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Enhanced sensitivity to naltrexone-induced drinking suppression of fluid intake and sucrose consumption in maternally separated rats

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Abstract

Early-life stress has been identified as a risk factor in the development of a host of disorders, including substance abuse; however the link between early postnatal stress and changes in measures of reward has not been thoroughly researched. The current study had two main objectives: 1) to determine the impact of maternal separation (an animal model of early-life stress) on the consumption of 10% and 2.5% sucrose solutions by Long–Evans rat dams and male and female offspring, and 2) to determine the effect of the opioid antagonist naltrexone (0.1–3.0 mg/kg) on drinking by each of those groups. Dam-pup separations occurred for varying lengths of time during the first two postnatal weeks. In Experiment 1, a two-bottle choice test (sucrose solution vs. water) was administered across five days to both nonhandled (NH) and maternally-separated (MS) offspring as adults and to dams 2–4 weeks post-weaning. In Experiment 2, naltrexone was administered prior to two-bottle choice tests. MS males and the dams of MS litters exhibited increased intake of total fluid and sucrose solutions, whereas results from females were less consistent. Naltrexone elicited a greater decrease in fluid intake and sucrose intake in male MS offspring compared to male NH offspring. These results indicate that early postnatal stress alters both sucrose consumption, a non-drug measure of reward, and apparently the brain opioid systems that mediate naltrexone-induced drinking suppression.

Keywords: Maternal separation; Stress; Sex difference; Postnatal; Sucrose; Naltrexone; Reward; Opioid

1. Introduction

Early life events can profoundly impact the development of both an organism's physiology and behavior. An ever-increasing number of studies point to incidents of early-life stress as risk factors in a multitude of illnesses ranging from cardiovascular disease to mental illness (Arellano, 1996; Caldji et al., 2000; Triffleman et al., 1995). While the link between early-life stress, depression, anxiety disorders, and post traumatic stress disorder has been relatively well researched, the link between early-life stress and the occurrence of substance abuse disorders is less clear. Most evidence connecting early-life stress and substance abuse disorders comes from clinical research establishing correlations between patients seeking substance abuse treatment and prior incidents of childhood abuse and neglect (Arellano, 1996; Ireland and Widom, 1994; Medrano et al., 1999). Little research has been conducted to investigate the effects such stress has directly on drug and non-drug measures of reward in animal models.

Periodic neonatal maternal separation in rats has been developed and used by investigators to model the effects early-life stressors have on adult physiology and behavior. During this procedure, neonatal rats are removed from the mother for several hours per day during the first few days to weeks of life (Kalinichev et al., 2002; Ladd et al., 2000; Plotsky and Meaney, 1993). The hallmark physiological effect of this manipulation is a prolonged disregulation of hypothalamic–pituitary–adrenal (HPA) axis reactivity to stress in adulthood (Kalinichev et al., 2002; Ladd et al., 2000; Plotsky and Meaney, 1993). Following a mild stress, previously separated offspring exhibit greater CRF depletion in the hypothalamus than do their non-separated counterparts, as well as greater plasma corticosterone levels. Furthermore, relative to their non-separated counterparts, these animals exhibit elevated basal levels of CRF mRNA in the

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hypothalamus and CRF in the median eminence, while also having distinctly different behavioral responses to stress.

Although the HPA axis comprises the main components of the stress response, stressors also appear to activate the endogenous opioid systems capable of inhibiting pain (Kehoe and Blass, 1986). Therefore, a stressor such as maternal separation might also alter the function of endogenous opioid systems. During the time of maternal separation, the distribution of both the opioid receptors and the opioid peptides is in flux, and manipulations that activate these systems may elicit long-lasting alterations in their function (Loughlin et al., 1985; Petrillo et al., 1987; Spain et al., 1985). Such effects have been observed behaviorally in tests of antinociception and pain responsiveness. Following both acute and chronic maternal separation, separated offspring show altered responses to exogenous opioid compounds (Kalinichev et al., 2001a,b; Kehoe and Blass, 1986). For example, maternally separated male offspring exhibit decreased responsiveness to morphine on the hot-plate test relative to non-separated males, but exhibit no difference on the tail-flick test, indicating alterations in endogenous opioid function.

The opioid systems are not only involved in stress and pain responsiveness, they also play key roles in reward as well as in fluid intake (Gerrits et al., 2003; Nestler, 1997; Van Ree et al., 2000; White and Holtzman, 2001; Yeomans and Gray, 2002). Like other drugs of abuse, opioids are self-administered, facilitate intracranial self-stimulation, and can be used to produce a conditioned place preference. It is believed that the actions of exogenous opioids on opioid receptors in the ventral tegmental area and nucleus accumbens are responsible for the reinforcing effect of opioid drugs (Gerrits et al., 2003; Pecina and Berridge, 2005). It follows from this that endogenous opioids working at those same receptors play a role in the reinforcing properties of other natural reinforcers such as sweetened milk or sucrose solutions (Pecina and Berridge, 2005).

Previous work in this laboratory has shown that early postnatal stress, as modeled by maternal separation, can alter the intake of a non-drug reward, a 10% sucrose solution, in male offspring. Maternally separated male offspring in this experiment had higher intakes of total fluid and sucrose solution than did their unseparated counterparts (Michaels and Holtzman, in press). In experiment one we extended these findings by determining the effect of maternal separation at multiple sucrose solution concentrations and in offspring of both sexes. As in our previous study, rat pups were stressed by separation from their dam during their first two postnatal weeks. As adults, maternally separated (MS) and nonhandled (NH) offspring were tested in a two-bottle choice procedure, comparing the consumption of a sucrose solution (10% or 2.5%) and water. Sucrose solution consumption is a well validated measure of non-drug reward and its intake is highly correlated with other commonly used measures of drug reward such as opiate self-administration, psychomotor stimulant intake, and alcohol consumption (Levine et al., 2003).

In experiment two we examined indirectly the role of endogenous opioids in the elevated consumption of sucrose solution in maternally separated rats through tests with the opioid antagonist naltrexone. Low doses of opioid receptor antagonists, such as naltrexone and naloxone decrease fluid consumption in rats (Brown and Holtzman, 1981a,b; Rowlett and Woolverton, 1995). Again rat pups of both sexes were tested in a two-bottle choice procedure, this time following administration of varying doses of naltrexone. Early postnatal stress also affects fluid consumption in the dams of litters that have undergone the maternal separation procedure (Michaels and Holtzman, in press), and apparently the endogenous opioid system of the dams as well (Kalinichev et al., 2000, 2002). Therefore, the dams from MS and NH litters were also tested using similar procedures to their offspring.

2. Materials and methods

2.1. Subjects

Subjects consisted of 24 adult Long–Evans hooded rat dams, 12 male offspring, and 12 female offspring (Blue-Spruce, Harlan Sprague Dawley Inc., Indianapolis, IN, USA). The same animals were used in both Experiment 1 and 2. Pregnant dams were shipped to our facility on day 12 of pregnancy. Pups were born and raised in our animal colony as described below, and following weaning housed in same-sex, same-litter groups of two or three until the time of testing. Testing began when the offspring reached approximately 90 days of age and weighed 300–400 g. Dams were housed individually and tested 2–4 weeks following weaning. During the period of testing, all offspring were individually housed in standard hanging polycarbonate cages and given access to food and water *ad libitum*. Animals were maintained on a regular 12:12 light-dark cycle, and tested during the light phase between 1:00 and 4:00 pm. This study was conducted in accordance with the National Academy of Sciences "Guide for the Care and Use of Laboratory Animals." In addition, all procedures were approved by the Institutional Animal Care and Use Committee of Emory University.

