned taste avoidance. Agents that are capable of inducing a conditioned taste avoidance can weaken acquisition of a conditioned taste avoidance induced by another agent when administered before acquisition and strengthen acquisition when administered after acquisition [36–38]. An intracerebroventricular dose of vasopressin that does not induce conditioned taste avoidance has already been identified [20]. Therefore, experiment 1 was designed to identify a peripheral dose of vasopressin that does not induce a conditioned taste avoidance.

## 2. General methods

### 2.1. Subjects

Male Sprague–Dawley rats (Simonsen Laboratory, Gilroy, CA), which weighed approximately 300 g at the beginning of the experiments, were used in this study. They were housed in pairs in cages that measured 58×38 cm and had a solid bottom covered with wood chips. A stainless steel divider was placed in the middle of each cage to separate each pair of rats. The vivarium in which the rats were housed was temperature- (21–22 °C), humidity- (51%), and light-controlled (a 12:12-h light:dark cycle with lights on at 1000 h and lights off at 2200 h). The rats were allowed at least 1 week to adapt to their living conditions before the experiments were initiated. Rats had ad libitum access to rat chow and tap water before behavioral testing was initiated. During conditioned taste avoidance testing, water was available 23 h a day. In previous studies, we found that rats given similar drinking schedules are essentially nondeprived [39–42]. The experiments were conducted according to the standards set by the National

Institutes of Health Guide for the Care and Use of Laboratory Animals (DHEW Publication 80-23, Revised 1985, Office of Science and Health Reports, DRR/NIH, Bethesda, MD) and the institutional guidelines of the University of Southern California.

## 2.2. Surgery

Before surgery, rats were anesthetized using sodium pentobarbital (60 mg/kg i.p.). After being secured in a stereotaxic instrument, a 20-gauge, stainless steel guide cannula with its dummy cannula (Plastics One) was placed 0.5 mm above the rat's right lateral ventricle and anchored to the skull using stainless steel screws and dental cement. The coordinates were: anterior–posterior (AP)  $-0.6$  mm, medial–lateral (ML)  $+1.3$  mm, and dorsal–ventral (DV)  $-4.0$  mm [43]. The analgesic buprenex and the antibiotic dualcillin (Western Medical, Arcadia, CA), were administered subcutaneously in the doses of 0.02 and 0.05 ml, respectively, to each rat the day of and the day following surgery. Animals were allowed 1 week to recover from surgery. Upon completion of the study, the localization of the tip of the cannulae was determined with an injection of methyl blue. Only animals with correct cannula placement were described in experiment 3.

## 2.3. Drugs and treatment procedure

Both LiCl and arginine vasopressin were purchased from Sigma (St. Louis, MO). LiCl was dissolved in distilled water to make a 0.15 M solution. A dose of 1.5 meq per kg of body weight (10 ml/kg of body weight) was injected intraperitoneally. The peptide was dissolved in normal saline (0.9% NaCl). Subcutaneous injections of the peptide were given in the doses of 3 and 6  $\mu\text{g}/\text{kg}$ . Control animals received an injection of saline. The injection amount for both peptide and saline was 0.2 ml. For intracerebroven-

tricular infusions, the dummy cannula was replaced with an injector cannula attached to a Hamilton syringe, via PE 50 tubing. The Hamilton syringes were mounted into a microinjector pump (CMA/100, Carnegie Medicin, Stockholm, Sweden). The pump infused the peptide in the amount of 1 ng vasopressin/0.025 ml saline/rat, with control animals receiving the same amount of the vehicle.

