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Behavioral cross-sensitization between morphine-induced locomotion and sodium depletion-induced salt appetite

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ABSTRACT

In general terms, *sensitization* refers to the capacity of a repetitive stimulus of fixed strength to produce a progressive increase in the magnitude of a response with each stimulation. In the addiction literature *cross-sensitization* is the capacity of an agent with abuse potential to sensitize a behavioral response induced by another stimulus. In the present experiments we examined the effects of morphine pretreatment on furosemide-induced saline intake and conversely sodium appetite induction on morphine-induced locomotion. In an initial experiment rats were pretreated with morphine (10 mg/kg, s.c.) or vehicle for 5 days. The rats were then sodium or sham depleted and 24 h later given a sodium appetite test. Sodium depleted rats pretreated with morphine increased saline intake compared to depleted rats initially pretreated with vehicle. In a second experiment rats that were previously depleted and repleted of sodium as compared to sham depleted animals showed enhanced locomotor activity in an open field test when challenged with morphine (1 mg/kg, s.c.). These studies demonstrate that the behavioral responses induced by sodium deficiency and morphine treatment cross-sensitize with one another and suggest that common neural substrates underlie the sensitization of behaviors associated with states induced by morphine and sodium appetite.

1. Introduction

Sodium appetite sensitization is a process where experience with multiple sodium depletions increases ingestion of sodium rich solutions usually in marked excess of what is needed for restoration of a sodium deficit (Bernstein, 2003). Sodium depletion presents a significant homeostatic challenge and induces a strong motivational state to rectify it. It has been hypothesized that sodium appetite sensitization is similar to drug sensitization. After repeated exposures to amphetamine or the state of sodium deficiency, changes in behavioral responses can last at least 4 months (Bernstein, 2003; Falk, 1966; Robinson and Becker, 1986; Sakai et al., 1987; Sakai et al., 1989). Drug sensitization usually occurs after repeated administration of drugs such as nicotine, amphetamine, cocaine, morphine, and caffeine and is behaviorally expressed as increased locomotion in an open field test or increased self-administration of a drug (Celik et al., 2006; Crombag et al., 2000; Crombag et al., 2002; Hall et al., 2008; Liu et al., 2005; Lodge and Grace, 2008; Morgan et al., 2006).

Repeated exposures to any of the aforementioned drugs causes animals to become particularly sensitive to the effects of another drug, a phenomenon known as cross-sensitization (Stewart and Badiani, 1993). This effect has been observed with amphetamine and cocaine and between morphine and amphetamine (Kalivas and Weber, 1988; Liu et al., 2007; Pierce and Kalivas, 1995; Vanderschuren et al., 1999a). These drugs not only cross-sensitize with other drugs but also cross-sensitize with naturally rewarding behaviors such as feeding and wheel running. For example, rats given access to running wheels for 1, 2, or 4 weeks show an increased preference for a 10% ethanol solution (Werme et al., 2002) demonstrating a cross-sensitization effect between running and ethanol preference. Food deprivation and drugs of abuse such as cocaine and amphetamine have also been shown to cross-sensitize (Cabeza et al., 2004; Carr, 2007). Hungry mice that were given a novel food (i.e., sweetened, Noyes® pellets) in a runway increased their activity in the presence and absence of this reward and also consumed more pellets when given ad libitum access in the runway where they had received the novel food. Exposure to cocaine (10 mg/kg) or morphine (20 mg/kg) increased activity in these mice, and the administration of naltrexone suppressed cross-sensitization to cocaine (Le Merrer and Stephens, 2006). Locomotor

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activity induced by amphetamine was significantly higher in rats exposed to an alternating schedule of 10% sucrose and food deprivation than in rats with ad libitum access to 10% sucrose (Avena and Hoebel, 2003a; Avena and Hoebel, 2003b).

Studies have shown that prior exposure to the psychostimulant, amphetamine, sensitizes the response to sodium depletion (Clark and Bernstein, 2004) producing behavioral plasticity indicative of cross-sensitization. The ability of other drugs to cross-sensitize with sodium appetite has not been tested. The current experiments were designed to address whether morphine, an opiate, will also cross-sensitize with sodium appetite-induced responses. The first experiment was designed to test if prior treatment with morphine produced enhanced intake of a hypertonic saline solution following a sodium depletion challenge. The second experiment explored the reciprocity of sensitization by first sodium depleting rats multiple times and then giving an open field test following a morphine injection. The final experiment was designed to see if morphine treatment interposed between two sodium depletions would further augment 0.3 M saline intake during the second sodium depletion challenge beyond that produced by the initial saline depletions. The primary hypothesis tested by these studies is that sodium appetite induced by a sodium deficit and the locomotor activation produced by morphine will show reciprocal cross-sensitization.

