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Age-related Declines in Thirst and Salt Appetite Responses in Male Fischer 344 x Brown Norway Rats

Robert L. Thunhorst^{1,4}, Terry Beltz¹, and Alan Kim Johnson^{1,2,3,4}

¹Departments of Psychology, University of Iowa, Iowa City, IA 52242-1407

²Health and Human Physiology, University of Iowa, Iowa City, IA 52242-1407

³Pharmacology, University of Iowa, Iowa City, IA 52242-1407

⁴the Cardiovascular Center, University of Iowa, Iowa City, IA 52242-1407

Abstract

The F344xBN strain is the first generational cross between Fischer 344 (F344) and Brown Norway (BN) rats. The F344xBN strain is widely used in aging studies as it is regarded as a model of “healthy” aging (Sprott, 1991). In the present work, male F344xBN rats aged 4 mo (young, $n = 6$) and 20 mo (old, $n = 9$) received a series of experimental challenges to body fluid homeostasis to determine their thirst and salt appetite responses. Corresponding urinary responses were measured in some of the studies. Following sodium depletion, old rats ingested less saline solution (0.3 M NaCl) than young rats on a body weight basis, but both ages drank enough saline solution to completely repair the accrued sodium deficits. Following intracellular dehydration, old rats drank less water than young rats, again on a body weight basis, and were less able than young rats to drink amounts of water proportionate to the osmotic challenge. Compared with young rats, old rats drank less of both water and saline solution after combined food and fluid restriction, and also were refractory to the stimulatory effects of low doses of captopril on water drinking and sodium ingestion. Age differences in urinary water and sodium excretion could not account for the age differences in accumulated water and sodium balances. These results extend observations of diminished behavioral responses of aging animals to the F344xBN rat strain and support the idea that impairments in behavior contribute more to the waning ability of aging animals to respond to body fluid challenges than do declines in kidney function. In addition, the results suggest that behavioral defense of sodium homeostasis is less diminished with age in the F344xBN strain compared to other strains so far studied.

Keywords

aging; diuresis; natriuresis; dehydration; hypovolemia

ADDRESS FOR CORRESPONDENCE Dr. Robert L. Thunhorst Department of Psychology University of Iowa 11 Seashore Hall E. Iowa City, IA 52242-1407 Office phone: (319) 335-0509 FAX: (319) 335-0191 thunhors@uiowa.edu.

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1. Introduction

Dehydration is a major health risk for the elderly (5, 46), involving decreased thirst sensations (20, 21, 28) and reduced abilities to conserve water (12, 23) and sodium (6). Thus, when faced with challenges to body fluid homeostasis, the elderly are more susceptible to fluid losses (46) and take longer to restore fluid balance (16, 21) than younger people. The success of rat models in examining age-related declines in renal (e.g., 1, 2, 3, 51) and cardiovascular (e.g., 8, 11, 30, 35) function is accompanied by a growing body of literature examining the effects of aging on thirst-related behaviors in rats (e.g., 4, 17, 24, 25, 36, 37, 38, 41, 42, 47). We have used the Brown Norway (BN) rat strain, a commonly studied alternative to the Fischer F344 (F344) and Sprague-Dawley (SD) strains for studies on aging, in an extensive series of studies on thirst and salt appetite responses during aging (36, 37, 38, 41, 42, 47). The BN strain lives longer relatively free of disease (13, 32) and accumulates less fat than other strains even into old age (22, 49). However, no single strain is without age-related pathologies that may confound results. To determine if observed declines in function are generic to the aging process or are strain specific it is beneficial to examine age-related phenomena across multiple strains. We now report the results of a series of experiments using the first generation cross of F344 and BN strains (i.e., F344xBN). Like the BN strain, the F344xBN rat has less age-related pathology (e.g., fewer tumors) than the F344 and SD strains (2, 32, 45), and also lives longer in absence of disease (13, 32). In addition, the F344xBN strain is less susceptible to hydronephrosis of the kidney (14, 31) and does not develop glomerular sclerosis (19), both of which may have consequences for studies of body fluid homeostasis. This work tests if the F344xBN strain has significant impairments in thirst- and salt appetite-related behaviors with age.

The considerable age differences involved in aging studies usually means that old rats are substantially heavier than younger rats which has consequences for administering experimental stimuli and for analyzing subsequent intakes. While intakes are typically adjusted for body weight (BW) in aging studies, there are differences of opinion when normalizing results according to BW is warranted (e.g., 48). In order to better approximate equivalent challenges for rats of diverse weights in the present work, we administered the treatments on a BW basis and focused the results and discussion on BW-adjusted values.

2. General methods

2.1. Animals

Male hybrid rats of the first generational cross between F344 and BN strains (i.e., F344xBN) were obtained from Harlan (Indianapolis, IN, USA) through services provided by the National Institute on Aging (NIA Bethesda, MD, USA). They were 4 mo (young; $n = 6$) and 20 mo (old; $n = 9$) at the beginning of testing. The rats were housed singly in hanging stainless steel cages in a room with constant temperature (23°C) and a 12:12 light:dark cycle (lights on at 7:00 am). They received *ad libitum* access to Purina rat chow, water and 0.3 M NaCl solution unless indicated otherwise. Intakes of water and saline solution from 100 ml graduated cylinders with attached stainless steel spouts fastened to the front of the cages were recorded daily for the duration of the studies described below. All work was conducted according to procedures approved by the University of Iowa Institutional Animal Care and

Use Committee and in compliance with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals*.

2.2. Drugs

Furosemide (Abbott Laboratories, N. Chicago, IL) was administered subcutaneously at 10 mg/kg BW. Captopril (SQ-14, 225; Bristol-Meyers-Squibb Pharmaceutical Research Institute, Princeton, NJ) was dissolved in tap water at 0.1 mg/ml.

2.3. Experimental protocols

Over a period of 11 weeks, the rats received a series of experimental challenges in the following order, with 6-12 days separating tests.