## 2.4. General conditioned taste avoidance procedure

The experimental testing procedure was divided into the following three periods: preconditioning, acquisition testing, and post-acquisition or extinction testing. All solutions used during testing were stored under refrigeration for 24 h before use and they were given to the rats at the beginning of the dark portion of the light/dark cycle. During preconditioning, the water bottle of each rat was replaced with one cylinder containing refrigerated tap water at the onset of the dark cycle. After 1 h, the cylinders were replaced with the regular water bottles. Preconditioning rats with cold water just after the lights switch off increases the likelihood that nondeprived rats will drink the novel sucrose solution used for conditioning. During acquisition testing, the water bottle of each rat was replaced with 1 cylinder containing a 10% (w/v) sucrose solution. One hour later, the amount of sucrose consumed was recorded, the cylinder was removed, and the regular water bottle was returned. Depending on the experiment, either vasopressin (experiment 1) or LiCl (experiments 2 and 3) was used as the conditioning agent and was injected after sucrose consumption. Post-acquisition or extinction tests were conducted in the same manner as the acquisition tests, except neither vasopressin nor LiCl was administered.

## 2.5. Statistical procedures

An alpha level of  $p < 0.05$  was used for determination of significance. For experiment 1, the amount of sucrose consumed across the two acquisition tests and the one post-acquisition test was analyzed with a two-factor (groups by tests) analysis of variance (ANOVA) with repeated measures on tests.

For experiments 2 and 3, the amount of sucrose consumed across acquisition and the first extinction test and the amount consumed across the extinction tests were analyzed with a two-factor (groups by tests) analysis of variance (ANOVA) with repeated measures on tests. In the case of significant interaction effects, this type of ANOVA also was used for paired comparisons. In addition, sucrose consumption during acquisition and extinction were compared for each group using dependent  $t$ -tests to determine when acquisition consumption levels were achieved during extinction. Because vasopressin influences the completion of extinction rather than the initiation of extinction, a two-factor (groups by tests) ANOVA with repeated measures on tests also was used to analyze sucrose consumption across the first three extinction tests [18,20].

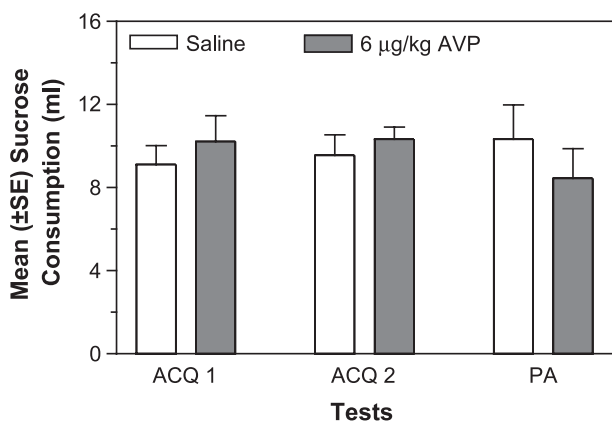


Fig. 1. Mean ( $\pm$ S.E.) sucrose consumption (ml) during two acquisition tests (ACQ 1–ACQ 2) and one post-acquisition test (PA) for rats injected with saline or 6  $\mu\text{g}/\text{kg}$  of vasopressin (AVP). On each acquisition day, injections were made subcutaneously 50 min after access to a sucrose solution. No significant differences were found.

### 3. Experiment 1

In our previous study, vasopressin successfully accelerated extinction of a LiCl-induced conditioned taste avoidance when it was infused intracerebroventricularly 50 min after LiCl injection. The dose of vasopressin that was infused could not itself induce conditioned taste avoidance when paired twice with a novel sucrose solution in the absence of LiCl [20]. The issue of whether vasopressin can induce conditioned taste avoidance is important because the avoidance induced by one agent can be strengthened by administration of a second agent that is capable of inducing conditioned taste avoidance [36,44–46]. In addition, a stronger conditioned taste avoidance is produced when two separate doses of 1.5 meq/kg LiCl are injected immediately and 35 or 70 min after sucrose consumption than when a single dose of 3.0 meq/kg LiCl is injected immediately after consumption [47]. Taken together, these results suggest that a dose of vasopressin capable of inducing conditioned taste avoidance would prolong extinction of a LiCl-induced conditioned taste avoidance when administered 50 min after LiCl injection.

