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Investigating the Motivational Mechanism of Altered Saline Consumption Following 5-HT_{1A} Manipulation

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The precise role played by serotonin (5-HT) in taste—an issue of great interest given the involvement of serotonin in human sensory and eating disorders—is a matter of considerable debate, perhaps because of the variety of methodologies that have been brought to bear by different researchers. Here, we use multiple methods to reveal the motivational mechanism whereby 5-HT_{1A} receptor activation modulates drinking behavior. Subcutaneous injections of the selective 5-HT_{1A} agonist 8-hydroxy-2-di-*n*-propylamino-tetralin (8-OH-DPAT), a drug that reduces 5-HT release by acting on presynaptic autoreceptors, dose-dependently increased consumption of 0.45M NaCl in a one-bottle test. In a two-bottle test, however, 8-OH-DPAT-treated animals (30 μg/kg/ml) demonstrated decreased NaCl preference—although our detection of this effect was obscured by adaptation to the drug across days. Rats' performance in a brief access test confirmed that 8-OH-DPAT decreased preference for saline by both increasing water consumption and decreasing NaCl consumption. Finally, taste reactivity tests demonstrated that the latter result does not reflect decreased NaCl palatability. Overall, the results suggest that 8-OH-DPAT-induced 5-HT hypofunction increases thirst without substantially affecting the palatability of NaCl.

Keywords: serotonin (5-HT), thirst, homeostasis, salt, palatability

The neurotransmitter serotonin (5-hydroxytryptamine, or 5-HT) drives activity in a wide range of neural systems. Its effects on sleep (Siegel, 2004), mood (Bell, Abrams, & Nutt, 2001; Shiah & Yatham, 2000), and development (Lauder, 1983; Mazer et al., 1997; Shemer, Azmitia, & Whitaker-Azmitia, 1991) are well documented. Less studied, but equally important, is 5-HT's involvement in a number of processes associated with feeding. For instance, 5-HT regulates sensory processing in the gastrointestinal tract and modulates the chemosensory responses of taste receptor cells (Ewald & Roper, 1994; Goodman, 1996; Herness & Chen, 2000; Huang et al., 2005; Imendra, Fujiyama, Miyamoto, Okada, & Sato, 2000; Neal & Bornstein, 2006).

Central 5-HT function has also been related to the control of appetite, most often to anorectic effects and speeding up of metabolism (Clifton, Lee, & Dourish, 2000; Leibowitz & Alexander, 1998; Menani, Thunhorst, & Johnson, 1996; Montgomery & Burton, 1986; Simansky & Nicklous, 2002). Systemic administration of 5-HT receptor agonists has been suggested to reduce meal size, the number of meals, and the rate of eating within a meal (Clifton et al., 2000). Direct, selective activation of 5-HT_{1B} receptors in the lateral parabrachial nucleus (LPBN) has similar effects (Simansky & Nicklous, 2002). Similarly, 5-HT agonism has been shown to decrease hypertonic saline consumption (Cooper & Barber, 1993; Menani et al., 1996; Neill & Cooper, 1989; Rouah-Rosilio, Orosco, & Nicolaidis, 1994).

Not all studies, however, suggest that 5-HT receptor activation causes hypophagia and hypodipsia. Specific 5-HT_{1A} agonists, such as gepirone, buspirone, and 8-hydroxy-2-di-*n*-propylamino-tetralin (8-OH-DPAT) have been suggested to elicit exactly the opposite result, for instance—hyperphagia and hyperdipsia. Furthermore, neither of these phenomena is substrate specific; increases in intake have been demonstrated for standard food pellets, saline, and even a diet consisting of sweetened condensed milk (Cooper, Fryer, & Neill, 1988; Neill & Cooper, 1988, 1989).

How does one explain this complexity of results? To start with, one must recognize the complexities of neurotransmission. There are numerous types of 5-HT receptors with specific functional characteristics. Activation of 5-HT_{1A} somatodendritic autoreceptors ultimately decreases overall serotonin neurotransmission (Curzon, 1991; Dourish, Hutson, & Curzon, 1985; Dourish, Hutson, Kennett, & Curzon, 1986; Gilbert & Dourish, 1987; Leibowitz & Alexander, 1998; Montgomery & Burton, 1986; Neill & Cooper, 1989; Simansky & Nicklous, 2002) whereas agonism of other 5-HT receptors (such as 5-HT_{1B} or 5-HT_{2C} receptors) increases transmission. Furthermore, two drugs commonly used to manipulate 5-HT function, dl-fenfluramine and buspirone, are known to have catecholaminergic side effects (Garattini, Mennini,

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& Samanin, 1987).Confounds such as these often combine to yield drastically different results.

