

Vasopressin content in select brain regions during extinction of a conditioned taste aversion

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ABSTRACT: Previous studies have shown that low levels of vasopressin during extinction of conditioned taste avoidance are associated with a faster extinction, that fluid deprivation differentially alters vasopressin levels in various neural areas, and that extinction of conditioned taste avoidance is accelerated in fluid deprived male rats. The following study was designed to identify areas of the brain in which vasopressin levels are different in fluid deprived and nondeprived males during extinction of conditioned taste avoidance. Arginine vasopressin content was determined by radioimmunoassay in the paraventricular nucleus (PVN), medial amygdala (AMe), bed nucleus of the stria terminalis (BNST), nucleus tractus solitarius (NTS), medial septum (MS), lateral septum (LS), and insular cortex (IC) of unconditioned nondeprived males and conditioned males that were maintained on a 23-h fluid deprivation schedule or that were nondeprived. Vasopressin content in the PVN of deprived and nondeprived males differed during extinction. Based on comparisons with unconditioned nondeprived males, this difference was due to an elevation in the vasopressin content of the nondeprived but not the deprived males. These results raise the possibility that a vasopressinergic system in the PVN plays a critical role in the differential extinction rate of fluid deprived and nondeprived males, which will need to be verified by manipulating vasopressin levels in this brain site during extinction of a conditioned taste avoidance.

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KEY WORDS: Brain vasopressin content, Conditioned taste avoidance, Extinction, Fluid deprivation.

INTRODUCTION

A conditioned taste aversion is acquired when ingestion of a novel substance is paired with the experience of illness and the animal associates the malaise with consumption of the novel substance. Learning is expressed as both avoidance of the food, indicated by reduction of consumption, and a shift in taste palatability, indicated by decreases in ingestive and increases in aversive orofacial responses [54]. The acquisition of taste avoidance in rats is robustly learned after just one trial, even when the time interval between the stimuli is minutes to hours, and the association can be maintained for an extended period of time, i.e., from weeks to months [35]. However, the avoidance can be extinguished eventually after

the rat has had repeated exposure to the substance without further malaise [35].

A number of factors have been found to influence the time it takes for an animal to extinguish a conditioned taste avoidance [19,20,23]. The state of fluid hydration is one factor that strongly modulates extinction in male rats. In previous studies, extinction was markedly shorter in deprived male rats compared to nondeprived male rats [13,22,61,63]. In these studies, the rats were fluid deprived for 22–23 h each day and the behavioral tests were of the two-bottle type, i.e., the rats were always given water in addition to the test solution during the 1–2 h daily test period. Therefore, the fast extinction in fluid deprived males was not a forced extinction due to thirst. In addition, fluid deprivation directly affects an extinction process. When male rats are fluid deprived during extinction but not during acquisition, extinction is accelerated [61].

A primary hormone involved in the maintenance of fluid balance is arginine vasopressin [73]. An initial physiological response to fluid imbalance, either by fluid restriction or salt loading, is increased synthesis and release of vasopressin from the posterior pituitary [58,79]. In addition to peripheral responses to fluid imbalance, there are changes in vasopressin levels in specific regions of the brain [31,33,44,53,79]. The direction of the changes in the vasopressin content of microdissected brain regions of fluid deprived rats differs depending on the region measured and the length of deprivation.

Not only is vasopressin a primary hormone in fluid homeostasis, this neuropeptide has been shown to increase resistance to extinction in a wide array of behavioral paradigms that range from passive avoidance behaviors to positively motivated tasks [3,29,30,68]. Of particular relevance to the study presented here is the modulation of extinction of conditioned taste avoidance by vasopressin. Systemic administration of vasopressin or a vasopressin agonist increases the length of time an animal takes to extinguish the conditioned taste avoidance [26,71]. The presence of these agents during extinction alone is sufficient to prolong extinction, i.e., these agents do not have to be present during acquisition to be effective [26,71]. Blocking vasopressinergic transmission has the opposite effects on extinction. Chronic central administration of a vasopressin antagonist significantly accelerates extinction [10]. Also, there is a difference in the extinction rate of male rats with a normally functioning vasopressinergic system (Long–Evans) and those that are essentially

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devoid of vasopressin due to a genetic defect in the gene coding for vasopressin (Brattleboro). The extinction rate of Brattleboro rats is faster than that of Long-Evans rats [11].

Thus, the evidence shows that (1) a decrease in vasopressinergic transmission in some area of the brain is associated with accelerated extinction of conditioned taste avoidance, (2) fluid deprivation accelerates extinction of conditioned taste avoidance, and (3) fluid deprivation alters vasopressin content in some areas of the brain. Taken together, these results raise the possibility that fluid deprivation accelerates extinction of conditioned taste avoidance by lowering vasopressinergic transmission in the specific areas of the brain that modulate extinction. As an initial step in testing this hypothesis, we conducted a study to identify areas of the brain in which fluid deprived and nondeprived males have different levels of vasopressin during extinction of conditioned taste avoidance.

There are a number of different brain regions that could be possible candidates for a vasopressinergic modulation of conditioned taste avoidance extinction. In this study, we focused on seven brain regions known to be part of the neural circuitry for conditioned taste aversions and/or to be part of the neural vasopressinergic system: the paraventricular nucleus (PVN), medial amygdala (AMe), bed nucleus of the stria terminalis (BNST), nucleus tractus solitarius (NTS), medial septum (MS), lateral septum (LS), and insular cortex (IC). There is evidence that the BNST, MS, and IC are involved in extinction of conditioned taste aversions but any involvement of the other brain areas remains unclear [24,47,48,59,64,75–78]. The PVN, AMe, and BNST are vasopressin synthesis sites, the NTS, MS, and LS are known target sites of a synthesis site, and low levels of vasopressin are present in the cortex [14,16,27,31–34,50,57,66,67,69]. This experiment, then, was designed to determine whether the vasopressin content of fluid deprived and nondeprived males would differ in at least one of these seven discrete areas of the brain during extinction of a conditioned taste avoidance.

