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Sensitization of Salt Appetite Is Associated With Increased "Wanting" but Not "Liking" of a Salt Reward in the Sodium-Deplete Rat

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To examine the role of incentive sensitization in the potentiation of salt appetite by prior depletions, the authors assessed the motivation to obtain salt ("wanting") and the palatability of salt ("liking") independently in salt-sensitized rats. Breakpoint on a progressive ratio reinforcement schedule was used to measure salt wanting and taste reactivity was used to measure salt liking in rats with and without a history of Na+ depletion. Salt-sensitized rats displayed higher breakpoints relative to controls. However, a history of Na+ depletion was not associated with a greater positive shift in taste reactivity measures. The data suggest that these components of reward are separable in this model and support the general proposition that sensitization may alter wanting but not liking.

Keywords: salt appetite, sensitization, progressive ratio, taste reactivity, motivation

The incentive sensitization hypothesis posits that sensitization contributes to addiction by selectively increasing drug craving without affecting drug liking (Berridge & Robinson, 1998). This hypothesis is based on the proposition that the motivational component of reward is separable from the hedonic component (i.e., "wanting" vs. "liking"; Berridge, 1996). Drug sensitization, an increased response to a drug after repeated administrations, is typically demonstrated in animals by increased psychomotor activation (Stewart & Badiani, 1993). Tests that reflect motivational components, such as conditioned place preference (Lett, 1989) and operant self-administration (Mendrek, Blaha, & Phillips, 1998), also show sensitization. A particularly interesting indicator of motivation or incentive is *breakpoint* (BP). This is the point at which animals, responding for drug or other rewards on a progressive ratio schedule of reinforcement, cease to respond. An increase in BP or the willingness to work harder for each successive reward has also been shown to accompany drug sensitization (Lorrain, Arnold, & Vezina, 2000).

Sensitization can be studied using natural as well as drug rewards. Sodium depletion is a homeostatic challenge that provokes a strong salt appetite in rats. A rat with a salt appetite displays a strong motivation to seek and ingest NaCl and will work to obtain salt in an operant task (McCutcheon & Levy, 1971). It is important to note that salt appetite shows behavioral sensitization as evidenced by an increase in salt intake after multiple depletions (Sakai, Fine, & Epstein, 1987). In other words, rats with a prior sodium depletion show significantly greater NaCl intake after a subsequent depletion than do rats depleted for the first time. This model has similarities to drug sensitization in that both are associated with changes in dendritic arborization in nucleus accumbens (Robinson & Kolb, 1997; Roitman, Na, Anderson, Jones, & Bernstein, 2002). In addition, a history of sodium depletion produces cross-sensitization to the psychomotor as well as the rewarding effects of amphetamine (Clark & Bernstein, 2004).

The sensitization of salt appetite is an intriguing model and has certain advantages with regard to testing the incentive salience hypothesis of sensitization. At this point, the most persuasive evidence for increased wanting but not liking as a component of drug addiction comes from self-report data with addicts (Lamb et al., 1991). Animal models using drug self-administration have been limited in their ability to assess this hypothesis because of the difficulty of independently assessing wanting and liking in such models (Berridge & Robinson, 2003). When amphetamine-sensitized rats display an elevated BP, this could reflect increases in the incentive salience (wanting) or increased liking of the reward (or both). Independent assessment of wanting and liking has frequently been performed with natural rewards such as sucrose or other taste stimuli without the limitations encountered when studying drug reward (for a review, see Berridge, 1996). For instance, Berridge, Venier, and Robinson (1989) and Berridge and Robinson (1998) showed that 6-hydroxydopamine lesions, which reduce dopamine levels in the striatum and render rats temporarily aphagic, do not influence palatability during this period. Liking roughly corresponds to palatability and is often assessed using taste reactivity (TR) or the oromotor reactions to a tastant delivered directly into the mouth. Sodium depletion is characterized by the avid ingestion of salt solutions and a shift in the liking or palatability of those solutions (Berridge, Flynn, Schulkin, & Grill, 1984). The question posed in the present studies is whether animals with a sensitized salt appetite display even greater increases in their liking of salt, as reflected in their TR response, increased wanting of salt, as reflected in their BP for operant responding for salt, or both. The benefit of salt appetite as a sensitization model is that wanting and liking can be assessed independently after the induction of sensitization.