Even these confounds cannot account for all of the contradictions found within the literature, however, perhaps because consumption reflects multiple processes that can be measured in multiple ways. The perceived palatability of a tastant and the need to maintain body homeostasis, a multivariate construct related to such factors as level of dehydration, sodium balance, postingestive caloric benefits, and hormone levels, interact in a complex manner to ultimately guide what and how the animal drinks (Barnfield, Parker, Davies, & Miles, 1994; Gray & Cooper, 1996; Treit & Berridge, 1990). These factors are frequently confounded in studies using animals in altered homeostatic states (e.g., water or sodium depletion, see Menani, Thunhorst, & Johnson, 1996), making it difficult to distinguish taste effects from a number of physiological effects. In addition, the results of studies that focus on single concentrations of a particular stimulus may not generalize to other concentrations and stimuli (in a single study, e.g., fenfluramine has been shown to both reduce and have no effect on saline consumption, depending on the salt concentration, Neill & Cooper, 1989). Furthermore, different taste paradigms, which can measure everything from "acceptance of" to "preference for" a given stimulus, are often treated as if they were identical (for review see Spector, 2000).

To resolve the nature of the 5-HT effect on consumption in the face of such complex issues, homeostatically normal animals must be tested in a battery of paradigms. Here, we brought four distinct drinking paradigms to bear on normal and hypo-serotonergic rats, specifically run without confounding effects of water- or Na-depletion. First, we replicated a previous study by Cooper et al. (1988), showing that 8-OH-DPAT elicits an increase in 0.45M NaCl acceptance in a one-bottle test. Second, we showed that 5-HT_{1A} activation decreases NaCl preference in a temporally dynamic manner in a two-bottle choice test. Third, we showed that these effects are neither artifacts of postingestive processes nor gender-specific, using a brief access test. Finally, we confirmed that the results were almost certainly not a reflection of changes in the palatability or perceived intensity of the stimuli, using an analysis of taste reactivity.

Overall, our results suggest that 5-HT_{1A} manipulation causes a "pure" increase in thirst by targeting homeostatic mechanisms in a temporally dynamic manner. These results have implications for our understanding of taste function, and for our understanding of clinical conditions, such as anorexia, bulimia, and autism, in which both abnormal serotonergic activity and dysfunctional responses to food stimuli have been observed.

Method and Materials

Animals

Subjects for the one-bottle, two-bottle, brief access, and taste-reactivity tests were 40 adult Long-Evans rats (Charles River Laboratories, Wilmington, MA, and Simonsen Lab, Gilroy, CA) weighing 220 to 285 g at the start of the experiments. All animals were housed individually with free access to standard food pellets. Water was available ad libitum except where noted below. The animals were maintained on a 12-hr light/12-hr dark cycle (lights on at 0700 h).

8-OH-DPAT Administration

8-OH-DPAT (Sigma, St. Louis, MO) was dissolved in sterile saline (0.9%) and administered subcutaneously (sc) 25 to 30 minutes before all testing. In control sessions, rats received sterile physiological saline (0.9%) vehicle. All rats received 1 ml/kg of fluid. In the one-bottle test, each animal served as its own control, with order of drug delivery (0, 10, 30, and 100 μg/kg) counterbalanced across subject ($n = 12$). For the two-bottle test, rats were randomly assigned to either the drug group or control group. Rats in the drug group ($n = 8$) received sc injections of 30 μg /ml/kg 8-OH-DPAT while rats in the control group ($n = 8$) received vehicle injections. For both the taste-reactivity and brief access tests, each animal served as its own control with order of drug delivery (30 μg /ml/kg 8-OH-DPAT and vehicle) counterbalanced across subject.

Experimental Protocol

Adaptation. At the start of each experiment, rats were adapted to handling and placed on a 22 hour water restriction protocol; this amount of restriction was sufficient to ensure experimental compliance, but not enough to confound gustatory discrimination or preferences (Scalera, 2000). Unless noted otherwise, this adaptation occurred during Days 1 through 4 of each experimental protocol. After all training and testing sessions, water was provided in the home cage such that rats had a total of 2 hours of access to fluid each day. Adaptation to water restriction was indicated by maintenance of at least 90% of the starting body weight. As no animal dropped below this mark, the process took at most 4 days.

One-bottle test. Male rats ($n = 12$) were placed in a test cage for 30 minutes a day with access to water from a single spout located at the front of the cage. This procedure was repeated until a steady baseline water consumption was established (3 days). On Day 8, rats were preexposed to 0.45M NaCl in the 30-min test cage session to prevent neophobia (Domjan, 1976). Saline injections were given on Days 7 and 8, adapting the rats to the injection procedure.

The test consisted of alternating "drug days" and "rest days." On the first drug day, rats were injected with either vehicle or a dose of 8-OH-DPAT and given 30 minutes of access to 0.45M NaCl in the test cage. The next day, rats received no injection and simply had 30 minutes of access to water in the test cage. This alternation of days continued, with different doses of 8-OH-DPAT being delivered on each drug day such that each rat received each drug condition. Order of concentration was counterbalanced across rat. The amount (ml) of 0.45M NaCl consumed was recorded for later analysis.

Two-bottle test. Female rats ($n = 16$) were placed in a test cage for 30 minutes per day with access to water from two bottle spouts located at the front of the cage. This adaptation proceeded until a steady baseline consumption of water in the test cage was established (at most, 3 days). On Days 5 and 6 the rats were accustomed to the injection procedure.