METHODS

Husbandry

The subjects were Fischer 344 male rats (Simonsen Laboratories, Gilroy, CA). All animals were allowed *ad lib.* access to laboratory rat chow throughout the experiment. Before the start of the experiment, all animals were maintained on *ad lib.* water but during the experimental test period, half of the animals were given *ad lib.* access to water and the other half were maintained on a daily 23-h fluid deprivation schedule. The animals were kept on a 12 h/12 h light/dark cycle throughout the experiment and they were housed four per cage in solid bottom cages (58 cm × 38 cm) that had wood chips as bedding material. Just prior to behavioral tests, the animals were removed in pairs and placed in adjacent test cages that were similar to their home cage. A divider was placed in the test cages to separate each pair during the test period. The behavioral test began at the beginning of the dark portion of the light/dark cycle.

Experimental Design

The rats were randomly assigned to one of six groups with 12 animals per group: these groups included two fluid balance states, nondeprived or fluid deprived, by three behavioral states (see Table 1). For each behavioral state, a nondeprived animal was paired with a deprived animal and the two animals were terminated by decapitation within minutes of each other and in random order. The first behavioral state was selected to ascertain the effect of acute fluid deprivation compared to nondeprivation conditions on vasopressin content. The deprived animals were terminated after one 23-h deprivation period and their designated nondeprived

TABLE 1

FLUID BALANCE STATE (FLUID DEPRIVED AND NONDEPRIVED) AND BEHAVIORAL STATE (NO-CTA, EXT-TEST-1, AND EXT-DAY) OF THE SIX GROUPS OF MALE RATS

	Fluid Deprived	Nondeprived
No-CTA	One 23-h deprivation period No conditioning	<i>Ad lib.</i> water No conditioning
Ext-Test-1	Ten 23-h deprivation periods One conditioning test One extinction test	<i>Ad lib.</i> water One conditioning test One extinction test
Ext-Day	>Ten 23-h deprivation periods One conditioning test >One extinction test	<i>Ad lib.</i> Water One conditioning test >One extinction test

Ext, extinction.

pairs were terminated at the same time. Neither group had been exposed to any behavioral tests, i.e., no acquisition of conditioned taste avoidance (No-CTA), at the time of termination. Because the unconditioned nondeprived males were on *ad lib.* food and water, had been adapted to their housing environment for 2 weeks, had been handled only for the purposes of cage cleaning, and had not been exposed to any conditioning procedures, it is reasonable to assume that the vasopressin levels in these males are representative of a homeostatic state. The second behavioral state was selected to determine the effect of acquisition of a strong avoidance in nondeprived males and a prolonged deprivation schedule (10 days) plus acquisition of a strong avoidance in deprived males. The behavioral experience for both deprived and nondeprived males included 7 days of preconditioning, acquisition of the conditioned taste avoidance, and one extinction test (Ext-Test-1). Animals were terminated 23 h after the extinction test. The third behavioral state was selected to determine vasopressin levels when the deprived animals were in the process of extinguishing their avoidance. The deprived animals and the paired nondeprived animals were terminated 23 h after the avoidance was at least 50% extinguished in the deprived animals (Ext-Day). Extinction was operationally defined as that time when an animal drank 100% of the amount of sucrose consumed during their acquisition test.

Conditioned Taste Avoidance Procedure

The experimental testing procedure was divided into the following five periods: preconditioning (one test per day on days 1–7), acquisition (one test on day 8), post-acquisition period (no test on day 9), first extinction test (one test on day 10), and remaining extinction tests (one test per day starting on day 11). All fluids used during testing were stored under refrigeration for 24 h before use. In the deprived No-CTA group, the water bottles were removed from the home cage 23 h before termination. The nondeprived No-CTA group was terminated at the same time but they remained on *ad lib.* water. For the remaining deprived groups, access to water was available only during each 1-h test period until termination but water was available *ad lib.* for the remaining nondeprived groups. At the beginning of each test period, the water bottles of the nondeprived animals were replaced with two fluid-filled cylinders and after each test period, the cylinders were replaced with the water bottles. During preconditioning, all the animals in the Ext-Test-1 and Ext-Day groups were adapted to general handling and testing procedures. Two cylinders containing tap water were given to these animals for 1 h and the amount consumed was recorded. On acquisition day, each rat was given one cylinder containing tap water and another cylinder containing a 10% (w/v)

sucrose solution for 1 h. The amount of water and sucrose consumed was recorded and then all rats were injected immediately with a LiCl solution (0.15 M, 10 ml/kg of body weight). Two days after acquisition, the first extinction test was given. The procedure was identical to that used for acquisition except no LiCl injections were given. Just prior to the second extinction test, the animals in the Ext-Test-1 groups were terminated. The animals in the Ext-Day groups continued to get daily extinction tests until the deprived rat of each pair of deprived and nondeprived rats began to consume at least 50% of its acquisition test consumption levels or until 28 extinction tests had been given.

Dissection and Biochemical Procedures

The animals were terminated by decapitation and the brains were removed immediately with the optic fiber tract carefully severed to protect the structural integrity of the ventral hypothalamic region. The brains then were frozen on powdered dry ice and stored at -70°C .