It has been demonstrated that when a 6  $\mu\text{g}/\text{kg}$  dose of vasopressin is administered peripherally, it does not induce a conditioned taste avoidance after two pairings with a taste solution [48]. In this experiment, male Wistar rats were given daily access to a sucrose solution for 8 days before pairing vasopressin with consumption of a sweetened milk solution. It is well established that even a single preexposure to a novel taste solution before pairing it with an aversive agent can weaken avoidance learning [49,50]. It is possible that the multiple preexposures to the sweet sucrose solution reduced the novelty of the sweet milk solution thereby compromising the ability of the rats to acquire a conditioned taste avoidance. Additionally, there are strain differences in the strength of conditioned taste avoidance induced by different doses of an agent [39]. Therefore, the following experiment was designed to verify that a 6  $\mu\text{g}/\text{kg}$  dose of vasopressin does not induce a conditioned taste avoidance when Sprague–Dawley male rats are not given preexposure to any sweet taste solutions.

#### 3.1. Method

Eighteen male rats were randomly assigned to one of two groups ( $n=9$  per group): injections of saline or 6  $\mu\text{g}/\text{kg}$  AVP. The rats were injected with saline or vasopressin 50 min after access to a sucrose solution for 1 h. A total of two acquisition tests and one post-acquisition test were given. Each test was given every other day.

#### 3.2. Results

Injections of 6  $\mu\text{g}/\text{kg}$  of vasopressin did not significantly reduce sucrose consumption when administered 50 min after the drinking period (see Fig. 1). The two groups did

not differ in the amount of sucrose consumed or in the change in consumption across the two acquisition tests and the one post-acquisition test ( $F(1,16)=0.00$ ,  $p=1.00$  for group main effect and  $F(2,32)=1.32$ ,  $p=0.28$  for interaction effect).

#### 3.3. Discussion

Even after two pairings, peripheral administration of 6  $\mu\text{g}/\text{kg}$  of vasopressin did not induce taste avoidance. This suggests that the failure to find a conditioned taste avoidance in previous work was not due to a latent inhibition effect [48]. Additionally, it suggests that any effect this dose of vasopressin might have on extinction is unlikely to be due to its aversive properties.

### 4. Experiment 2

Previous studies looking at the effects of vasopressin on extinction of LiCl-induced conditioned taste avoidance have reported a prolongation of extinction when peripheral injections of the neuropeptide are given before acquisition and an acceleration of extinction when central infusions are given after acquisition [18–20]. The following experiment was designed to determine whether this differential effect is due to the difference in route of administration by giving peripheral injections of vasopressin shortly after pairing a novel sucrose solution with LiCl.

#### 4.1. Method

Forty-eight males were randomly assigned to one of six groups: injection of saline ( $n=6$ ), 3  $\mu\text{g}/\text{kg}$  AVP ( $n=9$ ), or 6  $\mu\text{g}/\text{kg}$  AVP ( $n=9$ ) given 25 or 50 min after the LiCl injection on acquisition day. All of the rats were given one acquisition test and nine extinction tests. They were injected immediately with LiCl and then 25 or 50 min later, they received an injection of vasopressin (3 or 6  $\mu\text{g}/\text{kg}$ ) or saline. Starting 2 days later, the rats were given nine extinction test per day for 9 days. These tests were conducted in the same manner as the acquisition tests except LiCl and vasopressin were not administered.

#### 4.2. Results

Vasopressin treatment accelerated extinction and this effect was dependent on the dose and the timing of administration. Extinction was not affected when 3 and 6  $\mu\text{g}/\text{kg}$  of vasopressin were injected 25 min after acquisition (see Fig. 2). Sucrose consumption decreased across acquisition tests and the first extinction test in all males and the extent of the decrease was similar for all three groups ( $F(1,21)=183.71$ ,  $p<0.001$  for tests main effect and  $F(2,21)=1.86$ ,  $p=0.18$  for interaction effect). Sucrose consumption increased across the extinction tests and the