2.3.1. Experiment 1: extracellular fluid depletion—Rats were weighed in the morning and placed in standard metabolism cages with stainless steel funnels underneath. At 1:00 pm, furosemide was injected subcutaneously (sc; 10 ml/kg BW) to induce natriuresis and diuresis. One h later, water was provided in 100 ml graduated cylinders attached to the front of the cages. Food was not present. Water intakes were recorded the next morning, 20 h later. Both water and 0.3 M NaCl were then provided from 0.1-ml graduated chemical burettes with sipper spouts and intakes were recorded every 30 min for 4 h. The rats were then returned to the home cage where intakes of both solutions were recorded after another 20 h. Rats were given furosemide injections and then, 20 h later, were tested for water and salt intakes at 8-12 day intervals for a total of 3 times. In *test 1*, urine was collected into Nalgene® tubes (0.1 ml resolution) for the first h after furosemide injection. Urine for the remainder of the overnight period was collected into pre-weighed glass beakers and urine volume (UV) and water intakes were recorded in the morning. This UV was calculated as 1 gm = 1 ml. For *tests 2* and *3*, urine for the entire 20-h depletion period was collected into pre-weighed glass beakers. Samples were refrigerated for later analysis of sodium content. Urine was not collected during the salt appetite portion of testing, nor during the subsequent 20 h when the animals were returned to the home cage. Estimates of plasma sodium concentrations were derived from changes in sodium ingestion and excretion according to a formula that assumes similar basal plasma sodium concentrations between groups (33). The acute (4-h) period for measuring water and saline intakes the morning after depletion is referred to as the “test” period. Measures obtained before and after this period are referred to as “pretest” and “posttest” values, respectively. Similar nomenclature is used for discussing measures obtained in *Exp. 2* and *3*.

2.3.2. Experiment 2: intracellular fluid depletion—Two tests, separated by 1 week, studied the effects of intracellular fluid depletion. On test days, rats were weighed and placed in standard metabolism cages as above. The rats were injected sc with hypertonic saline (2 ml/kg BW, 1.0 M NaCl on *test 1* and 2.0 M NaCl on *test 2*), and water was provided immediately from glass burettes. Intakes were recorded every 30 min for 3 h. Urine was collected into Nalgene® tubes. Urine volume was measured at 3 h, and samples were refrigerated for later analysis of sodium content. To minimize potential discomfort from the sc injections, the solutions were made with 0.2% lidocaine (25, 42). The animals showed no signs of discomfort.

2.3.3. Experiment 3: overnight food and fluid restriction—At 10:00 am on test days, rats were weighed and placed in standard metabolism cages as above. Food, water and 0.3 M NaCl were not available. Urine was collected in pre-weighed glass beakers. The next morning, 23 h later, UV was measured, water and 0.3 M NaCl were provided from glass burettes, and intakes were recorded every 30 min for 3 h. Samples of urine were refrigerated for later analysis of sodium content.

2.3.4. Experiment 4: captopril adulteration of drinking water—Rats drink greater amounts of water or saline solutions when angiotensin-converting enzyme (ACE) inhibitors, such as captopril, are added to the drinking fluids or diet at low concentrations (24, 25, 40, 42). In this experiment, daily intakes of water and 0.3 M NaCl from 100-ml graduated cylinders with sipper tubes were recorded while captopril was added to the drinking water (0.1 mg/ml). In the first part of this experiment, intakes of water and 0.3 M NaCl were recorded for 3 days without captopril, for 4 days with captopril in the drinking water, and for 3 days after removal of captopril from the drinking water. In the second part, saline was removed and only water was available for drinking. Water intakes were recorded for 3 days without captopril, for 4 days with captopril in the drinking water, and for 3 days after removal of captopril from the drinking water.

2.4. Urine analysis

Urine was measured for volume (UV). Urinary sodium concentration (UNa) was determined by ion-specific electrodes (NOVA Biomedical, Waltham, MA) and was used for calculating urinary sodium excretion (UNaV). Relative water balances were calculated by subtracting UV from the total amount of fluid ingested (i.e., water or water + saline). Relative sodium balances were calculated by subtracting UNaV from sodium ingested in the form of 0.3 M NaCl. Fecal losses of water and sodium were not considered.

2.5. Statistical analysis

Data were analyzed by ANOVA with age as the between-subjects factor and with test and time (i.e., hours, days) as within-subjects repeated measures. Analysis of the drinking data was conducted on the raw, uncumulated measures at each time point (9). Planned comparisons were made with Fisher's least significant difference tests when the global F ratio was significant. All values are reported as significant at the $P < 0.05$ level.

Additionally, we note that, due to the sequential design of this study, we cannot account for possible effects of prior challenges on responses to subsequent challenges. All rats received the same order of testing, and so effects ascribed to age may include order effects.

3. Results

3.1. Body weights

Old rats weighed significantly more than young rats, but gained significantly less weight over the 11 weeks of testing than did young rats (~30 g vs. ~90 g, respectively, age x days interaction: $F_{7,91} = 37.52$, $P < 0.001$; **Fig. 1**). Because of the significant age-related difference in BW, the data are analyzed and presented as BW-adjusted measures.