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General Method

Subjects

Male Long–Evans rats weighing between 300 and 350 g, housed individually in hanging metal cages on a 12-hr light–dark cycle, lights off at 7 p.m., were used for all experiments. Unless otherwise noted, animals were maintained with access to water and Harlan Teklad rodent chow (Madison, WI) ad libitum. All procedures were done in accordance with Institutional Animal Care and Use Committee standards at the University of Washington.

Sodium Depletion

The diuretic furosemide was used to induce salt appetite with procedures modified from Wolf (1982). Prior to sodium depletion, food and water were removed from all cages. Rats in the deplete group were then injected with furosemide (10 mg/kg sc); control animals received saline. Weights were obtained before and 3 hr after treatment to confirm diuresis. Standard chow and water were returned to control animals; deplete animals were given distilled water and sodium-deficient chow (ICN Nutritional Biochemicals, Cleveland, OH). Food and water were removed from all cages 24 hr later, and rats were given access to a 3% NaCl solution for a 1-hr intake test. After testing, standard chow and water were returned to all animals.

Experiment 1

Rats with a history of sodium depletion display a sensitized salt appetite by increasing the amount of NaCl consumed and reducing the latency to consume it relative to animals depleted for the first time. To determine whether increased intake in sensitized animals reflects a change in motivation, we subjected sodium-depleted rats responding on a progressive ratio (PR) schedule for NaCl to a BP analysis. A PR schedule requires increasing amounts of work to obtain reward across trials. Performance on PR schedules has been shown to increase in a concentration-dependent manner with sucrose and saccharin and is dose dependently decreased by dopamine antagonists (Reilly, 1999), suggesting that it is a valid measure of the motivation to obtain natural rewards.

Method

Operant conditioning. Fourteen male Long–Evans rats were trained to lever press for water in sound-attenuated operant chambers (Coulbourn Instruments, Allentown, PA). A timeline for all procedures is presented in Figure 1. Prior to the beginning of training, all animals were given a 1-hr preexposure to a 3% NaCl solution to avoid neophobia. All rats sampled the solution but consumed less than 1 μl. On Days 1 and 2, rats were maintained on a water-deprivation schedule with access to water for 30 min in the morning (9:00–9:30 a.m.) and again in the afternoon (2:00–2:30 p.m.). Operant training consisted of 30-min magazine training for 2 days, to allow rats to autoshape, followed by lever pressing on a fixed ratio schedule of 1 (FR1) of reinforcement for 30 min/day for 4 days. The schedule was then extended to an FR3 schedule for 4 days to accustom the animals to performing multiple lever presses for a reward. After this, the PR3 schedule was introduced, with the number of presses required for reward increasing by 3 during the session (lever pressing was reinforced after the first, third, sixth, and so on lever press), and repeated for 4 days. Rats were given 3 min to complete the required number of lever presses for each ratio on the schedule. The BP was defined as the number of lever presses on the last completed trial. The last completed trial is the one prior to the trial on which a rat failed to press the required number of times to receive a reward within the 3-min time limit. Groups were matched on average BP for water across the 4 days of PR3 training. Half of the rats ($n = 7$) were depleted of Na+ according to methods described previously and half ($n = 7$) received sham treatment. After one day of recovery, rats were returned to the water-deprivation schedule for 2 days and given 4 additional days of PR3 training with water. All rats were then depleted of Na+ and given access to a 3% NaCl solution on the same PR3 schedule 24 hr later.

Statistics. Independent samples t tests were used to compare BP and total lever presses during the NaCl test session. Repeated-measures paired t tests were used to assess within-group changes in BP over PR3 trials.