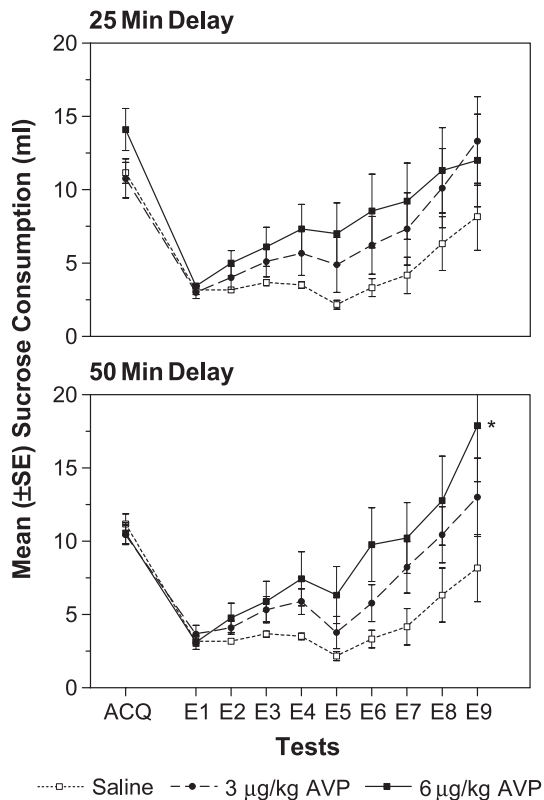


Fig. 2. Mean ( $\pm$ S.E.) amount of sucrose consumed during acquisition (ACQ) and extinction (E1–E9) of a conditioned taste avoidance. Intraperitoneal injections of 10 ml/kg of 0.15 M LiCl were given immediately after consumption of a sucrose solution on acquisition day and subcutaneous injections of saline or 3 or 6  $\mu$ g/kg of arginine vasopressin (AVP) were given 25 or 50 min after consumption. \*Significantly faster increase in sucrose consumption than the saline group during extinction,  $p=0.01$ .

amount of sucrose consumed and the extent of the increase was similar for all of the groups ( $F(8,168)=13.96$ ,  $p<0.001$  for tests main effect,  $F(2,21)=1.13$ ,  $p=0.34$  for group main effect, and  $F(16, 168)=0.76$ ,  $p=0.72$  for interaction effect).

Extinction was significantly accelerated in the rats injected with 6  $\mu$ g/kg of vasopressin 50 min after LiCl and the 3  $\mu$ g/kg dose had a marginal effect. Sucrose consumption decreased across acquisition tests and the first extinction test in all males and the extent of the decrease was similar for all three groups ( $F(1,21)=205.48$ ,  $p<0.001$  for tests main effect;  $F(2,21)=0.45$ ,  $p=0.64$  for interaction effect). Sucrose consumption increased across extinction in all of the groups but they differed in the rate of increase ( $F(8,168)=23.21$ ,  $p<0.001$  for tests main effect and  $F(16, 168)=1.77$ ,  $p=0.04$  for interaction effect). Paired comparisons revealed that the rate of increase in sucrose consumption was significantly faster in the animals treated with 6  $\mu$ g/kg vasopressin than those injected with saline across all nine extinction tests ( $F(8,104)=2.86$ ,  $p=0.01$  for interaction effect), but not across the first three extinction tests ( $F(2,26)=2.25$ ,  $p=0.126$  for interaction effect). The rate of increase in sucrose consumption for the rats injected with 3  $\mu$ g/kg vasopressin fell in between that of the 6  $\mu$ g/kg

and saline groups; it did not differ from either group ( $F(8,128)=1.15$ ,  $p=0.34$  and  $F(8,104)=1.21$ ,  $p=0.30$ , respectively, for interaction effects). Sucrose consumption reached acquisition levels during the fourth extinction test for the 6  $\mu$ g/kg group, the seventh extinction test for the 3  $\mu$ g/kg group, and the ninth extinction test for the saline group ( $t(9)=1.88$ ,  $p=0.10$ ,  $t(9)=1.23$ ,  $p=0.25$ , and  $t(6)=1.20$ ,  $p=0.28$ , respectively).

#### 4.3. Discussion

Peripheral administration of vasopressin accelerated extinction of conditioned taste avoidance when given 50 min after LiCl injection. This result is similar to our previous finding. Intracerebroventricular infusion of vasopressin 50 min after LiCl also accelerated extinction [20]. Thus, for this kind of effect, the route of administration is not a critical factor.