The experiment consisted of two blocks, each of which was made up of five testing sessions (one per saline concentration). On each day within a block, rats had 30 minutes of access to both

water and a single concentration of NaCl, via the two bottle spouts located at the front of the test cage. The concentration of NaCl (0.00M, 0.03M, 0.10M, 0.30M, 0.45M) was varied randomly so that rats experienced pairings of each concentration with water exactly one time per block. The NaCl bottle was positioned on alternating sides to ensure that rats did not develop a place preference. Only a single experimental session was run per day, thus a single block lasted 5 days. Block 1 was identical to Block 2. Order of presentation differed from rat to rat. Measurements from each session included total fluid intake and total NaCl intake.

Brief access test. Training and testing took place in the Davis MS-160 Lickometer rig (DiLog Instruments, Tallahassee, FL). This apparatus provides the rat periodic brief access to one of 16 stainless steel drinking tubes on a moveable carousel. Short tube presentations were automated via a microcomputer with tube access controlled by a motorized shutter in front of the carousel. Each contact with the drinking spout completed a low-current electrical circuit that was recorded as a single lick. See (Smith, 2001) for a more complete description.

After adaptation to water restriction (as described above), male and female rats (n = 4/gender) were habituated to the test cage over a period of 4 days. During the first 2 days, rats were placed in the Davis rig and allowed to drink water from a single tube continuously for 30 minutes. On the last 2 days of the habituation period, the rat was given practice injections, and was introduced to drinking water from the moving carousel for 30 minutes.

Each rat received two 30-min testing sessions under each drug condition. Between each pair of sessions, the rat was given 2 days of rest (during which it received vehicle injections and only water in the testing apparatus). During each session, solutions (0.00M, 0.03M, 0.10M, 0.30M, and 0.45M NaCl) were presented in a randomized order (sampling without replacement). Each solution was presented for 15 seconds from the time of the first recorded lick with 10 seconds between each presentation. If no first lick was recorded within 30 seconds of the tube presentation, the shutter closed and the tube holder moved on to the next tastant. Trials in which the rat failed to sample the available stimulus with at least one lick were dropped from the subsequent analysis.

Taste reactivity test. The surgical procedures for implantation of intraoral canulae (IOCs) are similar to those described previously (Phillips & Norgren, 1970). Female rats (n = 4) were anesthetized with a ketamine (100 mg/kg)/xylazine (5 mg/kg)/acepromazine (1 mg/kg) mixture (i.p.) and given small update doses (20–25% volume of initial dose) as needed to maintain deep anesthesia. The anesthetized rat was situated in a stereotaxic frame for insertion of skull screws and an initial application of dental acrylic. After the skull screws were properly positioned, thin polyethylene tubes were inserted bilaterally behind the first maxillary molar, through the masseter muscle and out through an opening in the scalp. The tubes were secured to the head via dental acrylic.

One IOC, designated for the delivery of water rinses only, was fitted with 18-gauge extra thin wall stainless steel tubing. The other IOC, designated for all other tastant deliveries, was fitted with a female luer-lock piece via stretched silicone. Immediately after surgery, the rat was injected with 4 ml of sterile saline (0.9%) for rehydration and 0.1 ml penicillin (300,000 units/ml) to prevent infection. Another penicillin dose was delivered 48 hours after surgery.

After surgery, rats were given at least a week to recover, during which time they were accustomed to being handled and to having their IOCs cleaned daily. After recovery, rats were started on a 22-hr water restriction protocol as described above. Once the rat had adapted to water restriction, the rat was placed in the test cage for habituation. Initially, the rat was left in the cage for 20 minutes. The duration of the habituation increased over a period of 3 days such that by the third day, the rat remained in the test cage for 45 minutes. After the rat was successfully habituated to the testing environment, the rat was adapted to receiving water rinses through the IOCs. Again, the duration of this adaptation increased from 20 minutes up to 45 minutes over the course of 7 days. Practice injections were given on the last 2 days of water rinse adaptation to familiarize the rat with the procedure.

A clear Plexiglas test cage was placed in a dark, sound attenuating box within an environmental isolation chamber. At 15-sec intervals, 40 μl aliquots of liquid were delivered directly into the rat's oral cavity via nitrogen pressurized tubes through the IOCs. Water rinse deliveries were given directly through the stainless steel IOC opening; randomized blocks of tastant deliveries (0.03M, 0.10M, 0.30M, and 0.45M NaCl) were given via a manifold through the luer-lock IOC. Water rinses were given between each tastant delivery. Digital recordings of the rat's orofacial responses to the taste deliveries were made via a low-light camera placed below the test cage facing a mirror that angled upward 45 degrees.

Each rat received three testing sessions of each drug condition—first, three 45 minute sessions over consecutive days under one drug condition, followed by 3 days under the other drug condition. Between each set of three sessions, the rat was given 2 days of rest (in which it received vehicle injections and water rinses in the testing apparatus).

Rats demonstrate taste-specific orofacial responses (Grill & Norgren, 1978). By recording and analyzing these behaviors after tastant delivery, the distinct palatability of a tastant can be quantified (Berridge & Aldridge, 2000). Two individuals blind to the conditions of the experiment coded the videos offline. Four dependent variables were extracted and quantified for each video session: (1) latency to respond, (2) duration of response, (3) number of lateral tongue protrusions (LTPs, the basic measure of palatability), and (4) number of gapes (the basic measure of aversiveness) (Grill & Norgren, 1978). Because the number of gapes was exceedingly small, however (a total of 16 were observed over all testing sessions, compared to 1154 LTPs), only LTPs were analyzed. Interrater reliability analyses (Cronbach's alpha) were performed for all dependent variables with the following results: *LTPs*: Cronbach's alpha = .997; *Latency:* Cronbach's alpha = .995; *Duration*: Cronbach's alpha = .997.