3.2. Experiment 1: extracellular fluid depletion

In this series of tests, the rats were depleted of extracellular fluid by injection of a diuretic/natriuretic (furosemide). Old rats weighed more on each test, but young rats gained weight across tests while old rats did not (age $\times$ test interaction: $F_{2, 26} = 30.17$, $P < 0.001$; **Table 1**). In the 20-h depletion period before the salt appetite test (i.e., pretest), old rats drank less water overnight in response to depletion compared with young rats (age main effect: $F_{1, 13} = 57.83$, $P < 0.001$; **Table 1**). Intakes during the 4-h salt appetite tests were analyzed as rates (i.e., milliliters per 30 min period). Old rats drank significantly less water than young rats. This effect was limited to the beginning of *test 1* (age $\times$ test $\times$ time interaction: $F_{14, 182} = 4.06$, $P < 0.001$; **Fig 2**). Old rats drank significantly less saline in 4 h than young rats (age main effect: $F_{1, 13} = 5.94$, $P < 0.05$), mostly in the first 30-60 min of saline access (age $\times$ time interaction: $F_{7, 91} = 4.23$, $P < 0.001$). With repeated testing, both ages increased saline intakes, especially from *test 1* to *test 2* (test main effect: $F_{2, 26} = 17.82$, $P < 0.001$). After the salt appetite tests, rats were returned to the home cage and intakes were monitored for another 20 h. Old rats drank significantly less water than young rats after being returned to their home cages, as well as cumulatively for the 24-h period after the start of the salt appetite test (age main effects: both $F_{1, 13} = 114.96$, $P < 0.001$). Water intake in 24 h averaged over the 3 tests for old vs young rats was 5.9 ± 0.2 vs. 9.1 ± 0.3 ml/100 g BW, respectively. Saline intakes over these 20 h were equivalent between ages, but cumulative 24-h saline intakes were significantly reduced—by nearly a third—for old rats compared with young rats (age main effect: $F_{1, 13} = 5.94$, $P < 0.05$). Saline intake in 24 h averaged over the 3 tests for old vs. young rats was 2.0 ± 0.1 vs. 2.9 ± 0.3 ml/100 g BW, respectively.

The pretest UV of old rats was significantly less than that of young rats (age main effect: $F_{1, 13} = 4.30$, $P < 0.001$; **Table 1**). At this time, old rats excreted significantly less sodium than young rats (age main effect: $F_{1, 13} = 20.34$, $P < 0.001$) and consistently had higher average pretest UNa compared with young rats (age main effect: $F_{1, 13} = 9.72$, $P < 0.01$). Urinary electrolyte measures for the extracellular depletion tests are presented in **Table 2**. Compared with young rats, old rats entered the salt appetite tests with reduced water balance (age main effect: $F_{1, 13} = 21.70$, $P < 0.01$). Old rats drank significantly less fluid overall during the salt appetite tests (age main effect: $F_{1, 13} = 7.48$, $P < 0.05$) and finished testing in significantly reduced water balance (age main effect: $F_{1, 13} = 16.76$, $P < 0.01$) compared with young rats. With repeated testing, the rats excreted significantly more UV in the pretest periods (from *test 1* to *test 3*), and also drank more overall fluid during the salt appetite tests and ended the tests in greater relative water balance, especially from *test 1* to *test 2* (test main effects: all $F_{2, 26} = 4.54$, $P < 0.05$). Old rats ingested significantly less sodium during the salt appetite test than did young rats (age main effect: $F_{1, 13} = 5.93$, $P < 0.05$). However, the groups finished testing with equivalent sodium balances. For both ages, pretest UNaV, test sodium ingestion, and posttest sodium balance increased significantly with repeated testing, especially from *test 1* to *test 2* (test main effects: all $F_{2, 26} = 5.27$, $P < 0.05$). In the first test, urine was collected one h after the injection of furosemide and again in the morning. In the first h, all measures, including UV, UNa and UNaV were equivalent between ages (**Table 3**).

Estimated plasma sodium concentrations were significantly higher for old rats compared with young rats (main effect: $F_{1,13} = 24.89$, $P < .001$) and were significantly higher posttest (i.e., after ingesting sodium) compared with pretest (i.e., following sodium depletion, main effect: $F_{1,13} = 49.29$, $P < .001$), especially after *test 2* (test $\times$ time interaction: $F_{2,26} = 4.61$, $P < .05$, **Table 2**).

3.3. Experiment 2: intracellular depletion

In these tests, the rats were depleted of intracellular fluid by sc injections of hypertonic saline (i.e., 2 ml/kg BW of 1.0 or 2.0 M NaCl). Water drinking in response was dose-dependent for both ages (dose main effect: $F_{1,13} = 19.86$, $P < 0.001$). Old rats drank significantly less than did young rats at both doses (age main effect: $F_{1,13} = 15.60$, $P < 0.01$; **Fig. 3**), i.e., about 1/3 less on average, after the first 30 min of water access (interaction: $F_{5,65} = 2.47$, $P < 0.05$). Drinking was essentially over by 1 h.

The amounts of sodium in the loads were administered on a BW-basis, and thus were equivalent between the ages (**Table 4**). The UV was significantly dose-related, and greater for old rats owing mostly to increased UV by old rats at the 2 M dose (both $F_{1,13} = 5.09$, $P < 0.05$). Old rats had significantly lower UNa (age main effect: $F_{1,13} = 9.37$, $P < 0.01$). The UNaV was significantly dose-related ($F_{1,13} = 20.05$, $P < 0.001$) and equivalent between ages. The ages excreted an equivalent percentage of their respective sodium loads during the 3 h of testing. Sodium balances were comparable between ages, and significantly higher after the 2 M dose ($F_{1,13} = 33.06$, $P < 0.001$).

The amount of ingested water needed to dilute the sodium loads to isotonicity with body fluids was estimated based on a formula from Wolf (50; **Table 5**). Since the rats received equivalent amounts of sodium on a BW-basis the ages also required equivalent, dose-related volumes of ingested water to dilute their loads to isotonicity. The “Post-excretion Water Need” takes into account the amounts of water and sodium excreted during the test, and was arrived at by modifying Wolf’s formula. Urinary sodium excretion had comparable effects in reducing the amount of water needed to dilute the remaining sodium load to isotonicity between ages. Water intakes were significantly reduced for old rats (age main effect: $F_{1,13} = 15.94$, $P < 0.01$). Water intakes were significantly dose-related, and higher at the 2 M dose, for both ages (both $F_{1,13} = 21.04$, $P < 0.001$). Old rats drank a smaller percentage of the water needed to dilute their sodium loads than did young rats (age main effect, $F_{1,13} = 16.31$, $P < 0.01$). In addition, both groups drank a smaller percentage of the water needed to dilute the 2 M load compared to the 1 M load (dose main effect: $F_{1,13} = 5.89$, $P < 0.05$). Water balance at the end of testing was significantly reduced in old rats compared with young rats (age main effect: $F_{1,13} = 24.30$, $P < 0.001$).