A complete posterior–anterior series of thick (300 μm) coronal sections were taken for micropunches using a cryostat microtome. Sectioning began at the brain stem and extended to the anterior commissure. The posterior–anterior range in millimeter, according to Paxinos and Watson [55], chosen for the punches for each brain region were as follows: PVN, $-2100/-1800$; AMe, $-3600/-2600$ (± 200); BNST, $-920/-260$ (± 150); NTS, $-14,300/-12,600$ (± 600); MS, $200/900$ ($\pm 100/300$); LS, $-400/900$ ($\pm 100/300$); IC, $-1400/200$ (± 200). The cryostat was maintained at -4°C in order to obtain optimal sections of both 300 and 50 μm thickness. Thin sections (50 μm) were taken, where appropriate, for histological staining with cresyl violet. These sections were used to identify and precisely localize the brain nuclei to be micropunched. A 0.5 or 1 mm (depending upon the brain region) stainless steel cannula fitted with a stylet was used for micropunches taken from the 300 μm sections. The micropunches were stored at -70°C until processed for assay.

Brain tissue from each micropunch was homogenized in 1 ml of hot 0.25% acetic acid using a probe sonicator. The homogenate was immediately placed in a boiling water bath for 2 min then removed and chilled on ice. The homogenate was titrated to neutral pH with NaOH and centrifuged at $10,000 \times g$ for 15 min at 4°C . The supernatant, which contained peptides isolated from the homogenate, was frozen at -70°C then lyophilized on a rotoevaporator vacuum and stored at -70°C . The pellet was stored at -70°C until protein content was determined later by the Lowry method [46].

Estimates of vasopressin recovery were made by adding a known amount of [^{125}I]-labeled arginine vasopressin ([^{125}I]-AVP) to samples of cortical micropunches. Recovery samples were handled and processed in a similar manner as the experimental samples. The percent of total radioactivity measured in the supernatant fraction following extraction indicated a 91% (10% peptide) recovery.

Vasopressin content was quantified by a highly sensitive radioimmunoassay. The antibody was freeze-dried rabbit antiserum to Arg8-vasopressin, which showed 50% cross-reactivity on a molar basis with lysine vasopressin and no reaction with oxytocin, vasotocin or any other peptides (Calbiochem). The assay buffer consisted of 60 mM sodium phosphate buffer (60 mM monobasic and 60 mM dibasic, anhydrous) containing 10 mM EDTA and 0.3% bovine serum albumin (BSA), pH 7.3. It was imperative that the buffer was brought to the proper pH after EDTA was added but before BSA was added. A standard curve was constructed by serial dilution (15–0.01 fmol) of a known amount of synthetic vasopressin (Peninsula Laboratories). The lyophilized tissue extracts were reconstituted in 3% NaOH. A 100 μl aliquot of [^{125}I]-AVP (Dupont, NEN; specific activity, 2200 Ci/mmol) was added to all

assay tubes to achieve approximately 1600–1800 cpm (1–2 fmol) per tube. The antiserum was reconstituted in 50 ml assay buffer (1:50,000 final titer) and 100 μl was added to each assay tube. Additional buffer was added to all vials to achieve a total assay volume of 700 μl . The radioimmunoassay reaction required a 5-day incubation to achieve maximal binding. Two control tubes, one containing antiserum and [^{125}I]-AVP and the other containing only [^{125}I]-AVP, were included for estimates of maximum binding and nonspecific binding, respectively.

Bound [^{125}I]-AVP was separated from free [^{125}I]-AVP by the addition of 250 μl of a dextran-coated charcoal solution (Dextran 60, charcoal Norit-A, Serva 4–7 μM). Assay tubes were then vortexed, incubated at $0-4^{\circ}\text{C}$ for 5–10 min and centrifuged at $1500 \times g$ for 15 min (Beckman TJ6). The amount of bound [^{125}I]-AVP in the supernatant was measured in a γ counter and plotted against known amounts of labeled AVP on a plot and analyzed by linear regression. AVP levels were expressed as pmol AVP/ μg protein for the PVN or fmol AVP/ μg protein for all other brain regions. The intraassay coefficient of variability was 1.6%.

Statistical Analyses

An α level of $p < 0.05$ was used for determination of significance in all statistical tests. Two separate two-factor (groups \times tests) ANOVA with repeated measures on tests were used to analyze the amount of sucrose consumed by the Ext-Test-1 and Ext-Day groups during acquisition and the first extinction test and the amount consumed by the Ext-Day groups during nine extinction tests. For vasopressin content, data were obtained from seven brain regions in all groups. The following data were excluded from analysis. Vasopressin data from deprived Ext-Day males that failed to extinguish the avoidance were excluded from analysis, as were data for their nondeprived Ext-Day pair. Outlier vasopressin values were determined for each brain region and were excluded from analysis [74]. All other exclusions were based on procedural losses during dissection, brain homogenization, vasopressin assay, or protein content determination. For each brain region, a one-factor (groups) ANOVA was used to determine group differences in vasopressin content. In the case of significant effects, Tukey HSD test for unequal n was done to determine differences among pairs of groups. Comparisons of interest were between (1) deprived and nondeprived males for each of the behavioral states, (2) the nondeprived No-CTA group (baseline vasopressin levels) and the nondeprived and deprived groups 23 h after the first extinction test (Ext-Test-1) and the last extinction test (Ext-Day), and (3) the first extinction test and the last extinction test for deprived and nondeprived groups.

RESULTS

Conditioned Taste Avoidance

Sucrose was given only to the Ext-Test-1 and Ext-Day groups. All of the deprived and nondeprived males in the Ext-Test-1 and Ext-Day groups consumed less sucrose during the first extinction test than during acquisition. The four groups did not differ in the extent of the decrease in sucrose consumption during the first extinction test [$F(3, 44) = 2.09, p = 0.11$]. The amount of sucrose consumed during the first extinction test was similar to amounts lost from passive dripping in an empty cage.