2. Methods

2.1. Animals

Male Sprague–Dawley rats weighing 250–300 g prior to experimentation were adapted to the laboratory for 1 week prior to testing. Rats were given ad libitum access to food (7013 NIH-31 modified rat diet, 0.25% NaCl) and water and were maintained on a 12:12 h light–dark cycle with lights on at 0600 h and off at 1800 h. Temperature was maintained at 22 °C. Rats were adapted to 0.3 M saline and sodium deficient chow (cat # 902902, MP Biomedicals, Santa Ana, CA) for at least 3 days prior to experimentation. Baseline daily intakes of 0.3 M saline were recorded. All experiments were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals, and approved by the University of Iowa Animal Care and Use Committee.

2.2. General methods

2.2.1. Induction of sodium appetite and sodium appetite test

Sodium appetite was induced by 2 subcutaneous injections of furosemide (10 ml/kg), a loop diuretic that promotes a rapid natriuresis/diuresis (Sakai et al., 1987; Sakai et al., 1989). Rats were either sodium or sham depleted using furosemide or isotonic saline, respectively, with 2 injections spaced 1 h apart. Body weights before and after injection and acute urine volumes (3 h postinjection) were recorded. Diuresis was confirmed by an animal losing at least 15 g of body weight. Rats were given access to sodium deficient chow and distilled water overnight and intakes of distilled water were recorded. Twenty-two h after the initial injection, rats were given 2 h access to distilled water and 0.3 M saline, and fluid intakes were recorded every 15 min for the first hour and then every 30 min thereafter.

2.3. Experimental protocols

2.3.1. Experiment 1: the effects of daily pretreatment of morphine for 5 days on sodium appetite

Rats were randomly assigned to one of four groups, $n=9$ per group: 1) vehicle + sham depletion (Ctrl + veh); 2) vehicle + furosemide (Ctrl + furo); 3) morphine + sham depletion (Mor + veh); 4) morphine + furosemide (Mor + furo). Rats were injected with isotonic saline vehicle or morphine (10 mg/kg, s.c., Sigma Aldrich, St. Louis, MO) once daily for 5 days prior to sham/sodium depletion. The dose of morphine was based on previously published work (Cunningham et al., 1997). Forty-eight h after the last morphine injection, rats were sham or sodium depleted. Twenty-four h after the first furosemide injection, rats were given a 2 h sodium appetite test and total saline and water intakes were compared between all groups.

2.3.2. Experiment 2: the effects of multiple sodium depletions on locomotor activity in rats given an acute morphine challenge

Rats were randomly assigned to one of four groups: 1) sham depletion + vehicle (SV), $n=10$; 2) sham depletion + morphine (SM), $n=9$; 3) furosemide + vehicle (FV), $n=8$; and 4) furosemide + morphine (FM), $n=7$. Rats were sham or sodium depleted 3 times with each sham or sodium depletion spaced one week apart. A 2 h sodium appetite test was given after each sham or sodium depletion. A criterion of a 30% increase (Na et al., 2007) in 0.3 M saline intake on the third sodium appetite test vs. the initial test was required to be included in the statistical analysis (see Fig. 1A and B for 0.3 M saline and water intakes during the sodium appetite tests). This criterion was based on previously published work demonstrating that rats depleted of sodium multiple times increased ingestion of 0.3 M saline by 30% (Na et al., 2007). Using this criterion, 3 rats from the FV group and 3 rats from the FM group were excluded from statistical analyses. Twenty-four h after the third sodium appetite test, rats

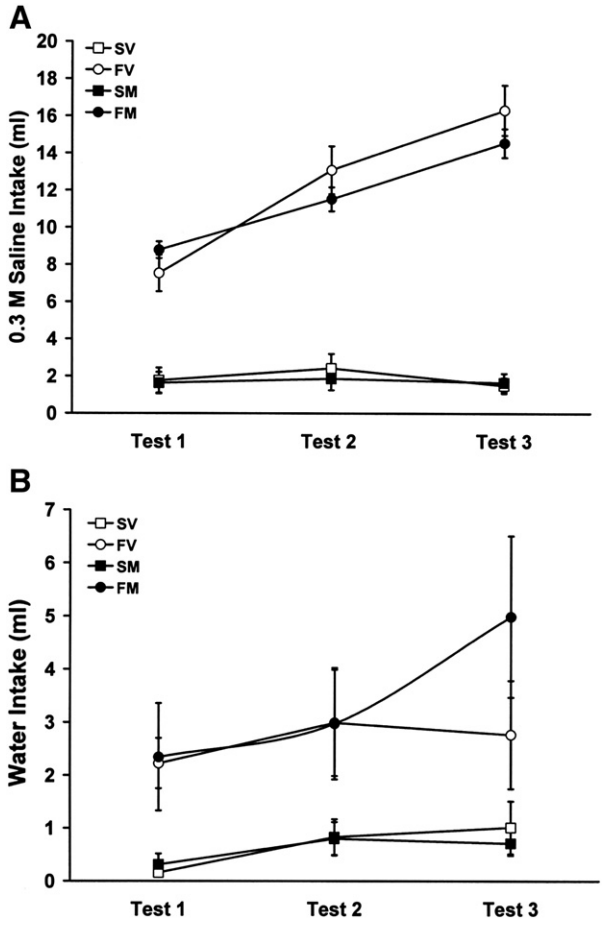

Fig. 1. Total 0.3 M saline and water intakes (±SEM) during a sodium appetite test in rats given multiple sham/sodium depletions. Rats were given 3 separate sham/sodium depletions and 3 subsequent sodium appetite tests after which rats were given either vehicle or morphine (1 mg/kg, s.c.) and then placed in an open field. A. A 30% increase in saline intake during the third sodium depletion challenge was the criterion to be included in further analyses. On average, FM and FV rats increased saline intake 69% and 145%, respectively, from the third sodium depletion to the first. B. Water intakes across three sham/sodium depletion challenges were recorded. SV: sham depletion + vehicle; FV: furosemide + vehicle; SM: sham depletion + morphine; FM: furosemide + morphine.