The accelerated extinction cannot be attributed to any delayed effect that vasopressin has on sucrose consumption. The results of experiment 1 and a previous experiment indicate that when vasopressin is administered peripherally or intracerebroventricularly 50 min after consumption of a sucrose solution, it has no effect on sucrose consumption 2 days later [20]. Immediate effects of vasopressin on sweet solution consumption also have not been found. Peripheral administration of vasopressin 1 h before consumption of a sodium saccharin solution has no effect on subsequent consumption levels [18].

Although the 6  $\mu$ g/kg dose of vasopressin was effective in accelerating extinction, the 3  $\mu$ g/kg dose had only a marginal effect. DeWied et al. [4] demonstrated that for retention of one-trial step-through shock avoidance, the ED50 in male Wistar rats was 3.56–4.07  $\mu$ g/kg. This suggests that those animals assigned the 3  $\mu$ g/kg dose of vasopressin in the conditioned taste avoidance study may not have received enough of the peptide to produce a robust effect. The fact that the rate of increase in sucrose consumption for the rats injected with 3  $\mu$ g/kg vasopressin fell in between that of the 6  $\mu$ g/kg and saline groups supports this suggestion.

#### 5. Experiment 3

The results of experiment 2 suggest that route of administration cannot account for the differential effects of vasopressin on extinction of LiCl-induced conditioned taste avoidance [18–20]. As indicated in the introduction, another difference between the study finding accelerated extinction and those reporting prolonged extinction is the timing of administration. In those studies reporting a prolonging effect of vasopressin on extinction, vasopressin was administered before acquisition training and in the studies finding accelerated extinction, vasopressin was administered after acquisition [18–20]. This experiment was designed to determine whether timing of administration can account for the differential effects by

administering vasopressin centrally before acquisition. If this is a critical factor, central administration of vasopressin before acquisition should result in a prolonged extinction, similar to the prolonging effect of vasopressin reported in previous studies using subcutaneous injection [18,19].

### 5.1. Method

All of the animals were implanted with a cannula aimed just above the right lateral ventricle. Thirteen males were randomly assigned to one of two groups: infusion of saline ( $n=8$ ) or 1 ng AVP ( $n=6$ ). The rats received one acquisition and seven extinction tests. Twenty minutes after receiving an infusion of vasopressin into the lateral ventricle on acquisition day, the animals were given access to a sucrose solution for 1 h, followed by an i.p. injection of LiCl. Starting 2 days later, the rats were given one extinction test per day for a total of 7 days. These tests were conducted in the same manner as the acquisition tests except LiCl and vasopressin were not administered.

### 5.2. Results

Histological examination of the brains confirmed that the tips of the cannulae were located within the lateral ventricle. The ranges for the locations of placements were as follows: the anterior–posterior range was +0.48 to –0.80 mm for vasopressin and +0.70 to –0.40 mm for saline and the medial–lateral range was 0.60 to 1.4 mm for vasopressin and 0.60 to 1.3 mm for saline.

Vasopressin did not affect the rate of extinction of a LiCl-induced conditioned taste avoidance (see Fig. 3). Sucrose