Results

Sodium-deplete animals lever pressed vigorously for 3% NaCl solution, a concentration that is avoided by nondeplete animals. Rates of lever pressing were comparable to those displayed by these same animals when thirsty and responding for water. BP

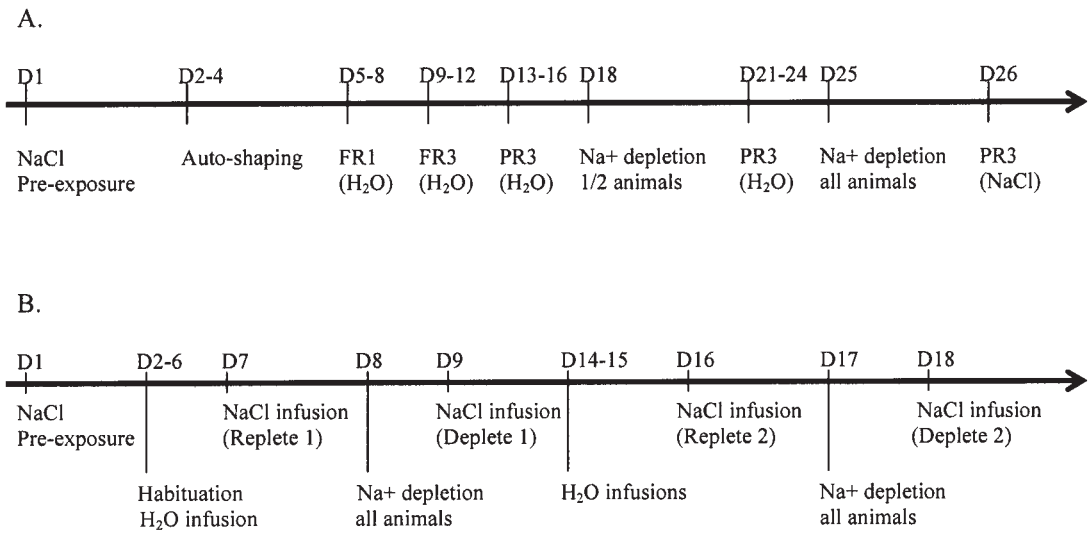

Figure 1. A: Experiment 1 timeline. B: Experiment 2 timeline. FR = fixed ratio; PR = progressive ratio.

analysis indicated that salt-sensitized rats were willing to work harder than control animals for the salt reward when subjected to a PR schedule of reinforcement. Mean BP data, the number of lever presses during the last completed trial, and total lever presses are shown in Figures 2A and 2B, respectively. Mean BP for animals with a history of sodium depletion (the history group) was significantly higher than that of controls, $t(11) = 2.63$, $p < .05$. Total lever presses during the test session were also significantly higher for the history group, $t(11) = 2.31$, $p < .05$. Additionally, this separation in BP cannot be accounted for by a general change in behavior for animals with a history of Na+ depletion. Mean BP for water during the sessions prior to depletion (38 lever presses) was not statistically different from the mean BP for water during subsequent sessions (41 lever presses), $t(6) = 0.532$, $p = .618$. Finally, the pattern of responses across trials (each ratio on the progressive schedule is considered to be an individual trial) on the NaCl test day was similar between groups until the rats approached BP. The mean time to complete the required number of lever presses on Trials 1–7 is presented in Figure 2C. Rats with a history of sodium depletion did not have a significantly shorter latency to finish a trial until the seventh trial, the point at which animals from the control group approached BP.