More than one extinction test was given only to the deprived and nondeprived Ext-Day groups. Two of the deprived males failed to reach at least 50% of their acquisition test consumption level by the 28th extinction test. The mean number of days it took the remaining deprived males to reach this consumption level was 20 days (with a range of 9–28 days) and the actual percentages of acquisition test consumption levels that were reached at the time

TABLE 2

MEAN \pm SE pmol OR fmol OF AVP/ μ g OF PROTEIN IN VARIOUS NEURAL AREAS OF UNCONDITIONED NONDEPRIVED AND 23-h FLUID DEPRIVED MALE RATS

	Nondeprived	Fluid Deprived
PVN (pmol)	9.62 \pm 1.41	34.67 \pm 6.12*
AMe (fmol)	8.86 \pm 2.01	3.08 \pm 0.22
BNST (fmol)	9.27 \pm 1.49	8.13 \pm 1.21
MS (fmol)	32.08 \pm 4.61	19.92 \pm 3.03
LS (fmol)	1.31 \pm 0.26	1.44 \pm 0.30
NTS (fmol)	5.44 \pm 0.71	3.46 \pm 0.41
IC (fmol)	1.03 \pm 0.39	0.55 \pm 0.05

* Significantly different than nondeprived, $p < 0.0002$.

of termination ranged from 50 to 75%. The nondeprived males were terminated at the same time as the deprived males with which they had been paired. None of the nondeprived males had begun to extinguish its avoidance at its time of termination. Analysis of sucrose consumption across nine extinction tests (the number of tests that all males received) revealed a faster rate of increase in sucrose consumption in the deprived group [$F(8, 176) = 2.16$, $p = 0.032$].

Vasopressin Content

Synthesis site: paraventricular nucleus. Twenty-three hours after the last extinction test, vasopressin content was lower in deprived males than nondeprived males. Comparisons with unconditioned nondeprived males revealed that the vasopressin levels of the deprived males after both extinction tests were similar but the levels of the nondeprived males were higher.

The six groups differed significantly in vasopressin content [$F(5, 51) = 11.04$, $p < 0.00001$]. Comparisons of the unconditioned deprived and nondeprived males showed that the deprived males had higher vasopressin content than the nondeprived males (see Table 2; $p = 0.0002$ for No-CTA). The reverse was found in comparisons of the conditioned deprived and nondeprived males; the vasopressin levels of the deprived and nondeprived groups were not different after the first extinction test but the content was lower in the deprived than the nondeprived groups after the last extinction test (see Fig. 1; $p = 0.29$ for Ext-Test-1 and $p = 0.002$ for Ext-Day). The baseline vasopressin levels of the unconditioned nondeprived group were similar to those of the conditioned deprived groups after each of the extinction tests but they were lower than those of the conditioned nondeprived groups ($p = 0.97$, 0.99 , 0.05 , and 0.01 for nondeprived No-CTA vs. deprived Ext-Test-1, deprived Ext-Day, nondeprived Ext-Test-1, and nondeprived Ext-Day, respectively). For both the conditioned deprived and nondeprived groups, the vasopressin levels after the first and last extinction tests did not differ ($p = 0.76$ in deprived and $p = 0.94$ in nondeprived for Ext-Test-1 vs. Ext-Day).

Synthesis site: medial amygdala. The deprived and nondeprived males had similar levels of vasopressin after each of the extinction tests. In addition, the vasopressin content of the unconditioned nondeprived males was similar to that of the deprived and nondeprived groups after both extinction tests.

The vasopressin levels of the six groups differed significantly [$F(5, 43) = 3.04$, $p = 0.019$]. However, the *post hoc* test failed to detect differences in the paired comparisons that were of interest. The unconditioned deprived and nondeprived males had similar levels of vasopressin (see Table 2; $p = 0.57$ for No-CTA). In the conditioned groups, the vasopressin content of the deprived and nondeprived males was similar after the first and last extinc-

tion tests (see Fig. 1; $p = 1.00$ for Ext-Test-1 and $p = 0.10$ for Ext-Day). The baseline vasopressin levels of the unconditioned nondeprived group were similar to those of both the conditioned deprived and nondeprived groups after each of the extinction tests ($p = 0.92$, 0.92 , 0.89 , and 0.50 for nondeprived No-CTA vs. deprived Ext-Test-1, deprived Ext-Day, nondeprived Ext-Test-1, and nondeprived Ext-Day, respectively). For both the conditioned deprived and nondeprived groups, vasopressin content after the two extinction tests was similar ($p = 1.0$ in deprived and $p = 0.09$ in nondeprived for Ext-Test-1 vs. Ext-Day).

Synthesis site: bed nucleus of the stria terminalis. The deprived and nondeprived males had similar levels of vasopressin after each of the extinction tests. However, the vasopressin levels of the unconditioned nondeprived males were lower than those of both the deprived and nondeprived conditioned groups after the last extinction test.

The vasopressin content of the six groups differed significantly [$F(5, 53) = 6.69$, $p = 0.00007$]. The unconditioned deprived and nondeprived males had similar levels of vasopressin (see Table 2; $p = 0.73$ for No-CTA). In the conditioned groups, the vasopressin content of the deprived and nondeprived males was similar after both extinction tests (see Fig. 1; $p = 1.0$ for Ext-Test-1 and $p = 0.84$ for Ext-Day). The baseline vasopressin levels of the unconditioned nondeprived group were lower than those of both the conditioned deprived and nondeprived groups after the last extinction test but not after the first extinction test ($p = 0.03$, 0.001 , 0.16 , and 0.29 for nondeprived No-CTA vs. deprived Ext-Day, nondeprived Ext-Day, deprived Ext-Test-1, and nondeprived Ext-Test-1, respectively). The vasopressin levels after the first and last extinction tests did not differ for both deprived and nondeprived males ($p = 0.96$ in deprived and $p = 0.18$ in nondeprived for Ext-Test-1 vs. Ext-Day).