2.2. Maternal separation procedure

A variation of the maternal separation procedure described by Plotsky and Meaney (1993) was used in this study. On postnatal day 2 (P2; birth day=P1) dams were removed from their cage and the pups were sexed and assigned in groups of 8–10 to a foster dam. Each litter contained half male offspring and half female offspring. Litters were randomly assigned to one of two groups, maternally separated (MS) or nonhandled (NH). On P3 offspring in MS litters were removed from their dam and placed in a bedding-lined container in an incubator (Veterinary water-lined warmer, ThermoCare Inc., Incline Village, NE, USA) for 24 h. The incubator was kept at 31° for the duration of the separation, and the litter was kept intact. On P4 no separation was conducted and from P5–P12 alternating 3 h and 6 h separations were conducted in the same manner. NH offspring were placed with their foster-dam following group assignment and left undisturbed until weaning, except for

required cage maintenance which included changing half of the cage bedding on P7, and replenishing food when needed. Pups were weaned on P22.

2.3. Experiment 1 — sucrose preference test

Consumption of three sucrose solutions (10%, 5%, and 2.5% sucrose) was observed in dams for 3 weeks, with each concentration being monitored for 5 consecutive days, and exposure randomized using a Latin square design. Offspring were tested similarly, however a Latin square design was not used, and only 2 concentrations were tested (10% and 2.5%). Males and females were divided into two separate groups (6 males, 6 females per group) with one given access to the 10% solution and the other given access to the 2.5% solution. Only one offspring of each sex was taken from each litter; the remaining offspring were used in other experiments.

Each testing day, dams and offspring were weighed and given 1 h access to two 100 ml calibrated water bottles (Wahmann Laboratories, Timonium, MD), one containing deionized water and a second containing one of the sucrose solutions (Sigma Chemical Co., St Louis, MO). At the end of the hour, water intake was determined to the nearest milliliter. Food was available during the testing sessions and each day bottles were alternated right-left to balance any possible side preferences.

2.4. Experiment 2 — naltrexone-induced drinking suppression

The suppressive effect of naltrexone (NTX) on drinking behavior was investigated in MS and NH offspring as well as in dams from both rearing conditions. Using a 10% sucrose solution yielded the largest differences between groups in dams during Experiment 1 and was therefore used to compare consumption in dams for Experiment 2. The same offspring used in Experiment 1 were kept grouped by concentration for testing in Experiment 2. For every group, NTX (Sigma Chemical Co., St. Louis, MO) was dissolved in sterile saline and injected subcutaneously in a volume of 1.0 ml/kg 15 min prior to testing. Saline and the following doses of NTX were tested in random order: dams −0.1, 0.3, and 3.0 mg/kg, offspring −0.1, 0.3, 1.0, 3.0 mg/kg, with one dose tested per day on 5 consecutive days. After 15 min, animals were again given access to two bottles; one containing the sucrose solution and a second containing deionized water for one hour. As in Experiment 1, intake was determined to the nearest ml at the end of the session and the position of the bottles was alternated between test sessions.

2.5. Data analysis

Water and sucrose solution intake were converted to ml/kg consumed. A sucrose preference ratio was calculated by dividing the ml/kg sucrose solution consumed by the total amount of liquid (sucrose solution + water) consumed. Data from both experiments were analyzed using a two-factor ANOVA with repeated measures on the second factor (either day or dose). Analysis of sex effects was performed separately from those of rearing effects, as well as analyzed within rearing groups. Additionally in Experiment 1, total fluid intake, sucrose intake, and sucrose preference ratio data were used to calculate the cumulative area under the curve (AUC) using Prism v4.0 (Graphpad Software, Inc., San Diego, CA). The AUC data were analyzed using a *t*-test (dams) or one-way ANOVA (offspring). When applicable, a Newman–Kuels test was used for multiple *post hoc* comparisons. Differences were considered significant if the p value was equal to or less than 0.05.

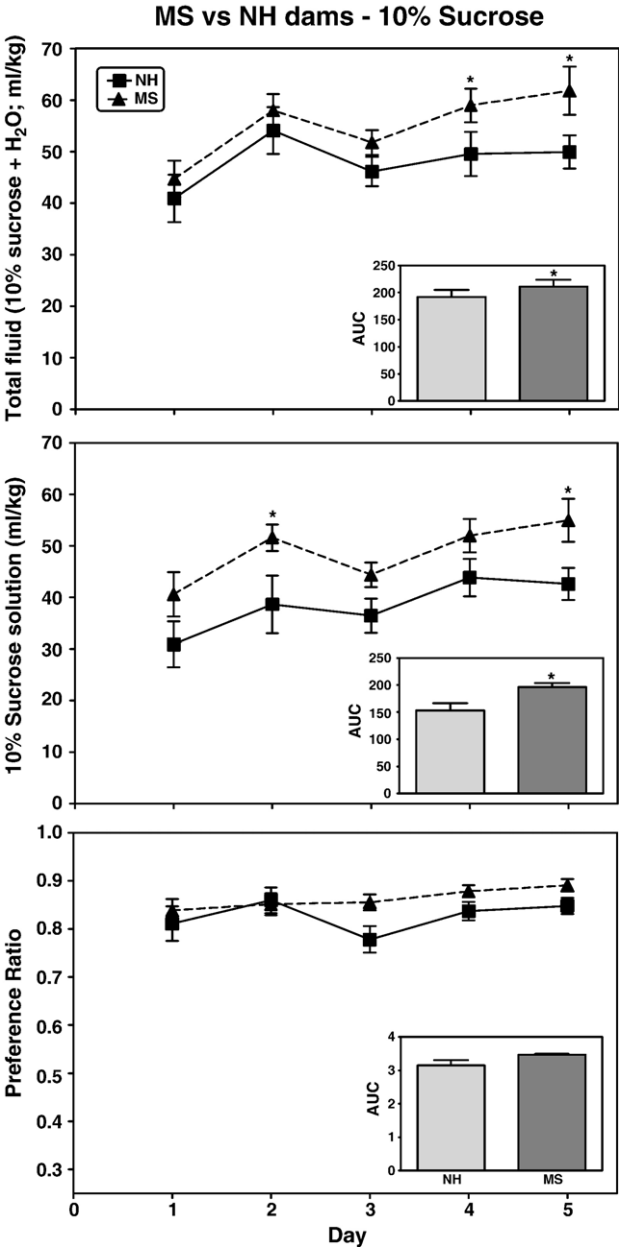

Fig. 1. Dams that underwent separation from their pups (MS) had a higher total fluid intake (top panel) and drank more 10% sucrose solution (middle panel) than did nonhandled dams (NH). However, there was no difference in preference ratio between the two groups (lower panel). Area under the curve (AUC) was also greater for MS dams for total fluid intake (top panel insert) and 10% sucrose intake (middle panel insert) but not for preference ratio (lower panel insert). Mean ± S.E.M.; $n = 12$/group. *$P < 0.05$ compared to NH dams.

3. Results

3.1. Sucrose preference

3.1.1. Dams

Overall dams that underwent the maternal separation procedure (MS dams) consumed more total fluid and more sucrose solution than dams that did not (NH dams) (Fig. 1). This was most apparent when comparing groups at 10% sucrose solution: there were significant effects of both rearing and day for total fluid intake [$F(1,21)=4.9$ and $F(4,88)=6.7$, respectively] and sucrose intake [$F(1,21)=8.5$ and $F(4,88)=6.5$]. Generally, MS dams consumed approximately 5–10 ml/kg more sucrose and total fluid when compared to NH dams on days 2 and 5. Nothing was significant at 2.5 and 5% sucrose with the exception of a day effect for sucrose intake at 5% sucrose [$F(4,92)=2.5$] (data not shown). Analysis of the AUC for dams from both rearing conditions revealed a significant effect of rearing on both total fluid intake [$t(20)=1.9$] and 10% sucrose intake [$t(20)=2.6$] (Fig. 1).