Results

One-Bottle Test

A previous study reported that the selective 5-HT_{1A} agonist 8-OH-DPAT elicits an increase in 0.45M NaCl consumption in a one-bottle test (Cooper et al., 1988). We replicated these results,

using a paradigm that differed only in that our single day of preexposure replaced the 10 days in Cooper et al.'s experiment. This allowed us to avoid any possible learning effects related to repeated taste exposure (Domjan, 1976) before the beginning of testing.

A one-way repeated measures ANOVA of the one-bottle data revealed a significant dose-dependent increase in 0.45M NaCl consumption, $F(3, 33) = 5.074$, $p < .01$. Figure 1 shows the expected dose-dependent drinking behavior. When given vehicle injections, rats consumed about 5 ml of 0.45M NaCl. Similar amounts of saline were consumed after an 8-OH-DPAT dose of 10 μg/kg/ml. As the dose increased, however, so did the amount of NaCl consumed, such that strong 5-HT_{1A} receptor activation significantly increased the intake of high-concentration saline over the control condition.

Although it seems that 8-OH-DPAT caused an increased preference for hypertonic saline, this dose-dependent increase in saline consumption could in fact reflect either an increase in preference or an increase in acceptance. That is, perhaps the rat simply drank more NaCl because it was the only tastant available. Dissociating these two possibilities requires a two-bottle choice test, in which a range of NaCl concentrations is presented with water. If preference for NaCl is truly increased after 8-OH-DPAT administration, then drug-infused rats will tend to choose saline over water more than control rats in this test.

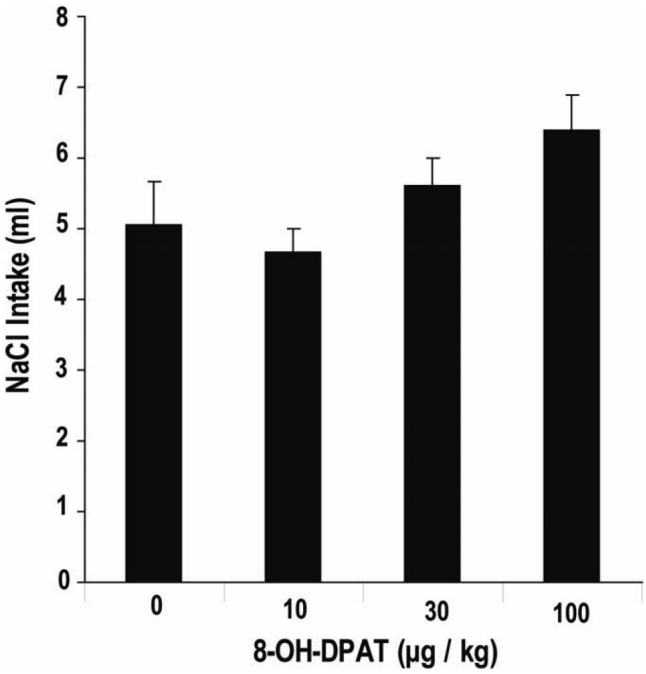

Figure 1. The *x*-axis represents the dose of 8-OH-DPAT delivered; the *y*-axis reflects the amount (ml) of 0.45M NaCl consumed in a 30-min one-bottle test. 8-OH-DPAT elicits a significant dose dependent increase in 0.45M NaCl consumption (one-way repeated measures ANOVA $p < .01$). Values are means ± *SEM* ($n = 12$).

Two-Bottle Test

Rats were presented with a range of NaCl concentrations (0.03M, 0.10M, 0.30M, and 0.45M), each paired with water in a single cage with two lick spouts. To measure preference in overall consumption, we calculated the percent of the total volume consumed that was NaCl, according to the formula: [NaCl Consumed (ml)/(NaCl + Water Consumed (ml))] × 100%.

Each saline concentration was compared to water in two sessions (one session per day), but out of concern over possible drug adaptation effects (Kreiss & Lucki, 1992, 1997) we divided the task into two 5-day blocks (in each of which all saline concentrations were compared to water once) and examined each block separately. Figure 2A presents the result of this analysis for the first block of sessions. The effect of concentration was significant in a two-way mixed-effects ANOVA, $F(4, 52) = 12.39$, $p < .001$, showing that low and middle concentrations of saline were preferred to high concentrations by both groups—that is, preferences dropped off with increases in saline concentration, forming the expected saline-preference curve (Flynn & Grill, 1988). For drug treated rats, the curve was similar in shape to that of controls, but there appeared to be a decrease in overall NaCl preference. The effect of 8-OH-DPAT did not quite reach significance, however, $F(1, 13) = 1.91$, $p > .05$, nor did the interaction ($F(4, 52) = 1.04$, $p > .05$).