3.4. Experiment 3: overnight food and fluid restriction

In this test, the rats were deprived of food, water and saline overnight. The next morning, old rats drank significantly less of both water and saline—less than half as much—than young rats in the first 30 min of fluid access (age $\times$ time interactions: both $F_{5,65} = 5.10$, $P < 0.01$; **Fig 4**). Ingestion was essentially completed by 1 h.

Food and fluid restriction caused equivalent loss of water between the ages (**Table 6**). The UNa, and UNaV were equivalent between ages. Old rats drank less fluid overall (i.e., water + saline) in the 3-h test than young rats, and as a result, also had significantly reduced water balance compared with young rats (both $F_{1,13} = 27.73$, $P < 0.001$). Although old rats consumed significantly less sodium in the 3-h test compared with young rats, the cumulative sodium balances were not different.

3.5. Experiment 4: captopril adulteration of drinking water

In this test, captopril was added to the drinking water in a low dose to stimulate water and saline consumption (40, 42). Water and 0.3 M NaCl were both available in the first 10 days of this experiment. Water intakes of old rats were significantly lower than those of young rats (age main effect: $F_{1,13} = 9.51$, $P < 0.01$; **Fig 5**). Water intakes increased for both ages on the first day of captopril treatment (days main effect: $F_{9,117} = 3.88$, $P < 0.001$). Saline ingestion increased significantly for both ages upon adulteration of the drinking water with captopril and more for young rats compared with old rats (age x days interaction: $F_{9,117} = 7.99$, $P < 0.001$). Saline intakes increased on the first day of captopril treatment for young rats but not until the second day of treatment for old rats.

In the second 10 days of this experiment, only water was available for drinking. Old rats drank significantly less water than young rats on both treatment and non-treatment days (age main effect: $F_{1,13} = 38.93$, $P < 0.001$). Upon adulteration with captopril, water ingestion increased (days main effect: $F_{9,117} = 7.15$, $P < 0.001$) due almost entirely to the increased intakes of young rats, which were significantly greater than those of old rats (age x days interaction: $F_{9,117} = 5.30$, $P < 0.001$).

3.6. Daily intakes of water and 0.3 M NaCl throughout experimental testing

The daily intakes of water and 0.3 M NaCl for *days 4-69* are presented in **Fig. 6** [intakes recorded during the last 20 days of experimental testing (*days 79-98*) are presented in **Fig. 5**]. For purposes of statistical analysis, intakes were averaged over blocks of 3 days before each experimental challenge, (e.g., *days 6-8* and *15-17*) yielding seven data points per group through *day 69*. The intakes were adjusted for BW using the BW determined on the day of each challenge. Old rats drank less daily water than did young rats (age main effect: $F_{1,13} = 121.94$, $P < 0.001$). The age differences in water drinking were greatest at the beginning of experimentation (days main effect: $F_{6,78} = 19.42$, $P < 0.001$). Old rats significantly increased daily water intakes as testing progressed while young rats drank significantly less water daily as testing progressed (age x days interaction: $F_{6,78} = 24.29$, $P < 0.001$). There were no significant effects pertaining to saline intake.

4. Discussion

There were a number of significant differences in the responses of young and old rats of the F344xBN strain: 1) The old rats were substantially heavier than the young rats, and generally drank less than the young rats in response to the challenges when BW was considered. 2) Following sodium depletion (*Exp. 1*), old rats drank less saline than young rats. Both young and old rats drank enough saline solution to completely replace the lost

sodium, and drank increasing amounts of saline upon repeated depletions. 3) Old rats drank less water than did young rats in response to osmotic challenge (*Exp. 2*). Old rats excreted more dilute urine in response to the hypertonic load, and finished testing in reduced water balance, compared with young rats. 4) Old rats drank significantly less of both water and saline solution during combined food and fluid restriction (*Exp. 3*) compared with young rats, despite having comparable renal (i.e., urinary excretion) responses to food and fluid restriction. Old rats also finished testing with significantly lower water and sodium balances, due solely to diminished water and sodium ingestion compared with young rats. 5) Old rats were refractory to the stimulatory effects of low-dose captopril treatment on daily intakes of water and saline compared with the intakes of young rats (*Exp. 4*). The results from this chronic procedure, together with the results from repeated sodium depletions (*Exp. 1*), reveal persistent age-related behavioral deficits in F344xBN rats.

4.1. Experiment 1: extracellular depletion

The first experiment compared the salt appetite and renal responses of young (4 mo) and old (20 mo) male F344xBN rats to repeated episodes of sodium depletion. When the depleted animals were given access to saline solution to drink, old rats consumed less sodium than young rats on a BW basis. Old rats drank less hypertonic saline solution, less fluid overall (i.e., water + saline) and finished testing in reduced water balance compared with young rats. Nonetheless, old rats drank sufficient fluid during testing to achieve positive water balance. Notably, old F344xBN rats had robust salt appetite responses on all tests and increased their ingestion of sodium upon repeated experiences with sodium depletion. In addition, old rats drank sufficient sodium to finish testing in similarly positive sodium balance with young rats on all tests. Thus, old F344xBN rats reliably compensated for their accrued sodium deficits. These findings contrast sharply with previous reports of greatly diminished (4, 25) and absent (42) depletion-induced salt appetite in old rats of other strains using the same depletion procedures. In those reports, old rats failed to drink enough saline solution to replace the lost sodium, and did not increase sodium ingestion upon repeated testing, while old F344xBN rats clearly do.

The acute losses of water and sodium in response to furosemide, i.e., urinary water and sodium excretion in 1 h, were equivalent between the age groups. However, by morning the losses of water and sodium were different between the groups. These differences are partly due to the behavioral responses during the initial 20 h of furosemide-induced depletion. Overnight, young rats drank more water than they excreted and entered during the salt appetite portion of testing in positive water balance, while old rats failed to drink enough water to match urinary losses and entered testing in negative water balance. The negative water balance of the old rats likely reduced their subsequent sodium ingestion due to osmotic inhibition from plasma sodium levels (34). In addition, old rats excreted significantly less water than young rats, probably because they drank less water, and excreted only 75% as much sodium as young rats. Therefore, old rats may have drunk less saline solution than young rats during the salt appetite portion of testing because they had less “need” for sodium than young rats. Old rats drank approximately 75% as much saline solution as did young rats, which is proportional to their loss of sodium compared with young rats.