3.1.2. Offspring

Males — in general MS male offspring consumed more 10% sucrose solution [$F(1,11)=6.8$], more total fluid [$F(1,11)=5.8$] and had a higher sucrose preference ratio [F

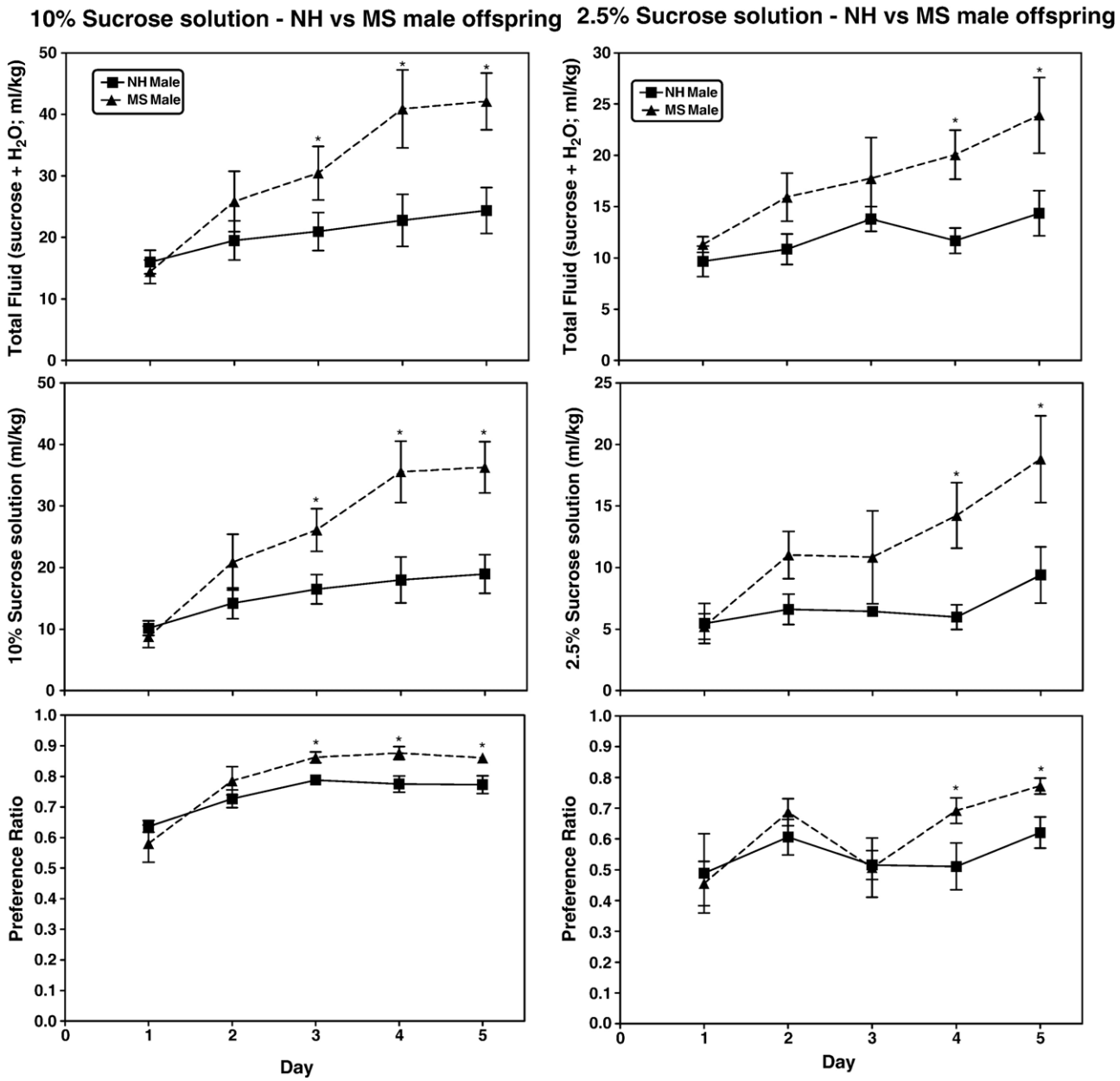

Fig. 2. Male MS offspring drank more total fluid (top panels) and sucrose solution (middle panels) at both sucrose concentrations (10% and 2.5%). MS males also had a higher preference ratio than NH males at 10% (lower-left panel) but not at 2.5% sucrose (lower-right panel). Mean ± S.E.M.; $n=6$/group. *$P<0.05$ compared to NH male offspring.

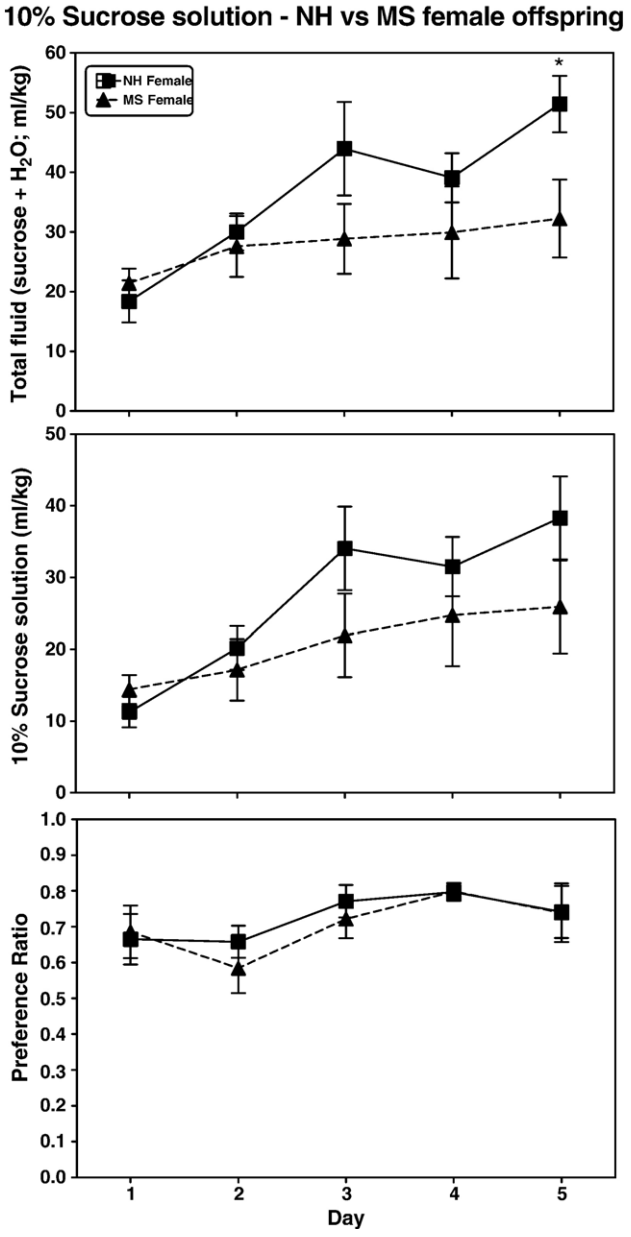

Fig. 3. There were no differences in total fluid intake (top panel), 10% sucrose intake (middle panel) or preference ratio (lower panel) when comparing MS females to NH female offspring. Mean±S.E.M.; $n=6$/group. *$P<0.05$ compared to MS females.