At the very least, the data from the two-bottle test demonstrate that in the one-bottle test, 8-OH-DPAT rats only increased their consumption of saline because no water was available; in fact, it seems more likely that the 5-HT manipulation reduced saline consumption. To more fully investigate this possibility, we examined intake of each fluid separately within context of the two-bottle test. This analysis reveals that 8-OH-DPAT significantly increases water consumption, $t(14) = 3.442$, $p < .005$. These data are presented in Figure 2B.

We suspected that the length of the two-bottle test—5 days per block of sessions—limited our ability to discern the effects of 8-OH-DPAT, which could vanish as drug adaptation occurs, possibly because of desensitization of raphe 5-HT_{1A} receptors (Kreiss & Lucki, 1992, 1997, but see Casanovas et al., 1999). Analysis of Block 2 data (see Figure 3) supports this concern, revealing that by the 6th to 10th day of drug treatment the effects observed in Block 1 (see Figure 2) are completely abolished. Just as in Figure 2A, Figure 3 depicts the preference for saline as a percent. Here, drug-treated rats (dashed line) fail to demonstrate even a trend toward decreased preference for saline. Clearly, 10 days of drug injections leads to adaptation, and a loss of effect (see Discussion), a fact that suggests that the results of the first 5-day block of tests may be weakened by progressive drug desensitization.

The two-bottle test reveals, contrary to expectation based on the one-bottle test, that 8-OH-DPAT may reduce saline consumption, while boosting water consumption. The power of the test to detect this effect, however, may have been limited by the across-day adaptation to 8-OH-DPAT, along with inherent between-subjects variability and possible postingestive effects related to the amount consumed over 30 minutes. It is also possible that the use of female rats on this test impacted our results; estrogen is known to affect taste behavior (Curtis, Davis, Johnson, Therrien, & Contreras, 2004), and to interact with 5-HT (Robichaud & Debonnel, 2004).

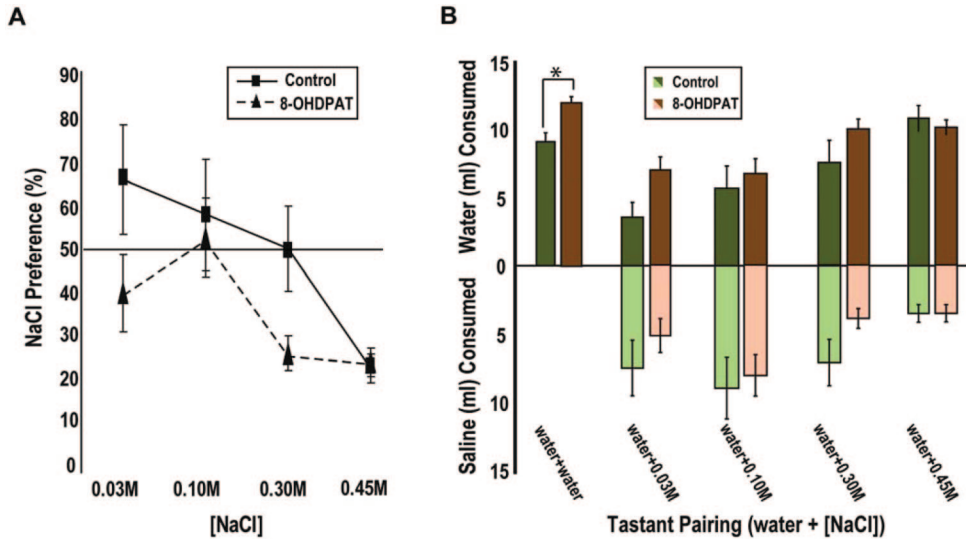

Figure 2. Saline preference for block 1 of a two-bottle test represented as a percent (A) and total amount consumed (B). *A*. The *x*-axis represents the concentration of saline paired with water in individual testing sessions. The *y*-axis reflects the percent of total fluid consumed that was saline. 8-OH-DPAT (dashed line) elicits a decrease in NaCl preference across tastant pairings (two-way ANOVA, $p < .06$). Chance consumption (50% mark) is indicated by the horizontal line. (B) Tastant pairings (water + [NaCl]) are represented on the *x*-axis. The *y*-axis, divided into upper and lower portions, reflects the amount of water and saline consumed (ml), respectively. Two columns are presented for each tastant pairing. Left columns (green scheme) reflect control data; right columns (brown scheme) represent 8-OH-DPAT data. 8-OH-DPAT significantly increases overall fluid consumption when only water is presented (two-sample *t* test, $^{*}p < .004$), but elicits no change in overall fluid intake otherwise. Values are mean ± *SEM* ($n = 16$).

To resolve these issues and solidify these results, we resorted to a third task, having both male and female rats perform a multibottle choice test with a within-subjects design: the brief access test.

Brief Access Test

Male and (estrous stage random) female rats were allowed brief access (15 seconds) to a range of saline concentrations, including 0.00M, 0.03M, 0.10M, 0.30M, and 0.45M, multiple times across 30-min experimental sessions. This design allowed us to repeat the comparisons of the two-bottle test, in a context that reduced the interfering effects of across-session drug desensitization, between-animal consumption differences, and postingestive processes that can change intake. Any effects observed in such a task can therefore be attributed to either preexisting states or orosensory processes.