With experience both age groups finished testing with increasingly positive sodium balance. The F344xBN rats ingested more sodium upon repeated depletions as is commonly observed in some other strains (7, 26). Both ages drank substantially more sodium on the second test than on the first test and drank the sodium more rapidly on the third test than on the second test. Both total fluid intake (water + saline) after depletion and posttest sodium balance increased from the first to the second tests for both groups. Interestingly, the increases in sodium consumption upon repeated testing were preceded by increases in pretest water and sodium excretion. The increases in pretest sodium loss were independent of BW changes (i.e., above what would be expected due simply to weight gain over the 3 weeks of testing) and were greatest from the first to the second test. The increases in pretest water excretion (UV) may be secondary to the increases in pretest water drinking upon repeated testing. Alternatively, renal responses to furosemide may have been greater upon repeated administration—the data do not permit a firm conclusion. Regardless, pretest water balances did not change across tests, so the level of pretest water depletion stayed the same. These findings are in agreement with our previous work involving young BN rats (42) but not with those of Sakai *et al.* (26) who did not observe increased urinary losses upon repeated depletions using SD rats. However, in each study the increases in salt appetite responses with repeated testing were clearly disproportionate to the urinary losses—at least for young rats—indicating greater changes to behavioral than to renal responses with experience.

4.2. Experiment 2: intracellular depletion

The second experiment examined drinking and renal responses to osmotic challenge following sc injection of 1M and 2 M saline in amounts estimated to increase body sodium concentrations by 2% and 4%, respectively. The osmotic challenge was delivered according to BW so that the age groups received proportional amounts of sodium and thus were obliged to consume proportional amounts of water to dilute the loads to isotonicity with body fluids. Old rats drank approximately 1/3 less water on average than did young rats. In turn, old rats drank, on average, only 50% of the water needed to dilute the sodium loads to isotonicity with body fluids (even taking excretion into account), while young rats drank, on average, 85% of the water needed to dilute the sodium load to isotonicity, including all of the water needed at the lower (1 M) dose. Compensatory sodium excretion reduced the estimated drinking required to dilute the remaining load to isotonicity with body fluids for both ages. In fact, the ages benefitted from excretion equally in this regard. Therefore, diminished water drinking, and not impaired renal excretion, is the reason old rats failed to compensate for osmotic loads as effectively as young rats. These results are consistent with other reports of diminished osmotic thirst in old rats (4, 17, 36, 42, but see also 25).

As a caveat, it could be argued that the osmotic challenge was relatively greater for the older animals. The proportion of fat to lean mass tends to increase with age. Therefore, since white fat is essentially inert as far as body fluid physiology is concerned, the osmotic loads — while proportional on a BW basis—would be distributed in an effectively smaller space in the old rats. With this consideration, the equivalent UV and U_{NaV} of old rats compared to young rats reflects an underperformance by the old animals, and the already diminished water intakes of the old rats become even more inadequate in the face of potentially higher

body sodium concentrations resulting from the load administered into a proportionately smaller volume.

4.3. Experiment 3: overnight food and fluid restriction

The third experiment examined age-related behavioral and renal responses to combined food and fluid restriction. The small losses of body water and sodium during combined food and fluid restriction produce a small, presumably renin-dependent, salt appetite response. In the present case, the groups had comparable urinary losses of body water and sodium. In response, old rats drank significantly less of both water and saline and ended testing in significantly reduced water balance compared with young rats. Thus, as with a purely osmotic challenge, diminished behavior (i.e., insufficient ingestion of water and sodium) was the main difference in how old and young rats responded to depletion. The losses of body water and sodium after combined food and fluid restriction likely accrued more gradually than did the larger deficits incurred abruptly after furosemide-induced diuresis/natriuresis (*Exp. 1*). It is notable that under these conditions both groups drank amounts of water and sodium that better matched their losses than after furosemide-induced depletion. However, in both situations, old rats finished testing in significantly reduced water balances compared with younger animals, and in equivalent sodium balances. This is further evidence that defense of sodium homeostasis remains relatively intact with age in the F344xBN strain.

4.4. Experiment 4: captopril adulteration of drinking water

Old rats were clearly refractory to the effects of administering captopril in the drinking water (*Exp. 4*). Adulteration of water or chow with low concentrations of ACE inhibitors such as captopril increases water drinking by rats when water alone is available, and preferentially increases sodium ingestion by rats when saline is available with water (24, 25, 40, 42). The increased ingestion of both substances is due to increased plasma renin levels resulting from the treatment-induced disruption of negative feedback suppression of renin secretion. In turn, this results in increased generation and delivery of angiotensin I (ANG I) to circumventricular organs of the brain where the ANG I is locally converted into angiotensin II (ANG II) by central ACE that is not blocked by the low doses of ACE inhibitor (25, 40). The increased water and saline drinking by young F344xBN rats are completely typical responses to this procedure. The deficient responding by old F344xBN rats is consistent with findings from other strains (24, 25, 42) with the exception of the small increases in daily saline intakes observed here. In previous work with BN rats (42), we speculated that the failure of old animals to increase fluid ingestion during the procedure resulted from impaired renin secretion. Old rats are noticeably deficient in secreting renin (1, 2, 3, 41). However, both reduced (24) and similar (25) levels of plasma renin have been found in old rats compared to young rats exposed to similar treatment (i.e., with low-doses of ACE inhibitor placed in the diet), so the cause of reduced ingestion by old rats under these conditions is not settled.

4.5. Daily intakes of water and 0.3 M NaCl throughout experimental testing

On a BW basis, daily water intakes initially differed with age before converging by 4-5 weeks. We noted a similar pattern in the daily water intakes of young and old BN rats (42), and attributed this difference to increased sensitivity of old rats to changes in conditions, and a need for more time by older animals to acclimate to new conditions. Additionally, the decline in daily water intakes by young rats could reflect maturational effects over this extended period of testing. Daily intakes of the concentrated saline solution were small for both groups, and there were no age-related differences in daily saline intakes during the course of experimentation, except for transient differences arising acutely in response to the sodium depletion tests or ACE inhibition. The reduced daily water intakes of old rats compared with young rats may simply be due to less metabolic need for water compared to young, rapidly-growing rats. However, it cannot be ruled out that the reduced daily water intakes may also reflect persistent deficits that underlie their diminished responses to challenges. Accordingly, the lack of group differences in daily sodium intake suggests the observed deficits to sodium depletion are specific to the challenge.