(1,11)=5.5] than NH male offspring (Fig. 2). On the first day of testing, offspring from both groups consumed similar amounts at 10% sucrose, but by day 5, MS males had increased their intake 280%, while NH males had increased theirs by only 70%. This resulted in a difference in intake of almost 20 ml/kg. A significant day effect was also found for each of the measures [$F(4,48)=11.9$, $F(4,48)=22.3$, and $F(4,48)=5.8$, respectively] as well as a significant day × rearing interaction for total fluid intake [$F(4,59)=3.8$] and sucrose intake [$F(4,59)=6.3$], but not for sucrose preference ratio. Similar results were observed when comparing male offspring at 2.5% sucrose (Fig. 2). Both rearing and day had significant effects on total fluid intake [F (1,11)=8.1, and $F(4,48)=4.6$, respectively] and sucrose intake, [$F(1,11)=11.6$, and $F(4,48)=4.5$], but had no effect on preference ratio. Intake for males of both rearing groups was again the same on day one, but by day 5, MS males had a 275% increase compared to only a 80% increase by NH males. No significant rearing × day interactions were determined using the 2.5% sucrose solution. Analysis of AUC for 10% sucrose solution showed a significant impact of rearing on total fluid intake [$F(1,20)=3.1$]

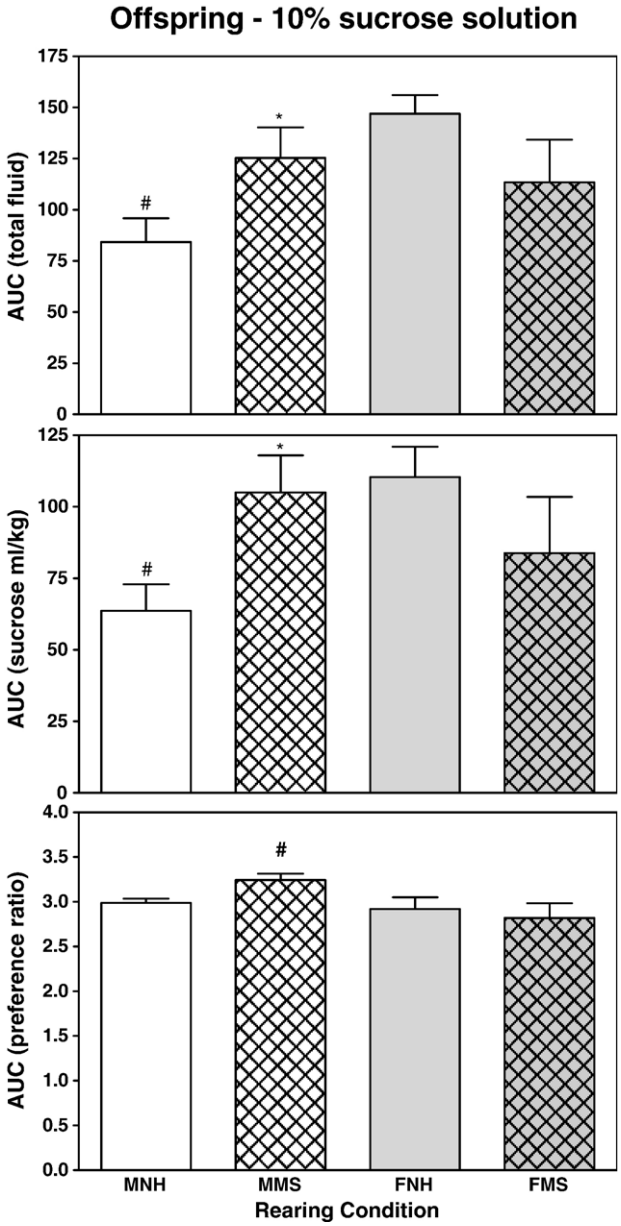

Fig. 4. Total fluid intake (top panel), 10% sucrose intake (middle panel), and preference ratio (lower panel) for male non-handled (MNH, white bars), male maternally-separated (MMS, hatched-white), female non-handled (FNH, grey), and female maternally-separated (FMS, hatched-grey) offspring across 5 consecutive days expressed as area under the curve (AUC). Mean±S.E.M.; $n=6$/group. *$P<0.05$ compared to same sex NH, $^{\#}P<0.05$ compared to females of the same rearing condition.

and 10% sucrose intake[$F(1,20)=2.9$], but no alterations in preference ratio (Fig. 4).

Females — rearing did not affect total fluid intake, sucrose intake, or sucrose preference ratio when comparing MS and NH female offspring at 10% (Fig. 3) or 2.5% (data not shown) sucrose. Additionally no day × rearing interactions were observed at either concentration. A significant day effect was found for all three measures with the 10% sucrose solution, [$F(4,59)=5.9$, $F(4,59)=9.2$, and $F(4,59)=3.2$, respectively], but not with the 2.5% solution. AUC analysis for 10% sucrose revealed no rearing effect on total fluid intake, 10% sucrose intake, or preference ratio (Fig. 4).

3.2. Sex comparisons

NH offspring — sex differences were apparent at both sucrose concentrations, but less dramatic at the lower concentration (2.5% sucrose). For both sucrose concentrations, sex had a significant impact on total fluid intake [$F(1,11)=22.7$ for 10%, and $F(1,11)=32.4$ for 2.5%], sucrose intake [$F(1,11)=12.0$, and $F(1,11)=28.0$], and sucrose preference ratio[$F(1,11)=7.2$, and $F(1,11)=16.2$] (Fig. 5). At 10% sucrose concentration, NH females increased their consumption by 180% by testing day 5 compared to 50% for NH males. Day also impacted all three measures, but only