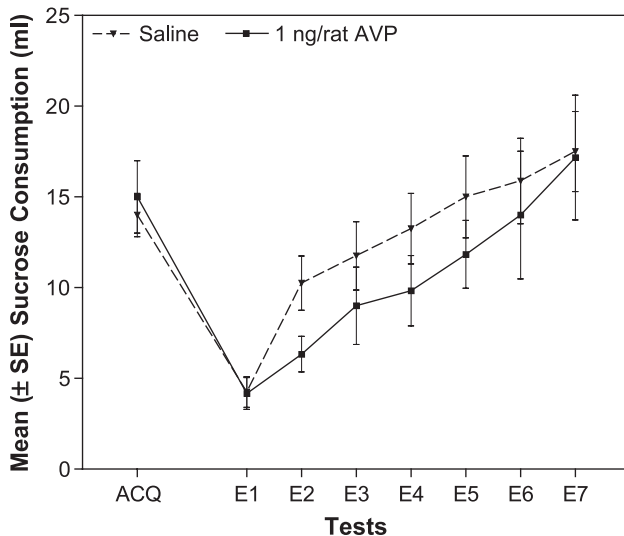


Fig. 3. Mean ( $\pm$ S.E.) amount of sucrose consumed during acquisition (ACQ) and extinction (E1–E7) for rats given an intracerebroventricular infusion of saline or vasopressin (AVP, 1 ng/rat) 20 min before the acquisition test, which consisted of access to a sucrose solution followed immediately with intraperitoneal injections of 10 ml/kg of 0.15 M LiCl. No significant differences were found.

consumption for all animals in both groups decreased across acquisition and the first extinction test and the extent of the decrease was similar for both groups ( $F(1,12)=14.69, p<0.01$  for tests main effect;  $F(1,12)=3.24, p=0.10$  for interaction effect). Both the vasopressin and saline groups increased their consumption of sucrose across the first three and all seven extinction tests ( $F(2,24)=5.09$  and  $F(6,72)=16.38, p<0.001$  for tests main effects). The two groups did not differ in the amount of sucrose consumed and the extent of increase in consumption;  $F(1,12)=0.86-2.20, p=0.16-0.37$  for groups main effect;  $F(2,24)=0.16$  and  $F(6,72)=0.41, p=0.85-0.87$  for interaction effect). Sucrose consumption reached acquisition levels during the second extinction test for the saline group and the third extinction test for the vasopressin group ( $t(7)=1.58, p=0.16$  and  $t(5)=1.60, p=0.17$ , respectively).

### 5.3. Discussion

Central administration of vasopressin had no effect on extinction of a LiCl-induced conditioned taste avoidance when given before acquisition. Although one previous study reported prolonged extinction of conditioned taste avoidance when vasopressin was given before acquisition, another study failed to find an effect [18,19]. In that study, young fluid deprived male rats did not show a significant reduction in saccharin consumption or a slower extinction rate when they were injected with lysine vasopressin before acquisition of a LiCl-induced conditioned taste avoidance [19]. However, there was a tendency for these males to consume less saccharin. There also was a tendency for the males in experiment 3 to consume less sucrose. The vasopressin-treated males took 1 day longer to reach acquisition levels than the saline-treated males. In addition, the mean sucrose consumption was lower for the vasopressin-treated males across most of the extinction tests and the difference between the vasopressin and saline males approached significance on the second extinction test ( $t(12)=2.05, p=0.06$ ).

It is unclear why some studies have found prolonged extinction when vasopressin is administered before acquisition and others have found no effect. The study finding prolonged extinction used fluid deprived Wistar male rats, DG-LVP, a one-bottle test, a saccharin solution, LiCl, three acquisition tests, and an 80-min interval between administration of vasopressin and injection of LiCl. The studies finding no effect used fluid deprived and nondeprived Sprague–Dawley male rats, lysine vasopressin and arginine vasopressin, a two-bottle test, a saccharin and sucrose solution, LiCl, one acquisition test, and an 80- and 90-min interval between administration of vasopressin and injection of LiCl. It is possible that the methodological differences in strain of rat, form of vasopressin, type of bottle test, or number of acquisition tests could account for the differences in the effect of vasopressin. There is some evidence that animal strain is a factor that can influence the effect of vasopressin in learning tasks. Different strains of mice are differentially affected by vasopressin in a two-way shuttle

box task. Vasopressin accelerates acquisition in one strain of mice, disrupts acquisition in a second strain, and produces no effect in four other strains [51]. In the conditioned taste avoidance studies, the same study that failed to find a reduction in saccharin consumption during extinction in young fluid deprived Sprague–Dawley males after one acquisition test did find a reduction in old fluid deprived Sprague–Dawley males after one acquisition test, although rate of extinction was not significantly prolonged in either the young or the old males [19]. This suggests that differences in methodology might not account for the differences in the effects of vasopressin when administered before acquisition. However, it is possible that more reliable effects of vasopressin on extinction are found when Wistar rather than Sprague–Dawley rats are used.