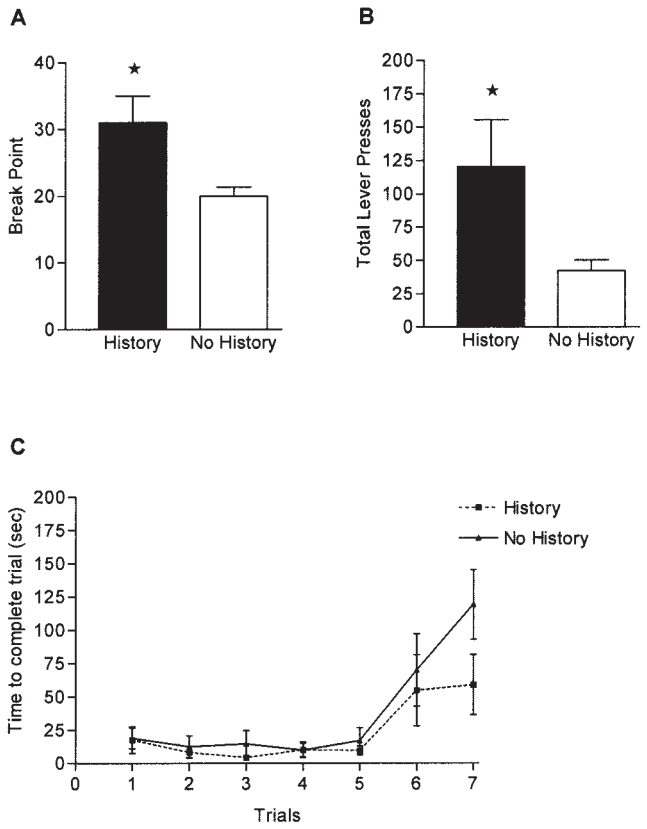

Figure 2. A: Mean (± *SEM*, indicated by the error bars) breakpoint (number of lever presses during the last completed trial) for 3% NaCl. B: Mean (± *SEM*, indicated by the error bars) total lever presses for animals with and without a history of sodium depletion. C: Mean (± *SEM*, indicated by the error bars) time to complete Trials 1–7 for both groups. The stars indicate $p < .05$ between history and no-history groups.

Experiment 2

Previous data and the results from Experiment 1 show that rats with a history of Na+ depletion not only drink more of an NaCl solution after depletion but will also work harder for it as defined by significantly higher BP on a PR schedule. It remains to be determined whether their higher BP is attributable to a change in the motivation to obtain salt, a change in the palatability of salt, or a combination of both. Previous work with the TR paradigm has shown a striking shift in palatability of highly concentrated NaCl solutions from primarily aversive to primarily ingestive when rats are depleted of Na+ (Berridge et al., 1984). Examination of data in that article failed to provide evidence that this shift was more pronounced after repeated depletions, although the study was not designed to detect such effects. To clarify this issue, in the next study, we used TR testing to determine whether positive shifts in palatability after Na+ depletion are potentiated by repeated depletions. Potentiation would suggest that sensitization of salt appetite is associated with greater palatability (liking) of salt solutions.

Method

Surgery. Five male Long–Evans rats were implanted with intraoral (I/O) cannulas under Ketamine (170 mg/kg) and Xylazine (17 mg/kg) anesthesia. The I/O cannula, made of PE-100 tubing, was inserted using a 19-gauge sharpened stainless steel probe. The placement of the cannula was anterolateral to the maxillary molar, and the cannula exited at the scapular area behind the head (Schafe, Thiele, & Bernstein, 1998). Rats were handled and their cannulas flushed daily for 5 days prior to the start of TR testing.

TR. A timeline for all procedures is presented in Figure 1. Prior to the start of TR procedures, all rats were given 1-hr access to a 3% NaCl solution to avoid neophobia. All rats sampled the solution but drank less than 1 μl. The 3% NaCl solution was chosen because rats find this concentration aversive when Na+ replete but palatable when Na+ deplete, and it was the same concentration as was used in Experiment 1. All TR procedures were performed according to methods modified from Grill, Specter, Schwartz, Kaplan, and Flynn (1987). Animals were habituated to the test chambers and infusion procedures for several days. After habituation, all rats were tested when replete and deplete by being passively infused with a 3% NaCl solution for 1 min (1 μl/min). One week later, this procedure was repeated so that rats received two NaCl sessions while sodium replete and two sessions while sodium deplete. All sessions were videotaped for analysis using a camera trained on a mirror mounted below the cylindrical chambers to capture the facial movements of the rats.