were adapted two times for 15 min each to an open field (99.1 cm by 66 cm by 30.5 cm). Following adaptation, rats were given either a vehicle (isotonic saline, s.c.) or morphine (1 mg/kg, s.c.) injection; the morphine dose was chosen based on the work of Kalinichev et al. (2004). Kalinichev et al. (2004) demonstrated that an acute systemic injection of 10 mg/kg of morphine depressed locomotor activity while 1 mg/kg revealed locomotor sensitization after rats received daily treatments of 10 mg/kg of morphine. Total distance traveled and rearing were monitored for 3 h (Trujillo et al., 2004; Vanderschuren et al., 1997; Vanderschuren et al., 2001) using Ethovision software version 3 (Noldus Information Technology, Wageningen, The Netherlands). The last 80 min of the test were analyzed based on the work of Powell and Holtzman (2001) who discovered that peak responding occurred between 1.5 and 2 h of a 6 h test. Cumulative distance and cumulative rearing were analyzed and compared between groups.

2.3.3. Experiment 3: the effects of morphine treatment between sodium depletions on sensitization of sodium appetite

Rats were randomly assigned to one of four groups, $n=5$ per group: 1) vehicle + sham depletion (Ctrl + veh); 2) vehicle + furosemide (Ctrl + furo); 3) morphine + sham depletion (Mor + veh); 4) morphine + furosemide (Mor + furo). Rats were sham or sodium depleted and given a 2 h sodium appetite test the next day. Forty-eight h after sham or sodium depletion, rats were injected daily with either vehicle or morphine (10 mg/kg, s.c.) for 5 days. Twenty-four h after the last vehicle or morphine injection, rats were given a second sham/sodium depletion and given a sodium appetite test the following day.

2.4. Data analysis

Mean 0.3 M saline and water intakes for Experiment 1 were compared using a one-way ANOVA. Planned comparisons were analyzed using a Fisher's LSD test for post-hoc analyses. Mean distance traveled and rearing for Experiment 2 were compared using a one-way ANOVA. Post-hoc comparisons were analyzed using a Fisher's LSD. Mean 0.3 M saline and water intakes were compared for Experiment 3, in which rats were depleted multiple times, using a repeated measures ANOVA with time as the within group factor and treatment as the between group factor. Paired *t*-tests were used to compare intake between the first and second sodium depletions, if the interaction effect was significant. A probability value of $p<0.05$ was considered statistically significant. Additional *t*-tests with a Bonferroni correction were used to compare 0.3 M saline and water intakes between morphine treated sodium depleted rats and vehicle treated sodium depleted rats.

3. Results

3.1. Experiment 1: the effects of daily pretreatment of morphine for 5 days on sodium appetite

3.1.1. Total 0.3 M saline intake after furosemide or sham depletion in rats pretreated with vehicle/morphine (10 mg/kg) for 5 days

0.3 M saline intake after sodium or sham depletion in rats pretreated with vehicle or morphine is displayed in Fig. 2A. 0.3 M saline intake was significantly different after morphine pretreatment, $F(3,35)=82.381$, $p<0.05$. There were significant differences between sodium depleted rats given daily morphine pretreatment and all other groups [$t(16)=14.95$, $p<0.01$ vs. Ctrl + veh; $t(16)=2.82$, $p<0.01$ vs. Ctrl + furo; $t(16)=11.41$, $p<0.01$ vs. Mor + veh], with Mor + furo rats drinking significantly more 0.3 M saline during the 2 h intake test. Also, as would be expected, Ctrl + furo rats drank significantly more than Ctrl + veh, $t(16)=10.12$, $p<0.01$ and Mor + veh, $t(16)=7.53$, $p<0.01$ groups. There were no significant differences in saline intake between Ctrl + veh and Mor + veh groups.