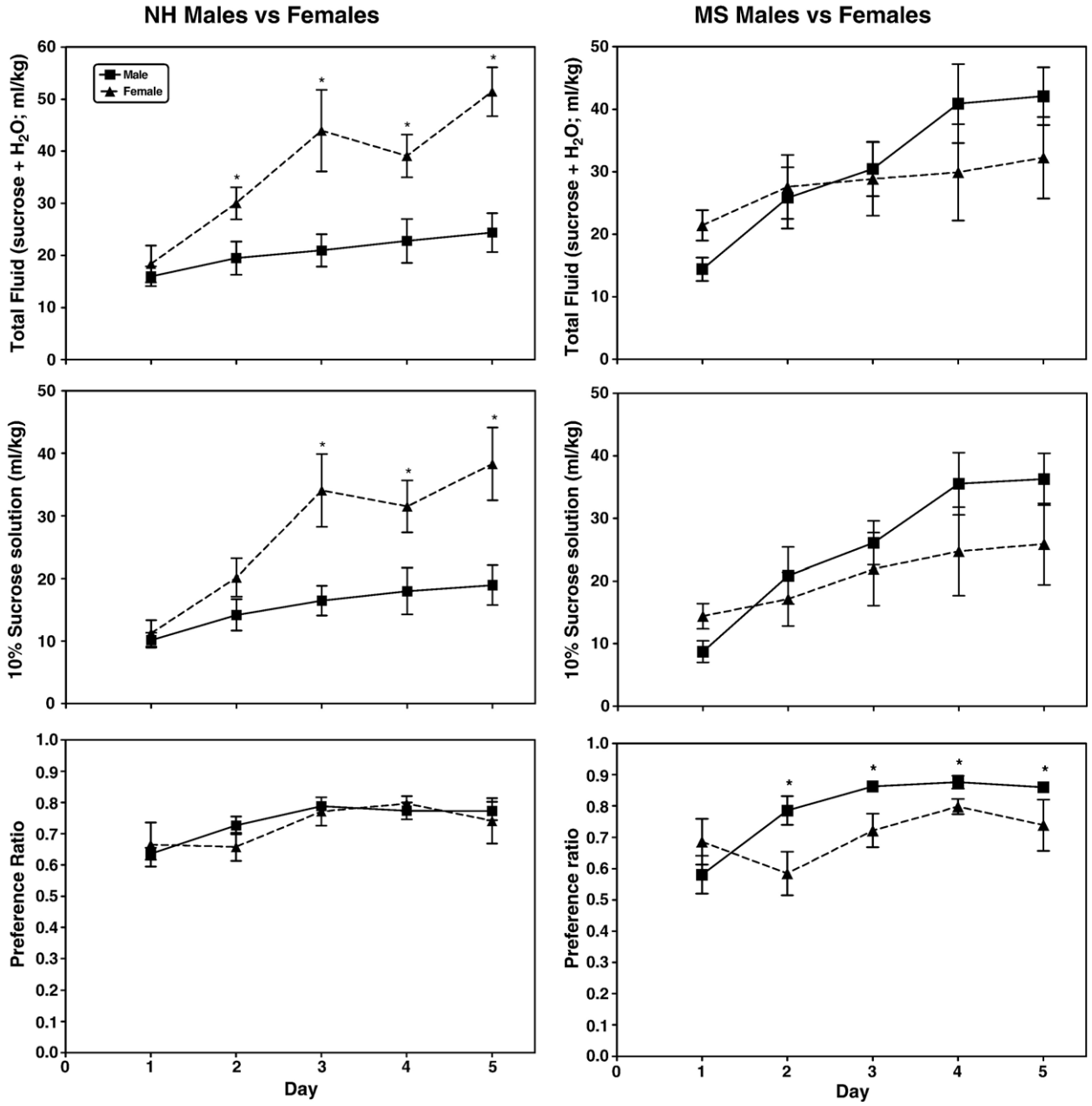

Fig. 5. Sex difference between male and female NH and MS offspring. NH females drank more total fluid (top-left panel) and 10% sucrose solution (middle-left panel) than NH males, but did not exhibit a difference in preference ratio (lower-left panel). MS males had a significantly higher preference ratio (lower-right panel) compared to MS females, but did not differ in total fluid intake (top-right panel) or 10% sucrose solution intake (middle-right panel). Mean ± S.E.M.; $n=6$/group. *$P<0.05$ compared to females of the same rearing condition.

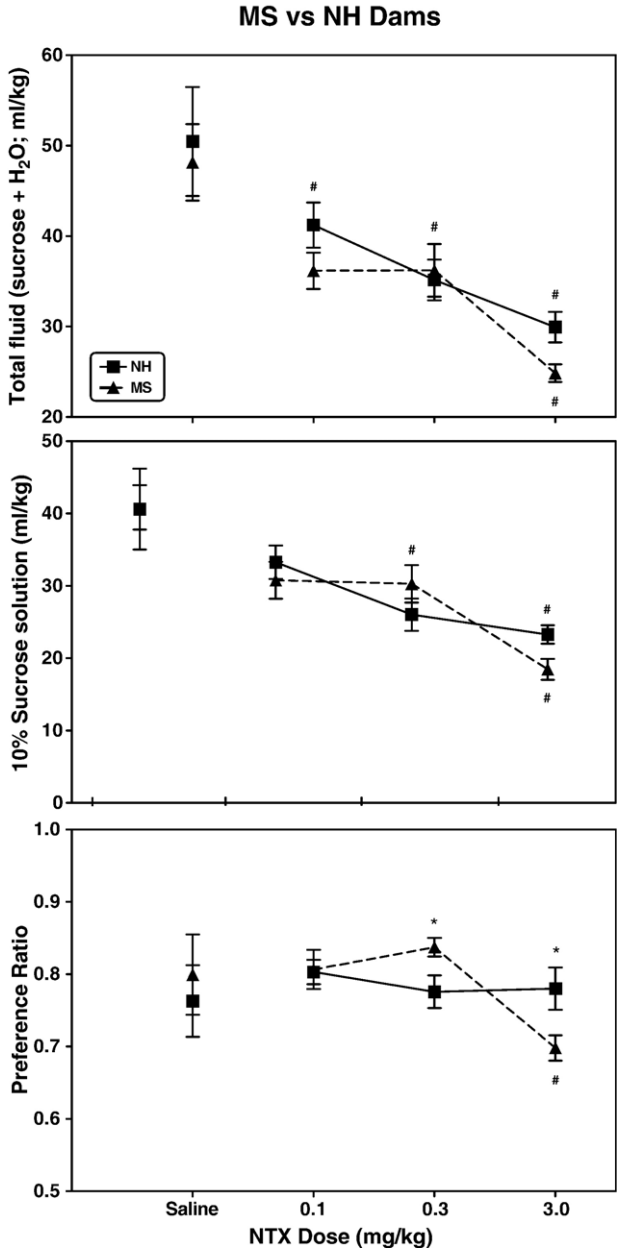

Fig. 6. The effects of NTX administration on total fluid intake (top panel), 10% sucrose intake (middle panel) and preference ratio (lower panel) in NH and MS dams. As NTX dose increased, total fluid and 10% sucrose intake decreased in dams of both rearing conditions. A significant dose × rearing interaction was found for preference ratio, with MS dams having a higher ratio at 0.3 mg/kg NTX and NH dams having a higher ratio at 3.0 mg/kg. Mean ± S.E.M.; $n=6$/group. *$P<0.05$ compared to NH dams, #$P<0.05$ compared to saline control of the same rearing condition.

when using 10% sucrose solution [total fluid intake, $F(4,48)=10.2$; sucrose intake, $F(4,48)=12.0$; preference ratio, $F(4,48)=9.3$], and not when using a 2.5% solution. A significant sex × day interaction was also found at both concentrations for total fluid intake, [$F(4,59)=3.9$ for 10% and $F(4,59)=2.6$ for 2.5%], only at 10% sucrose for sucrose intake, [$F(4, 4,59)=3.0$] and at neither concentration for preference ratio. Sex differences also were detected at 10% sucrose by AUC analysis, with a significant sex effect for total fluid intake [$F(1,20)=6.5$] and 10% sucrose intake [$F(1,20)=4.7$], but not for preference ratio (Fig. 4).

MS offspring — at both 10%, and 2.5% sucrose solution, sex appeared to have no affect on total fluid intake, or sucrose intake. Sucrose preference ratio differed significantly between groups at 10% sucrose [$F(1,11)=3.7$], but not at 2.5% sucrose (Fig. 5). A significant day effect was found for total fluid intake [$F(4,48)=9.9$ for 10%, and $F(4,48)=5.9$ for 2.5%], sucrose intake [$F(4,48)=15.1$, and $F(4,48)=6.4$] and preference ratio (10% only) [$F(4,48)=8.2$]. Sex × day interactions were observed for both sucrose intake and preference ratio [$F(4,59)=$

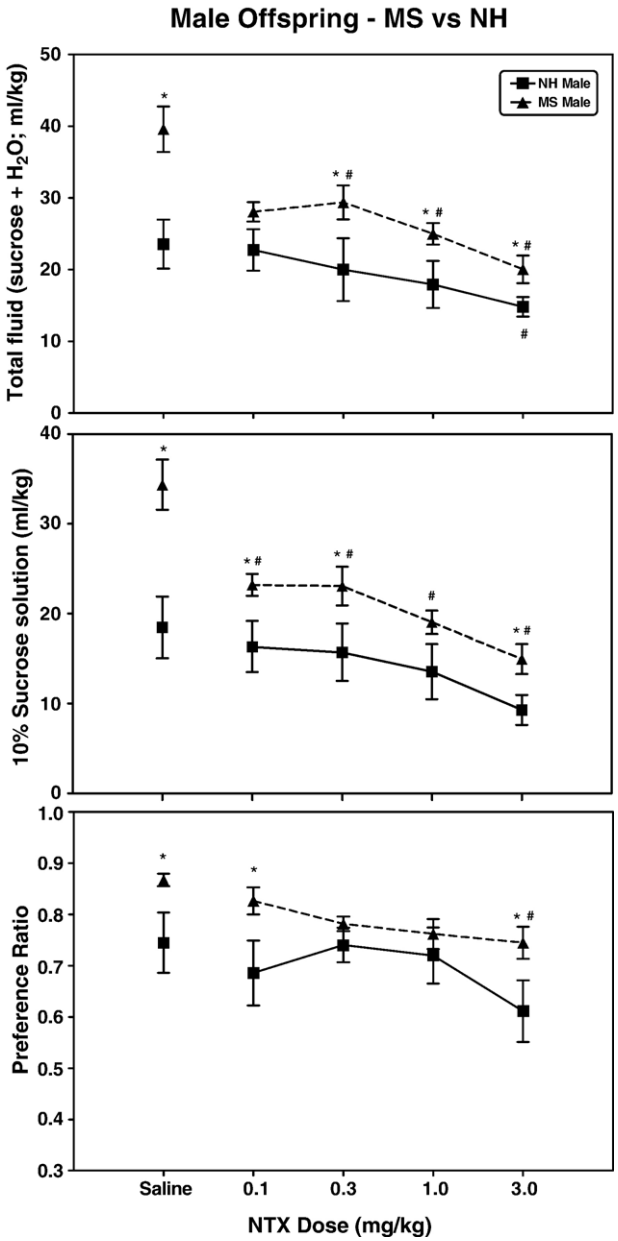

Fig. 7. Dose response to NTX administration in male MS and NH offspring. MS males drank more total fluid (top panel), more 10% sucrose solution (middle panel) and had higher sucrose preference ratios (lower panel) than their NH counterparts. Mean ± S.E.M.; $n=6$/group. *$P<0.05$ compared to same sex NH offspring, #$P<0.05$ compared to saline control of same rearing condition.