Video analysis. Videotapes from each of the four sessions were scored blind according to methods modified from Berridge et al. (1984). Each 1-min session was viewed in slow motion and normal speed twice: once to score aversive responses and a second time to score ingestive responses. Aversive responses included gapes, chin rubs, head shakes, face washing, forelimb flailing, and locomotion (as measured by rearing). Ingestive responses included rhythmic mouth movements, tongue protrusions, lateral tongue protrusions, and paw licking. Paw licks, mouth movements, tongue protrusions, and face washing were considered to be continuous events and were therefore scored in bins. If the duration of one of these behaviors was 5 s, it was scored as a single occurrence. All other behaviors were scored as discrete events. The individual episodes from each behavior type were then added up within a response category (aversive and ingestive) to create composite scores. All analyses were done on the composite scores.

Statistics. All data were analyzed with repeated-measures paired *t* tests.

Results

Rats showed the predicted shift in responses from primarily aversive during the replete state to primarily ingestive during the deplete state in both sets of tests. However, a history of Na+ depletion did not alter this pattern. Aversive responses and ingestive responses are shown in Figures 3A and 3B, respectively. Mean aversive responses during the first replete session were significantly higher than the responses in the first deplete session, $t(3) = 4.17$, $p < .05$. Alternatively, mean ingestive responses were significantly lower in the first replete session than the responses in the first deplete session, $t(3) = -4.60$, $p < .05$. The second set of tests followed the same pattern, $t(3) = 5.17$, $p < .05$, for mean aversive responses between the second replete session and the second deplete session, and $t(3) = -4.91$, $p < .05$, for mean ingestive responses between the second replete session and the second deplete session. Neither the aversive nor the ingestive responses during the second depletion test were different from those of the first test. Thus, TR testing provides no evidence that rats find 3% NaCl more palatable after a second Na+ depletion. In addition, the measures from the first replete test were not different from those of the second replete test. This finding indicates that a prior depletion does not alter the need-free palatability of highly concentrated NaCl solutions.

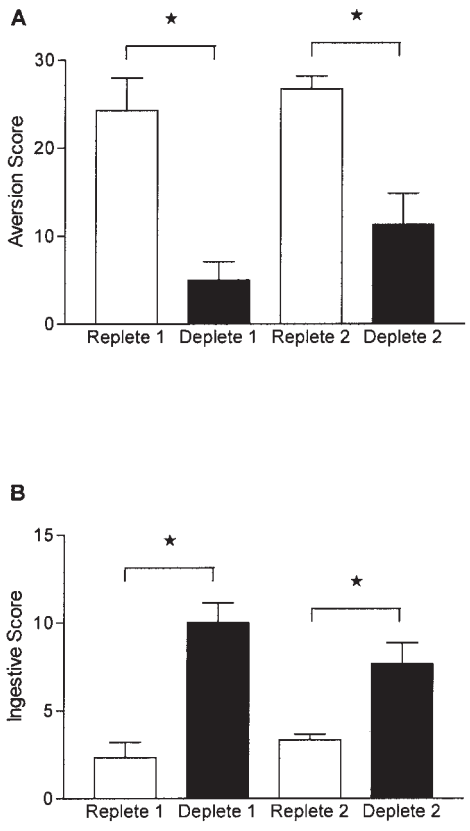

Figure 3. A. Mean (± *SEM*, indicated by the error bars) composite aversion scores. B: Mean (± *SEM*, indicated by the error bars) composite ingestive scores for each of the four test sessions. The stars indicate $p < .05$.

Discussion

A history of sodium depletion sensitizes salt appetite and cross-sensitizes the response to psychostimulants. With a BP analysis, we compared the incentive motivation for NaCl in sodium-depleted rats with and without a prior depletion history and found significantly higher BPs in the sensitized group. In contrast, with a TR analysis, we found no evidence for enhanced NaCl palatability in the sensitized animals. Thus, these studies provide support for the incentive sensitization hypothesis by demonstrating the association of enhanced wanting but not enhanced liking of salt in animals with sensitized salt appetite.