4.6. General Discussion

One of the general concerns in aging research is that a given strain of rat is likely to show a higher prevalence for a specific disorder than other strains. For example, compared to many strains, the SD rat is prone to tumors which increase in incidence over the life span (2, 32). Such concerns have led to the use of specific strains in aging studies because of the low probability of confounding diseases (13, 45). However, no single strain is totally without some potentially complicating pathology that may interact with the aging-related endpoints under study. Consequently, in order to be able to make generalizations about specific age-related effects, one strategy is to use multiple aging strains, each with relatively low age-related pathologies. With the use of multiple strains of rats, patterns of diminished responding with age are beginning to emerge. The present studies add to this emerging consensus by demonstrating a number of significant differences in the responses of young and old rats of the F344xBN strain.

The present findings that old rats of the F344xBN strain drank less sodium in response to the sodium depletion (*Exp. 1*) and combined food and fluid deprivation (*Exp. 3*) challenges than did young rats are consistent with reports from other strains (24, 25, 42). Sodium intake following sodium depletion and, presumably, food and fluid depletion, depends on renin secretion and subsequent formation of angiotensin (10, 27, 39). Since old rats typically secrete less renin than young rats in response to most stimuli (2, 3, 41), they may ingest less sodium than young rats during these procedures because they secrete less renin. In addition, furosemide treatment produces dilute urine, indicating that the loss of water and sodium during diuresis is accompanied by increased body fluid osmolality. Old rats are relatively insensitive to increased osmolality and do not drink as much as do young rats in response to osmotic stimuli (17, 36, 42; *Exp 2*). It is plausible that reduced osmotic sensitivity of the old rats prevented them from drinking as much water and excreting as much sodium as young rats during the depletion period. In turn, the negative water balances of the old rats, combined with their reduced sodium excretion compared with young rats, suggests they must have been relatively more concentrated than young rats at the start of salt appetite

testing. This conclusion is supported by the significantly increased estimated extracellular sodium concentration in old rats compared with young rats at the start of salt appetite testing. As noted above, old rats plausibly experienced osmotic inhibition of salt intake from elevated plasma sodium levels (34). Therefore, old rats may have ingested less sodium after depletion than young rats both because they had less need and also were less “dilute” than young rats.

Silver *et al.* (29) previously examined age-related fluid ingestion in the F344xBN strain using 3, 12, 20 and 24 mo-old rats. In their study, daily water intakes declined with age when analyzed on an absolute basis. This result differs from our present work in which older rats had equivalent daily water intakes on an absolute basis (data not shown) and smaller daily water intakes compared with young rats only upon adjusting for BW. However, old rats in our studies had simultaneous access to saline solution to drink, while rats in the studies by Silver *et al.* (29) did not. Silver *et al.* (29) found age-related reductions in water intake both after a period of water deprivation and in response to feeding after a period of food deprivation. In both of these cases, water intake is likely driven primarily by osmotic factors. Thus, these previous findings dovetail with our current findings that old F344xBN rats are refractory to pure osmotic stimulation (*Exp 2*). Lastly, Silver *et al.* (29) found that water drinking in response to sc injections of the dipsogen, ANG II was significantly reduced at all ages >3 mo, but only on a BW basis. Thus, refractory responding to ANG II, along with diminished capacity for secreting renin, may explain some of the reduced thirst and salt appetite responses observed in our present work (e.g., *Exp 1, 3 and 4*).

There are notable differences in some characteristics of the F344xBN strain and their parent strains. First, F344xBN rats grow more rapidly than F344 or BN rats (31). In our experience, young (4 mo) rats of the F344xBN and BN strains weigh the same (42 and present work), while old (20 mo) F344xBN rats are much (~90 g) heavier than BN rats of the same age. Second, both parent strains have less robust appetite responses (18, 42). Following similar means of sodium depletion as employed here (i.e., furosemide), both F344 (18, 25) and BN (42) rats require several hours—or longer—to drink sufficient volumes of saline solution to replace the lost sodium. In contrast, F344xBN rats drank enough hypertonic saline solution in just 2 hours to achieve positive sodium balance. Finally, the depletion-induced salt appetite responses of old BN rats are essentially absent (42). In contrast, old F344xBN rats demonstrated a robust salt appetite response to sodium depletion.

The present work extends observations of age-related changes in thirst and salt appetite to rats of the F344xBN strain, and includes corresponding renal responses. The work suggests that young F344xBN rats have a markedly greater salt appetite response than their peers from either parent strain, and that old F344xBN rats have far less age-related reductions in salt appetite compared to the diminished and near-absent salt appetite responses of old rats of the parent strains. While these possibilities can be established only by direct strain comparisons, it is interesting to speculate if these different levels of responding correspond to strain differences in peripheral renin and aldosterone secretion, central neural responses to challenge, or other volume-related factors. For example, F344 and SD rats develop severe renal pathology with age (2, 32). BN rats have increased incidence of hydronephrosis (13, 31), although this is not age-related. All three strains have age-related reductions in ability to

secrete renin (2, 3, 15). On the other hand, the F344xBN rat, which maintains a robust salt appetite response with age, has less incidence of renal pathology (19, 43, 44, 45). Regardless, the waning ability of aging F344xBN rats to respond to body fluid challenges was due more to impairments in behavior than in kidney function.

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Highlights

The effects of age on water and sodium ingestion were tested using male F344xBN rats.

Old male F344xBN rats had decrements in thirst- and salt appetite-related behaviors.

Behavior declined with age more than did kidney function.

Sodium homeostasis diminishes less with age in the F344xBN strain than in other strains.

Figure 1.