2.6, and $F(4,59)=3.8$] with the 10% solution, but not for total fluid intake (Fig. 5). With the 2.5% sucrose solution, no interactions were detected. AUC for 10% sucrose differed significantly between sexes for sucrose preference ratio [$F(1,20)=3.1$] but not for total fluid intake or 10% sucrose intake.

3.3. Effect of naltrexone

3.3.1. Dams

There was no main effect of rearing on any measure of consumption between MS and NH dams (Fig. 6). A significant effect of dose was observed for total fluid intake, total sucrose intake, and for sucrose preference ratio, [$F(3,72)=18.9$, $F(3,72)=19.5$, and $F(3,72)=7.2$, respectively] (Fig. 6). As the dose of naltrexone increased, total fluid intake and sucrose intake decreased in both groups. Additionally, there was a dose × rearing condition interaction for sucrose preference ratio [$F(3,95)=5.2$], with MS dams consuming a higher proportion of sucrose at 0.3 mg/kg NTX compared to NH dams, and NH dams consuming a higher proportion of sucrose then MS dams at a dose of 3.0 mg/kg. No interactions were observed for total fluid intake or sucrose intake.

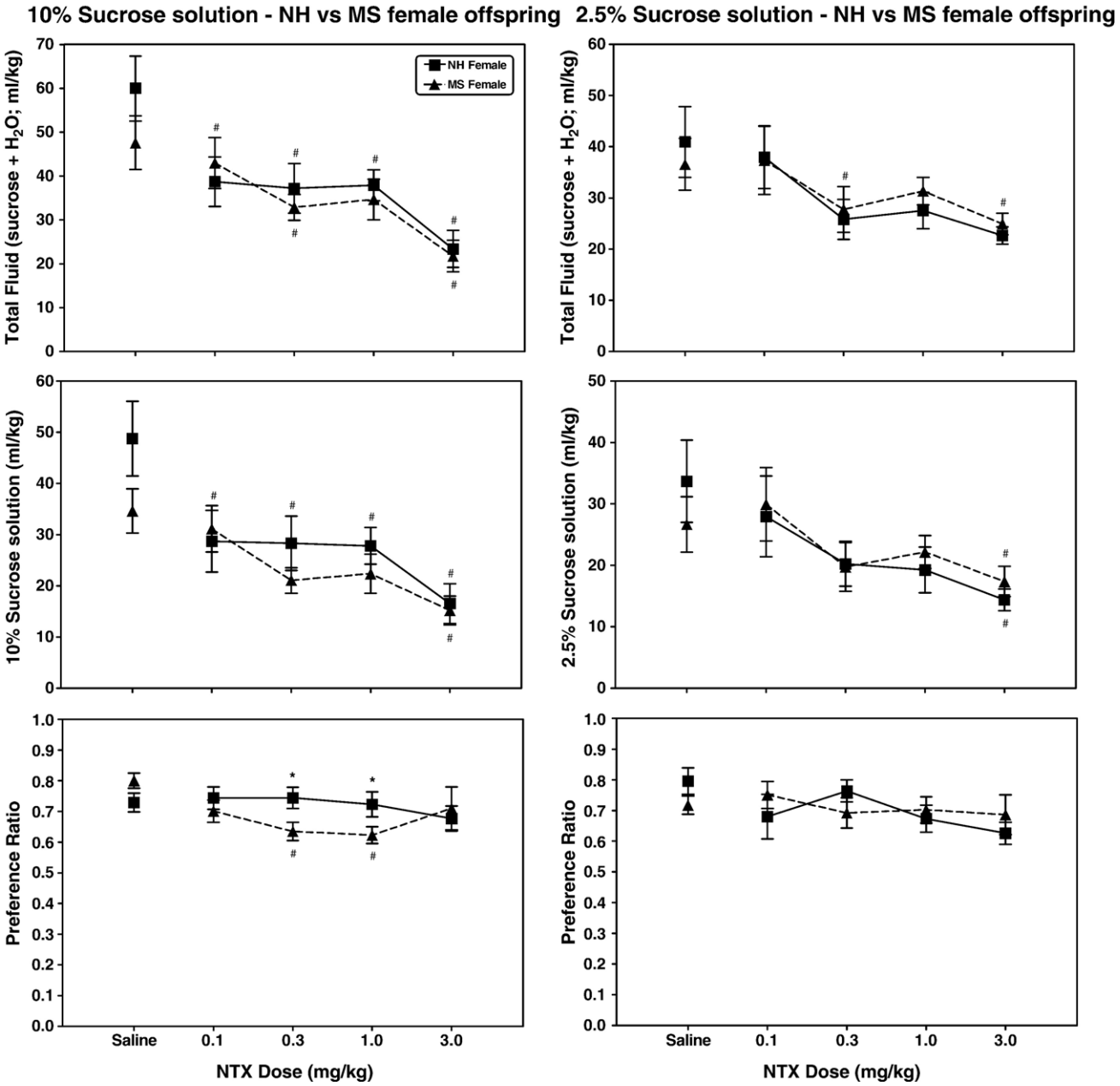

Fig. 8. Dose response to NTX administration in female MS and NH offspring. Results with 10% sucrose solution are on the right, 2.5% sucrose solution left. Females from both groups drank similar amounts of total fluid (top panels) and sucrose solution (middle panels). NH females had higher sucrose preference ratios than MS females at 10% sucrose solution (lower-left panel). Mean ± S.E.M.; $n=6$/group. *$P<0.05$ compared to same sex NH offspring, $^{\#}P<0.05$ compared to saline control of same rearing condition.

3.3.2. Offspring

Males — at both 10% and 2.5% sucrose concentrations, MS male offspring consumed more total fluid [$F(1,11)=10.5$ for 10%, and $F(1,11)=5.6$ for 2.5%], sucrose solution [$F(1,11)=10.2$, and $F(1,11)=4.8$], and had higher sucrose preference ratios [$F(1,11)=4.9$, and $F(4,124)=5.6$] than their NH counterparts across all doses of naltrexone (Fig. 7, only 10% data shown for simplicity). MS male offspring consumed approximately 5–10 ml/kg more 10% and 2.5% sucrose solution, regardless of naltrexone dose. Naltrexone dose-dependently decreased total fluid intake [$F(4,48)=13.8$ for 10%, and $F(4,48)=8.6$ for 2.5%] and sucrose intake [$F(4,48)=17.2$, and $F(4,48)=5.7$] and preference ratio [$F(4,48)=4.9$, and $F(4,48)=3.8$] at both concentrations. Additionally a significant rearing × dose interaction was detected for 10% sucrose intake, [$F(4,59)=3.0$], but not 2.5%. No rearing × dose interaction was found for total fluid intake or preference ratio at either concentration.