In the majority of studies that have been conducted, vasopressin facilitates the ability of animals to maintain a learned shock avoidance, as measured by retention and extinction tests [3–17]. However, contradictory results have been obtained for this learning task as well [52,53]. In these studies, assessment of the performance of individual animals, which were tested under identical methodological conditions, has revealed striking differences in the effect of vasopressin on their ability to maintain a shock avoidance. For most animals, vasopressin facilitated maintenance but for some, it disrupted maintenance. In the present experiment, only one of the eight saline animals (12%) took more than 5 days to reach acquisition day consumption levels, four of the six vasopressin-treated animals (67%) took more than 5 days. A chi-square analysis of these data reveals that this difference is significant ( $\chi^2(1)=4.38$ ,  $p=0.036$ ). This suggests that vasopressin prolonged extinction of a conditioned taste avoidance for most of the animals.

Sahgal and his colleagues [52,53] have suggested that vasopressin heightens arousal and that the initial arousal state of the animal may determine the kind of effect vasopressin will have. If the initial arousal levels are low, vasopressin will enhance maintenance and if they are high, it will disrupt maintenance. Other investigators have proposed that inconsistencies are simply a reflection of genetic variation in the substrate upon which vasopressin acts [51,54–56]. For example, inconsistencies in the effect of vasopressin on maintenance of shock avoidance behavior in Brattleboro rats, which are virtually void of endogenous vasopressin, have been tied to genetic drift between the different breeding colonies [56]. At this time, one can only conjecture as to whether arousal state, genetic variation, or some other factor accounts for the failure to reliably obtain prolonged extinction when vasopressin is administered before acquisition of a conditioned taste avoidance.

## 6. General discussion

Previous studies have found that vasopressin has differential effects on extinction of a LiCl-induced conditioned

taste avoidance. It prolongs extinction when it is administered peripherally before acquisition of the avoidance and it accelerates extinction when it is administered centrally after acquisition [18–20]. In the present experiments, it was found that central administration of vasopressin before acquisition of a LiCl-induced conditioned taste avoidance had no significant effect on extinction, although there was a tendency for a majority of the animals to show prolonged extinction. Peripheral administration of vasopressin after acquisition of a LiCl-induced conditioned taste avoidance accelerated extinction. All of the results taken together suggest that administering vasopressin after acquisition of a LiCl-induced conditioned taste avoidance is critical for producing an acceleration of extinction and that the differential effects of vasopressin on extinction is due to the timing of administration.

Other hormones also have been shown to have differential effects on extinction of conditioned taste avoidance depending on when they are present [36–38]. For example, estradiol accelerates extinction of a LiCl-induced conditioned taste avoidance when it is continuously present a week before acquisition or a week before extinction but it prolongs extinction when it is continuously present during extinction [36–38]. The evidence supports the hypothesis that the differential effects of estradiol can be accounted for by one mechanism, the aversive properties of estradiol. Agents with aversive properties can produce differential effects when administered at different times during the acquisition and extinction processes and those effects are almost entirely consistent with what has been found for estradiol. However, it is unlikely that the differential effect of vasopressin is due to any aversive properties of this peptide. First, the doses of arginine vasopressin and DG-LVP that were administered before or after acquisition do not themselves induce conditioned taste avoidance [20,21,57,58]. Second, when an aversive agent is administered just before acquisition of a conditioned taste avoidance induced by the same agent, the conditioned taste avoidance is weakened [59–61]. Additionally when an aversive agent is administered after acquisition of a conditioned taste avoidance induced by the same agent or another aversive agent, the conditioned taste avoidance is strengthened [62,63]. These results are opposite to those found when vasopressin is administered before or after acquisition of a LiCl-induced conditioned taste avoidance.