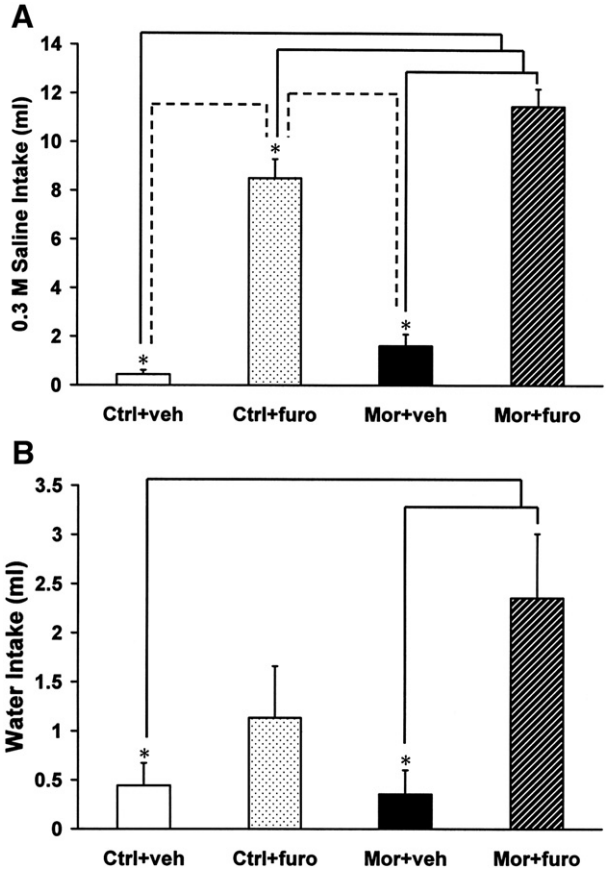

Fig. 2. Total 0.3 M saline and water intakes (±SEM) after 5 days of morphine pretreatment in sham/sodium depleted rats. Rats were given vehicle or morphine (10 mg/kg, s.c.) daily for 5 days and then were either sodium or sham depleted. Total water and 0.3 M saline consumed during a 2 h sodium appetite test was recorded and compared across groups. A. Total 0.3 M saline intake (±SEM) after furosemide or sham depletion in rats pretreated with vehicle/morphine (10 mg/kg) for 5 days. Sodium depleted rats given morphine pretreatment consumed more saline than the other 3 groups. B. Total water intake (±SEM) after furosemide or sham depletion in rats pretreated with vehicle/morphine (10 mg/kg) for 5 days. Sodium depleted rats given morphine pretreatment consumed more water than sham depleted groups. Ctrl + veh: vehicle + sham depletion; Ctrl + furo: vehicle + furosemide; Mor + veh: morphine + sham depletion; Mor + furo: morphine + furosemide. *$p<0.05$ as compared to Mor + furo group.

3.1.2. Total water intake after furosemide or sham depletion in rats pretreated with vehicle/morphine (10 mg/kg) for 5 days

Water intake in sodium/sham depleted rats pretreated with morphine or vehicle are displayed in Fig. 2B. Water intake was significantly different in rats pretreated with vehicle or morphine, $F(3,35)=4.186$, $p<0.05$ with Mor + furo rats consuming more distilled water during a 2 h intake test than either sham depleted groups, $t(16)=2.77$, $p<0.01$ vs. Ctrl + veh group; $t(16)=2.87$, $p<0.01$ vs. Mor + veh group. However, sodium depleted rats pretreated with morphine did not differ from sodium depleted rats given saline vehicle injections indicating that morphine pretreatment preferentially increased 0.3 M saline intake.

3.2. Experiment 2: the effects of multiple sodium depletions on locomotor activity in rats given an acute morphine challenge

3.2.1. Total distance traveled in an open field in rats sham or sodium depleted 3 times and then given morphine or vehicle prior to an open field test

Total distance over 3 h in an open field test was analyzed in rats given three sodium/sham depletions followed by a morphine or vehicle challenge (Fig. 3A). A one-way ANOVA indicated that there was a significant difference among groups, $F(3,33)=10.275$, $p<0.05$.