Target site: nucleus tractus solitarius. The deprived and nondeprived males had similar levels of vasopressin after each of the extinction tests. In addition, the vasopressin content of the unconditioned nondeprived males was similar to that of the deprived and nondeprived groups after both extinction tests.

The six groups differed significantly in vasopressin levels [$F(5, 56) = 5.11$, $p = 0.0006$]. However, the *post hoc* test failed to detect differences in the paired comparisons of interest. The unconditioned deprived and nondeprived males had similar levels of vasopressin (see Table 2; $p = 0.60$ for No-CTA). In the conditioned groups, the vasopressin content of the deprived and nondeprived males was similar after both extinction tests (see Fig. 2; $p = 1.0$ for Ext-Test-1 and $p = 0.88$ for Ext-Day). The baseline vasopressin levels of the unconditioned nondeprived group were similar to the levels of the conditioned deprived and nondeprived males after the first and last extinction tests ($p = 1.0$, 0.09 , 1.0 , and 0.56 for nondeprived No-CTA vs. deprived Ext-Test-1, deprived Ext-Day, nondeprived Ext-Test-1, and nondeprived Ext-Day, respectively). For the conditioned deprived and nondeprived groups, vasopressin content after the two extinction tests was similar ($p = 0.19$ in deprived and $p = 0.57$ in nondeprived for Ext-Test-1 vs. Ext-Day).

Target site: medial septum. The deprived and nondeprived males had similar levels of vasopressin after each of the extinction tests. In addition, the vasopressin content of the unconditioned nondeprived males was similar to that of the deprived and nondeprived groups after both extinction tests.

The vasopressin content of the six groups differed significantly [$F(5, 41) = 4.19$, $p = 0.004$]. The unconditioned deprived and nondeprived males had similar levels of vasopressin (see Table 2; $p = 0.11$ for No-CTA). In the conditioned groups, the vasopressin content of the deprived and nondeprived males was similar after both extinction tests (see Fig. 1; $p = 1.0$ for Ext-Test-1 and