Each rat was tested in two sessions under each drug condition (8-OH-DPAT, 30 μg/kg/ml, and vehicle 1 ml/kg); we recorded the number of licks made to each tastant. We observed the expected significant main effect of concentration in a three-way (drug × concentration × gender) repeated measures ANOVA, $F(4, 143) = 19.73$, $p < .01$, demonstrating that high saline concentrations were less palatable than lower. This effect was similar for rats of both genders (p of gender × concentration interaction >0.05). In addition, a significant drug by taste interaction, $F(4, 143) = 2.718$, $p < .04$ can be seen in Figure 4; when rats were treated with 8-OH-DPAT (dashed line), the average number of licks to water (two-sample *t* test, $p < .03$) increased and the average number of licks to 0.10M NaCl (two-sample *t* test, $p < .04$) decreased, compared to control rats (solid line). Gender also had no impact on this effect (F of three-way interaction <1).

The brief-access test makes it clear that 8-OH-DPAT does in fact both decrease the preference for NaCl and increase preference for water similarly for male and female rats. These two behavioral effects may be simply explained in terms of a single motivational effect—increased thirst—but it is conceivable that the drug alters perceptual properties of NaCl (i.e., palatability) in addition to altering thirst levels, and that this alteration of palatability caused the specific reduction of salt consumption. To investigate this possibility, we assessed the impact of the drug on taste reactivity.

Taste Reactivity Test

Rats demonstrate stereotypical orofacial behaviors that can be used to identify the perceived hedonic value of a given stimulus (Berridge & Aldridge, 2000; Grill & Norgren, 1978). Using direct tastant infusions into the oral cavity via surgically implanted IOCs, we were able to record these orofacial behaviors to a range of saline concentrations (0.03M, 0.10M, 0.30M, and 0.45M), and to quantify them offline (Berridge & Aldridge, 2000; Phillips & Norgren, 1970). We assessed a basic measure of palatability: lateral tongue protrusions (LTPs). The main effect across the saline concentrations used was significant, $F(3, 21) = 7.666$, $p < .005$, with LTPs increasing with concentration as expected based on previous data (Grill & Norgren, 1978). We observed no significant drug effects, however—neither the main effect of drug nor the

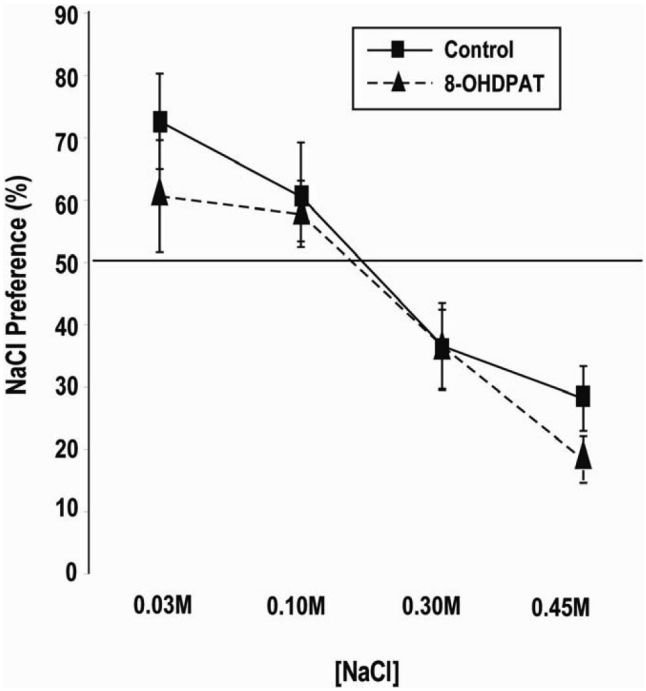

Figure 3. Saline preference for Block 2 of a two-bottle test represented as a percent. The *x*-axis represents the concentration of saline paired with water in individual testing sessions. The *y*-axis reflects the percent of total fluid consumed that was saline. The decrease in NaCl preference observed in Block 1 (Figure 2A) is abolished in Block 2 of a two-bottle choice test. Values are mean ± *SEM* ($n = 16$).

drug x concentration interaction approached $p < .05$. That is, although the number of lateral tongue protrusions increased monotonically with increasing NaCl concentration (see Figure 5), there were no significant differences between drug-treated (dashed line) and control (solid line) groups. Overall, these results converge with results of the one-bottle, two-bottle, and brief access tests to say that 8-OH-DPAT exerts its effects by targeting homeostatic, and not perceptual, processes.

Discussion

We brought a battery of tasks to bear on the question of exactly how manipulation of 5-HT_{1A} receptors modulate a change in acceptance or preference for NaCl. The results—increased intake in a one-bottle test, increased water intake and marginally decreased preference in a 5-day two-bottle test, increased intake of water with decreased intake of NaCl in a brief access test, and the lack of effect on orofacial behaviors in a taste reactivity test—strongly suggest that 8-OH-DPAT increases thirst by targeting nontaste mechanisms.