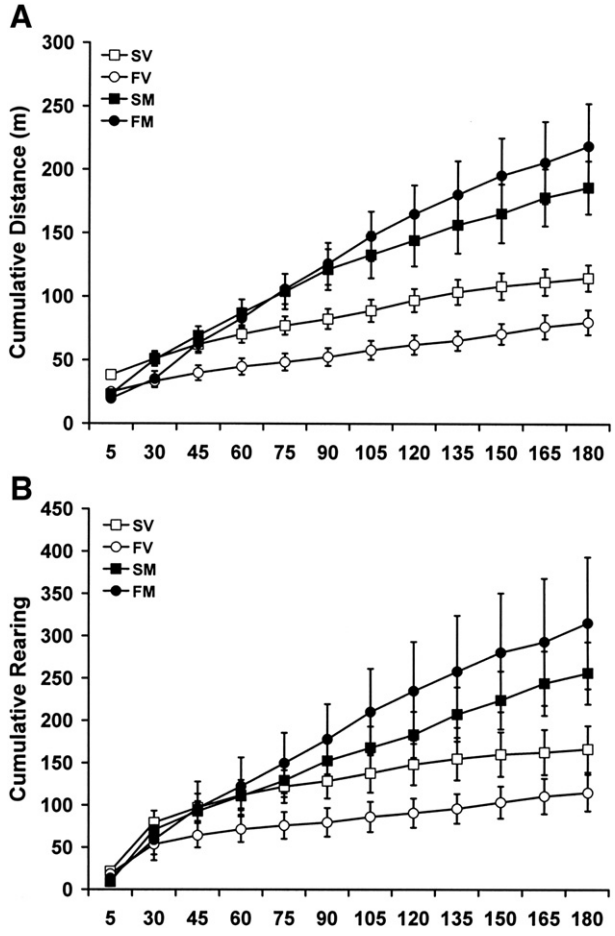

Fig. 3. Total cumulative distance and rearing (±SEM) in 3 h open field test after morphine/vehicle treatment in sham/sodium depleted rats. Rats were given 3 separate sham/sodium depletions and 3 subsequent sodium appetite tests after which rats were given either vehicle or morphine (1 mg/kg, s.c.) and then placed in an open field for 3 h. Total rearing and distance (m) behavior were recorded in 5 min bins over the 3 h period. A. Total cumulative distance (m) traveled over 3 h (±SEM). Distance over the 3 h period was not significantly different between SM and FM groups. B. Total cumulative rearing over 3 h (±SEM). Rearing over the 3 h period was not significantly different between SM and FM groups. SV: sham depletion + vehicle; FV: furosemide + vehicle; SM: sham depletion + morphine; FM: furosemide + morphine.

Post-hoc analyses revealed that there was a significant difference between rats treated with vehicle vs. morphine treated rats ($p<0.05$) but that there was no difference between rats with a history of sodium depletions vs. those without a history of sodium depletions ($p<0.05$). A separate analysis of the last 80 min of the test was conducted as past studies have shown that peak responding to morphine (1 mg/kg) occurs 1.5–2 h after administration (Kalinichev et al., 2004; Powell and Holtzman, 2001; Vanderschuren et al., 1997; Vanderschuren et al., 2001). There was a significant difference in cumulative distance traveled during the last 80 min of the test, $F(3,33)=9.437$, $p<0.05$. Rats with a history of sodium depletions traveled a greater distance than the other three groups after morphine administration, $t(15)=3.83$, $p<0.01$ vs. SV; $t(13)=3.58$, $p<0.01$ vs. FV; $t(14)=1.55$, $p<0.04$ vs. SM (Fig. 4A and B). Sham depleted rats given morphine also traveled a greater distance than SV, $t(17)=3.42$, $p<0.02$, and FV, $t(15)=3.34$, $p<0.01$, groups.

3.2.2. Total rearing in an open field in rats sham or sodium depleted three times and then given morphine or vehicle prior to an open field test

A one-way ANOVA revealed significant differences in rearing, $F(3,33)=4.438$, $p<0.05$. There were significant differences between morphine treated rats and vehicle treated rats, FM vs. SV $t(15)=3.17$, $p<0.01$; FM vs. FV $t(13)=2.74$, $p<0.01$; SM vs. SV $t(17)=2.96$, $p<0.02$; SM vs. FV $t(15)=2.52$, $p<0.03$. Additional analyses did not reveal significant differences between rats with a history of sodium depletions and those without a history of sodium depletions (Figs. 3B, 5A, and B).

3.3. Experiment 3: the effects of morphine treatment between sodium depletions on sensitization of sodium appetite

3.3.1. Total 0.3 M saline intake after daily treatment with vehicle or morphine in sham depleted or furosemide depleted rats

A repeated measures ANOVA revealed a significant group (e.g., furosemide vs. sham depletion) by time (e.g., 0.3 M saline intake during the first sodium appetite test vs. 0.3 M saline intake during the second sodium appetite test) interaction effect, $F(3,16)=3.897$, $p<0.03$. Paired *t*-tests revealed that both groups of sodium depleted rats increased 0.3 M saline intake from the first to the second sodium depletion, $t(4)=3.73$, $p<0.01$ for FM and $t(4)=3.37$, $p<0.01$ for SM rats. However, sodium depleted rats given morphine interposed between the 2 sodium depletions did not drink significantly more than sodium depleted rats given vehicle between the 2 sodium depletions (Fig. 6A).