Females — NH female offspring had a higher sucrose preference ratio than MS female offspring with 10% [$F(1,11)=4.1$] but not 2.5% sucrose solution (Fig. 8). No differences, based on rearing, were observed at either concentration for total fluid intake or sucrose intake. Naltrexone dose dependently decreased total fluid intake [$F(4,48)=9.5$ for 10%, and $F(4,48)=7.5$ for 2.5%] and sucrose intake [$F(4,48)=6.1$, and

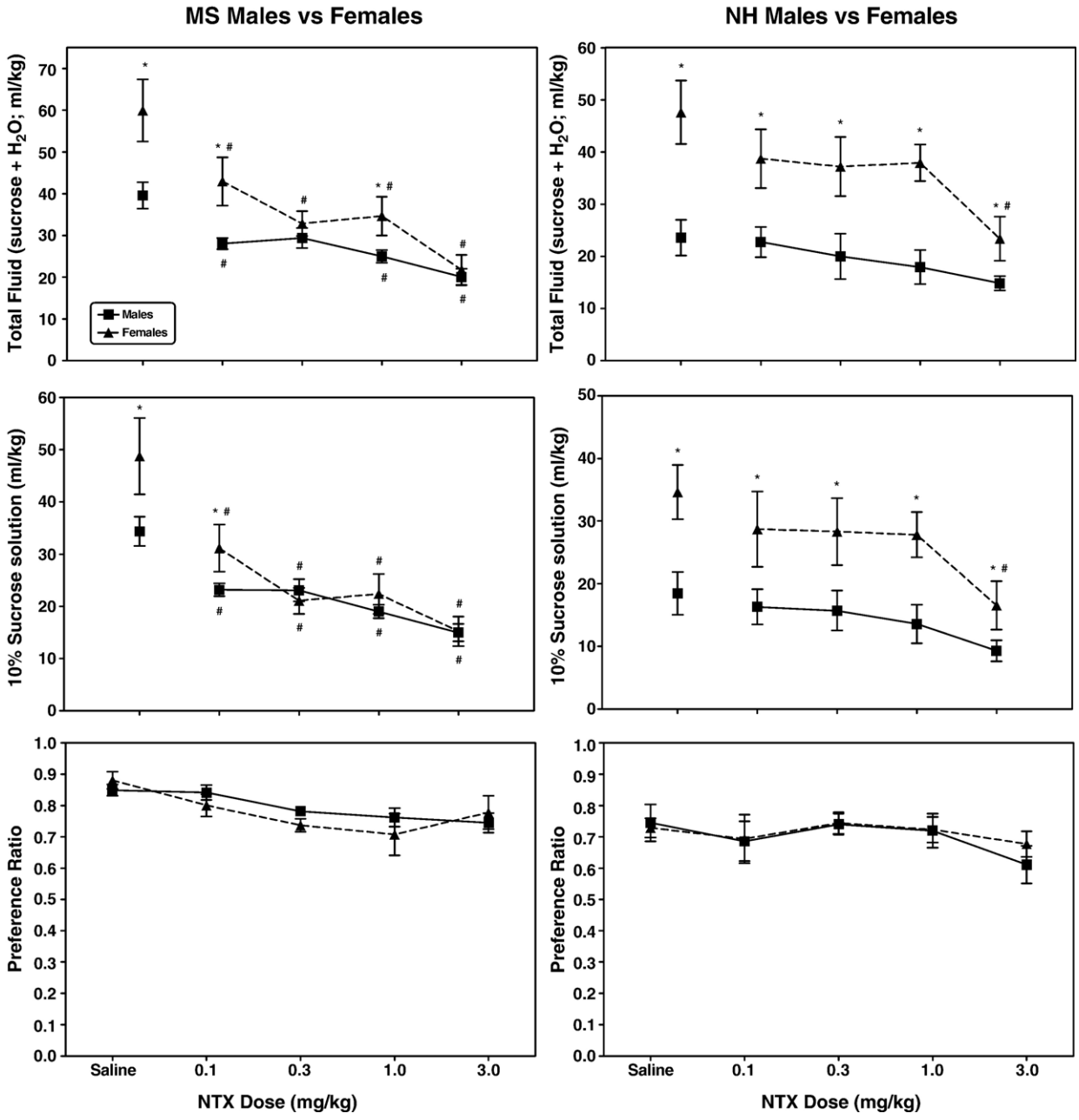

Fig. 9. Sex differences across NTX doses ranging from 0 mg/kg–3.0 mg/kg. MS and NH females drank more total fluid (top panels) and 10% sucrose (lower panels) compared to males of the same rearing condition. No differences were found for sucrose preference ratio (lower panels). Mean ± S.E.M.; $n=6$/group. $^{*}P<0.05$ compared to NH of the same rearing condition, $^{\#}P<0.05$ compared to saline control of the same rearing condition.

$F(4,48)=8.1$] in all groups at both concentrations. As the dose of naltrexone increased from 0 mg/kg to 1.0 mg/kg, total fluid intake decreased approximately 50% at 10% sucrose and 40% at 2.5% sucrose in both MS and NH females similarly. No rearing × dose interactions were observed at 10% or 2.5% sucrose.

3.3.3. Sex comparisons

Significant sex differences appeared when comparing drinking behavior across all doses of naltrexone. Regardless of rearing condition, female offspring consumed more total fluid [$F(1,11)=23.7$ for MS, and $F(1,11)=19.1$ for NH] as well as 10% sucrose [$F(1,11)=10.4$, and $F(1,11)=11.9$] and 2.5% sucrose [$F(1,11)=10.7$, and $F(1,11)=15.2$] than male offspring (Fig. 9; for simplicity, only the data with 10% sucrose solution are shown). Sucrose preference ratio however was unaffected by sex at 10% or 2.5% sucrose. A NTX dose effect was found for total fluid intake [$F(4,48)=13.5$, for MS, and $F(4,48)=6.4$, for NH] as well as 10% sucrose solution [$F(4,48)=14.9$, and $F(4,48)=5.1$] and 2.5% [$F(4,48)=4.9$, and $F(4,48)=7.7$] sucrose solution, but not for preference ratio. Additionally, no significant sex × dose interactions were detected at 10% or 2.5% sucrose.

4. Discussion

In general, rearing condition had an effect on total fluid intake, sucrose intake and sucrose preference ratio in male offspring, but not in females, and group differences were greater with the higher concentration (10%) of sucrose than with the lower one (2.5%). In contrast to NH male offspring, NH females consumed significantly more total fluid and sucrose solution and again results were more dramatic comparing intake of the 10% sucrose solution. Results from the dams were similar to those of male offspring, with MS dams consuming more sucrose solution then NH dams, and with the most dramatic differences observed at the highest sucrose concentration (10%). By comparing total fluid intake, sucrose solution intake and converting those data to a preference ratio we were able to examine not only the intake of a non-drug reward, but also whether preference for that reward (sucrose solution) was affected. Water intake remained stable between both groups across days, with the differences in total fluid intake between groups generally reflecting differences in their intake of the sucrose solutions.

4.1. Sucrose preference

The results of Experiment 1 are in accordance with work previously completed in this laboratory and by Vazquez et al. (2005a,b). We previously found that maternal separation caused an increase in total fluid intake and 10% sucrose solution intake in male offspring only (Michaels and Holtzman, in press). In this study we added to these findings, determining that results were similar, but less dramatic at lower sucrose concentrations in males. Females appear to be less affected by the maternal separation manipulation than are males, which is consistent with previous reports on ethanol drinking in maternally-separated rats (Moffett et al., in press). Our results for male offspring were similar to those of Vazquez et al. (2005a,b), in which maternally deprived male offspring exhibited increased consumption compared to those that had not been deprived. Although Vazquez et al. (2005a,b) used a low concentration of sucrose (0.25%) for their experiments they also deprived their animals for 20 h prior to testing. This may explain why they exhibited consistent results at a lower concentration, while our results at lower sucrose concentrations were less profound. Additionally our research supports what was known about sex differences in NH animals, that female rats exhibit increased fluid intake per kilogram weight, compared to male rats (Lancaster and Spiegel, 1992; Paino et al., 2005). These sex differences also carried over to our experiments involving NTX.