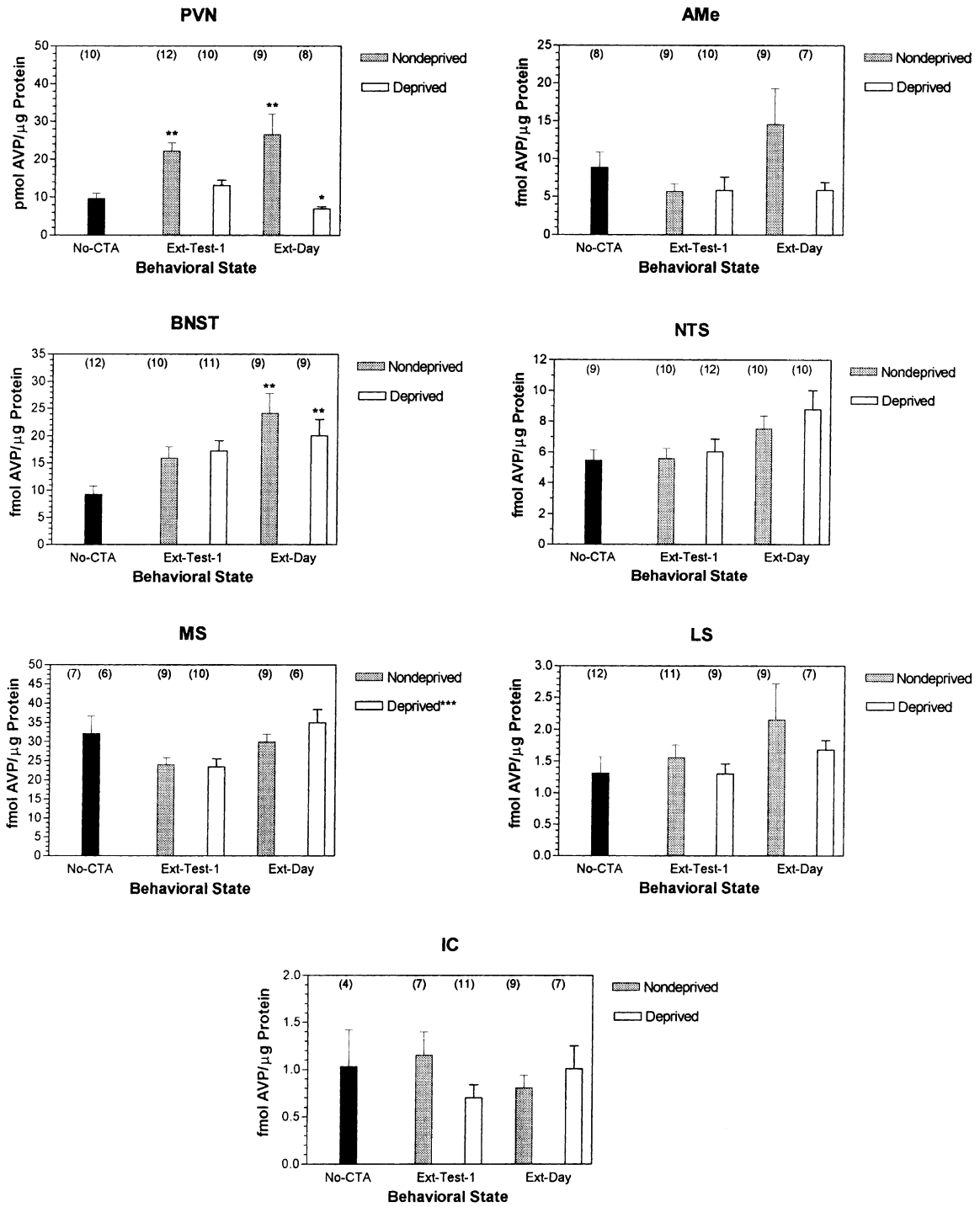


FIG. 1. Mean (\pm SE) pmol of AVP/ μ g of protein in the PVN and fmol AVP/ μ g of protein in the AMe, BNST, NTS, MS, LS, and IC of unconditioned nondeprived males (No-CTA) and of conditioned nondeprived and deprived males terminated 23 h after the first extinction test (Ext-Test-1). In the Ext-Day groups, each nondeprived male was paired with a deprived male and each pair was terminated 23 h after the deprived male of the pair began to consume at least 50% of the amount of sucrose solution consumed on acquisition day. None of the nondeprived Ext-Day males had begun extinguishing its conditioned taste avoidance at the time of termination. The number of animals in a particular group is indicated in parentheses above the bar for that group. (*) Significant difference between Ext-Day deprived and Ext-Day nondeprived males, $p \leq 0.05$. (**) Significantly different from nondeprived No-CTA males, $p \leq 0.05$. (***) Significant difference between Ext-Test-1 deprived and Ext-Day deprived males, $p < 0.05$.

$p = 0.88$ for Ext-Day). The baseline vasopressin levels of the unconditioned nondeprived males were similar to those of the conditioned deprived and nondeprived groups after both extinction tests ($p = 0.43, 0.99, 0.50,$ and 1.0 for nondeprived No-CTA vs. deprived Ext-Test-1, deprived Ext-Day, nondeprived Ext-Test-1, and nondeprived Ext-Day, respectively). For the conditioned nondeprived groups, the vasopressin content after the two extinction tests did not differ ($p = 0.79$ for Ext-Test-1 vs. Ext-Day) but for the conditioned deprived groups, the vasopressin content was higher after the last extinction test ($p = 0.04$ for Ext-Test-1 vs. Ext-Day).

Target site: lateral septum. The vasopressin content of the six groups did not differ significantly [$F(5, 52) = 1.13, p = 0.35$; see Table 2 and Fig. 1].

Low vasopressin site: insular cortex. The vasopressin content in the IC was measurable, albeit near the limit of detection, and no significant differences were found [$F(5, 35) = 0.71, p = 0.62$; see Table 2 and Fig. 1].

DISCUSSION

This study yielded a number of significant results. First, after one 23-h period of fluid deprivation, vasopressin content was higher in the PVN of fluid deprived unconditioned males than nondeprived unconditioned males. Second, the vasopressin content in the BNST of both the fluid deprived and nondeprived males was elevated during extinction when compared to the homeostatic levels of the unconditioned nondeprived males. Third, comparisons between fluid deprived and nondeprived males revealed differences in vasopressin content in the PVN during extinction. Based on comparisons with unconditioned nondeprived males, this difference was due to elevations in the vasopressin content of the nondeprived but not the deprived males. Finally, there was a faster rate of increase in sucrose consumption in fluid deprived males than nondeprived males during extinction.

23-h Fluid Deprivation Effects

After 23 h of fluid deprivation, the vasopressin content in the PVN of the unconditioned fluid deprived males was higher than that of the unconditioned nondeprived males. Vasopressin content in the PVN has not been examined after 23 h of fluid deprivation in previous work. However, induction of *c-fos*-like immunoreactivity, an anatomical marker of neuronal activity, has been observed in this nucleus after 5, 16, and 24 h of fluid deprivation [51]. In addition, plasma levels of vasopressin are elevated after 24 h of fluid deprivation [49]. Taken together, these data suggest that the activation of cells projecting to the neurohypophysis and releasing vasopressin into the general circulation are the source of at least some of the elevated vasopressin content in the unconditioned fluid deprived males.

After conditioning and extinction testing, the vasopressin levels of fluid deprived males were not elevated after conditioning and one extinction test. The levels of these males were significantly lower than those of the unconditioned fluid deprived males ($p < 0.0002$) and they were not different than the homeostatic levels of the unconditioned nondeprived males. Whether this change is due to prolonged exposure to a 23-h fluid deprivation schedule, conditioning and extinction experiences, or an interaction of the two will need to be addressed in future studies. But clearly, levels of vasopressin after 23 h of fluid deprivation cannot be used as an indicator of what levels will be after exposure to these experiences.

Vasopressin Content in Bed Nucleus of the Stria Terminalis

Fluid deprived and nondeprived males did not differ in vasopressin content in the BNST during extinction. Thus, if a vasopressin mechanism mediates the extinction rate difference between

fluid deprived and nondeprived males, the site of action of this mechanism is not this neural structure. However, for both deprived and nondeprived males, vasopressin levels in the BNST were higher after the last extinction test when compared to the homeostatic levels of unconditioned nondeprived males.

There are a number of possibilities that can be generated to account for this effect. First, because the unconditioned males had not been exposed to sucrose solution prior to vasopressin measurement, it is possible that the elevated levels in the conditioned males are a simple response to exposure to the sucrose solution. However, if this were the case, then one would expect significant elevations in vasopressin after the first extinction test, and the elevations were not significant. In addition, vasopressin measurements were made 23 h after exposure to sucrose during the extinction tests. Therefore, a simple exposure to sucrose hypothesis would require either prolonged elevation of vasopressin after exposure or delayed elevation.