5-HT_{1A} receptor activation is auto-inhibitory. The ultimate 5-HT hypofunction and reduction in neuronal firing caused by this auto-inhibition elicits an increase in 0.45M NaCl intake when no other fluid is available. These results are not wholly unexpected, given previous evidence (Curzon, 1991; Dourish et al., 1985, 1986; Gilbert & Dourish, 1987; Leibowitz & Alexander, 1998; Montgomery & Burton, 1986; Neill & Cooper, 1989; Simansky & Nicklous, 2002). However, 8-OH-DPAT actually elicits a decreased preference for NaCl at certain concentrations when the animal has the option of consuming water. This shift in preference reflects both an increase in the rat's desire for water and a decrease in the rat's desire for NaCl, a fact that was somewhat obscured in the two-bottle test (probably because of across-session drug adaptation, perhaps centered on the raphe nuclei, see Kreiss & Lucki, 1992, 1997) but confirmed in the brief-access test. Future experiments comparing these results to those obtained using 5-HT_{1A} antagonists (such as WAY 100635) will confirm and extend the results (although the fact that 5-HT_{1A} agonism causes thirst does not necessarily imply that 5-HT_{1A} antagonism will reduce thirst).

The overall pattern of results suggests the following motivational mechanism, diagrammed in Figure 6: Thirsty rats will drink any single fluid stimulus (NaCl or water) that is available, but if multiple stimuli are available, they will adjust their drinking ratio, such that they rehydrate most efficiently. While increasing water intake is, of course, an efficient way to alleviate thirst, NaCl increases thirst (and subsequent water intake) via both ingestive and postingestive mechanisms (Manesh, Hoffmann, & Stricker, 2006; Stricker, Hoffmann, Riccardi, & Smith, 2003). Additionally, oral-sensory cues have been shown to directly interact with postingestive cues (Poothullil, 2005), a finding that suggests the possibility that 8-OH-DPAT-treated (and hence thirsty) animals may reject NaCl based on only oral-sensory cues.

This interpretation highlights the importance of using multiple tasks and multiple concentrations of NaCl when studying the

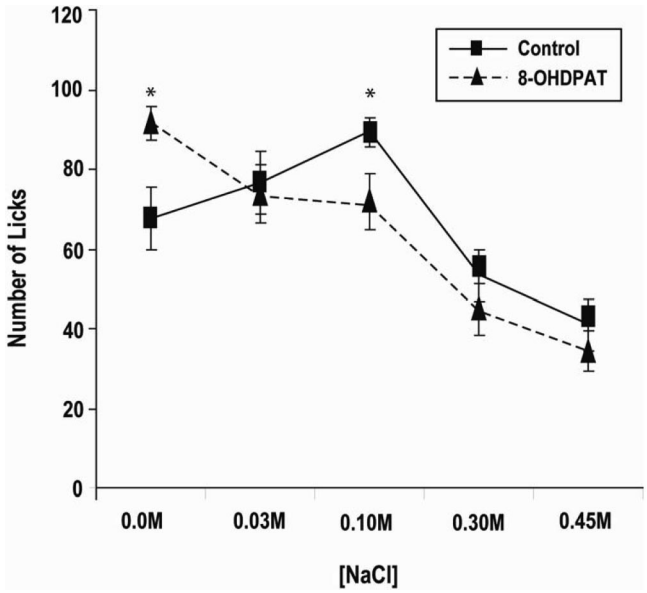

Figure 4. Saline preference measured as the average number of licks in a brief access test. The *x*-axis represents the concentration of saline; the *y*-axis reflects the number of licks to each tastant made in 30-min testing sessions. 8-OH-DPAT elicits significantly more licks to water and less licks to NaCl (two-way repeated measures ANOVA, drug × concentration interaction, $p < .04$). Significant differences (two sample *t* tests, $^{*}p < .04$) were seen for both water and 0.1M NaCl intake. Values are mean ± *SEM* ($n = 8$).

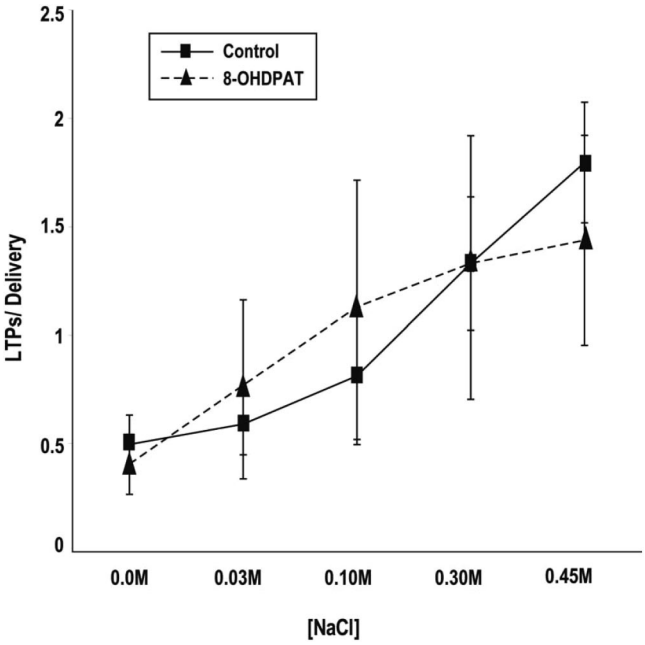

Figure 5. Palatability measured as the number of LTPs per tastant delivery in a taste reactivity test. The X-axis is concentration of saline; the *y*-axis is the number of lateral tongue protrusions per delivery of tastant. The number of LTPs increases with increasing concentration of NaCl in a taste reactivity test ($p < .0013$); however, 8-OH-DPAT does not elicit a significant difference from controls ($p > .80$). Values are mean $\pm$ *SEM* ($n = 4$).