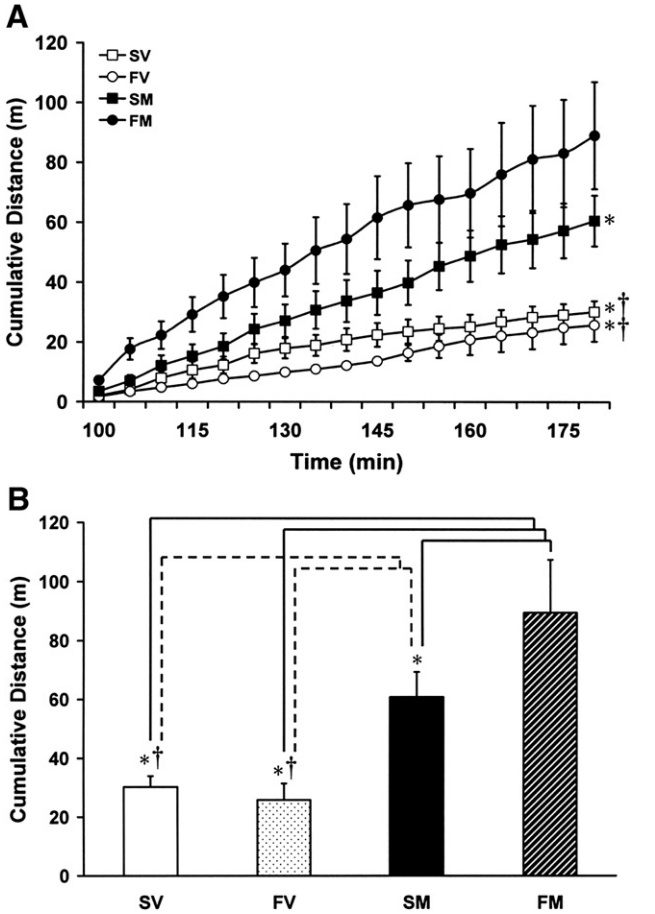

Fig. 4. Total distance traveled (±SEM) during the last 80 min of 3 h open field test in sham/sodium depleted rats given morphine/vehicle treatment. Rats were given 3 separate sham/sodium depletions and 3 subsequent sodium appetite tests after which rats were given either vehicle or morphine (1 mg/kg, s.c.) and then placed in an open field for 3 h. A. Rats depleted of sodium multiple times showed increased locomotion during the last 80 min of the open field test after a systemic morphine injection compared to sham depleted rats given morphine. B. Bar graph depicting total distance (±SEM) traveled during the last 80 min of the test. *$p<0.05$ compared to FM rats. †$p<0.05$ compared to SM rats. SV: sham depletion + vehicle; FV: furosemide + vehicle; SM: sham depletion + morphine; FM: furosemide + morphine.

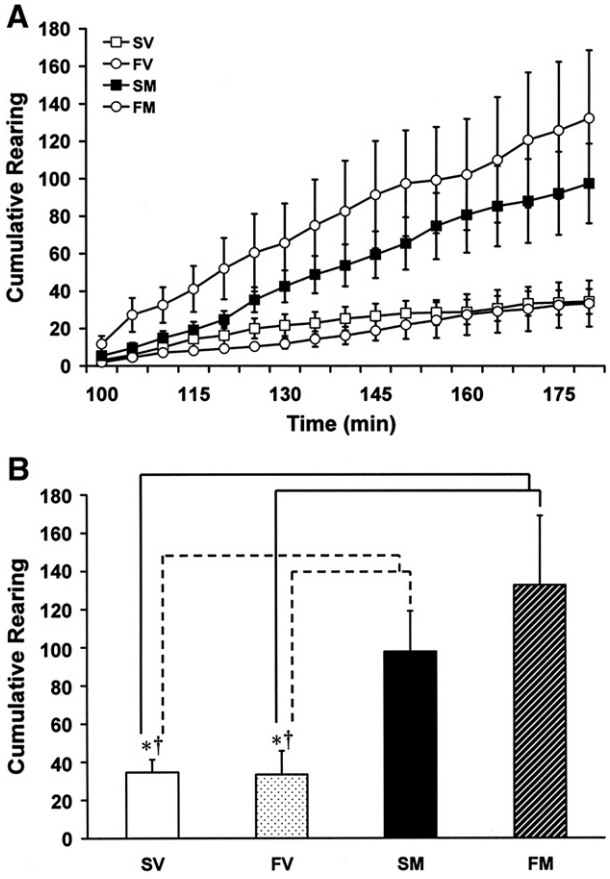

Fig. 5. Total cumulative rearing (±SEM) during last 80 min of 3 h open field test in sham/sodium depleted rats given morphine/vehicle treatment. Rats were given 3 separate sham/sodium depletions and 3 subsequent sodium appetite tests after which rats were given either vehicle or morphine (1 mg/kg, s.c.) and then placed in an open field for 3 h. A. Rearing over the last 80 min of the test was not significantly different between SM and FM groups. B. Bar graph depicting the mean (±SEM) cumulative rearing for each group during the last 80 min of the test. FM and SM rats reared more than SV and FV groups, *$p<0.05$ compared to FM; †$p<0.05$ compared to SM. SV: sham depletion + vehicle; FV: furosemide + vehicle; SM: sham depletion + morphine; FM: furosemide + morphine.

3.3.2. Total water intake after daily treatment with vehicle/morphine in sham depleted or furosemide depleted rats

There were no significant differences in acute water intake after multiple sodium or sham depletions between groups (Fig. 6B).

4. Discussion

The present experiments examined cross-sensitization between morphine treatment and depletion-induced sensitization of sodium appetite. The results from Experiments 1 and 2 indicate that sodium appetite sensitization reciprocally cross-sensitizes with morphine as evidenced by both 0.3 M saline intake tests and locomotor activity tests. Pretreatment with morphine for 5 days resulted in a significant increase in hypertonic saline consumption after an initial sodium depletion (Experiment 1). Rats that expressed sodium appetite sensitization demonstrated increased locomotion during a subsequent morphine challenge but only during the last 80 min of a 3 h test (Experiment 2). Finally, treatment with morphine between two sodium depletions did not impact saline intakes during a second sodium depletion (Experiment 3), which indicates that behavioral sensitization induced by the drug and by sodium deficient states are not additive. Taken together the current studies extend the findings of Clark and Bernstein (2004) by demonstrating that another class of drugs with abuse potential, the opioid analgesics, and sodium appetite reciprocally cross-sensitize.