The results we observed for dams in Experiment 1 were also in accordance with those of our previous work; however the differences between MS and NH dams were not as striking at 10% sucrose and not existent at 5% or 2.5% sucrose. This may be explained by our use of a Latin square design. By using such a design, each dam was randomly exposed to all concentrations of sucrose tested. Our experimental design may have unintentionally altered the results by incorporating an order effect of sucrose exposure and thus made differences less apparent.

Clinically, early-life stress is linked to both depression and substance abuse in later life. Depression and substance abuse disorders are often co-morbid; therefore, it is not surprising that an animal model of early-life stress could be used to explore both conditions (Arellano, 1996; Brady and Verduin, 2005; Nunes and Rounsaville, 2006; Triffleman et al., 1995). While we have used maternal separation to investigate potential changes in reward, others have used it to model depressive phenotypes. Sometimes in these latter studies, sucrose solution consumption is used to assess not reward *per se*, but anhedonia, a condition associated with depression and characterized by inability to experience pleasure from normally pleasurable stimuli. In many of these studies, consumption of sucrose solution (or sucrose) was reduced, an outcome similar to that resulting from chronic mild stress, another procedure used to model depressive phenotypes (Grippo et al., 2003; Matthews et al., 1995; Papp et al., 2003). The results of our study are in contrast to the findings of those studies. It is likely that early-life stress impacts many brain systems during development and produces multiple outcomes, depending upon the type, duration, and timing of the stressor, and the age at which the subjects are tested. Regardless, the results of the present study are entirely consistent with those of our previous one (Michaels and Holtzman, in press). Thus, with our particular experimental parameters, early maternal separation results in reproducible long-lasting increases in the intake of and preference for sucrose solution, particularly in male offspring.

4.2. Effect of naltrexone

In dams, rearing condition appeared not to affect total fluid or sucrose solution consumption at any dose of NTX; however a dose × rearing interaction was found, with MS

dams having a higher sucrose preference ratio at 0.3 mg/kg NTX, but NH dams having a higher ratio at a dose of 3.0 mg/kg NTX. NTX dose-dependently decreased almost all dependent measures in all groups tested. Amongst offspring, NTX decreased fluid and sucrose solution intake in most cases, although there were some differences between groups. NTX appeared to cause a greater decrease in MS males compared to NH males, while rearing appeared to have little effect on female offspring, with no rearing difference for total fluid or sucrose solution intake. Sex differences also were apparent. MS males consumed more than MS females across all NTX doses, and NH females consumed more across all doses compared to NH males. These findings are in accordance with previous work, in which opioid antagonists such as naloxone and NTX caused dose-dependent decreases in fluid intake. In both our study and that of Brown and Holtzman (1981a), as NTX dose increased, fluid consumption decreased. Brown and Holtzman (1981a) examined water intake only, following a 24 h water deprivation. Our results are also in agreement with the results of Baker et al. (2004) and Locke et al. (1982), in which consumption of palatable solutions (such as sweetened condensed milk) was suppressed following NTX administration, without water deprivation.

NTX induced drinking suppression in the dams during Experiment 2 may have been impacted by the same problem as in Experiment 1, the previous use of a Latin square design for sucrose exposure. Although only a 10% sucrose solution was used in constructing the NTX dose response curve, all animals had previously been exposed to all sucrose concentrations, and this may have altered the reinforcing properties of the 10% solution (Taha et al., 2006). It is also possible that the effects of the maternal separation procedure on dams dissipated by the time of Experiment 2 (which was conducted 3 weeks after Experiment 1), as they are known to dissipate over time (Kalinichev et al., 2000). What experiments could be conducted with the dams was also limited by the small number of subjects available (1 dam/litter) compared to the number of offspring available (10 offspring/litter).

One of most interesting findings was the sex difference amongst both MS and NH offspring. This was not the first time sex differences were observed when examining processes related to endogenous opioid systems in maternally separated offspring. In tests of antinociception, male MS offspring exhibited a decreased responsiveness to morphine-induced antinociception, whereas MS females did not (Kalinichev et al., 2001b). Differences in the effectiveness of opioid compounds between sexes are well documented and highlight the possibility that the distribution and functionality of the endogenous opioid systems differs between males and females (Kest et al., 2000). Sex-based differences also appear to be common to measures of reward other than sucrose consumption, such as intracranial self-stimulation and IV drug self-administration (Cicero et al., 2003; Stratmann and Craft, 1997). Maternal separation could be enhancing an underlying sex difference in endogenous opioids or in other components of brain reward systems, accounting for the results observed here.

4.3. Opiates and changes in reward

The observed differences in response to NTX between MS and NH offspring gives added support to the theory that early postnatal stress, as modeled by maternal separation, can elicit long-lasting changes in the endogenous opioid systems. While NTX blocks all three opioid receptor subtypes, it is the antagonism at the mu (for H_2O and sucrose) and kappa opioid receptors (sucrose only) that underlie the drug's suppressive effects, either directly suppressing drinking, or by altering the palatability (i.e., rewarding value) of the liquid (Beczkowska et al., 1992; Cichelli and Lewis, 2002). NTX-induced suppression of drinking also appears to be centrally mediated: direct administration of NTX into the nucleus accumbens, a brain site involved in both drug and nondrug reward (Koob, 2000; Wise, 1998), decreases sucrose intake (Kelly et al., 1996; Ukai and Holtzman, 1987). Additionally, maternal separation appears to alter the function or distribution of the mu opioid receptor as evidenced by increased ED50s for MS offspring on tests of antinociception as well as increased global withdrawal scores in these animals (Kalinichev et al, 2001b). Such an alteration would be consistent with our finding that MS and NH offspring differed in NTX induced drinking suppression.

The mu-opioid systems may not be the only endogenous opioid system affected by maternal separation; components of the enkephalenergic system, which includes the delta opioid receptor, also appears altered. In the nucleus accumbens, maternal separation results in a decrease in pre-proenkephalin mRNA and lower basal levels of Met-enkephalin-like immunoreactivity (Ploj and Nylander, 2003; Vazquez et al., 2005b). These findings along with our own raise the possibility that maternal separation alters the functionality and/or distribution of multiple opioid systems in the brain, any or all of which could result in the observed changes in reward.

Although this study has focused on endogenous opioid systems, when discussing brain reward systems, the impact of the mesolimbic dopamine system should not be overlooked. Maternal separation appears to alter the function of this system, increasing dopamine release from the ventral tegmental area (VTA) while reducing dopamine transporter density in the nucleus accumbens (Meaney et al., 2002; Moffett et al., in press). Alterations within the mesolimbic dopamine system could alter the rewarding properties of sucrose, thus altering its intake. There are known interactions between the two neurotransmitter systems (opioids and dopamine) within the mesolimbic system, and it is believed that the two systems work in tandem to contribute to different aspects of reward (Berridge and Robinson, 2003). Therefore, the changes in intake of sucrose solution could be the result of alterations in neurotransmission mediated by both dopamine and opioids, including possible interactions between them.

In summary, these data extend what was previously known about the effects of early postnatal stress on non-drug reinforcers by showing that the changes in intake of total fluid and sucrose solution hold-up across a range of concentrations. Additionally, they also add to our knowledge of the impact of early-postnatal stress on the endogenous opioid systems, with

the opioid antagonist NTX having a differential effect on consumption in offspring as a function of rearing condition. Sex also appears to play a role, with rearing altering NTX-induced drinking suppression more dramatically in male offspring than in female offspring. These data suggest that early postnatal stress can affect the reinforcing properties of non-drug reinforcers, and also alter the functioning of endogenous opioid systems. These results could be clinically relevant for humans who have suffered early-life trauma or neglect, and exhibit substance abuse disorders as adults.

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