Body weights of young (4 mo) and old (20 mo) rats. Each point is the average BW of rats on the day of a test. Old rats were significantly heavier at each point. Values are means $\pm$ SE. In this figure, the SE are smaller than the symbols.

Figure 2.

Cumulative intake of water and 0.3 M NaCl in response to overnight sodium depletion in young (4 mo) and old (20 mo) rats in *tests 1, 2 and 3*. Rats drank more water on *test 1* than on *tests 2 and 3*, due mainly to the intakes of young rats. Rats drank more 0.3 M NaCl on *tests 2 and 3* compared to *test 1*. Values are means $\pm$ SEM. *Significantly different from old rats, $P < 0.05$.

Figure 3.

Cumulative intake of water in response to subcutaneous injections of hypertonic saline (2.0 ml/kg bw) in young (4 mo) and old (20 mo) rats. First test, 1.0 M NaCl. Second test, 2.0 M NaCl. Values are means $\pm$ SE. *Significantly different from old rats, $P < 0.05$.

Figure 4.

Cumulative intakes of water (left) and 0.3 M NaCl (right) in response to overnight food and fluid restriction in young (4 mo) and old (20 mo) rats. Values are means $\pm$ SE.

*Significantly different from old rats, $P < 0.05$.

Figure 5.

Daily intakes of water (top) and 0.3 M NaCl (bottom) in young (4 mo) and old (20 mo) rats. Captopril (cap, 0.1 mg/ml) was added to the drinking water on some days. NaCl was not available for drinking on days 11-20. Values are means $\pm$ SE. *Significantly different from days before and after cap, $P < 0.05$. **Significantly different from old rats and from days before and after cap, $P < 0.05$.

Figure 6.

Daily intakes of water (top) and 0.3 M NaCl (bottom) in young (4 mo) and old (20 mo) rats. Gaps reflect days of saline restriction. Sodium depletion tests, D1, D2, D3. Hypertonic saline (sc) tests, H1, H2. Food and fluid restriction test, FF. Daily intakes during captopril experiment are not included here. Values are means $\pm$ SE.

Table 1

Body weight, UV, water intake and water balance in young (4 mo) and old (20 mo) rats in extracellular depletion studies.

Age	Test	Body Weight [*]	Pretest UV [*]	Pretest Water Intake [*]	Pretest Water Balance [*]	Test Fluid Intake [*]	Posttest Water Balance [*]
		g	ml/100 g	ml/100 g	ml/100 g	ml/100 g	ml/100 g.
4 mo	1	311 ± 13	6.4 ± 1.0	7.2 ± 1.1	0.9 ± 0.3	2.1 ± 0.3	3.0 ± 0.5
	2	322 ± 14	6.9 ± 0.5	7.9 ± 0.7	0.9 ± 0.4	2.7 ± 0.4	3.7 ± 0.7
	3	337 ± 13 [‡]	7.2 ± 0.6 [‡]	7.7 ± 0.5	0.4 ± 0.4	2.7 ± 0.4 [‡]	3.1 ± 0.6 [‡]
20 mo	1	537 ± 12	3.2 ± 0.2	2.6 ± 0.3	-0.6 ± 0.1	1.4 ± 0.1	0.8 ± 0.2
	2	537 ± 11	3.9 ± 0.2	3.4 ± 0.3	-0.5 ± 0.1	1.9 ± 0.1	1.4 ± 0.2
	3	540 ± 11	4.3 ± 0.2	3.7 ± 0.3	-0.6 ± 0.1	2.0 ± 0.1	1.4 ± 0.2 [‡]

Values are means ± SEM. Pretest urine volume (UV), pretest water intake, and pretest water balance in the 20 h preceding the salt appetite test, total fluid intake (i.e., water + saline) during the 4-h salt appetite test, and relative water balance at the end of testing. Urine was not collected during the salt appetite portion of testing.

* Significant main effect of age, $P < 0.05$.

[‡] Significant change across tests, $P < 0.05$.

Table 2

Urinary sodium concentration and excretion, sodium intake, balances and estimated plasma sodium concentration in young (4 mo) and old (20 mo) rats in extracellular depletion studies.

Age	Test	Pretest UNa* $\mu\text{mol/ml}$	Pretest UNaV* $\mu\text{mol/100 g}$	Pretest Estimated PNa* $\mu\text{mol/ml}$	Test Na Intake* $\mu\text{mol/100 g}$	Posttest Na Balance $\mu\text{mol/100 g}$	Posttest Estimated PNa* $\mu\text{mol/ml}$
4 mo	1	59 $\pm$ 11	341 $\pm$ 54	143 $\pm$ 1	462 $\pm$ 87	121 $\pm$ 69	146 $\pm$ 1
	2	57 $\pm$ 4	386 $\pm$ 15	142 $\pm$ 1	690 $\pm$ 103	304 $\pm$ 100	147 $\pm$ 1
	3	52 $\pm$ 4	369 $\pm$ 8 [‡]	146 $\pm$ 1	700 $\pm$ 35 [‡]	331 $\pm$ 39 [‡]	148 $\pm$ 1
20 mo	1	69 $\pm$ 7	219 $\pm$ 27	148 $\pm$ 1	360 $\pm$ 31	142 $\pm$ 43	150 $\pm$ 1
	2	81 $\pm$ 5	310 $\pm$ 14	147 $\pm$ 1	501 $\pm$ 36	191 $\pm$ 42	150 $\pm$ 1
	3	71 $\pm$ 3	297 $\pm$ 7 [‡]	149 $\pm$ 1	526 $\pm$ 33 [‡]	229 $\pm$ 31 [‡]	150 $\pm$ 1

Values are means $\pm$ SEM. Pretest urinary sodium concentration (UNa), excretion (UNaV) and estimated sodium concentration (PNa) in the 20 h preceding the salt appetite test, sodium intake during the appetite test, and posttest sodium balance and PNa at the end of testing.

* Significant main of age, $P < 0.05$.

[‡] Significant change across tests, $P < 0.05$.

Table 3

Urine volume, UNa, and UNaV in 1 h after furosemide injection in young (4 mo) and old (20 mo) rats in extracellular depletion studies.