In the preceding discussions, an assumption was made that it is the presence of vasopressin before or after acquisition that is the critical timing factor. But another timing possibility should be taken into consideration when attempting to explain the modulatory effects of vasopressin on extinction. The effects of LiCl gradually increase in intensity, peak approximately 30 min after injection, and remain elevated for 0.5–1.5 h before slowly dissipating [64,65]. Some of the behavioral effects of LiCl, such as decreased ingestive and increased aversive taste reactions to a novel sucrose solution, have been observed 15 and 20 min,

respectively, after injection [66]. It has been suggested that the acquisition process begins at this time and it probably continues at least as long as LiCl is producing aversive stimulus effects. This means that when vasopressin is given before the acquisition test it is present during the initial stages of the acquisition process but when it is given 50 min after the acquisition test it is present during the later stages of the acquisition process. The critical timing factor, then, may not be whether vasopressin is present before or after acquisition but whether it is present during the early or late stages of the acquisition process. There is evidence in other learning paradigms that vasopressin can differentially modulate learned behaviors depending on whether it is present during the initial or later stages of the learning process [67]. When vasopressin was infused into the ventral hippocampus after the first learning session of an appetitive visual discrimination task, performance was slightly delayed during subsequent testing, but when infused after the second learning session, performance was enhanced.

As indicated in the discussion of experiment 3, it has been suggested that vasopressin influences learning and memory processes by acting on arousal systems in the brain [52,53]. In the present studies, it is possible that vasopressin given before acquisition produced optimal levels of arousal for mnemonic processing, which led to increased salience of the taste stimulus or experimental context and thus facilitated the acquisition process and prolonged extinction. On the other hand, exogenous vasopressin administered after acquisition, combined with the stress-evoked endogenous release of vasopressin [68–70], could have led to heightened levels of arousal that disrupted mnemonic processing and resulted in an acceleration of extinction.

Instead of acting indirectly on neural areas mediating conditioned taste avoidance via an arousal system, however, it may be that vasopressin acts directly on these areas. We had previously suggested that when vasopressin is present after LiCl injection, it acts on the acquisition process by reducing the illness-inducing effects produced by LiCl and thereby accelerates extinction [20]. Vasopressin can reduce illness responses produced by other agents. The cytokine interleukin-1 (IL-1) reduces social exploration of conspecific juveniles by adult animals, a behavioral response that is very sensitive to illness-inducing agents [71]. Infusion of vasopressin into the lateral ventricle attenuates these responses while infusion of a vasopressin antagonist exacerbates them. In the conditioned taste avoidance studies that administered vasopressin after acquisition, vasopressin accelerated the completion and not the initiation of extinction [20]. Recent work in our lab indicates that a similar pattern is found when comparisons are made between two different doses of LiCl (10 and 20 ml/kg of a 0.15 M solution) [72]. The weaker dose accelerated the completion but not the initiation of extinction. These results lend support to the hypothesis that vasopressin effectively reduces the dose of LiCl. Vasopressin treatment could alter the illness inducing effects of LiCl by reducing the neural

response to the aversive properties of LiCl. The brain entry route critical for acquisition of conditioned taste avoidance induced by LiCl is the area postrema [73–77] and V<sub>1</sub> receptors have been identified in this circumventricular structure [78–82].

There is some evidence that vasopressin stimulates neural activity in forebrain areas mediating conditioned taste avoidance. In studies looking at fos-like protein immunoreactivity in unconditioned mice after intracerebroventricular infusion of vasopressin, expression was found in the basolateral amygdala, an area essential for acquisition of conditioned taste avoidance [83,84]. This expression was time-dependent and peaked as soon as 15–120 min after infusion. Vasopressin receptors have been found in different nuclei in the amygdala (e.g., medial and central), but there are no reports of finding receptors in the basolateral nucleus [85,86]. This suggests that activation of this area is achieved indirectly. This indirect action could occur via arousal areas or other areas mediating conditioned taste avoidance. In either case, these findings support the possibility that vasopressin treatment before acquisition could result in changes in neurophysiology, e.g., neurotransmitter release, that alter any subsequent processing of taste-illness information. Future research will need to assess the importance of the area postrema and basolateral amygdala in the modulatory effects of vasopressin on extinction of conditioned taste avoidance.

### Acknowledgements

The research described in this article was part of a doctoral dissertation submitted by UnJa L. Hayes to the University of Southern California. It was supported by BNRF and Sigma Xi GIAR.

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