A second possibility is that the elevated vasopressin levels represent a simple reaction to the LiCl administration; the unconditioned animals were not exposed to LiCl. Although the effects of LiCl on brain vasopressin levels are unknown, LiCl administration does cause systemic elevations in vasopressin levels [72]. LiCl is neither protein bound nor metabolized by any means and its biological role is determined by its bioavailable concentration [60]. Plasma levels of LiCl are negligible 24 h after administration of the same dose used in the present study and brain levels are negligible 48 h after [52]. In the present study, vasopressin content was measured 3 days (Ext-Test-1) or longer (Ext-Day) after LiCl administration, which suggests that LiCl is no longer present in the general circulatory system or the brain. Thus, a simple exposure to LiCl hypothesis would require prolonged elevation of vasopressin after LiCl is no longer present. If this were the case, then one would expect significant elevations in vasopressin after the first extinction test, and the elevations were not significant.

A third possibility is that the elevation in vasopressin is an anticipatory response based on a circadian rhythm. These animals had been adapted for 10 days (Ext-Test-1) or longer (Ext-Day) to a schedule in which they were given chilled fluids every 24 h. The vasopressin levels were measured 23 h after the last exposure, which was 1 h before the next scheduled exposure. It is well known that animals display anticipatory responses based on a circadian rhythm of drinking or feeding [4–6]. The neural sites of the pacemaker and its targets for this circadian rhythm remain unknown, although the pacemaker probably is not the suprachiasmatic nucleus [7].

A final possibility is that the elevated vasopressin levels are a marker of the extinction process or are in some way involved in the continued expression of taste avoidance during extinction. Both deprived and nondeprived males showed a complete avoidance during the first extinction test and although the degree of avoidance differed, both the deprived and nondeprived males still exhibited taste avoidance during the last extinction test given before termination. The nondeprived males had not begun extinguishing and the deprived males were still in the process of extinguishing. Ibotenic lesions of the BNST or the stria terminalis before acquisition of conditioned taste avoidance do not affect the ability of a rat to acquire an avoidance, but electrolytic lesions after acquisition abolish the acquired avoidance [75,76]. This suggests that this area is important for retention or retrieval of conditioned taste avoidance and as such is probably involved in the extinction process.

Vasopressin Content in Medial Septum, Lateral Septum, Insular Cortex, and Medial Amygdala

The MS, LS, and IC are clearly involved in extinction of conditioned taste avoidance. Lesions of the MS or LS facilitate extinction of conditioned taste avoidance [64] and extinction is faster

in animals with ibotenic lesions of the gustatory IC than controls [75,77]. In the present study, however, no differences in the vasopressin content of any of these areas were found between deprived and nondeprived males during extinction. Thus, if a vasopressin mechanism mediates the extinction rate difference between fluid deprived and nondeprived males, the site of action of this mechanism is probably not the MS, LS, and IC. It should be noted that the low vasopressin levels found in the IC are consistent with previous reports of the cortex in general [31,33].

Given that the amygdala and its major target, the ventral hippocampus, are brain structures long known to be involved in memory processes [8,9,16,25,28,42], it is reasonable to suggest that it could be an obligatory component to maintenance of a learned association such as occurs in the conditioned taste avoidance paradigm. Although the involvement of the AMe in extinction of conditioned taste avoidance remains relatively unexplored, there is evidence that the hippocampus is involved. Excitotoxic lesions of the whole hippocampus do not affect the ability of an animal to acquire taste avoidance, but they do increase the rate at which an animal extinguishes the avoidance [78]. It is worth noting that although the Tukey *post hoc* test failed to reveal a significant difference in the AMe of deprived and nondeprived males during extinction, less conservative tests did (LSD and Newman-Keuls, $p < 0.02$). Because the statistical power of the sample analyzed was low, further investigation of the contribution of the vasopressinergic neurons in the AMe to the extinction process is warranted.

Vasopressin Content in Paraventricular Nucleus

Vasopressin levels were higher in the PVN of nondeprived males than deprived males after the last extinction test. When compared to the homeostatic vasopressin levels of the conditioned nondeprived males, this difference was based on an elevation in the levels of the conditioned nondeprived males. After both the first and last extinction tests, the levels were higher in the conditioned nondeprived males than the unconditioned nondeprived males and the levels of the conditioned deprived males were at baseline.

These results raise a number of issues that will need to be addressed in future studies. The first issue is focused on the stimulus conditions that induced the higher vasopressin levels in the nondeprived males during extinction as compared to the unconditioned nondeprived males. Regardless of the reason for the higher vasopressin levels in the conditioned nondeprived males, however, fluid deprivation prevented vasopressin levels from being elevated during extinction.

A second issue is whether the difference between deprived and nondeprived males is causally connected to the differences found in extinction of a conditioned taste avoidance. Although the nondeprived males clearly had higher vasopressin levels than the deprived males after the last extinction test, the results for the data after the first extinction test were ambiguous. The vasopressin levels of the deprived males fell in between those of the unconditioned nondeprived males and the conditioned nondeprived males; they were not different than either of these groups and these two groups differed from one another. A failure to find a significant difference between deprived and nondeprived males after the first extinction test does not present a problem for an hypothesis suggesting a causal relationship between vasopressin levels in the PVN and extinction of conditioned taste avoidance. In most of the experiments examining the effects of vasopressin or fluid deprivation on extinction of conditioned taste avoidance, vasopressin, vasopressin antagonist, or fluid deprivation treatments were present during all extinction tests [10,13,22,61,63,71]. In one study, however, the first of several vasopressin injections was given prior to the fifth extinction test and extinction was prolonged [26]. At that time, the animals had not initiated extinction. This demonstrates that vasopressin levels

do not have to be elevated from the time of the first extinction test in order for prolonged extinction to be observed; a slower extinction can occur if vasopressin levels are elevated at any time prior to initiation of extinction.

It should be stressed that the difference in the vasopressin content of the conditioned deprived and nondeprived males after the last extinction test cannot be due to the fact that the deprived animals had nearly extinguished the avoidance and none of the nondeprived animals had even begun extinguishing. If this were the case, one would expect to see a difference in vasopressin levels between the first extinction test and the last extinction test in deprived males but not in nondeprived males. However, for both nondeprived and deprived males, the differences between the first and last extinction tests were not significant.