The significantly higher BPs in salt-sensitized rats are similar to those that have been described for animals self-administering drugs. Prior drug sensitization has been shown to increase mean BP for amphetamine by ≈60%–90% (Lorrain et al., 2000; Mendrek et al., 1998). A single prior episode of sodium depletion in this study increased the BP for NaCl by 65%. This extends the parallel between sensitization to artificial or drug rewards and natural rewards. Thus, both multiple sodium depletions and repeated drug administrations are capable of inducing behavioral sensitization. In addition, each has been associated with experience-dependent plasticity in nucleus accumbens, an area thought to be critical to both types of reward. Finally, sensitization appears to enhance the motivation to obtain reward in both models.

Of course, it could be argued that a higher BP for NaCl in rats with a history of sodium depletion was due in part to their learning a positive association between the taste of salt and the rewarding postingestive consequences (repletion) of NaCl ingestion after the first depletion. Such learning could enhance the incentive salience of NaCl in animals in the history group. The contribution of NaCl intake to the sensitization of salt appetite was examined by Sakai et al. (1987). They found that the experience of NaCl consumption was not necessary for the development of sensitization. Enhanced intake after the second depletion was seen whether rats were repleted with a saline injection or given the opportunity to drink NaCl after the first sodium depletion. This suggests that an association between the taste of NaCl and the postingestive consequences is not necessary for the induction of salt appetite sensitization. It should also be noted that in the present study, rats with a prior depletion history had never lever pressed for NaCl, and that both groups displayed comparable response patterns until the BP. The mean amount of time required to complete a trial and receive reward was not different between groups over the first six trials. The groups began to diverge only when rats from the control group approached BP on the seventh trial. The absence of higher initial response rates in the rats with a history of sodium depletion suggests that they were not quicker to learn these associations. However, the possibility remains that previous NaCl intake is necessary for this effect and is worthy of future examination.

The lack of evidence for enhanced NaCl palatability in salt-sensitized rats is supportive of the postulated dissociation between wanting and liking in sensitization or addiction models. Unlike those models, however, liking was measured directly and independently of wanting. One possibility is that the failure to find elevated liking was simply due to a ceiling effect in the TR responses. The mean numbers of ingestive and aversive responses found for 3% NaCl in this study are lower than those found with sucrose and with quinine in previous studies (Wyvell & Berridge,

2000), making a ceiling effect unlikely. Although depletion itself shifted NaCl palatability from aversive to ingestive, as previously demonstrated by Berridge et al. (1984), we found no evidence for an increased positive shift or a decreased negative shift associated with repeated depletions.

The small number of animals in the TR study could make the statistical reliability of a negative finding tenuous. However, the pattern of results shows no trend toward greater palatability responses after the second than after the first depletion and, in fact, is in the opposite direction. Thus, the absence of evidence for increased liking is unlikely to be due to lack of statistical power. The availability of both negative and positive categories of responses provides two opportunities to detect palatability shifts should they occur.

Our data are in agreement with previous work suggesting that these components of reward are separable in animals with a salt appetite and that the neural underpinnings of liking and ingestion differ. Galaverna et al. (1993) found that lesions to the central nucleus of the amygdala abolished depletion-induced salt appetite as measured by voluntary intake. However, lesions had no effect on the shift in palatability from aversive to ingestive induced by sodium depletion as measured by the TR paradigm. Thus, the amygdala appears necessary for the incentive properties of NaCl and related cues to induce consummatory behaviors in rats depleted of sodium but does not appear necessary for the affective evaluation of salt under the same conditions.

Overall, these experiments support the general proposition that sensitization alters the wanting component but not the liking component of reward. Salt appetite sensitization produced an increase in wanting of a salt reward without affecting liking. Previous studies concerning this hypothesis have focused on the effects of prior sensitizing drug treatments on the motivation to obtain natural rewards (Wyvell & Berridge, 2001). The present studies are the first to demonstrate this dissociation within the same model of a reward system that shows behavioral sensitization.

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