complex panoply of factors underlying ingestion (for a review making this same point, see Spector, 2000), particularly when studying rats in states of extreme Na- and H_2O-deprivation. Na-depletion itself perturbs multiple physiological systems, and determining of the source of NaCl drinking differences in animals becomes difficult when these systems and the serotonergic system are manipulated. Differences between the hydrating properties of sucrose and saline, two of the most common taste stimuli used in such studies (Barnfield et al., 1994; Cooper et al., 1988), further add to the interpretive complexities; sucrose solutions do not dehydrate animals to the same degree that NaCl does.

One possible neural mechanism by which 5-HT might increase thirst is discussed in De Gobbi et al. (2005), who suggested that infusions of 8-OH-DPAT into the LPBN prevent feedback mechanisms that normally inhibit further drinking. Chromatography data demonstrate that overall 5-HT levels rise in the lateral parabrachial nucleus (LPBN) of acutely fluid and sodium deprived rats when they have access to drinking stimuli (Tanaka et al., 2004). Rats under the same condition have been shown to ingest more water and 0.3M NaCl when treated with 8-OH-DPAT directly into the LPBN (De Gobbi et al., 2005). These authors suggest that normally, as the rats begin drinking NaCl and water, levels of 5-HT in the LPBN rise, which normally inhibits further drinking (and, presumably, subsequent activation of 5-HT_{1A} receptors resets the system such that drinking can begin anew). When these rats are treated with 8-OH-DPAT, 5-HT levels in the LPBN are kept low by the activation of somatodendritic autoreceptors and thus drinking continues.

This work has potentially important implications for current investigations into human neuropsychological disorders. Previous work has implicated abnormalities involving the 5-HT_{1A} receptor in both anorexic humans and rats (Kaye et al., 2005; Shimizu, Hori, Ogino, Kawanishi, & Hayashi, 2000). It is possible that 5-HT_{1A} activation modulates homeostatic mechanisms that affect drinking behavior, which may help to explain the abnormal ingestion patterns seen in patients with anorexia and bulimia nervosa. In addition, these results have implications for the study of autism and related developmental disorders. Autism, a disorder characterized by hyper-sensitivity to sensory stimuli, has been linked to hyperserotonemia (Abramson, 1989; Baranek, 2002; Chugani, 2004; Khalfa et al., 2004; Leventhal, Cook, Morford, Ravitz, & Freedman, 1990; O'Neill & Jones, 1997; Rogers, Hepburn, & Wehner, 2003; Tecchio et al., 2003). Although it is possible that boosting circulating 5-HT levels (or lowering them in particular locations not affected by 8-OH-DPAT) will change taste percep-

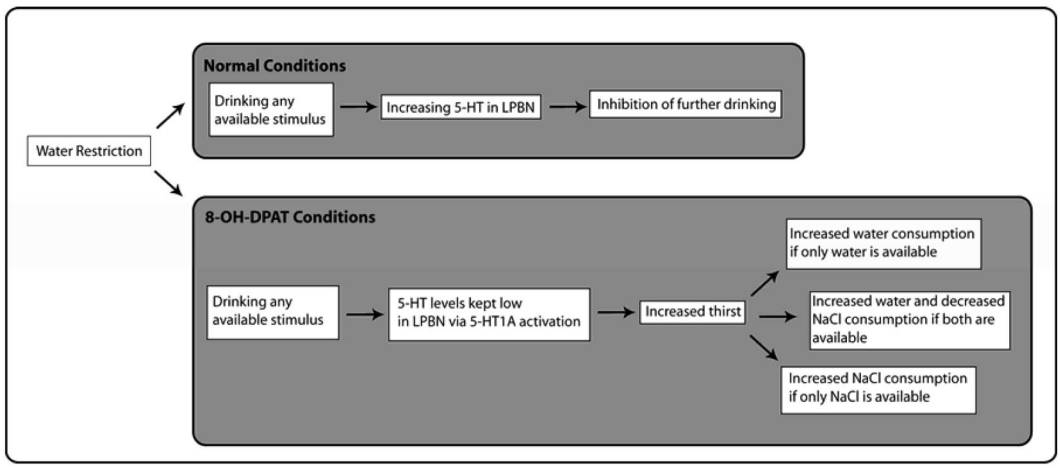

Figure 6. Proposed mechanism of action used by the 5-HT_{1A} receptor in the control of NaCl and water consumption.

tion in a way consistent with autistic symptomatology—increasing perceived intensity or reducing perceived palatability—our data show that reducing 5-HT release via systemic manipulation affects neither intensity nor palatability. Further investigations of particular 5-HT receptor subtypes may both help to explain the autistic sensory deficits and to define the disorder itself.

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