Age	Test	1-h	1-h	1-h
		UV ml/100 g	UNa $\mu\text{mol/ml}$	UNaV $\mu\text{mol/100 g}$
4 mo	1	1.9 ± 0.3	106 ± 5	194 ± 31
20 mo	1	1.5 ± 0.2	99 ± 5	145 ± 16

Values are means $\pm$ SEM. Body weight-adjusted values are in units/100 g BW. Urine volume, (UV). Urinary sodium concentration, (UNa). Urinary sodium excretion, (UNaV).

Table 4

Body weights, sodium load, UV, UNa and UNaV, fractional sodium excretion and sodium balances in young (4 mo) and old (20 mo) rats after hypertonic saline challenge. .

Age	Dose	Body Weight [*] g	Na load [*] μmol	Na load $\mu\text{mol}/100\text{g}$	UV [*] $\mu\text{l}/100\text{g}$	UNa [*] $\mu\text{mol}/\text{ml}$	UNaV $\mu\text{mol}/100\text{g}$	Percent Na load Excreted	Posttest Na Balance. $\mu\text{mol}/100\text{g}$
4 mo	1M	346 $\pm$ 11	688 $\pm$ 23	199 $\pm$ 1	0.3 $\pm$ 0.1	221 $\pm$ 8	74 $\pm$ 5	37 $\pm$ 2	125 $\pm$ 5
	2M	354 $\pm$ 12 [‡]	1417 $\pm$ 50 [‡]	401 $\pm$ 1	0.5 $\pm$ 0.1 [‡]	251 $\pm$ 11	125 $\pm$ 17 [§]	31 $\pm$ 4	276 $\pm$ 17 [‡]
20 mo	1M	542 $\pm$ 12	1083 $\pm$ 22	200 $\pm$ 1	0.3 $\pm$ 0.1	199 $\pm$ 13	65 $\pm$ 12	33 $\pm$ 6	135 $\pm$ 12
	2M	550 $\pm$ 11 [‡]	2200 $\pm$ 43 [‡]	400 $\pm$ 1	0.9 $\pm$ 0.1 [‡]	209 $\pm$ 8	178 $\pm$ 22 [‡]	44 $\pm$ 6	222 $\pm$ 22 [‡]

Values are means $\pm$ SEM. Na load is amount of injected sodium. Urine volume, (UV); urinary sodium concentration, (UNa); urinary sodium excretion, (UNaV). "Percent Na load excreted" is UNaV/Na load $\times$ 100.

* Significant main effect of age, $P < 0.05$.

[‡] Significant effect of dose, $P < 0.05$.

Table 5

Estimated water intakes required to dilute sodium load, the same measure taking urinary Excretion into account, water intakes, fractional water intakes and water balances in young (4 mo) and old (20 mo) rats after hypertonic saline challenges.

Age	Dose	Water Need* ml	Water Need ml/100 g	Post-Excretion Water Need ml/100 g	3-h Water Intake* ml/100 g	Percent of needed Water Ingested*	Posttest Water Balance* ml/100 g
4 mo	1M	3.9 ± 0.1	1.1 ± 0.0	1.0 ± 0.0	1.0 ± 0.1	98 ± 15	0.6 ± 0.1
	2M	8.7 ± 0.3 [‡]	2.5 ± 0.0 [‡]	2.1 ± 0.1 [‡]	1.4 ± 0.1 [‡]	66 ± 6 [‡]	0.9 ± 0.1
20 mo	1M	6.1 ± 0.1	1.1 ± 0.0	1.0 ± 0.0	0.6 ± 0.1	58 ± 5	0.3 ± 0.1
	2M	13.6 ± 0.3 [‡]	2.5 ± 0.0 [‡]	2.1 ± 0.1 [‡]	1.0 ± 0.1 [‡]	46 ± 5 [‡]	0.1 ± 0.2

Values are means ± SEM. The “water need” is the estimated amount of water required through ingestion to dilute the sodium load to isotonicity before taking excretion of the load into account. The formula is from Wolf (50), “water need” = sodium load/150 – water load, where the sodium load is the administered sodium (μmol), 150 is the “isotonic” concentration of sodium in plasma, and water load (ml) is the amount of water the sodium load was administered in. The amount of ingested water required to dilute the loads decreases for both groups after urinary excretion is factored in, as shown by “post-excretion water need”. For this measure, we modified Wolf’s formula to take into account the amount of sodium and water excreted in the urine during the 3 h of testing, (sodium load – UNaV)/150 – water load + UV. “Percent of needed water ingested” is the 3- h water intake/ post-excretion required water intake x 100, a measure of how much of the needed water rats ingested.

* Significant main effect of age, $P < 0.05$.

[‡] Significant main effect of dose, $P < 0.05$.

Table 6

Body weights, UV, UNa and UNaV in young (4 mo) and old (20 mo) rats after overnight food and fluid restriction, and subsequent total fluid and sodium intakes and cumulative water and sodium balances.

Age	Body Weight g	Pretest UV ml/100 g	UNa $\mu\text{mol/ml}$	Pretest UNaV $\mu\text{mol/100 g}$	Test Fluid Intake ml/100 g	Posttest Water Balance ml/100 g	Test Na Intake $\mu\text{mol/100 g}$	Posttest Na Balance. $\mu\text{mol/100 g}$
4 mo	381 $\pm$ 15	0.5 $\pm$ 0.1	316 $\pm$ 62	160 $\pm$ 38	1.7 $\pm$ 0.2	1.2 $\pm$ 0.2	90 $\pm$ 32	-70 $\pm$ 43
20 mo	564 $\pm$ 11 *	0.7 $\pm$ 0.1	183 $\pm$ 39	126 $\pm$ 29	0.6 $\pm$ 0.1 *	-0.1 $\pm$ 0.1 *	23 $\pm$ 9 *	-103 $\pm$ 36 .

Values are means $\pm$ SEM. Urine volume, (UV); urinary sodium concentration, (UNa); urinary sodium excretion, (UNaV).

* Significant main effect of age, $P < 0.05$.