These data suggest that morphine and sodium appetite may affect common brain mechanisms to induce these changes in behavior. One mechanism that may mediate the reciprocal cross-sensitization between morphine and sodium appetite is the neurochemical modification of dopaminergic signaling in brain regions important for the processing of rewarding stimuli, e.g., the nucleus accumbens (NAc). Cocaine and amphetamine treatment increase extracellular levels of dopamine in the NAc (Carboni et al., 1989; Weiss et al., 1992) as does the administration of morphine in drug naïve rats and in rats chronically treated with morphine (Spanagel et al., 1993). Hungry rats consuming food as well as satiated rats given access to palatable solutions (e.g., 0.3 M sucrose) or foods such as short cakes or Fonzies® (cheese snacks similar to Cheetos®) show elevated levels of dopamine release in the NAc (Hajnal and Norgren, 2001; Martel and Fantino, 1996; Radhakishun et al., 1988; Tanda and Di Chiara, 1998). Similarly, sodium depleted rats consuming hypertonic saline also show evidence of elevated levels of dopamine release in the NAc (Hoebel et al., 1989). Morphine binds preferentially to μ-opioid receptors and stimulation of μ-opioid receptors in the ventral tegmental area (VTA) results in increased extracellular dopamine in the NAc (Spanagel et al.,

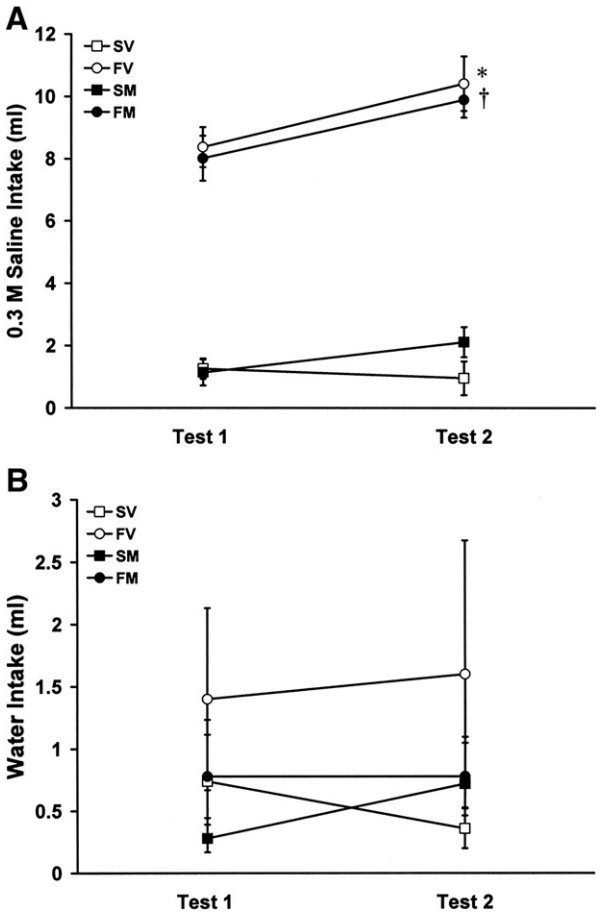

Fig. 6. Total 0.3 M saline and water intakes (±SEM) in rats given 5 days of morphine/vehicle between 2 sham or sodium depletions. Rats were sham or sodium depleted and given a sodium appetite test. Rats were then given daily injections of vehicle or morphine (10 mg/kg, .s.c) for 5 days. Rats were given a second sham/sodium depletion 48 h after the last morphine/vehicle injection and then were given a sodium appetite test. Total water and 0.3 M saline intakes (±SEM) over 2 h were recorded. A. Both groups of sodium depleted rats drank significantly more 0.3 M saline during the second sodium appetite test than during the first sodium appetite test. *$p<0.05$ for FV rats; †$p<0.05$ for FM rats. However, sodium depleted rats given morphine between 2 sodium depletions did not drink significantly more than sodium depleted rats given vehicle between 2 sodium depletions. B. There was no significant change in acute water intake after multiple furosemide treatments in rats given daily morphine injections between sodium depletions. SV: sham depletion + vehicle; FV: furosemide + vehicle; SM: sham depletion + morphine (10 mg/kg); FM: furosemide + morphine (10 mg/kg).

1993). The doses of morphine used in the current experiments while administered systemically were sufficient to act centrally as experiments have shown that morphine is actively transported across the blood brain barrier at a 10 mg/kg dose (Bouw et al., 2000). Thus, in the context of the present experiment, morphine may have indirectly increased extracellular dopamine by binding centrally to μ-opioid receptors thereby inducing neurochemical sensitization within nuclei associated with the mesolimbic dopamine system. The increased saline intake in rats pretreated with morphine was similar to that seen in rats depleted of sodium multiple times (Experiment 1). Sodium depleted rats were not under the influence of morphine during the sodium appetite test which may be indicative of relatively long-term changes in the central nervous system occurring as a result of morphine pretreatment. Thus, morphine and sodium appetite may interact at neural sites associated with the mesolimbic dopamine system to induce sensitized behavioral responses.