Administration of a vasopressin antagonist in the lateral ventricle of male rats has been shown to produce more rapid extinction when compared to saline treated males [10]. This strongly suggests that taste avoidance extinction is modulated by alterations in vasopressinergic transmission at some neural site. Future work that manipulates vasopressin levels in the PVN will need to be conducted to establish whether the PVN is that site. This is especially critical because the involvement of the PVN in conditioned taste avoidance remains unexplored. It is also unclear whether the elevated vasopressin levels in conditioned nondeprived males represent an increase in vasopressin release and vasopressinergic transmission. The interpretation of increases in vasopressin content is not straightforward and does not necessarily reflect increased release [45].

A final issue is focused on the neural system (or systems) within the PVN that is responsible for the differences in vasopressin levels between deprived and nondeprived males during extinction. The projection sites of the different vasopressin-producing cells in the PVN vary. These sites include the neurohypophysis from which vasopressin is released into the general circulation, the median eminence, and the brain stem [1,70]. One established target in the brainstem is the NTS, which reciprocally communicates back to the PVN [14,27,41]. There is evidence to suggest that the NTS plays a role in the extinction process. The pattern of activity of sucrose-best neurons in response to a sweet taste changes after rats acquire an aversion to this taste so that the activity pattern more closely resembles that of bitter tastes [24]. Also, after acquisition of a conditioned taste avoidance, *c-fos*-like-immunoreactivity is induced in the intermediate and caudal areas when animals are first exposed to the conditioned sweet solution, but after extinction there is little or no induction in these areas [39,59]. Although extinction has been examined after lesions in the NTS, only three tests were given and neither the sham nor lesioned animals showed any evidence of initiating extinction during these tests [36]. In the present study, vasopressin content in deprived and nondeprived males differed in the PVN, but they did not differ in the NTS. This suggests that the vasopressin-producing cells in the PVN that project to the NTS are not involved in the differences in vasopressin content between nondeprived and deprived males. The role of other paraventricular projection sites remains to be determined.

Behavioral Extinction

Extinction of conditioned taste avoidance in Long-Evans rats under high fluid deprivation (23-h schedule) is accelerated when compared to extinction under low deprivation (10-h schedule) [37]. In addition, a weaker avoidance has been reported in fluid deprived (24 h) Charles River female and male rats when compared to nondeprived rats [56]. In our laboratory, a faster extinction rate in fluid deprived (22- to 23-h schedule) as compared to nondeprived male rats has been replicated seven times in Sprague-Dawley and three times in Fischer 344 male rats [13,22,61,63]. In studies of Fischer

344 males given the same dose of LiCl as the present study, all of the deprived males ($n = 22$) and only four of the nondeprived males ($n = 4$ of 22) had extinguished their avoidance by the 28th daily extinction test. The range of extinction times for the four nondeprived males was 14–25 extinction tests [13]. Clearly, this is a strong and highly replicable effect. By the ninth daily test in the present study, significant differences in sucrose consumption were already evident.

It is unlikely that the accelerated extinction in deprived males represents weaker learning. The presence of fluid deprivation conditions during acquisition has no effect on extinction rate. When the fluid deprivation state during acquisition and extinction is varied independently, rapid extinction occurs only when animals are fluid deprived during extinction [61]. These results are consistent with the hypothesis that extinction is not just a reflection of the acquisition process and with the evidence that the neural areas mediating acquisition and extinction of conditioned taste avoidance are different [2,15,20,40,78].

In previous papers, we suggested that fluid deprivation accelerates extinction of a conditioned taste avoidance by decreasing testosterone availability through a reduction in serum testosterone levels [13,19,22,23,61,63]. There is a considerable amount of evidence to support this hypothesis in Sprague–Dawley male rats [17,18,21–23,61–63]. Circulating testosterone, however, does not play a modulatory role in the taste avoidance extinction of deprived Fischer 344 males. Fluid deprivation does not decrease testosterone levels and although exogenous testosterone treatment can prolong extinction in fluid deprived males, it takes extremely high doses to do so [12,13]. This suggests that for fluid deprived Fischer 344 male rats, there is a testosterone-independent mechanism that modulates extinction. A similar mechanism also may exist in Sprague–Dawley rats along with the testosterone-dependent mechanism. When circulating levels of testosterone are controlled *via* exogenous treatment in gonadectomized Sprague–Dawley males, testosterone is less effective in prolonging extinction in fluid deprived males than nondeprived males even though testosterone levels are similar [22]. The data from the present study raise the possibility that the PVN is part of a testosterone-independent mechanism that modulates extinction in fluid deprived rats. Although androgen receptors are found in the PVN, the integrity of the vasopressinergic systems is not dependent on testosterone [28,38,65].

CONCLUSION

Our study was designed to identify differences in neural vasopressin content of deprived and nondeprived male rats that, with the exception of fluid restriction, shared the same behavioral experiences. It was not designed to assess what specific aspects of the behavioral experience contribute to the differences in vasopressin. Future work will need to address this issue. It is worth noting, however, that after acquisition of a one-test passive shock avoidance task, decreases in hippocampal vasopressin have been found after the retention test [43]. This decrease has been shown to be due to acquisition of the shock-avoidance association and not to other factors tied to the learning situation. In the present study, it seems likely that (1) the elevated levels of vasopressin in the PVN of the conditioned nondeprived males during extinction are a result of the conditioning experience and subsequent extinction process and represent a facilitation of vasopressinergic transmission, and (2) fluid deprivation reduces the ability of vasopressinergic neurons in the PVN to respond normally during extinction.

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