Rats that expressed a sensitized sodium appetite after multiple sodium depletions showed increased locomotion during the last 80 min of a 3 h test compared to rats without a history of sodium depletions (Experiment 2). Kalinichev et al. (2004) found that peak responding to this dose of morphine occurred 1.5–2 h after the beginning of a 6 h test ($t_{1/2} = 115$ min). Thus the current data are consistent with these past findings. In addition, the locomotor sensitization seen in rats depleted of sodium multiple times is consistent with reports demonstrating an increase in locomotion in response to an amphetamine challenge (Clark and Bernstein, 2004; Roitman et al., 2002).

Another possible explanation that could account for the cross-sensitization between morphine and sodium appetite is that sodium depletion may be a significant "stressor" for the animal. Cross-sensitization between stressors and drugs of abuse is a well established phenomenon (Covington and Miczek, 2005; Piazza et al., 1990). However, stress does not necessarily have a global effect on sensitization as different stressors have variable effects on behavioral responses to drugs. For example, Covington and Miczek (2005) have demonstrated that social defeat stress induces sensitization to cocaine and amphetamine as demonstrated by self-administration and locomotor activity paradigms, respectively. However, rats that were exposed to aggressive bouts did not express sensitization-like behavior to cocaine or amphetamine. Tantamount to these findings is that plasma corticosterone levels were elevated after exposure to social defeat stress and aggressive behavior, underscoring the idea that not all stressors exert the same behavioral or physiological effects nor do all stressors reliably cross-sensitize with drugs of abuse. In addition, arguably, sodium depletion may not be a stressor in a traditional sense as corticosterone, a hormone used as an indicator of stress, is not elevated in response to sodium depletion (Roitman et al., 1999).

While morphine was effective at inducing elevated saline intake in naïve rats subsequently challenged with sodium depletion, morphine treatment interposed between the two sodium depletions did not result in enhanced saline intake over that of vehicle treated rats (Experiment 3). Because rats demonstrated an increased saline intake after morphine pretreatment, it may be necessary that rats are naïve in order to enhance the cross-sensitized saline drinking response. Another possibility is that the saline intake in both groups of sodium depleted rats during the second sodium depletion may have reached a response ceiling thereby obscuring any additional effect morphine treatment might have had on sodium appetite.

Past studies have demonstrated cross-sensitization between amphetamine and morphine with morphine pretreatment enhancing amphetamine-induced locomotor activity. Vanderschuren et al. (1999b) found that systemic pretreatment with amphetamine does not induce cross-sensitization with morphine although morphine pretreatment enhances locomotor activity to subsequent amphetamine challenges. These data suggest that an order effect may be a determining factor in the development of sensitization. It may be necessary to expose the animal first to morphine to produce cross-sensitization to amphetamine. This may be relevant to the current findings since we found that rats exposed first to morphine have a sensitized sodium appetite (Experiment 1) unlike those rats exposed first to a sodium depletion (Experiment 3). Route of administration could impact the development of sodium appetite sensitization. When amphetamine is infused into the VTA, rats show cross-sensitization to morphine-induced locomotor activity (Bjijou et al., 1996; Vezina and Stewart, 1990). Thus, directly infusing morphine into the VTA may be necessary to potentiate the development of sodium appetite sensitization.

Taken together these data indicate that a significant homeostatic challenge such as sodium depletion cross-sensitizes with morphine. Researchers that have explored the sensitization between amphetamine and sodium appetite have posited that natural and synthetic rewards act on the same neural substrates to invoke these changes in behavior (Clark and Bernstein, 2004; Roitman et al., 2002). Past studies have demonstrated a change in the dendritic morphology of medium spiny neurons in the NAc of rats with a history of multiple sodium depletions similar to those changes seen in animals sensitized to amphetamine (Roitman et al., 2002). Morphine however induces a decrease in dendritic branching and the number of dendritic spines on medium spiny neurons in the NAc shell suggesting that morphine and amphetamine affect synaptic reorganization in different ways (Robinson and Kolb, 1999a; Robinson and Kolb, 1999b).

In prior work we found a substantial increase in Fos-immunoreactivity in the NAc as well as in the basolateral amygdala in response to multiple sodium depletions (Na et al., 2007). Both the NAc and the basolateral amygdala have been heavily implicated in the neural plasticity underlying drug sensitization. These data indicate that nuclei associated with processing information related to reward may be implicated in facilitating the behavioral plasticity seen in rats depleted of sodium multiple times. Based on these past findings, it would not be unlikely that the cross-sensitization between sodium appetite as produced by multiple sodium depletions and morphine could also alter the neurochemistry with the NAc and/or the basolateral amygdala. Additional studies will need to be conducted to clarify this matter. The current data demonstrate that morphine, similar to amphetamine, cross-sensitizes with sodium deficiency-induced sodium appetite to alter locomotion and saline ingestion.

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